UNIVERSITÉ DE FRIBOURG - FACULTÉ DES SCIENCES
DÉPARTEMENT DE MÉDECINE
UNITÉ DE PHYSIOLOGIE

Développement d'un Modèle Expérimental d'Implant Cochléaire Incorporant des Propriétés d'Adaptation Neuronale.

THÈSE D'AGRÉGATION

présentée à la Faculté des sciences de l'Université
de Fribourg (Suisse)
pour l'obtention de la venia legendi

par

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Né le 5 juin 1972 à Nantes (France)

Fribourg, 2006

Acceptée par la Faculté des sciences de l'Université de Fribourg.

Fribourg, le 29 novembre 2006

Le doyen, Prof. Titus A. Jenny

REMERCIEMENTS

Les travaux présentés dans cette thèse ont été effectués à l'Unité de Physiologie du Département de Médecine de l' Université de Fribourg en Suisse. Je remercie son directeur le Prof. M.D. Jean-Pierre Montani ainsi que le responsable du laboratoire Prof. Dr. Eric-Michel Rouiller pour leur soutien et la confiance qu'ils me témoignent depuis mon arrivée au sein de cet institut. Les excellentes conditions mises à ma disposition ont permis à des travaux originaux de voir le jour et ont concouru à la réalisation de cette thèse.

Plus anciennement, j'aimerais remercier le Dr. Catherine de Waele du Laboratoire de Neurobiologie des Réseaux Sensorimoteurs de Paris (France) pour m'avoir fait découvrir la recherche fondamentale en neurosciences. J'ai pu développer avec son concours des aptitudes scientifiques que j'utilise chaque jour. J'exprime également toute ma gratitude au Dr. Jean-Marie Aran du Laboratoire de Biologie Cellulaire et Moléculaire de l'Audition de Bordeaux (France) pour ses conseils éclairés et son soutien constant au cours de mes travaux en audiologie. Enfin, je tiens à remercier le Dr. Pierre Campo du Laboratoire de Neurotoxicité et Immunotoxicité de Vandœuvre (France) pour m'avoir proposé de travailler en toxicologie. Tous m'ont apporté des compétences scientifiques durables dans un champ interdisciplinaire.

Je voudrais particulièrement remercier le Dr. Marco Pelizzone du Centre Romand d'Implants Cochléaires de l'Hôpital Cantonal Universitaire de Genève (Suisse) qui m'a fait découvrir le monde des implants cochléaires. J'espère que nos échanges et collaborations continueront.

Mes remerciements s'adressent également à tous mes collègues de Suisse et de France avec qui j'ai eu l'occasion de travailler ainsi qu'aux étudiants et au personnel technique et administratif pour leur aide quotidienne si précieuse.

Enfin, mes plus profonds remerciements s'adressent à mes parents, à mon frère et à mon épouse pour le soutien constant et les encouragements, ainsi qu'à ma fille Angélique pour sa joie communicative.

SOMMAIRE

RÉSUMÉ

Les travaux de recherche rapportés dans la présente thèse s'intéressent à la fonction auditive qu'elle aborde sous l'angle de la réhabilitation à l'aide d'une prothèse implantée dans la cochlée : l'implant cochléaire. Ce type de prothèse est utilisé depuis une vingtaine d'années et fournit d'ores et déjà à de nombreux patients des conditions d'écoute satisfaisantes, exceptées dans des ambiances bruyantes. Dans l'optique d'améliorer les performances de ces appareils dans le bruit, nous avons mis au point un modèle expérimental chez le rat adulte qui permet d'identifier les fonctions importantes perdues avec les systèmes électriques actuels. Grâce à une comparaison entre les réponses produites par une stimulation naturelle de l'oreille (acoustique) et celles issues de stimulations artificielles (électriques), nous avons montré que les phénomènes d'adaptation neuronale communs à tous les systèmes sensoriels et qui se manifestent par une diminution de l'activité neuronale pendant une stimulation ne sont peu ou pas reproduits lorsque la cochlée est stimulée électriquement. Une nouvelle stratégie de stimulation électrique a donc été développée et est présentée dans ce travail. Sa validation définitive est en cours sur un second modèle expérimental, le singe adulte. Les résultats préliminaires laissent prévoir sur le plan clinique une amélioration significative de la compréhension de la parole en conditions d'écoute difficile chez les patients implantés.

Mots clés : Adaptation, Noyau cochléaire, Implant cochléaire, Potentiel auditif évoqué.

The research projects reported in the present thesis deal with the auditory function and more specifically the topic dedicated to auditory rehabilitation with a prosthesis implanted into the cochlea: the cochlear implant (CI). Such prosthesis was used for about twenty years and already provides to numerous patients satisfactory conditions, excepted in noisy environment. From the point of view to improve the performance of these devices in noise, we developed an experimental model in the adult rat in order to identify normal hearing functions that are lost in electro-auditory hearing with present CI. Based on the comparison between responses obtained by natural stimulation of the ear (acoustic) and those obtained by artificial stimulation (electric), we demonstrated that neuronal adaptation phenomena common to all sensory systems and represented by a decrease of neuronal activity during the stimulation are little or none reproduced when the cochlea is electrically stimulated. Therefore, a new strategy of electrical stimulation has been developed and is described in details in the present work. The final validation is under process in a second experimental model, the adult monkey. From a clinical point of view, preliminary results suggest that a significant improvement in speech intelligibility in noise may be achieved for cochlear implanted patients.

Keywords : Adaptation, Auditory evoked potential, Cochlear implant, Cochlear nucleus.

CHAPITRE 1

INTRODUCTION : Généralités sur les stimulations acoustique et électrique (artificielle) de l'oreille interne

1.1. Stimulation acoustique

Les sensations procurées par les systèmes sensoriels permettent aux êtres vivants de communiquer, avec leur milieu intérieur, avec leurs semblables et avec leur environnement. Ces sensations résultent toujours de l'action d'un stimulus (physique ou chimique) sur un récepteur spécifique et spécialisé. L'évaluation qu'en fait ensuite le système nerveux permet d'accéder à la connaissance de ce stimulus (sensation). Pour le système auditif, le stimulus est une onde acoustique propagée dont les caractéristiques sont modifiées au cours de leur cheminement dans les parties externe et moyenne de l'oreille en vue d'une optimisation de la stimulation. Le récepteur sensoriel proprement dit est situé dans les milieux liquides de l'oreille interne (cochlée) elle-même enchâssée dans l'os temporal où s'effectuent l'analyse et la transduction des vibrations mécaniques. La fonction d'analyse est réalisée grâce à une membrane, la membrane basilaire, qui s'enroule le long de la cochlée et est capable de vibrer en un point précis selon la fréquence du stimulus (codage spatial). Sur cette même membrane, des cellules spécialisées appelées cellules ciliées internes sont responsables de la transduction ou transformation de l'énergie mécanique en influx nerveux. De cette transformation par des processus électrochimiques complexes résultent des courants électriques qui stimulent les fibres du nerf auditif au nombre de 18000 chez le rat[1], 31000 chez le singe[2] et 36000 chez l'homme[3]. Les courants prennent alors la forme de potentiels d'action qui sont émis en une séquence particulière comme par exemple de façon synchronisée avec la phase du stimulus (principe de la périodicité[4]) pour coder la fréquence d'un son. Il est important de noter que ce principe de codage n'est respecté que pour les composantes fréquentielles basses (inférieures à 4000Hz) lorsqu'il s'agit de sons purs[5] ou de sons complexes[6] et pour des fréquences de répétition de train de brèves impulsions (clics) inférieures à 3000Hz[4, 7]. Au-delà de ces limites fréquentielles, le codage de l'information auditive ne repose probablement que sur la sélectivité tonale des fibres individuelles du nerf auditif (codage spatial). Cependant, à l'heure actuelle, on ne connaît toujours pas la part exacte du codage temporel ou spatial que le cerveau utilise. Dans tous les cas, le mode de décharge est identique pour toutes les fibres du nerf auditif et consiste, en réponse à un son pur, en une brusque augmentation du nombre de potentiel d'action au début du stimulus (Fig. 1), suivie d'une diminution progressive puis de l'atteinte d'une valeur constante (pla-

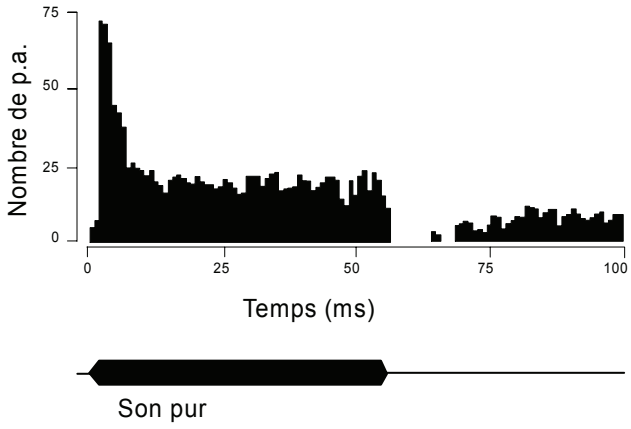

Fig. 1 Histogramme PST (post-stimulus-time-histogram) montrant la distribution des potentiels d'action (p.a.) en réponse à un son pur d'une durée de 50ms. D'après Brown, 1999.

teau) tant que la stimulation acoustique est maintenue[8]. Cette diminution caractéristique du nombre de décharges correspond au phénomène d'*adaptation* dont l'origine est principalement située à la synapse entre les cellules ciliées internes et les afférences dendritiques du nerf auditif[9, 10]. En réponse à un train de clics (brèves impulsions acoustiques), les fibres du nerf auditif présentent également ce phénomène d'adaptation[7, 11] mais avec des pics de décharges distincts pour chaque clic perçu (Fig. 2). En ce qui concerne le codage de l'intensité d'un son, il a été montré[4, 12] que le nombre de potentiels d'action est proportionnel au niveau sonore jusqu'à une certaine intensité à partir de laquelle le taux de décharge est saturé. Compte tenu du fait qu'une fibre seule ne peut coder tout le domaine des intensités audibles (gamme dynamique d'environ 120dB chez l'homme), il apparaît raisonnable de penser que le codage de l'intensité résulte de l'activité d'une population de fibres. Une fois ce premier codage effectué, le pattern d'excitation neuronal correspondant à la nature du stimulus est ensuite transmis au noyau cochléaire qui est le premier relais central des voies auditives. Dans ce noyau, on trouve une grande variété de types cellulaires qui, au contraire des fibres du nerf auditif, présentent une grande variété de réponses[13, 14, 15]. Seule une population de cellules situées dans le noyau cochléaire antéroventral présente un type de réponse semblable à celui des neurones auditifs primaires et ont été désignés pour cela comme type « primaire » (primary-like en anglais). Ces neurones sont particulièrement intéressants à étudier dans notre contexte de recherche puisqu'ils présentent eux aussi des phénomènes d'adaptation[16, 17], de synchronisation avec la phase du stimulus pour des sons purs de basse fréquence[18] et des pics multiples en réponse à des trains de clics[13]. Par ailleurs, ces derniers se distinguent facilement des neurones auditifs primaires par leur latence de réponse qui est d'environ 1ms plus longue, c'est-à-dire comprise entre 2 et 3ms[13]. Enfin, l'information neuronale poursuit son chemin et est transmise à travers différents relais (complexe de l'olive supérieure, noyau du lemnisque latéral, colliculi inférieur et supérieur, thalamus) pour finalement parvenir au cortex auditif où elle est interprétée comme un son. Les phénomènes d'adaptation et de synchronisation existent également dans ces relais mais ils demeurent difficiles à mettre en évidence compte tenu de l'augmentation du nombre de neurones de la périphérie vers le cortex et de la complexité croissante d'étage en étage du codage de l'information.

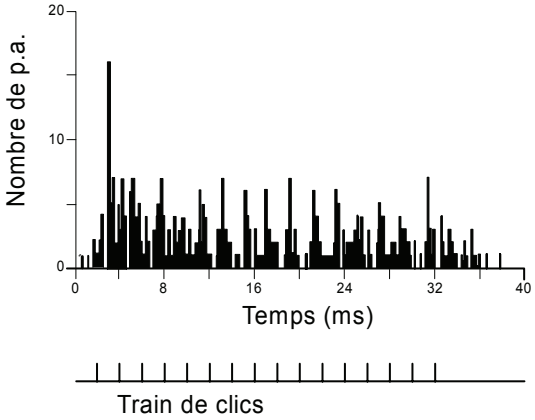

Fig. 2 Histogramme PST (post-stimulus-time-histogram) montrant la distribution des potentiels d'action (p.a.) en réponse à un train de clics d'une durée de 30ms (intervalle entre clic : 2ms). Modifié d'après Wickesberg et Stevens, 1998.

L'étude des différentes composantes de la sensation engendrée par un son (intensité, hauteur tonale, timbre) relève du domaine de la psychoacoustique et a fait l'objet de nombreux travaux normatifs chez l'homme pour établir des valeurs représentatives de l'audition « normale » et ainsi faciliter le diagnostic des déficiences auditives. Cet handicap qui affecte environ 17% de la population mondiale à des degrés divers sans tenir compte des personnes âgées de plus de 65 ans, résulte soit d'une affection de l'oreille externe ou de l'oreille moyenne (surdité de transmission), soit d'une altération de l'oreille interne (surdité de perception), d'un mélange de ces deux types (surdité mixte), soit enfin d'une altération des voies nerveuses auditives (surdité nerveuse). Dans une optique de réhabilitation de la fonction auditive, différents moyens ont été développés et visent de manière générale soit à rétablir la

conduction du son (chirurgie réparatrice de l'oreille externe ou de l'oreille moyenne) soit à modifier le stimulus de telle façon que sous sa nouvelle forme il soit apte à stimuler le système auditif. C'est cette deuxième approche qui nous intéresse puisqu'elle fait appel à des dispositifs prothétiques. Ces derniers peuvent être classés selon la nature de la stimulation et le niveau du système auditif où elle est délivrée :
- dans le conduit auditif externe on délivre un stimulus acoustique amplifié (aide auditive),
- dans l'oreille moyenne on transmet un stimulus mécanique vibratoire pour, soit mettre en mouvement la chaîne tympano-ossiculaire, soit stimuler les parois de l'oreille interne (conduction osseuse),
- dans l'oreille interne on utilise des stimuli électriques pour exciter le système nerveux au moyen d'électrodes placées à proximité des fibres du nerf auditif (implant cochléaire) ou du noyau cochléaire (implants du tronc cérébral).
Dans ce dernier cas, la nature d'une stimulation acoustique est profondément modifiée par un codage sous forme d'impulsions électriques ce qui entraîne pour le sujet appareillé une rééducation souvent longue et requérant une bonne plasticité cérébrale pour s'adapter à un stimulus nouveau.

1.2. Stimulation électrique

Le principe d'un implant cochléaire est basé sur le contournement de différentes structures normalement impliquées dans l'audition telles que l'oreille externe, l'oreille moyenne et une partie de l'oreille interne comprenant les cellules ciliées internes. Cet ensemble est remplacé par 1) un microphone placé à hauteur de l'oreille externe qui a pour fonction de capter les signaux issus de l'environnement sonore et de les transformer en signal électrique, 2) un processeur responsable de la mise en forme du signal (filtrage, intensité) et de la production d'un code en temps réel sous la forme d'impulsions électriques, 3) une ou plusieurs électrodes insérées dans le premier tour cochléaire de manière à être le plus proche possible des fibres afférentes du nerf auditif et de leurs corps cellulaires (ganglion spiral). Le principal défi consiste bien évidemment à produire un code électrique capable de stimuler les neurones auditifs de façon efficace pour générer une information qui sera interprétée comme un son au niveau du cerveau. A l'heure actuelle, ce type de prothèse parvient plus ou moins à restituer l'amplitude et la fréquence d'une stimulation acoustique. Pour l'amplitude, le codage se base sur l'intensité de la stimulation électrique et sur sa durée puisqu'une quantité minimale de charge (intensité du courant multiplié par sa durée) est requise pour modifier le potentiel d'une membrane d'une fibre nerveuse[19] et déclencher des potentiels d'action. Chez le chat, il a été montré[20] que les seuils de réponse les plus faibles étaient obtenus avec de brèves impulsions électriques biphasiques d'une durée de 100µs par phase. Une connaissance de ce paramètre particulier de stimulation est essentielle puisque la gamme dynamique électrique (courbe de croissance du nombre de potentiels d'action en fonction de l'intensité de la stimulation du seuil au maximum) est considérablement réduite (environ 4dB[21]) par rapport à celle obtenue en réponse à une stimulation acoustique (de 20 à 50dB[13]). Ceci a pour conséquence de disposer d'un nombre limité d'intensités et au cas où un mauvais choix de paramètres aurait été effectué, la discrimination pourrait être affectée. En ce qui concerne le codage de la fréquence, les deux mécanismes (temporel et spatial, voir §1.1.) ont été reproduits. Le premier, qui s'appuie sur la synchronisation des décharges neuronales avec la phase du stimulus, a pu être développé à partir d'une stimulation électrique pulsée à la même période que la stimulation acoustique. Pour ce faire, une seule électrode intracochléaire (stimulation monopolaire) est implantée en un point de la cochlée et le courant injecté diffuse pour stimuler une large population de neurones auditifs. Toutefois, les données montrent

qu'une stimulation électrique sinusoïdale ou pulsée au-dessus de 500Hz ou 500 pulses par secondes (pps) ne produit pas les mêmes patterns de décharges que ceux obtenus en réponse à une stimulation acoustique de la même fréquence[22]. Ce résultat peut être relié à la période réfractaire absolue des fibres du nerf auditif qui impose un intervalle de temps minimum (0,5 à 1ms[23, 24]) entre deux stimulations pour pouvoir générer des potentiels d'action synchronisés. La conséquence pour la majorité des patients implantés avec ce type d'implants (House/3M[25] et Vienna/3M[26]) dans les années 70 a été de ne pas pouvoir extraire d'information fréquentielle au-dessus de 1000Hz alors qu'il existe des informations importantes dans le langage jusqu'à 4000Hz. On peut noter cependant que comme ces patients étaient capables de discriminer les voyelles des consonnes, d'avoir une idée de la prosodie et de différencier certaines voyelles et certaines consonnes, le langage fut reconnu et une forme de communication rétablie[27, 28]. Une deuxième stratégie de codage de la fréquence fondée sur le code spatial a motivé le développement d'implants cochléaires multicanaux afin de stimuler différentes fibres du nerf auditif à différents endroits de la cochlée. Pour cela un groupe d'électrodes (de 4 à 22 dans les dispositifs actuels) sont implantées le long du canal cochléaire dans une configuration monopolaire (électrode de référence à l'extérieur de la cochlée) ou bipolaire (électrodes active et de référence proches l'une de l'autre) et reçoivent une stimulation en fonction de la fréquence du signal : les électrodes situées à la base de la cochlée sont stimulées avec les signaux de haute fréquence tandis que celles localisées à l'apex sont stimulées par les signaux de basse fréquence. En ce qui concerne le signal proprement dit, deux types de stratégies ont été mis au point. La première utilise un processeur qui décompose le signal d'entrée en ses différentes composantes fréquentielles puis délivre une enveloppe sous forme analogique (le signal acoustique sous forme électrique) ou pulsée (trains de brèves impulsions électriques) aux électrodes appropriées. C'est le principe des processeurs commercialisés par les sociétés Advanced Bionics[29] et MED-EL[30]. La seconde stratégie charge le processeur de reconnaître certaines caractéristiques essentielles du langage comme par exemple la fréquence fondamentale (F0) et les premiers formants (F1 et F2) qu'il envoie ensuite sous forme de trains de brèves impulsions électriques sur les électrodes appropriées : c'est le principe des processeurs commercialisés par la société Cochlear[31]. Les études d'évaluation menées chez les patients implantées avec les systèmes multiélectrodes font état d'une compréhension de la parole acceptable pour la majorité d'entre eux[32, 33] et une augmentation significative de leur qualité de vie[34]. Cependant, à l'heure actuelle, le bénéfice apporté par les implants cochléaires est sérieusement limité par le bruit et, la mauvaise reconnaissance de la parole dans une ambiance bruyante (rue, restaurant, réunion familiale, etc.) reste la principale raison d'insatisfaction pour la majorité des utilisateurs[35]. Différentes hypothèses de travail peuvent être envisagées comme par exemple l'implantation cochléaire bilatérale dont les premiers résultats sont très prometteurs[36] ou la restauration de propriétés physiologiques encore absentes dans les stratégies de codage comme le sont les phénomènes d'adaptation neuronale. Pour notre part, nous pensons que la restitution des fonctions d'adaptation normalement présentes à la synapse entre les cellules ciliées internes et les afférences dendritiques peut améliorer de façon significative la perception en condition d'écoute difficile. En effet, nous postulons que ce phénomène réduit le nombre de décharges des neurones auditifs pendant la durée d'une stimulation et permet à ces mêmes neurones d'être rapidement mobilisés dans le cas de l'apparition d'un nouveau stimulus. Les travaux réalisés chez l'animal[37, 38, 39, 40] montrent qu'en réponse à une stimulation électrique ce type d'adaptation est complètement absent lorsque les cellules ciliées internes sont détruites et il ne subsiste alors qu'une adaptation d'origine nerveuse étroitement dépendante de la fréquence de pulsation des stimuli électriques. Ainsi, aux basses fréquences (100-200pps) les réponses sont relativement bien synchronisées avec chaque pulse électrique et il y a peu ou pas d'adaptation tandis que lorsque la fréquence augmente, on s'approche de

la période réfractaire et les phénomènes d'adaptation deviennent plus marqués. Ce comportement a été retrouvé chez les patients implantés cochléaire[41] et pourrait expliquer la très mauvaise discrimination de la parole (F0□200Hz) dans les environnements sonores bruyants.

Le présent travail rend compte dans un premier temps du développement d'un modèle expérimental destiné à approfondir l'étude des propriétés d'adaptation neuronale dans le noyau cochléaire en réponse à des stimulations acoustiques ou électriques dont on fait varier l'intensité et/ou la fréquence. Les techniques d'enregistrement faisant appel à des moyens invasifs (électrodes intracérébrales chroniques), le recours à un modèle animal, le rat Long-Evans, a été indispensable. L'implantation cochléaire a été pratiquée de façon similaire à ce qui est réalisé chez l'homme. Compte tenu de la similarité des protocoles entre les deux modes de stimulation (acoustique ou électrique) d'une même oreille, nous avons pu dans un second temps quantifier les deux types d'adaptation obtenue et les comparer. Dans un troisième temps, nous nous sommes employés à les faire correspondre au mieux et nous avons ainsi trouvé qu'en utilisant des stimuli électriques répétitifs d'intensité exponentiellement décroissante (équation de décroissance fournie par les résultats de l'adaptation acoustique de l'oreille avant implantation) il était possible de restaurer les phénomènes normaux d'adaptation neuronale.

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Hearing Research 171 (2002) 72–81

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Neural adaptation to pulsatile acoustical stimulation in the cochlear nucleus of the rat

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Received 19 October 2001; accepted 26 March 2002

Abstract

This study, carried out in adult Long–Evans rats, was designed to investigate the adaptive properties of the cochlear nucleus to pulsatile acoustical stimuli. To achieve this purpose, near-field evoked potentials were picked up from the ventral cochlear nucleus in awake animals. Individual auditory thresholds were measured and responses to 250 ms trains of repetitive clicks with pulse rates ranging from 100 to 2000 pulses per second were collected. The amplitude of the first negative (N_1) component of the evoked potentials to consecutive individual pulses in the train was measured by using a subtraction method. As expected, a rapid amplitude decrement of the responses in the train was obtained and a three phase adaptation was described. The decrease of individual N_1 component amplitude was fitted for each rate of stimulation with exponential decrease equations and time constants were calculated. Such an analysis allowed us to characterize three distinct adaptive processes which were discussed. The results were comparable to those obtained in previous studies in the auditory nerve and suggest that the adaptation recorded in the ventral cochlear nucleus by using near-field evoked potentials reflects the adaptive properties of auditory nerve fibers.

Key words: Adaptation; Cochlear nucleus; Auditory evoked potentials; Rat; Repetitive stimulation

1. Introduction

Adaptation is a common feature of sensory receptors and generally means a decrease of sensitivity in the presence of a constant stimulus. In the auditory nerve, different experimental protocols have been used to study the adaptation of cochlear afferents. A first approach consisted of describing neuronal responses to simple long stimuli such as tone-bursts (about 50–100 ms duration) and analyzing the evoked spike trains with peristimulus time histograms (e.g. Kiang et al., 1965). The responses of primary auditory neurons showed a three-staged adaptation: the initial stage was a rapid exponential decay from an initial onset response and had a time constant of a few milliseconds (*rapid adaptation*; Westerman and Smith, 1984; Yates et al., 1985; Chimento and Schreiner, 1991); the second stage had a longer time constant in the order of 10 ms (*short-term adaptation*, Smith and Zwislocki, 1975); the third stage was a very gradual reduction in the firing rate and lasted approximately 10 s (*long-term adaptation*; Kiang et al., 1965; Javel, 1996).

A second commonly used approach to study the adaptation properties was the forward-masking paradigm, where the effects of a preceding masker (conditioning tone) on the response to a subsequent probe tone are quantified as a function of the time interval between the mask and the probe. It has been used in many psychophysical experiments (Zwicker, 1974), and also in electrophysiological experiments based on compound action potentials (CAP) or single-nerve fiber responses (Harris and Dallos, 1979). With such an ap-

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Abbreviations: CAP, compound action potential; CN, cochlear nucleus; CNP, cochlear nucleus auditory evoked potential; ICP, inferior colliculus auditory evoked potential; N_1, first negative component of auditory evoked potential; P_1, first positive component of auditory evoked potential; pps, pulse per second; SPL, sound pressure level; VCN, ventral cochlear nucleus

0378-5955/02/$ – see front matter
PII: S0378-5955(02)00394-5

proach, the study of adaptation by recording CAP amplitude (Eggermont and Spoor, 1973b; Abbas and Gorga, 1981; Gorga and Abbas, 1981; Abbas, 1984; Chimento and Schreiner, 1990, 1992) or auditory nerve discharge rate (Smith, 1977, 1979; Chimento and Schreiner, 1991) in response to a probe showed an exponential decrement which reflects a decrease of activity of fibers sensitive to the masker. Furthermore, the time constants for the rapid and short-term adaptive phases were comparable when derived from CAP or single nerve fiber responses (Eggermont and Spoor, 1973b; Chimento and Schreiner, 1991). Therefore it was concluded that the adaptation properties of the CAP of the auditory nerve reflected the adaptive properties of single nerve fiber population.

Finally, a third strategy consisted of describing adaptation of the auditory nerve responses to short repetitive acoustic stimuli (Peake et al., 1962a,b; Eggermont and Spoor, 1973a; Huang, 1981). These studies have first demonstrated a close relation between the amplitude of the response and the stimulus repetition rate. Second, responses are time-locked to the individual acoustic pulses for repetition rates as high as 3000 pulses per second (pps; Peake et al., 1962a). However, it was shown that conventional averaging to derive CAP responses was contaminated by a temporal overlap of response waveforms at repetition rates of 300 pps and higher. Actually, only very recent studies tried to develop new procedures in order to obtain non-contaminated responses at higher repetition rates (Burkard, 1994).

However, adaptation is not restricted to the auditory nerve and it may be present at different levels of the auditory pathways (Abbas, 1984). For example, Huang and Buchwald (1980) showed in the cat that evoked potentials in response to a repetitive tonal stimulation resulted in a similar amplitude decrement in both auditory nerve and cochlear nucleus (CN). In the CN, more detailed studies conducted at the single unit level reported a large variety of response patterns (e.g. Kiang, 1965; Pfeiffer, 1966; Bourk, 1976) and adaptation differences between cell types (for a review see Evans, 1975). In the chinchilla for instance, primary-like, primary-like with notch and chopper units showed an adaptation to tone bursts which reflected well the adaptation pattern of auditory nerve fibers as seen in peristimulus time histograms (Boettcher et al., 1990; Kaltenbach et al., 1993). In contrast, pauser, buildup and onset units showed a strong alteration of adaptive properties as compared to auditory nerve fibers. These results suggest that the properties of adaptation established in the auditory nerve can be modified in the CN, indicating that the CN is not simply a relay of the stereotyped auditory nerve activity. In the rat, Møller (1969) demonstrated that some CN neurons, referred to as 'transient units', could follow repetitive acoustic pulses (clicks presented in trains of 50 ms duration) up to a rate of 500 pps. At higher rates (from 600 to 1000 pps), these units produced only a single action potential at the beginning of each train presentation and the failure to respond to the subsequent pulses was interpreted as an adaptation.

Nevertheless, although comparisons between auditory nerve fibers and CN units have been provided (Kiang, 1965; Møller, 1976; Huang and Buchwald, 1980), few quantitative data are yet available to describe the adaptive properties of the ventral cochlear nucleus (VCN) as precisely as it has been done for the auditory nerve. Therefore, the aim of the present investigation was to specify the time course and time constants of VCN neural adaptation. To achieve this purpose, CN near-field auditory evoked potentials were recorded in adult rats in response to pulsatile acoustical stimuli by using chronic electrophysiological recordings. Thus, in contrast to most previous studies, all the present experiments were performed on unanesthetized rats. The results were compared to the quantitative adaptation properties previously established for the auditory nerve.

2. Methods

2.1. Animals

Male adult Long–Evans rats (approximately 300 g and 3 months old) were purchased from Janvier Laboratories (France) and were housed in individual cages ($425 \times 266 \times 150$ mm) from the beginning of the experiment. Food pellets (Kliba-Mühlen, Basel, Switzerland, ref.: 343) and tap water were available ad libitum. The temperature in the animal quarters was maintained at 20 ± 1°C and light was on from 7.00 to 19.00 h. The experiments were conducted in accordance with the guidelines of the US NIH and the Declaration of Helsinki for animal care, and were approved by the Swiss veterinary authorities.

2.2. Experimental procedures

2.2.1. Animal preparation

Just before the surgery, rats ($n = 8$) were deeply anesthetized with pentobarbital (Vetanarcol®, 40 mg/kg) and received atropine sulfate (0.05 mg/kg) to minimize respiratory distress. Then, the animals were placed in a stereotaxic apparatus (Muromachi Instrument, Model 1404, Japan) equipped with special ear bars to prevent damages to the tympanic membrane. One custom-built perforated earbar was used for acoustic stimulation. A midline incision was made on the scalp to expose the

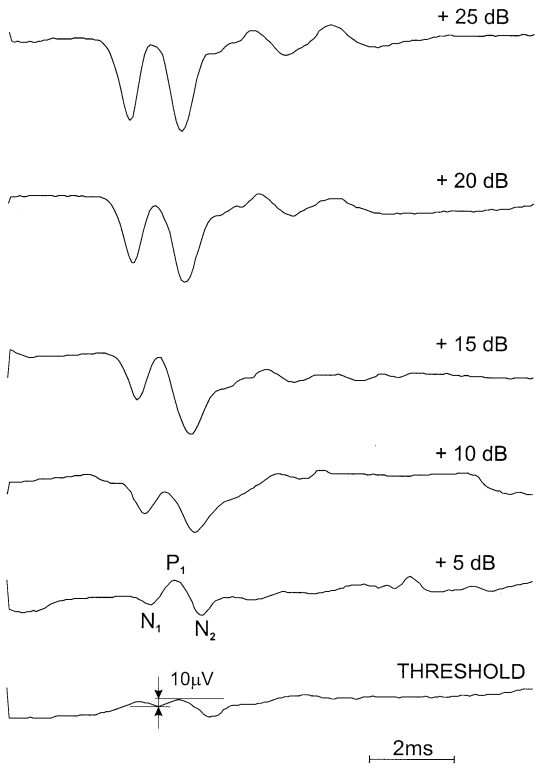

Fig. 1. Typical VCN auditory evoked potentials (CNP) elicited by 4 kHz tone bursts (rat #4). The vertical bar in the threshold curve corresponds to a 10 μV through-to-peak N_1–P_1 amplitude, taken as the criterion for response. Negative polarity is downward.

skull reference point bregma. From this landmark, and based on the Paxinos and Watson's atlas (Paxinos and Watson, 1998), stereotaxic coordinates of the left VCN were accurately determined (AP = −9.80 mm; ML = 4.30 mm; DV = 8.00 mm). A tungsten electrode of 2–4 MΩ impedance was implanted in the left VCN to record auditory evoked near-field potentials and a ground electrode was placed in the rostral cranium on the dura mater. The optimal location for the recording electrode was assessed by observing potentials (two negative deflections known as N_1 and N_2, Fig. 1) when the electrode was advanced into the CN (DV = 7.99 ± 0.35 mm). Then, the two electrodes were soldered to a socket and fixed with dental cement to the skull (adapted from Henderson et al., 1973). The animals were allowed to recover for 2 weeks before chronic recording.

2.2.2. Audiometry

Each rat was first tested with pure tones for measuring the audiometric thresholds in our experimental conditions. Testing was performed in an audiometric room (IAC, Model AC-1 Chamber, Germany) on awake rats placed in a restraining device (adapted from Campo et al., 1997). Acoustic signals were synthesized digitally using SigGen32 software (Tucker-Davis Technologies System II), fed into a programmable attenuator and after D/A conversion, transduced by a speaker positioned 10 cm away from the left pinna. The calibration of the system was carried out with a sound level meter (B&K, 2231) by measuring the sound pressure level (SPL; root mean square, re: 20 μPa) emitted by the speaker when it was driven by a pure tone signal at 9 V peak level. The calibration microphone (B&K, 4155, prepolarized free-field 1/2″) was positioned at the location occupied by the middle central point of the animal's head. The CN evoked potentials (CNP) were amplified (10^3), bandpass filtered between 30 Hz and 3 kHz (Ithaco®, 1201), then fed into an A/D converter (Tucker-Davis Technologies System II). The data acquisition software BioSig32 (Tucker-Davis Technologies) was used to automate CNP averaging over 250 presentations and storage for off-line analysis. During the experiments, the ambient noise level within the audiometric room did not exceed 69 dB SPL when considering the overall spectra linear level and 38 dB SPL when considering the frequencies above 1 kHz.

Audiometric test stimuli were tone bursts of 3 ms duration (0.5 ms for the rise and fall time) shaped by cosine-squared functions, presented alternately in phase and repeated at a rate of 10 per second. The analysis window lasted 10 ms. CNP thresholds were determined for 11 tone frequencies ranging from 1 to 30 kHz by varying the stimulation intensity in 5 dB SPL increments. The amplitude of the N_1 component of the CNP (10 μV criterion) was measured as the voltage difference between the negative (N_1) and positive (P_1) peaks (Fig. 1).

2.2.3. Repetitive acoustic stimulation

Five rats were tested with short rectangular-pulse clicks (50 μs) delivered in train of 250 ms duration followed by a pause (silence) of 250 ms before the next train (Fig. 2A). The intra-train pulse rates ranged from 100 to 2000 pps (inter-pulse intervals ranging from 10 to 0.5 ms). According to mean audiometric thresholds (Fig. 3), the intensity of repetitive acoustic stimulation was set to 70 dB SPL for all animals in order to recruit a large number of primary auditory neurons. CNP were amplified (2×10^3), bandpass filtered between 30 Hz and 3 kHz and averaged over 50 presentations. The analysis window started 10 ms before delivery of each train and lasted 260 ms. At high repetition rates (> 500 pps) temporal overlap of CNP to individual clicks was unavoidable, thus preventing the measure of the N_1–P_1 amplitude of each consecutive CNP. To circumvent this problem, the recording method consisted in presenting trains containing an incremented number of clicks during a constant period of 250 ms (Fig. 2A). Then, the CNP obtained in response to a train of n clicks (C_n) was subtracted from the CNP derived in response to a train of n+1 clicks (C_{n+1}) at

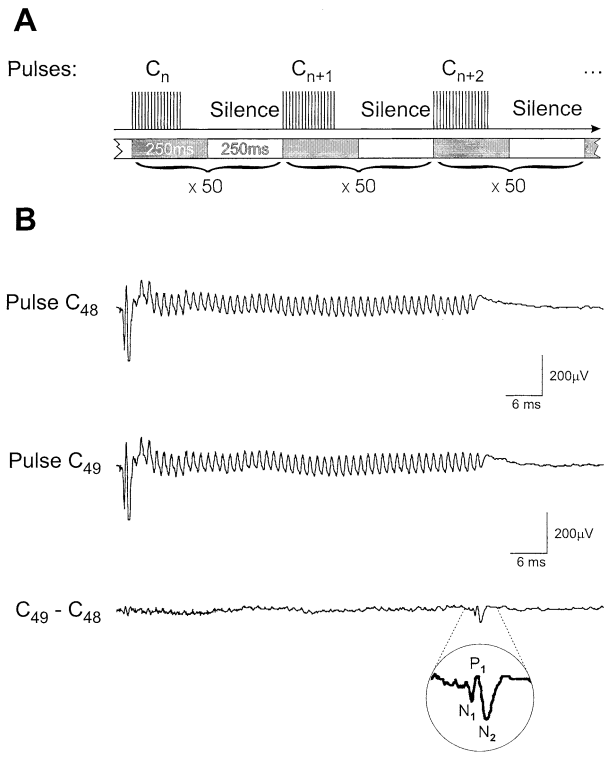

Fig. 2. (A) Time structure of the pulsatile acoustic stimuli. Each incremented click train (C_n) was presented 50 times during a constant period of 250 ms and followed by a pause of equal duration. (B) Illustration of the recording method at step C_{49} with a 800 pps train stimulation. The averaged brainstem (CN) evoked potentials obtained with 48 clicks (pulse C_{48}) and 49 clicks (pulse C_{49}) were subtracted ($C_{49}-C_{48}$) from each other in order to obtain the averaged evoked potential corresponding to the last click in pulse C_{49}.

the same repetition rate. The resulting curve thus exhibited the CNP to the n+1 click in the train without contamination by the CNP of the preceding clicks (Fig. 2B). However, the disadvantage of this method is the large number of trains needed to separate the CNP to each click at high rates. Therefore, to avoid an overstimulation of the auditory nerve during a given recording session, CNP were collected using the subtraction method for all consecutive clicks during the initial 20 ms of the train, then for one click every 10 ms during the next 60 ms of the train and for only three individual clicks during the last 160 ms of the train. Results were presented as N_1–P_1 amplitude normalized relative to the highest CNP observed in the train (usually the CNP to the first click) and plotted as a function of the position of the corresponding click in the train expressed in milliseconds.

2.2.4. Histology

In order to verify the location of the recording electrode in the VCN, brains of all rats were prepared for histology at the end of the experiment (3 months after the implantation). Each animal was deeply anaesthetized with an overdose of pentobarbital and perfused through the heart with normal saline followed by a 3% glutaraldehyde solution in phosphate buffer (0.1 M at pH 7.4). After decapitation, the brains were removed, postfixed one night in the fixative solution and kept the next day in a 30% sucrose solution at 4°C until further use. Frozen coronal sections (40 µm thick) were cut, washed in phosphate buffer and mounted on slides. Finally, sections were counterstained with cresyl violet (Fluka, 0.06%), coverslipped with Eukitt and observed in light microscopy.

3. Results

The histological section taken from the brainstem of rat #1 (Fig. 4) is a typical illustration of the location of the recording electrode used to derive the CNP in the VCN. As in the other animals, the chronic recording electrode has been inserted in the CN at a depth where its tip was clearly located in the VCN.

3.1. CNP thresholds to pure tone stimulus

To assess the auditory sensitivity of the rats included in the study, pure tones were presented at 11 frequencies for which the CNP threshold was established. Fig. 3 shows the mean CNP thresholds derived from the eight animals. The highest sensitivity (25 dB) was at 16 kHz and decreased at higher and lower frequencies (∼34 dB at 30 kHz and ∼74 dB at 1 kHz). The shape of the curve (squares) is comparable to that obtained with evoked potentials recorded from the inferior colliculus in Long–Evans adult rats (circles; data from Campo et al., 1997), although thresholds of CNP were higher than inferior colliculus evoked potential

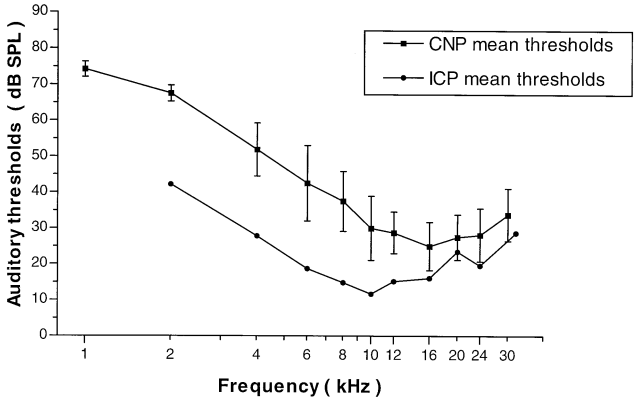

Fig. 3. Mean auditory thresholds of CNP recorded in eight adult Long–Evans rats (squares). The circles represent the mean auditory thresholds of the ICP in 85 adult Long–Evans rats (according to Campo et al., 1997). In the CNP curve, the error bars represent the 95% confidence intervals.

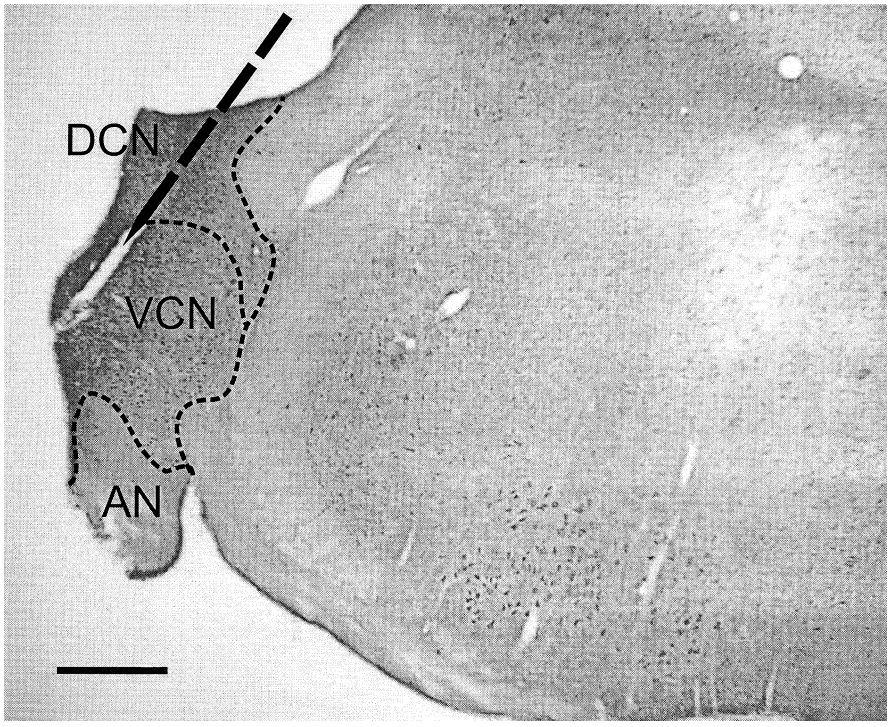

Fig. 4. Photomicrograph of the left CN of an implanted rat (rat #1). AN: Auditory nerve; DCN: dorsal cochlear nucleus. The dotted arrow indicates the trajectory and orientation of the electrode track. Scale bar: 500 μm.

(ICP) thresholds. In addition, ICP threshold was lowest at 10 kHz whereas it was at 16 kHz for CNP.

3.2. CNP to repetitive clicks

In Figs. 5 and 6, normalized N_1–P_1 amplitudes of individual CNP were plotted as a function of time position of the corresponding individual clicks along the train for two animals: rat #1 (Fig. 5A,B) and rat #5 (Fig. 6A,B). Similar data were obtained for the other three rats (not shown). For each rate tested, the normalized amplitude decreased exponentially with time. There appeared to be an initial rapid adaptation followed by a slower adaptation and a plateau. To verify this three-phased adaptation, data were fitted with functions that were consistent with adaptation properties of primary auditory neurons (Westerman and Smith, 1984; Chimento and Schreiner, 1991). The equation used was:

$$\mathrm{NP}(t) = \mathrm{NP}_1 e^{-t/K_2} + \mathrm{NP}_2 e^{-t/K_2} + \text{plateau}$$

where NP_1, NP_2 are the y intercepts of the rapid and short-term components respectively. Plateau is equal to the N_1–P_1 amplitudes during the steady state response, and K_1, K_2 are the decay time constants of the two postulated adaptation components. An illustration of the fit is shown in Fig. 7A. In some instances, in particular at the highest click rate (2000 pps), data were best fitted with a one time constant adaptation equation:

$$\mathrm{NP}(t) = \mathrm{NP} e^{-t/K} + \text{plateau}$$

For both equations, the curve fitting was run by using the Levenberg–Marquardt method with GraphPad Prism® 3.02 software and the deviation from model was assessed by considering the correlation coefficient ($R^2 = 0.90$) and by testing the Gaussian distribution of the residuals around the curve ($P > 0.1$). For each animal ($n = 5$), rapid (K_1) and short-term (K_2) time constants were extracted and plotted in Fig. 7B,C. Both time constants exhibited a progressive exponential decrease as a function of stimulus rate and reached a plateau. For example, the mean value of K_1 was 6.7 ms at 100 pps, decreased down to 0.54 ms at 600 pps and stabilized around this value at higher rates. For K_2, the mean value was 41.1 ms at 100 pps, decreased down to 1000 pps and stabilized around 5 ms at higher rates. The largest inter-individual variability was observed at low repetition rates. Mean values were calculated for all animals ($n = 5$) and are summarized in Table 1.

Data shown in Figs. 5 and 6 indicate that the adaptation reached a plateau after the rapid and short-term phases. The magnitude of this plateau was calculated at all repetition rates for each animal in the last 150 ms of the train for rates ranging from 100 to 1000 pps and during 10 ms (corresponding to the click position be-

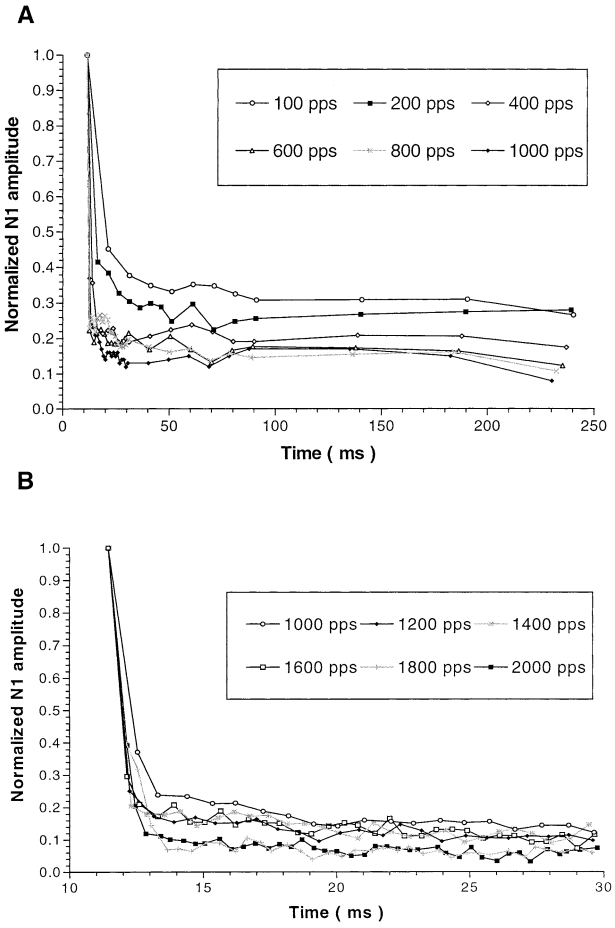

Fig. 5. (A) Normalized amplitudes of ventral CNPs in rat #1 displayed as a function of the position of the stimulating click in the train. Click rates ranged from 100 to 1000 pps. The abscissa (250 ms) corresponds to the duration of a train beginning after 10 ms of silence. (B) Amplitudes of CNPs in the same animal (rat #1) displayed in the same fashion as in (A) for click rates ranging from 1000 to 2000 pps. The abscissa was expanded (first 30 ms) for clarity's sake.

tween 20 and 30 ms) for rates ranging from 1000 to 2000 pps. Mean values of plateau amplitude are plotted in Fig. 8 with standard deviations as a function of click repetition rates. This figure shows that normalized amplitude of the CNP at plateau level exponentially decreased. At low stimulation rates (100, 200 and 400 pps), doubling the repetition rate led to a decrease of the plateau amplitude of about 10%. At higher frequencies, the decrease continued more progressively with increasing rates (about 5% between 600 and 2000 pps).

3.3. Extension to a model

The experimental data shown in Table 1 could be arbitrary distributed in three clusters: the first cluster ([a]) corresponded to the shortest time constants (below 1 ms), the second cluster ([b]) gathered together the second time constants for high rates and the first time constants for lower rates (ranging from 1 to 20 ms) and the third cluster (open circles) was estimated somewhere in the 20–40 ms range for low rates. Therefore, three mean time constants (τ_1, τ_2, τ_3) were calculated within each of these three clusters and were assumed to be descriptive of three different adaptive processes (Table 2). By fitting our data with these fixed time constants and the following equation:

$$NP(t) = NP_1 e^{-t/\tau_1} + NP_2 e^{-t/\tau_2} + NP_3 e^{-t/\tau_e} + \text{plateau}$$

it was possible to estimate NP_1, NP_2 and NP_3 and therefore the importance of the particular adaptive processes for each repetition rate. Results are summarized in Table 2. At low repetition rates (100, 200 and 400 pps) the adaptation consisted of two slow processes (τ_2, τ_3) without rapid component. In contrast, a rapid component (τ_1) was present above 600 pps as well as a short-term component (τ_2), except at the highest rates tested (1800 and 2000 pps) where the adaptation was limited to a single rapid component. As far as the y intercepts are concerned, NP_1 drastically increased above 1000 pps whereas NP_2 ranged between 4.26 and 1.5 and NP_3 was estimated around 0.1.

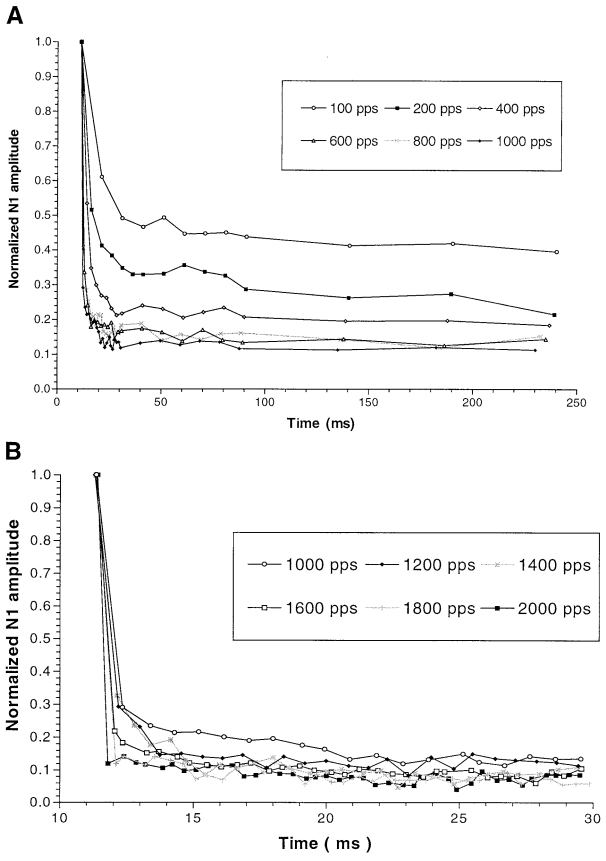

Fig. 6. (A,B) Data derived from rat #5 displayed in the same fashion as in Fig. 5.

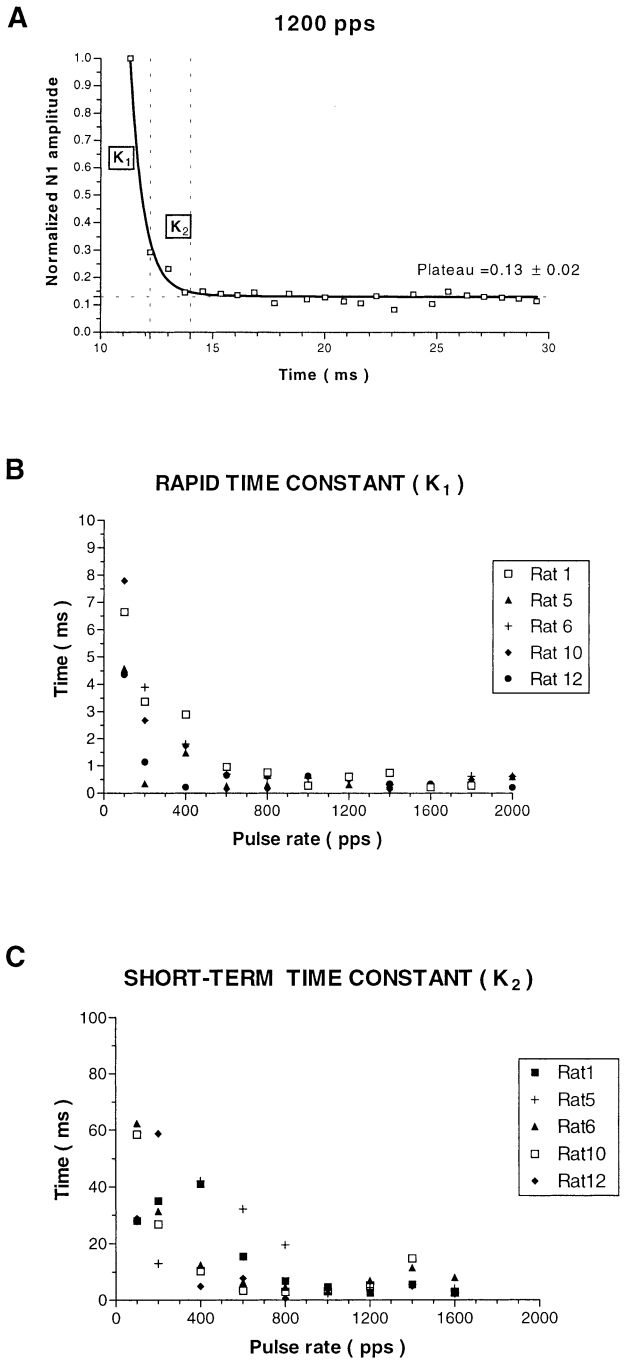

Fig. 7. (A) Demonstration of the fit of CNP-normalized amplitudes by a two phase exponential decay model (plots are from rat #1 at 1200 pps stimulation rate). K_1 and K_2 are the corresponding time constants to rapid and short-term adaptation phases. Coefficient of correlation for fitted curve is 0.98. (B) Time constants of the rapid adaptation phase (K_1) plotted for five animals as a function of stimulation rate. (C) Time constants of the short-term adaptation phase (K_2) plotted for five rats as a function of stimulation rate.

4. Discussion

The present study is based on recordings of CNP in the rat by using a chronic electrophysiological method.

Table 1
Time constants of CNPs adaptation as a function of stimulation rate

Stimulation rate (Hz)	Rapid time constant (K_1; ms)		Short-term time constant (K_2; ms)	
	mean	S.D.	mean	S.D.
100	6.70[b]	2.40	41.10[c]	17.64
200	2.28[b]	1.50	32.95[c]	16.64
400	1.63[b]	0.96	22.16[c]	17.90
600	0.54[a]	0.33	13.00[b]	11.58
800	0.48[a]	0.24	6.95[b]	7.39
1000	0.41[a]	0.16	3.36[b]	0.97
1200	0.48[a]	0.11	4.64[b]	1.58
1400	0.37[a]	0.22	8.45[b]	4.42
1600	0.28[a]	0.05	4.00[b]	2.38
1800	0.38[a]	0.16	–	–
2000	0.51[a]	0.20	–	–

K_1, K_2: Time constants of adaptive phases one and two obtained with non-linear regression fitting curve (see text for method). Mean values and standard deviations (S.D.) were calculated from data obtained in five rats. Symbols indicate the arbitrary grouping of time constant values in three clusters.
[a]Time constants below 1 ms, with an average value of 0.48 ms (τ_1 in Table 2).
[b]Time constants ranging from 1 to 20 ms, with an average value of 5.72 ms (τ_2 in Table 2).
[c]Time constants above 20 ms, with an average value of 32.06 ms (τ_3 in Table 2).

The latency of the N_1 response (2.9 ms at 4 kHz and 25 dB above threshold; Fig. 1) is consistent with latencies of VCN single unit discharges (2.79 ms at 4 kHz, 25–35 dB above threshold; Young et al., 1988). The present technique has allowed repeated stimulation sessions in the same conditions without anesthetics and thereby the possible side effects of anesthesia on the responses were eliminated. This recording technique was modified from previous works (Henderson et al., 1973; Campo et al., 1997) and CNP thresholds were generally comparable to those previously obtained for ICPs (Campo et al., 1997) at frequencies ranging from 16 to 30 kHz (Fig. 3). In this figure, the gap between the two curves in the low- and mid-frequencies (from 1 to 12 kHz) is likely due to two main reasons. First, different acoustic stimuli were used: filtered clicks for ICPs recordings versus tone bursts for CNP recordings. Second, some variability of the recording electrode location may affect more the CN than the inferior colliculus because of a more segregated distribution of distinct cell types in the CN than in the central nucleus of the inferior colliculus. In fact, tone bursts which exhibited spectra similar in shape across frequencies (Burkard, 1984) were preferred in the present paper to validate the technique and it allowed us to determine the required intensity of stimulation to recruit a large number of primary auditory neurons without causing long-term threshold shifts.

Concerning the adaptation properties of the CN, it is important to note that no fatigue was detected since a

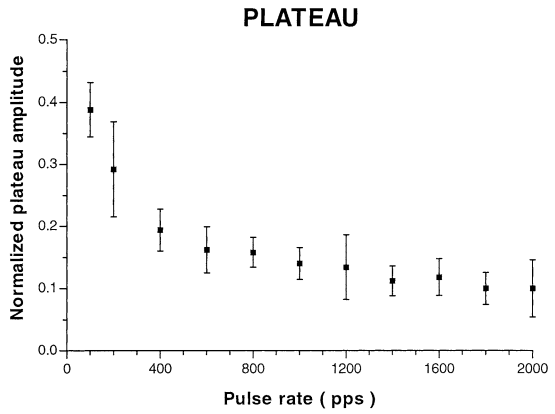

Fig. 8. Mean normalized plateau amplitudes obtained for five animals plotted as a function of stimulus rate. The bars represent standard deviations.

sudden variation of stimulus intensity in a train always produced a larger CNP response than the current one (data not shown). Figs. 5 and 6 illustrate two important adaptation properties. First, for each rate tested (from 100 to 2000 pps), the progressive decrement of CNP amplitude was a function of the position of the stimulating click in the train. The exponential decay of the curve is comparable to the adaptation observed in the auditory nerve (Eggermont and Spoor, 1973a; Westerman and Smith, 1984) and exhibits three similar phases: a rapid, a short-term and the beginning of a long-term adaptation. Second, the larger and faster decrease of CNP amplitude for increasing rates is in agreement with adaptive responses of the auditory nerve (Peake et al., 1962a). Therefore, these qualitative results demonstrate that adaptation taking place in the VCN in our experimental conditions well reflects the adaptation present in the auditory nerve.

The quantitative analysis based on the description of adaptation with a sum of two exponential processes (see Section 3) allowed us to examine the time course of adaptation in VCN, especially in supplying values for the adapted phase (plateau) and for time constants of the first two phases (Table 1). First, at low stimulating rate (100 pps), it is important to note that the rapid time constant (6.7 ms; Table 1) is roughly consistent with single auditory nerve data in gerbils (2.1 ms; Westerman and Smith, 1984) or in cats (5.5 ms; Chimento and Schreiner, 1991) and with CAP data (4.8 ms in cats; Chimento and Schreiner, 1990). In contrast, the short-term time constant (41.1 ms, Table 1) is shorter in the VCN as compared to single auditory nerve data (58 ms in gerbils, 93.7 ms in cats) and to CAP data (73.5 ms in cats). Second, at stimulating rates higher than 100 pps, the time constants of both adaptive phases (rapid and short-term) progressively decreased (Fig. 7) and reached a plateau. The shortest time constants (rapid adaptation) were found at rates above 600 pps and equaled approximately 0.5 ms (K_1 in Table 1). This result was expected since it is in line with reports that the absolute refractory period of the auditory nerve is around 0.85 ms in the guinea pig (Mulheran, 1999) and between 0.56 and 0.86 ms in the cat (Li and Young, 1993). It can be noted that during this period, a new stimulation of any intensity was inefficient and the rhythm of nervous discharges can not be higher than 1000 spikes per second without a saturating effect (Smith, 1979).

As far as the remaining K_1 and K_2 time constants are concerned ($K > 0.5$ ms in Table 1), they seemed to segregate in two clusters independently of the stimulating rate with time constants around 5 and 30 ms (respectively τ_2 and τ_3 in Table 2). Therefore, two new adaptive processes could be described and their importance was estimated (NP_2 and NP_3 in Table 2). The values are low ($1.5 < NP_2 < 4.26$; $NP_3 \approx 0.1$) by comparison with the values of the refractory mechanism (NP_1). Based on previous investigations (Eggermont, 1985), it is likely that these two processes relate to pre- and post-synaptic properties at the synapse between inner hair cells and primary auditory neurons. However, the present attempt of modelization does not allow us to infer more precisely the location of the adaptation mechanisms. Moreover, it is also possible that one of the adaptive process could be related to the relative refractory period of the auditory nerve fibers. In the CN of the rat, a previous study showed that some units exhibited an adaptation to trains of repetitive clicks (Møller, 1969). The adaptation was detected for rates higher than 500 pps, an observation consistent with our results since a drastic adaptation is settled around 400–500 pps (Fig. 8).

The results of the present study demonstrate that the adaptive properties of the VCN obtained by recording evoked potentials are comparable to those usually ob-

Table 2
Respective characteristics of the three postulated adaptive processes as a function of stimulating rate

Stimulation rate (Hz)		100	200	400	600	800	1000	1200	1400	1600	1800	2000
$\tau_1 = 0.48$ ms	NP_1	–	–	–	5.09	50.00	1E+10	1E+10	1E+10	1E+10	1E+10	1E+10
$\tau_2 = 5.72$ ms	NP_2	3.79	4.12	4.26	3.85	3.38	2.03	1.72	1.51	1.50	–	–
$\tau_3 = 32.06$ ms	NP_3	0.12	0.14	0.10	–	–	–	–	–	–	–	–

τ_1, τ_2, τ_3: Fixed time constants (mean values calculated from data obtained in five rats and for each of the three clusters defined in Table 1); NP_1, NP_2, NP_3: y intercepts of each adaptive process obtained with non-linear regression fitting curves (see text for method).

served in primary auditory neurons. Therefore, the VCN does not seem to generate new adaptive properties but reflects rather well the auditory nerve adaptation. These results are in line with the temporal coding of periodic stimuli (e.g. phase-locking) which has been reported to be very similar in the VCN and in the auditory nerve (Joris et al., 1994). However, it is important to note that the behavior of some cell types in the VCN may not be reflected by the present evoked potential recordings and that further studies are needed at the single unit level to determine possible specific adaptation properties of various cell types in the VCN.

Acknowledgements

The authors would like to thank Dr. A. Babalian (Division of Physiology, University of Fribourg, Switzerland) for helpful discussions and comments. They also wish to thank V. Moret, B. Aebischer, E. Regli and A. Gaillard for their technical assistance and J. Corpataux and B. Morandi for taking care of the rats in the animal room. This research project was supported by the Swiss National Science Foundation (Grant No. 32-56352.99; TANDEM) and the National Center for Competence in Research (NCCR) 'Neural plasticity and Repair'.

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Exp Brain Res (2003) 153: 436–442
DOI 10.1007/s00221-003-1689-9

RESEARCH ARTICLE

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Effects of intensity of repetitive acoustic stimuli on neural adaptation in the ventral cochlear nucleus of the rat

Received: 6 December 2002 / Accepted: 27 March 2003 / Published online: 22 October 2003

Abstract To study neural adaptation as a function of stimulus intensity, auditory near-field evoked potentials were recorded from the ventral cochlear nucleus in awake Long Evans rats. Responses to 250-ms trains of repetitive clicks (pulse rates ranging from 100 to 1000 pulses per second) were collected at stimulus intensities of 5, 10, 30, 50 and 70 dB SPL. The amplitude of the first negative (N_1) component of the average evoked potentials to individual pulses in the train was measured by using a subtraction method. The N_1 responses were normalized with respect to the highest cochlear nucleus potential observed in the train, and then plotted as a function of click position in the train. As expected, the general trend of the curves was an exponential decay reaching a plateau more or less rapidly as a function of both intensity and rate of stimulation. Fitting these curves with exponential decay equations revealed that the rapid time constant decreased for increasing stimulus intensities whereas the short-term time constant is relatively independent of intensity. The amount of adaptation (expressed as the ratio of the plateau to the first peak amplitude) was substantially less prominent at low intensities (5–10 dB SPL) and low rates (100–200 pulses per second) than at higher intensities and high rates. These results indicate that adaptation patterns obtained in the ventral cochlear nucleus by using near-field evoked potentials exhibit properties comparable to those already present at the level of the auditory nerve.

Keywords Unanesthetized · Brainstem · Auditory evoked potentials · Click

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Introduction

From a psychophysical point of view, the subjective intensity of a pure-tone that lasts more than a few seconds and does not exceed 30 dB SL (sensation level) decreases during stimulation. This loudness decrease, referred to as adaptation, is due to the reduction of neural response to continuous stimulation over time (Gelfand 1997). In profoundly deaf patients, however, the current generation of cochlear implants does not reproduce adaptation properties, so imparting a loss of information which may contribute, at least in part, to the commonly reported low level of speech intelligibility in noisy conditions.

Experimental studies on auditory adaptation at the neural level have generally been conducted in animal models (cat and rodent) by recording compound action potentials (Eggermont and Spoor 1973; Abbas 1984; Chimento and Schreiner 1990, 1992) or action potentials from single auditory nerve fibers (Smith and Zwislocki 1975; Smith 1977, 1979; Harris and Dallos 1979; Westerman and Smith 1984; Rhode and Smith 1985; Yates et al. 1985; Javel 1996). In the auditory nerve (AN), the compound action potential (CAP) in response to a transient sound is characterized by a negative deflection, reflecting the synchronized discharges of several individual fibers. In response to a variety of different stimulus types such as high frequency tones (Gorga and Abbas 1981; Abbas 1984), low frequency tones (Chimento and Schreiner 1990, 1991, 1992), short repetitive tone-bursts (Peake et al. 1962a, 1962b; Eggermont and Spoor 1973; Müller and Robertson 1991), or click trains (Kiang et al. 1965; Wickesberg and Stevens 1998), both techniques (CAP and single unit recordings) yielded similar results, namely an initial rapid decrease of CAP amplitude or firing rate during the first few milliseconds (*rapid adaptation*), followed by a slower decay over tens of milliseconds (*short-term adaptation*), and then an even slower decrease over several seconds (*long-term adaptation*) or minutes (*very long-term adaptation*). The similarity of adaptation patterns to pure tones and to series of clicks could possibly be attributed to the fact that

at high repetition rates, the latter stimulus is close to a cosine-phase harmonic complex (Hafter and Richards 1988). Nevertheless, small differences were reported at the single unit level, especially between high and low spontaneous rate AN fiber populations (Rhode and Smith 1985; Müller and Robertson 1991), suggesting that different mechanisms may underlie adaptation. Moreover, it is important to note that the measures of adaptation were essentially performed in two ways: either over the duration of the stimulus (Peake et al. 1962a, 1962b; Eggermont and Spoor 1973; Westerman and Smith 1984; Müller and Robertson 1991; Javel 1996) or during the recovery period (Gorga and Abbas 1981; Abbas 1984; Chimento and Schreiner 1990, 1991, 1992). However, both approaches are closely related, and thus lead to comparable results (rapid, short-term, and long-term adaptation). Similar to the AN, the near-field evoked potential recorded from the cochlear nucleus (CN) in response to transient sounds is characterized by a negative deflection (with a longer latency), believed to reflect the synchronized discharges of secondary auditory neurons (Møller 1983). With this approach, the measurements of adaptation in the ventral division of the CN (VCN), carried out with short repetitive tone-bursts (Møller 1969; Huang and Buchwald 1980; Huang 1981) or click trains (Møller 1969; Loquet and Rouiller 2002), showed an adaptive pattern similar to that of the AN, with at least three distinct decay components. At the single unit level, only two of the three major response types described in the VCN (primary-like and chopper) exhibited adaptation patterns similar to those reported in the auditory nerve fibers in response to tones (Evans 1975). The same conclusion was reached in studies using a forward-masking paradigm (Boettcher et al. 1990; Shore 1995).

As to the mechanisms involved, adaptation phenomena are still not fully understood. On the one hand, for the auditory nerve, authors agree to consider adaptation to be the result of neural refractory properties and depletion of available transmitter at the hair cell–nerve fiber synapse (for review see Eggermont 1985; Javel 1996). Therefore, the multi-component adaptation may be attributed to a multi-stage transmitter depletion (Smith and Brachman 1982), with transmitter release depending to some extent on the nature of the stimulus. For example, there would be an increase of adaptation in AN fibers when the intensity of a tone stimulation increased (Peake et al. 1962b; Westerman and Smith 1984; Yates et al. 1985; Rhode and Smith 1985; Müller and Robertson 1991). On the other hand, in CN, adaptation was expected to be more complex, mainly because of (1) the variety of afferent inputs, (2) the intrinsic membrane characteristics of each cell type, and (3) inhibitory inputs. Nevertheless, at the single unit level, it was reported that adaptation patterns in firing rate of primary-like and chopper units when the intensity of a tone was increased were similar to those observed in single AN fibers (Boettcher et al. 1990; Shore 1995; Burkard and Palmer 1997). In contrast, there are no studies addressing this issue at the whole CN level based on near-field evoked potentials.

In a recent report (Loquet and Rouiller 2002), we demonstrated that the dynamic properties of adaptation to trains of repetitive clicks in VCN were comparable to those of the AN at a fixed intensity. Whether this similarity persists at various intensity levels needed further investigation. To address this question, VCN near-field evoked potentials were chronically recorded in an animal model (unanesthetized adult rats) in response to pulsatile acoustical stimuli of varying intensities (ranging from 5 to 70 dB SPL) and pulse rates (ranging from 100 to 1000 pulses per second, pps). The results are compared to adaptive properties previously established for the auditory nerve.

Materials and methods

Animals

Experiments were conducted on male adult Long-Evans rats (Janvier Laboratories, Le Genest-Saint-Isle, France) weighing approximately 300 g and aimed at recording auditory evoked near-field potentials from a chronic electrode implanted in the left VCN. The experimental procedure was approved by the Swiss veterinarian authorities and was performed in accordance with the *Principles of laboratory animal care* (US NIH Publication No. 86-23, revised 1985) and the 1964 Declaration of Helsinki for animal care. The procedure was described in detail in a recent report (Loquet and Rouiller 2002) and will only be summarized here. Briefly, before surgery, the animals (n=6) were treated with atropine sulfate (0.05 mg/kg s.c.) to minimize respiratory distress, and with a nonsteroidal anti-inflammatory drug (Carprofen, 4 mg/kg i.m.) to reduce inflammation and pain. Then, they were deeply anesthetized with pentobarbital (Vetanarcol, 40 mg/kg i.p.) and placed in a stereotaxic apparatus (Model 1404; Muromachi Instruments, Japan) in order to implant the chronic recording tungsten electrode (2–4 MΩ impedance) in the left VCN (coordinates: AP=−9.80 mm, ML=4.30 mm, DV=7.99±0.35 mm from bregma). A ground electrode was placed in the rostral cranium on the dura mater, and the two electrodes were soldered to a socket and fixed to the skull with dental cement. The animals were allowed to recover for 1 week before beginning chronic recording. The location of the recording electrode in the VCN was histologically verified in the brains of all rats at the end of the experiment (see Loquet and Rouiller 2002).

Acoustic stimulation

Testing was performed with Tucker-Davis Technologies System II (TDT, Alachua, FL, USA) equipment in a sealed sound proof booth (IAC, Niederkrüchten, Germany) on awake rats placed in a restraining device (Loquet and Rouiller 2002). Acoustic signals were synthesized digitally using TDT SigGen32 software and, after digital-to-analog conversion, fed into a programmable attenuator before delivery to a speaker positioned 10 cm away from the left pinna of the rat. The calibration of the system was carried out with a sound level meter (B&K, model 2231) by measuring the sound pressure level (SPL root mean square re 20 μPa) emitted by the speaker when it was driven by a pure tone signal at 9 V peak level. The sound field was calibrated by positioning the microphone (B&K, model 4155, prepolarized free-field 1/2 inch) at the point normally occupied by the center of the animal's head. Within the audiometric room, the ambient noise level did not exceed 69 dB SPL with regard to the overall spectra linear level, and 38 dB SPL with regard to frequencies above 1 kHz. Cochlear nucleus near-field potentials (CNP) were amplified (2×10^3), bandpass filtered between 30 Hz and 5 kHz and then fed into an analog-to-digital converter.

The TDT data acquisition software BioSig32 was used to automate CNP averaging over 50 presentations and for offline analysis.

Acoustic stimulation consisted of repetitive short condensation-rectangular-pulse clicks (100 μs) delivered in a train of 250 ms duration followed by a pause (silent period) of 250 ms before the next train. The intra-train pulse rates varied from 100 to 1000 pps, and five intensities were tested: 70, 50, 30, 10 and 5 dB SPL. The analysis window stretched over 250 ms, and the amplitude of the N_1 component of the CNP was measured as the voltage difference between the first negative (N_1) and the first positive (P_1) peak. At repetition rates greater than 400 pps, responses to individual clicks started to overlap so that the amplitude of a certain response was influenced by the preceding one. To circumvent this contamination, the 250-ms trains were presented in an order so as to always increment click number in the train by one. The average CNP obtained in response to a certain train (*n* clicks) was then subtracted from the CNP to the following train (*n*+1 clicks), the resulting curve exposing solely the *n*+1 click (see Loquet and Rouiller 2002). Thus, individual clicks could be studied free of contamination. Finally, N_1–P_1 amplitudes were normalized with respect to the highest CNP observed in the train (usually the CNP to the first click), and were plotted as a function of the position of the corresponding click in the train.

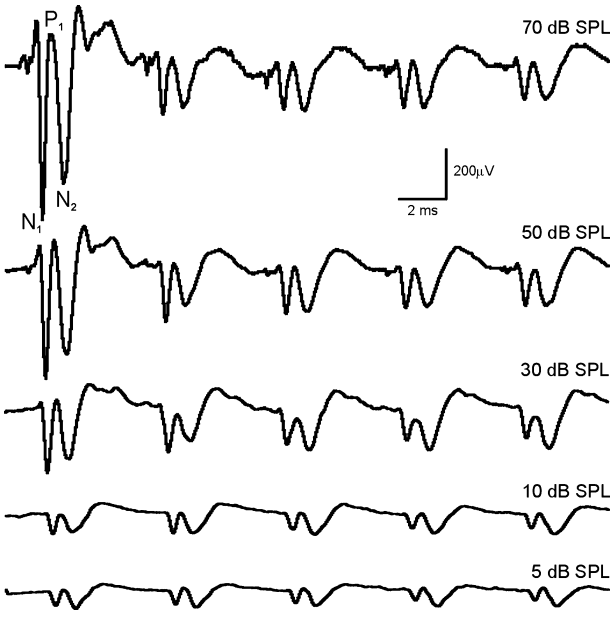

Fig. 1 Typical ventral cochlear nucleus near-field evoked potentials (CNP) elicited by 250-ms trains of repetitive clicks presented at a rate of 200 pps (Rat #6). Only the first 25 ms of the train are shown in order to easily identify the N_1, P_1 and N_2 deflections (negative polarity is downward). Note that the decrease of N_1–P_1 amplitude as a function of time became more obvious at high levels of stimulation. Stimulus intensity is given to the *upper right* of each trace

Results

As shown in Fig. 1, CNP amplitude measured as the voltage difference between the negative peak N_1 and the subsequent positive peak P_1 varied as a function of both intensity of stimulation and position of the stimulating click in the train. In Fig. 1, one of the most obvious effects of an augmentation in stimulus intensity is the increase of the first through-to-peak N_1–P_1 amplitude and the decrease of its latency. Then, it can also be noticed that increasing the intensity of stimulation induced an accentuation of amplitude differences between responses to the first click and the subsequent ones. In other words, these qualitative data show that adaptation was more pronounced when stimulus intensity increased.

Amplitudes of individual CNPs to consecutive clicks in the train were plotted for each rat; one representative animal is depicted in Fig. 2. In general, curves tend to display an exponential decrease of the normalized CNP amplitudes as a function of time (position of the corresponding stimulating click in the train). Most curves exhibit an initial rapid adaptation followed by a slower adaptation and a plateau, except for those obtained at low repetition rates (100 and 200 pps) and low intensities (5–10 dB SPL) where one phase was sometimes missing. In addition, for each rate tested (100–1000 pps), adaptation became more pronounced when intensities were increased from 5 to 70 dB SPL, as represented by a decrease of the plateau level and a shortening of the rapid and short-term adaptive components. This latter assumption was verified by determining the decay time constants of the two

Table 1 Time constants of cochlear nucleus auditory-evoked potentials as a function of stimulus rate and intensity. K_1, rapid, and K_2, short-term adaptation time constants were determined with non-linear regression fitting curves (see Results section). The few curves that were better described by a one-component time-constant equation are not included in this table. Mean values and standard deviations (SD) were calculated from data obtained from five rats

Stimulus rate	Value	Time constant at stimulus intensity									
		5 dB		10 dB		30 dB		50 dB		70 dB	
		K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2	K_1	K_2
100 pps	Mean	15.5[a]	46.6[a]	11.4	61.2	12.2	49.9	5.3	50.6	2.4	48.1
	SD			3.8	17.1	5.5	12.1	3.4	8.1	3.2	12.6
200 pps	Mean	6.5	74.4	9.7	64.0	4.6	54.8	2.8	41.4	2.4	44.0
	SD	1.5	25.7	4.3	37.1	1.4	16.0	1.2	21.1	1.6	34.1
400 pps	Mean	4.4	64.5	4.6	66.4	2.2	54.6	1.4	36.4	0.5	6.3
	SD	2.3	25.5	1.4	21.2	0.7	15.5	0.5	36.9	0.5	2.7
600 pps	Mean	3.8	52.2	3.1	45.9	1.2	30.2	0.8	46.4	0.2	6.8
	SD	1.2	15.1	1.6	25.4	0.6	23.4	0.2	49.4	0.2	0.9
800 pps	Mean	2.6	100.0	1.6	38.5	0.7	28.6	0.2	28.1	0.1	4.9
	SD	1.4	100.8	1.0	24.9	0.2	21.8	0.2	18.8	0.1	2.7
1000 pps	Mean	1.5	54.5	1.1	49.7	0.3	15.0	0.3	8.8	0.2	5.1
	SD	1.6	62.5	0.8	55.3	0.3	11.8	0.3	5.4	0.1	0.9

[a]Adaptation curve of only one animal fitted by a two-component time-constant equation

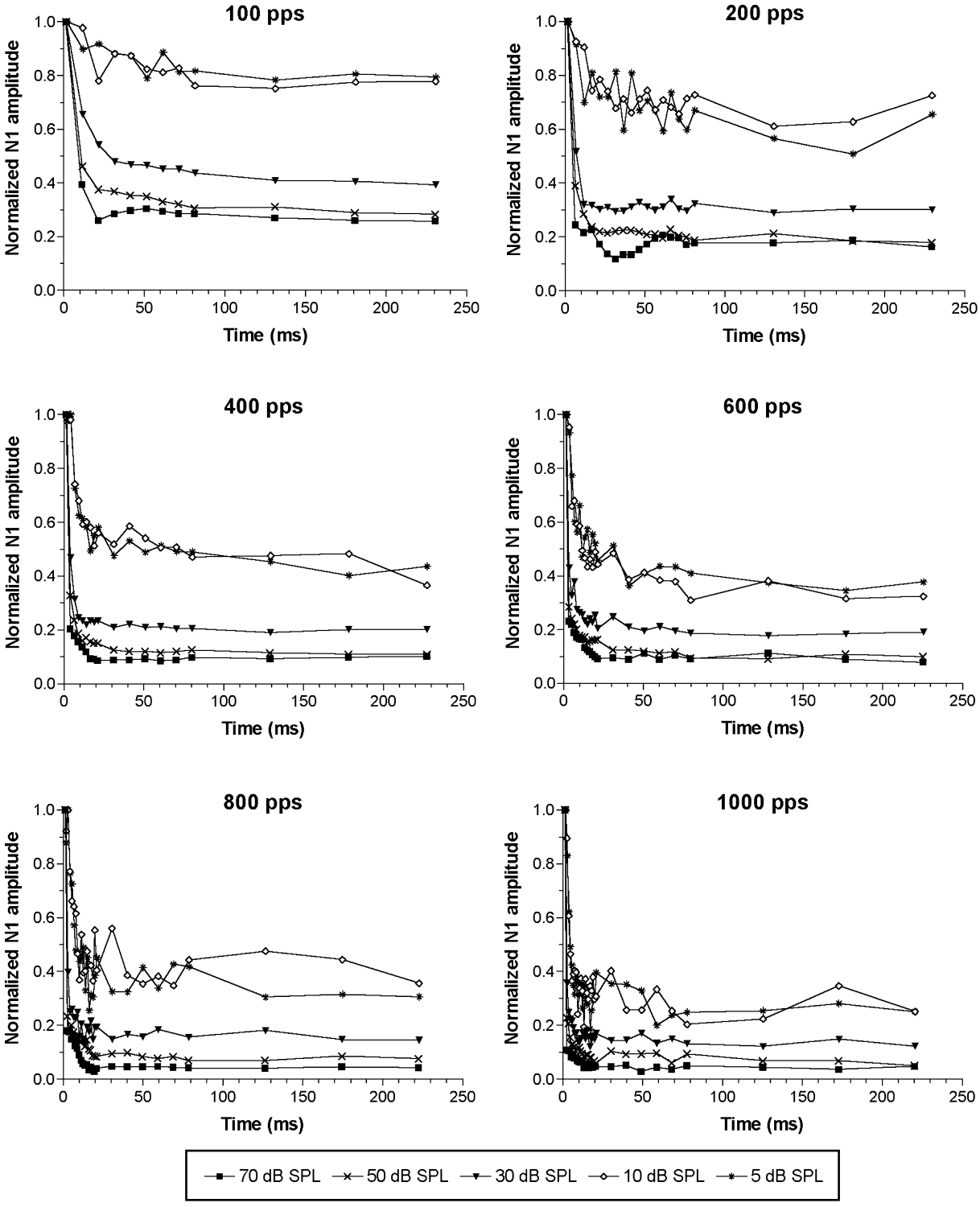

Fig. 2 Normalized amplitudes of ventral cochlear nucleus near-field evoked potentials in Rat #6 displayed as a function of the position of the stimulating clicks in the 250 ms train. Five intensities ranging from 70 to 5 dB SPL are presented for each of the click rates ranging from 100 to 1000 pps

adaptive components using exponential decrease equation fittings that we previously demonstrated to be consistent with adaptation properties of VCN near-field potentials (Loquet and Rouiller 2002). The equation used was:

$$NP(t) = NP_1 e^{-t/\kappa_1} + NP_2 e^{-t/\kappa_2} + Plateau$$

where NP_1, NP_2 are the y-intercepts of the rapid and short-term components respectively; *Plateau* is equal to the

N_1–P_1 amplitudes during the steady state response, and K_1, K_2 are the decay time constants of the two postulated adaptation components. In some instances, in particular at the lowest intensities of stimulation (5–10 dB SPL), data were best fitted with a one-component time constant adaptation equation (NP_2=0). The curve-fitting was run using the Levenberg-Marquardt method with GraphPad Prism v3.02 software and the deviation from the model was assessed by considering the correlation coefficient ($R^2 \geq 0.70$) and by testing the Gaussian distribution of the residuals around the curve ($P>0.1$). Mean values of K_1 and K_2 were calculated from data obtained in five animals; they are summarized in Table 1 and depicted in Fig. 3 (results of fittings using only one-component time constant have not been included in the data presented). The rapid time constants (K_1) exhibited a progressive decrease as intensity of the stimulus increased. A maximum reduction of 13.1 ms (from 15.5 to 2.4 ms) was obtained at 100 pps, and was less pronounced at higher pulse rate (1.3 ms at 1000 pps). The short-term time constants (K_2) appeared to be roughly independent of intensity over an intensity range of 45 dB (from 5 to 50 dB SPL). In contrast, at 70 dB SPL, a marked decrease of K_2 was observed at 400 pps and above. Large inter-individual variabilities were observed at rates of 800 and 1000 pps.

For each rat, the total extent of adaptation was estimated by the ratio of the plateau amplitude to the highest CNP amplitude in the train. The magnitude of the plateau was determined by averaging the CNP amplitude values obtained during the last 150 ms of the train. These data were then averaged across the six rats and plotted in Fig. 4. On the right of the figure, an adaptation indicator has been introduced to underline the fact that the smaller the ratio, the more pronounced the adaptation. The curves, progressively declining from left to right, thus indicate an increase in adaptation for higher levels of stimulation. Indeed, increasing the intensity from 5 to 70 dB SPL led to an augmentation of adaptation of about 50% at 100 pps, and 40% at 200 pps. Interestingly, one can notice that at all higher repetition rates (400 to 1000 pps) this augmentation of adaptation with intensity remained around 30%. This suggests a separation of the data into two groups: at high repetition rates (400, 600, 800, 1000 pps) the change of adaptation with intensity is comparable, whereas at low pulse rates (100, 200 pps), the progressive increase of adaptation with intensity is rate-dependent and, in addition to that, more marked than at high repetition rates.

Discussion

CNP waveforms obtained in the present study in response to click stimuli (Fig. 1) are similar to those elicited by tone bursts that were reported recently (Loquet and Rouiller 2002). The 2.11 ms latency of the N_1 response at 5 dB SPL is consistent with previously observed values for the CN ranging between 2.0 and 3.0 ms (Huang and Buchwald 1980; Møller 1983), as opposed to shorter latency values obtained from the AN ranging between 0.5 and 1.8 ms (Møller 1983). However, as to the exact origin of the N_1 deflection within the CN, latency data do not allow for further conclusions since a large overlap exists between anteroventral, posteroventral and dorsal CN latencies (Godfrey et al. 1975a, 1975b). Nevertheless, under our experimental conditions, the N_1 response most likely reflects synchronized discharges in the VCN for the following reasons. Firstly, the histological verification in the brainstems of all rats (data not shown) demonstrated that the tip of the chronic recording electrode was clearly located in the anteroventral part of the CN. Secondly, the contribution of units located in the dorsal CN (pauser and buildup units) is unlikely, considering their inhibitory response patterns and their low ability to follow repetitive clicks (Rhode and Smith 1986).

The exponential decrease of CNP amplitudes in response to repetitive stimuli shown in Fig. 2 is in

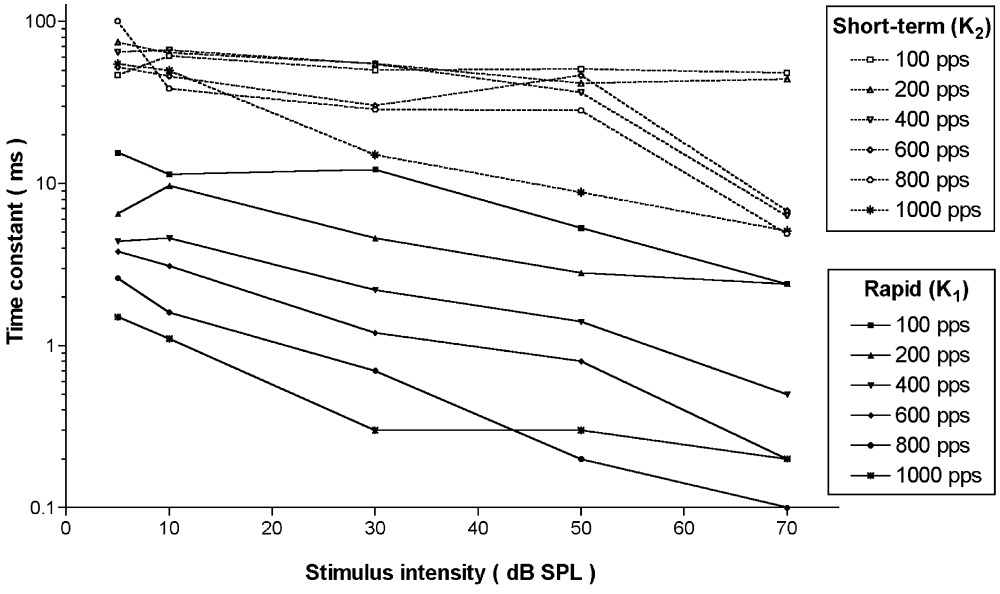

Fig. 3 Mean fitted decay time constants displayed as a function of stimulus rate and intensity. Time constants for rapid (K_1) and short-term (K_2) adaptation components were obtained from five animals by fitting normalized N_1–P_1 amplitude curves (see "Results" section). Standard deviations of data points are omitted for the sake of clarity but can be found in Table 1

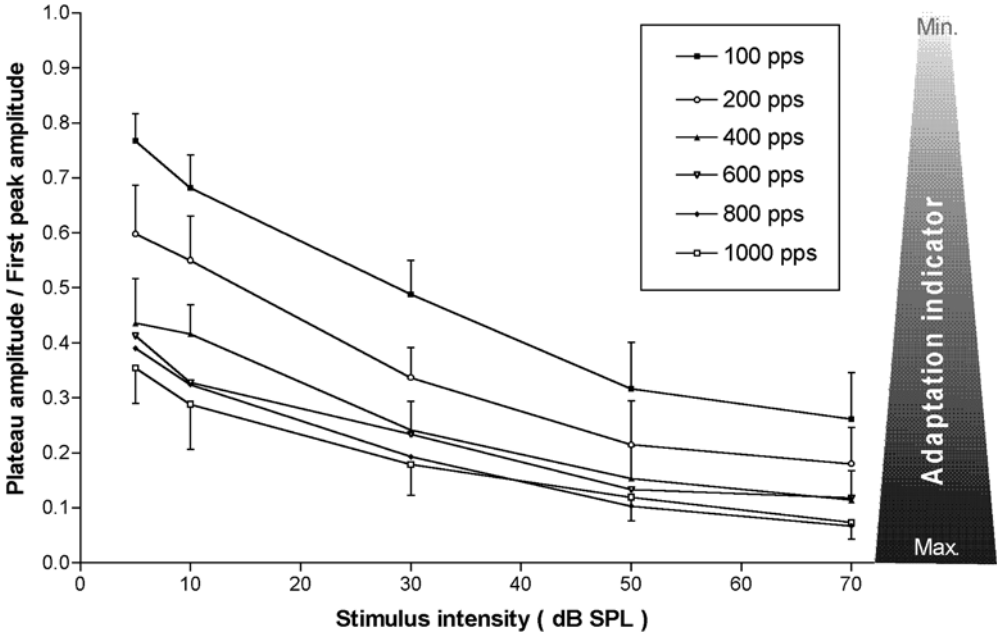

Fig. 4 Amount of adaptation expressed as the ratio of the plateau to the first peak amplitude, as a function of stimulus intensity. Mean values were derived from six animals, the *bars* representing 95% confidence intervals. At two stimulation rates (600 and 800 pps), the bars are omitted for the sake of clarity. An adaptation indicator is depicted on the *right* of the graph

agreement with the data of Huang (1981) and Loquet and Rouiller (2002). Such an adaptation generally follows a two-phase exponential decline (rapid [K_1] and short-term [K_2] adaptive components) to finally reach a plateau. This pattern varies as a function of stimulation rate with a faster (decreasing time constants) and larger (decreasing plateau level) decay as frequency increases. This repetition-rate effect on neural adaptation in the CN is in agreement with our previous report (Loquet and Rouiller 2002) and with data obtained in the AN (Peake et al. 1962a), for which the response amplitude decreased as soon as stimulus rate exceeded 10 pps. Unexpectedly, however, the present study demonstrates that the augmentation of the amount of adaptation as a function of increased stimulus intensity (Fig. 4) does not depend strongly on the repetition rate at 400 pps and above. The reason for such a segregation into two groups (high and low repetition rates) is not known but one may speculate that it is related to the refractory period. At 100, 200 and 300 pps, the unit is not affected by refractory mechanisms, in contrast to high rates (400 pps and above) for which the period between two consecutive pulses falls within the relative refractory period.

Concurrently with the rate effect, the present data indicate that adaptation in VCN depends on the level of stimulation. Indeed, increasing the stimulus intensity from 5 to 70 dB SPL led to a more prominent exponential decrease of CNP amplitudes, irrespective of the rate tested (Fig. 2). As mentioned in earlier studies (Westerman and Smith 1984; Yates et al. 1985), this effect is mainly attributable to the rapid adaptation time constant (K_1), which shows a constant decrease with increasing intensity (Table 1 and Fig. 3). In contrast, the short-term adaptation time constant (K_2) shows little intensity dependence (except for the interval between 50 and 70 dB SPL), an observation in line with data on AN fibers (Smith and Zwislocki 1975; Westerman and Smith 1984). The effects of intensity on VCN adaptation is also well characterized by the level of the plateau (steady-state component), whose decrease was at its most significant between 10 and 30 dB SPL (Figs. 2 and 4). At high stimulus intensities (50–70 dB SPL), average amounts of adaptation were similar to those found in high-spontaneous-rate nerve fibers (Brown 2001). Therefore, in our experimental conditions (awake rats), VCN near-field evoked responses appear to be essentially the same as those recorded in the AN, as far as adaptation is concerned.

Physiological mechanisms

In line with the present study, authors presenting simple short tone bursts, either as described by Blackburn and Sachs (1989) or in the context of a forward-masking paradigm (Boettcher et al. 1990), observed a more pronounced adaptation with increasing intensity in some CN neurons, namely the bushy cells (which produce primary-like and primary-like-with-notch responses) and stellate cells (usually associated with chopper responses), than in cell types generating other response patterns (pauser, buildup, onset). More recently, response decrement differences were found within VCN (Shore 1995), in particular between primary-like, primary-like-with-notch, sustained chopper, transient chopper, low-intensity chopper, onset and on-chopper responses. The author suggested that both adaptation and inhibition were involved in producing these differences. In the present study, it is likely that adaptation recorded in the VCN by using near-field evoked potentials (summation of individual neurons) mainly reflects the adaptive properties of primary-like units, which have themselves properties close to those of AN fibers, in particular comparable adaptive time constants. As far as chopper units are concerned, their contribution seems to be substantially less than that of primary-like units according to Shore (1995) and Burkard

and Palmer (1997). Moreover, it can be noticed that AN and CN adaptations are likely to mirror still more peripheral changes, primarily located at the hair cell-nerve fiber synapse (Norris et al. 1977; Furukawa et al. 1978). At this stage, adaptation was suggested to involve depletion of neurotransmitter in a cascade of reservoirs (Eggermont 1985).

Overall, despite the fact that adaptation is complex and may depend on specific properties of each relay along the auditory pathway, the present study supports the idea of a reconciliation between single unit data and near-field evoked potentials. Indeed, in the same way that adaptation of VCN primary-like units is essentially the same as that of AN fibers, CNP adaptation obtained in VCN was found to be comparable to CAP adaptation obtained in AN. Therefore, it seems reasonable to hypothesize that our CNP data mainly reflect properties of primary-like units in VCN. As a consequence, the present animal model can be used to better understand auditory neural adaptation phenomena and to transpose these features to cochlear implants that aim at restoring, as accurately as possible, normal physiological hearing in profoundly deaf patients. Such studies could lead to direct clinical applications. They could, for example, be used to improve current stimulation paradigms in order to achieve better speech recognition in ambient noise by cochlear implant patients.

Acknowledgements The authors would like to thank B. Aebischer, E. Regli and A. Gaillard for their technical assistance, F. Tinguely for the histology, and J. Corpataux and B. Morandi for taking care of the rats in the animal room. This research project was supported by the Swiss National Science Foundation (Grant no. 32-56352.99; TANDEM) and the National Center for Competence in Research (NCCR) "Neural Plasticity and Repair".

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Original Paper

Audiology & Neuro-Otology

Audiol Neurootol 2004;9:144–159
DOI: 10.1159/000077266

Received: June 18, 2003
Accepted after revision: December 10, 2003

Matching the Neural Adaptation in the Rat Ventral Cochlear Nucleus Produced by Artificial (Electric) and Acoustic Stimulation of the Cochlea

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Key Words

Click · Cochlear implant · Cochlear nucleus · Latency · Near-field potential · Repetitive pulses

Abstract

To investigate neural adaptive properties, near-field evoked potentials were recorded from a chronically implanted electrode in the ventral cochlear nucleus in awake Long-Evans rats exposed to acoustic stimuli or receiving intracochlear electric stimulation. Stimuli were 250-ms trains of repetitive acoustic clicks (10, 30 and 50 dB SPL) or biphasic electric pulses (30, 50 and 70 μA) with intratrain pulse rates ranging from 100 to 1000 pulses per second (pps). The amplitude of the first negative (N_1) to positive (P_1) component of the average evoked potentials was measured for each consecutive individual pulse in the train. While a progressive exponential decrease in N_1–P_1 amplitude was observed as a function of the position of the pulse within the train for both types of stimulation, the decrement of electric responses (adaptive pattern) was substantially less prominent than that observed for acoustic stimuli. Based on this difference, the present work was extended by modifying electric stimuli in order to try to restore normal adaptation phenomena. The results suggest the feasibility of mimicking acoustic adaptation by stimulation with exponentially decreasing electric pulse trains, which may be clinically applicable in the auditory implant field.

Introduction

As a result of sustained efforts made over the past 30 years to define features of speech coding for cochlear prostheses, many profoundly deaf patients demonstrate nowadays very high levels of speech intelligibility in quiet with their cochlear implant [Tyler et al., 1995]. However, most of the patients still complain about unsatisfactory performance in background noise [Fetterman and Domico, 2002]. Among several reasons for this, one major cause is a deterioration of the ability to process dynamic aspects of speech such as abrupt changes in intensity, special transitions (vowel-consonant) and different spectral components. Indeed, although the most basic characteristics of the normal auditory system such as frequency selectivity and tonotopy [Parkins and Anderson, 1983], nonlinear compression and temporal coding [Wilson, 1991] are well reproduced by an accurate temporal and spatial delivery of electric stimuli to the cochlea, several natural features are still not reproduced by present electric stimulation paradigms. The features not yet taken into consideration

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include, for instance, differences between auditory nerve (AN) fiber subpopulations in terms of spontaneous firing rates, a property correlated with threshold [Liberman, 1978], or the decline in the AN fibers' discharge rate following the onset of a tone burst referred to as a phenomenon of adaptation. Adaptation has been observed, in normal hearing conditions, at several levels of the auditory pathway: first, at the hair cell level, during translation of the stimulus into hair bundle deflection and then in the transduction of the bundle deflection into a receptor potential (data from the frog reviewed in Eatock [2000]); second, at the hair cell-AN fiber synapse, during transmitter release and postsynaptically [Furukawa and Matsuura, 1978]; third, more centrally, in the population of primary auditory neurons [Eggermont and Spoor, 1973; Westerman and Smith, 1984; Yates et al., 1985; Rhode and Smith, 1985; Müller and Robertson, 1991; Javel, 1996] and in the cochlear nucleus (CN) [Møller, 1969; Evans, 1975; Huang, 1981; Burkard and Palmer, 1997; Loquet and Rouiller, 2002]. Based on numerous studies, authors seem now to agree in locating the major source of auditory adaptation at the hair cell-AN synapse, due to a depletion of available synaptic vesicles [Furukawa and Matsuura, 1978; Furukawa et al., 1982]. Adaptation observed more centrally probably reflects a combination of the adaptation taking place in the organ of Corti as well as in the central nervous system, and one could argue that it is a signaling cascade in which peripheral sites reverberate their effects more centrally. This view is supported by data [Gerken, 1979] demonstrating that auditory peripheral damages affect responses evoked by central electric stimulation. In practice, in response to a tone burst (typically 50 ms duration), but also to short repetitive tone bursts [Müller and Robertson, 1991] or click trains [Wickesberg and Stevens, 1998], AN fibers and CN units (primary-like and chopper response types) exhibit an abrupt increase in firing rate at stimulus onset followed by a rapid decrease during the next 10–30 ms to reach a plateau maintained until stimulus offset. We may postulate that such an adaptive response pattern is likely to prevent auditory neurons from constantly discharging (in some cases at saturation) along the entire stimulus duration, in order to make them quickly sensitive again to a subsequent stimulus occurring during the ongoing initial stimulus or to a rapid change of the latter. Adaptation might therefore emphasize the contrast between novel stimuli relative to background stimuli and thus significantly contribute to improve speech perception.

However, adaptation phenomena observed during acoustic stimulation of a normal ear clearly differ from those elicited by current electric stimulation devices which still code a tone burst of constant amplitude as a current burst of constant amplitude. For example, previous studies in human subjects [Wilson et al., 1997] and in animals [Kiang and Moxon, 1972; Hartmann et al., 1984; van den Honert and Stypulkowski, 1984, 1987; Javel et al., 1987; Parkins, 1989; Haenggeli et al., 1998; Matsuoka et al., 2000] have established that evoked potentials or discharges of AN fibers in response to electric stimuli are excitatory responses, precisely phase-locked to sinusoidal or pulse train stimuli for low stimulation rates (<1 kHz or 100–200 pulses per second, pps). Furthermore, increasing stimulus intensity produced a greater degree of synchronization but no adaptation. In contrast, for higher rates, an adaptation effect has been reported in the AN in both humans [Wilson et al., 1997] and animals [Javel et al., 1987; van den Honert and Stypulkowski, 1987; Haenggeli et al., 1998; Matsuoka et al., 2000], where the response was maximal at the beginning of a pulse train, followed by a progressive decay to reach a plateau after 30–40 ms from stimulus onset. However, if the interpulse interval of the electric pulse train is within the relative refractory period of the stimulated fibers, then the response to the second pulse in the train is reduced and a pattern of amplitude alternation or oscillation to successive pulses was observed [Wilson et al., 1997; Matsuoka et al., 2000]. This phenomenon was more pronounced in humans than in animals and made adaptation difficult to estimate. At very high rates of pulsatile electric stimulation (4000 pps and above), due to a rapid adaptation, the response reached a plateau quickly during the first milliseconds of stimulation. Based on these data, it was suggested that speech perception could be enhanced by using such high stimulation rates in cochlear prostheses [Wilson, 1997]. In summary, adaptation in the AN appears strongly dependent on the rate of pulsating electric stimuli, although some studies revealed a considerable interfiber variability in the time course of adaptation [Dynes and Delgutte, 1992; Killian et al., 1994; Litvak et al., 2001].

In the CN, adaptation phenomena [Møller, 1969; Evans, 1975; Huang and Buchwald, 1980; Huang, 1981; Boettcher et al., 1990; Shore, 1995; Burkard and Palmer, 1997; Loquet and Rouiller, 2002; Loquet et al., 2003] are more difficult to study than in the AN because of the presence of a large variety of cell types [Osen, 1969] processing in parallel the stereotyped incoming acoustic information. In the past, numerous studies have described in detail the variability of discharge patterns across CN neurons when stimulated with tones [Kiang et al., 1965; Pfeif-

fer, 1966; Evans and Nelson, 1973; Godfrey et al., 1975], but there have been fewer studies aimed at investigating the effects of repetitive electric stimulation of the cochlea in the different subregions of the CN [Shofner and Young, 1985; Glass, 1985; Maffi et al., 1988; Wiler et al., 1989; O'Leary et al., 1995b; Paolini and Clark, 1998; Babalian et al., 2003]. In the ventral part of the CN (VCN), pulsatile electric stimulation of the cochlea led to a reduction of the diversity of discharge patterns as compared to acoustic stimulation. A high degree of synchronization in response to continuous constant-current sinusoids [Glass, 1984; Clopton and Glass, 1984] or to pulse trains [Maffi et al., 1988] was observed for frequencies up to at least 12 kHz or for rates up to 800 pps, respectively. In response to electric AN stimulation, intracellular recordings [Babalian et al., 2003] demonstrated that VCN cells followed with high probability each pulse in a train at low stimulation rates (200–300 pps) whereas at high stimulation rates (500–1000 pps), the usual response patterns (primary-like, onset) were evoked. In the dorsal part of the CN, neurons exhibited primary-like, onset, buildup and pause response patterns, but none synchronized its activity to repetitive pulses even at low rates, as demonstrated on the basis of extracellular [O'Leary et al., 1994, 1995a, b] and intracellular [Babalian et al., 2003] recordings.

To study neural adaptive properties further, a way could be to stimulate the same ear with both acoustic and electric stimuli in order to perform a direct comparison between the two modes of stimulation. Such an approach has been used in few studies, based on acute AN evoked compound action potential [Prijs and Eggermont, 1975; Simmons and Glattke, 1972; Prijs, 1980] or single unit recordings [Parkins, 1989]. In the present study, we used pulsatile acoustic and electric stimuli of varying intensities and repetition rates and recorded near-field evoked potentials from a chronic electrode implanted in the VCN. This approach was chosen in order (1) to obtain stable recordings along repeated (acoustic, then electric) stimulation sessions in unanesthetized adult rats; (2) to perform measures from the same ear before and after cochlear implantation allowing then direct comparisons between acoustic and electric data. First, the present study confirmed that neural adaptation to repetitive electric pulses is much less prominent than in response to acoustic repetitive pulses. Second, the comparison of acoustic and electric CN evoked potentials allowed us to better infer the location of this adaptation. Finally, a paradigm of electric stimulation of the cochlea was developed yielding a neural adaptation in VCN mimicking that observed with acoustic stimulation.

Methods

Animal Preparation

Male adult Long-Evans rats (Janvier Laboratories, France) weighing approximately 300 g at the beginning of the experiment underwent two surgeries at a 1-month interval in order to record near-field potentials in the CN in response to successive stimulation of the same ear with acoustic and electric pulses. Surgical and experimental procedures were approved by the Swiss veterinary authorities and were conducted in accordance with the guidelines of the US NIH and the Declaration of Helsinki for animal care.

The first surgery, aimed to implant a chronic recording electrode in the left VCN, was described in detail in a previous report [Loquet and Rouiller, 2002] and is only briefly summarized here. Firstly, rats (n = 4) were deeply anesthetized with pentobarbital (Vetanarcol®, 40 mg/kg, i.p.) and treated with atropine sulfate (0.05 mg/kg, s.c.) to minimize respiratory distress and carprofen (4 mg/kg, s.c.) to reduce pain. Then, they were placed in a stereotaxic apparatus, and 1 tungsten electrode (2–4 MΩ impedance) was chronically implanted in the left VCN, whereas a second electrode was inserted in the rostral cranium to serve as the ground electrode. The 2 electrodes were soldered to a socket, then fixed to the skull with dental cement, and finally the animals were allowed to recover for 1 week before sessions of chronic recording in response to acoustic stimuli took place.

About 1 month later, the second surgery was conducted in the same rats in order to implant a chronic stimulating electrode in their left cochlea. To achieve this purpose, the animals were anesthetized (Vetanarcol, 40 mg/kg, i.p.), treated with atropine sulfate and carprofen and then placed in a custom-made surgical table. A heating pad was used to maintain the body temperature within a range of 37–39 °C. Through a retroauricular approach on the left side without damaging the facial nerve, the otic capsule was opened to expose the round window and, under visual control with an operating microscope, an intracochlear stimulating electrode was inserted 4 mm inside the round window (approximately half of the basilar membrane length). The electrode was a 100-µm diameter platinum-iridium Teflon®-coated wire, flamed to a ball at the tip (approx. 0.15 mm) and 200 kΩ impedance (tested in saline with a 1-kHz sine wave). The electrode was then secured with connective tissue, and the bulla was closed with dental cement. For the return (monopolar stimulation), a PtIr ball electrode (ball diameter: ±0.5 mm, 100 kΩ impedance) was inserted and ligated in the cleidomastoideus muscle. Then, the 2 electrodes were soldered to the skull socket and fixed with dental cement. The electric stimulation sessions began the next day and lasted up to 4 days after implantation.

Stimulation

Acoustic stimuli were generated with a Tucker-Davis Technologies system II equipment (SigGen32 software, programmable attenuator and D/A converter) and delivered via a speaker (JBL®, 2405H) positioned 10 cm away from the left pinna of the rat. Stimuli consisted of 250-ms trains of rectangular condensation clicks (100 µs) followed by a 250-ms pause before the next train. For such a duration (100 µs), the click has a frequency spectrum ranging from 0.25 to 10 kHz, tonotopically corresponding to about 40% of the basilar membrane length from the apex (~3.2 mm, according to Greenwood [1996]). The intra-train pulse rates varied from 100 to 1000 pps, and 3 intensities were tested: 50, 30 and 10 dB SPL. The system was calibrated with a Bruël & Kjær 12.7-mm microphone by measuring the sound pressure level (RMS, re: 20 µPa) emitted by the

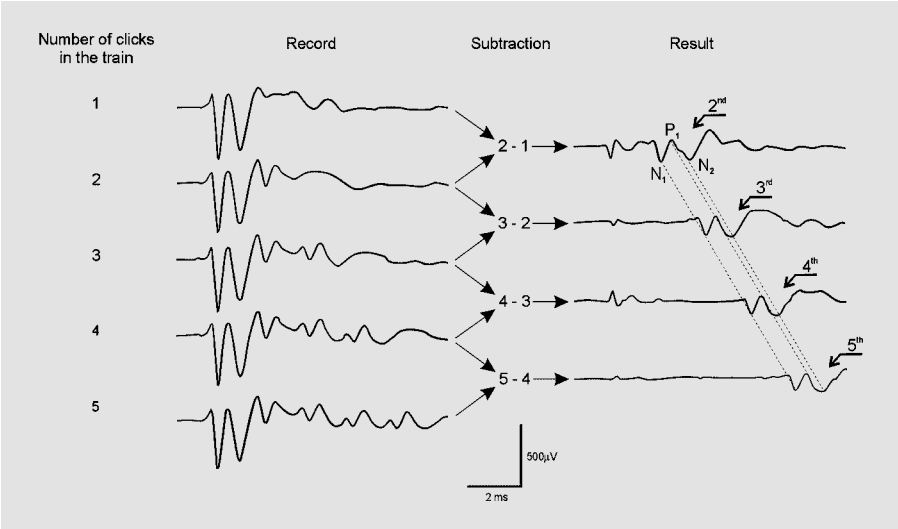


Fig. 1. Illustration of the subtraction method used to measure the amplitude of the response (N_1–P_1) in near-field evoked potentials to individual clicks delivered in a train. The columns from left to right show the number of clicks in the train, the raw responses evoked by the corresponding number of clicks ('record'), the subtraction performed (e.g. trace 2 – trace 1) and the resulting waveforms ('result'). Data are from rat No. 4 and were obtained with clicks presented at the rate of 600 pps, at an intensity of 50 dB SPL. The first 10 ms of the train are shown, and negative polarity is downward. The dashed line points to the 'uncontaminated' response to the 2nd, 3rd, 4th and 5th clicks in the train, from top to bottom in the rightmost column.

speaker when it was driven by a train of clicks at a repetition rate of 1000 pps. The calibration microphone was positioned at the location occupied by the central point of the animal's head.

Electric stimuli were generated by a programmable numerical speech processor referred to as 'Geneva Wearable Processor' [Pelizzone et al., 1999] and developed by the Cochlear Implants Center at the Cantonal University Hospital of Geneva. The system was built by using a Motorola application development system which consists of an application development module containing a 40-MHz Motorola 56002 DSP (digital signal processor) and a software controlling the application development module. An interactive MATLAB subroutine was added to allow the user to set the characteristics of the electric stimuli (intensity, pulse rate, train duration). At the output of the system, numerical signals were converted (D/A at 20 kHz, 12 bits) into trains of biphasic pulses routed to custom optoisolated current generators and delivered directly to the implanted electrodes in the cochlea. Single anodic-first biphasic pulses (50 μs/phase), identical to those used in human cochlear implants [Pelizzone et al., 1999], were first produced at increasing intensity levels (from 0 to 100 μA in steps of 10 μA) in order to establish the growth function of electric CN evoked potentials. Based on the monotonic functions obtained, 3 intensities were chosen, namely 30 μA (close to threshold intensity), 50 μA (intensity giving about 50% of the maximal response) and 70 μA (close to the intensity of saturation). Adaptation was tested in a way similar to acoustic stimulation, and therefore the electric stimuli consisted in pulse trains of 250 ms followed by a 250-ms pause before the next train with intratrain pulse rates varying from 100 to 1000 pps. The in situ monopolar impedance values of the intracochlear electrode ranged from 6 to 17 kΩ.

Recording

Recordings were performed in an audiometric room (IAC, Germany) on awake rats placed in a restraining device [Loquet and Rouiller, 2002]. The acoustic and electric CN evoked potentials (aCNP and eCNP, respectively) were amplified (2×10^3), bandpass filtered between 30 Hz and 5 kHz, then fed into an A/D converter (Tucker-Davis Technologies system II). The data acquisition software BioSig32 was used to automate CNP averaging over 50 presentations, for offline analysis, and also to trigger the electric stimulation (56002 DSP). The analysis window stretched over 250 ms. CNP latencies were measured from stimulus onset to the peaks N_1 and N_2 of the response, whereas CNP amplitude was measured as the voltage difference between the first negative (N_1) and the first positive (P_1) peaks. Because responses to individual clicks overlapped at repetition rates higher than 400 pps, CNP responses were derived using a subtraction method (fig. 1). The method consists of deriving the response to the nth click in a train by subtracting a record which had 1 click less in its train. The resulting waveform thus exhibited the CNP to the nth click without contamination by the CNP of the preceding clicks. This method was used by other authors [Wilson et al., 1997; Rubinstein et al., 1999] and was previously validated with acoustic stimulation [Loquet and Rouiller, 2002]. The subtraction method was applied to all repetition rates tested, and the N_1–P_1 amplitude was normalized relative to either the largest CNP observed in the same train (usually the CNP to the first pulse) or to the largest CNP obtained at the highest stimulus level for a given repetition rate. The normalized CNP was then plotted as a function of the position of the corresponding stimulating pulse in the train expressed in milliseconds from stimulus onset.

Histology

The location of the recording electrode in the VCN was verified at the end of the experiment (3 months after the implantation) in all implanted rats. To achieve this purpose, the animals were deeply anesthetized with an overdose of pentobarbital (Vetanarcol, 80 mg/kg, i.p.), and a continuous, positive current (10 μA) was passed through the recording electrode for 10 min. The rats were then perfused through the heart with normal saline followed by a 4% paraformaldehyde solution in phosphate buffer (0.1 *M* at pH = 7.4). After decapitation, the brain was removed, postfixed for 1 night in the fixative solution and kept the next day in a 30% sucrose solution at +4°C for cryoprotection. Frozen coronal sections (50 μm thick) were cut, washed in phosphate buffer and mounted on slides. Finally, sections were counterstained with cresyl violet (Fluka, 0.06%), coverslipped with Eukitt and observed by light microscopy.

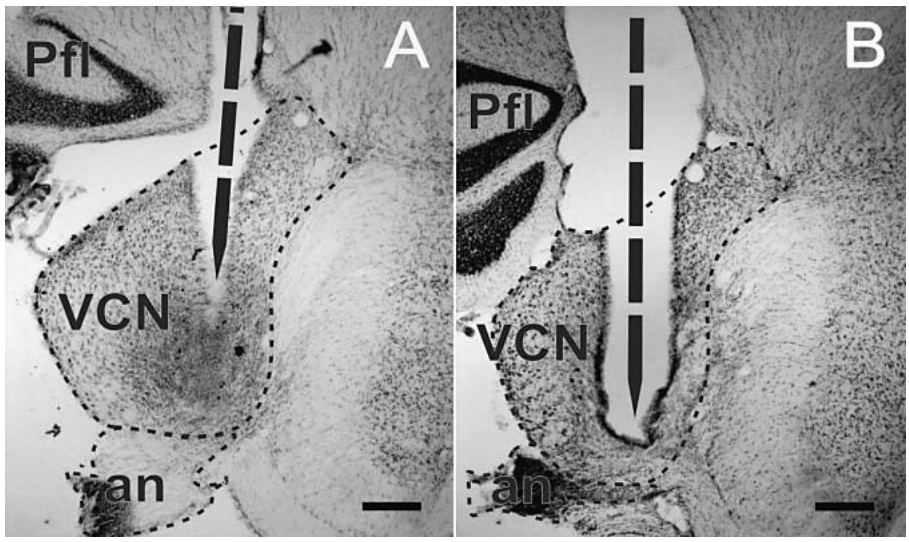


Fig. 2. Photomicrographs of frontal sections through the brainstem showing the location of the tip of the chronic recording microelectrode in the left VCN of 2 implanted rats: rat No. 1 (**A**) and rat No. 2 (**B**). an = Auditory nerve; Pfl = paraflocculus. The dashed arrow represents the electrode track. Scale bar = 300 μm.

Results

The photomicrographs presented in figure 2 show the location of the tip of the chronic recording electrode used to derive CNP from the VCN for 2 animals. A variation of insertion depth was observed but did not exceed 300 μm across animals. In all 4 animals, the electrode was located in the VCN.

CNP in Response to Steady Acoustic or Electric Pulse Trains

Examples of typical acoustic and electric CNP responses derived from the same chronic VCN recording electrode are presented in figure 3A and B, respectively, for the first 35 ms of 250-ms records. Very stable aCNP responses were observed over several weeks, whereas the thresholds of the eCNP increased slightly starting at 5 days after implantation. The response to individual 100-μs rectangular acoustic clicks was characterized by 2 negative deflections of comparable magnitude referred to as N_1 and N_2, separated by a peak of opposite polarity (P_1). At the rate of 100 pps, aCNP magnitudes were uniform along the train for low stimulation intensities (10 dB SPL). In contrast, increasing the intensity of stimulation (30 and 50 dB SPL) resulted in an increase in the trough-to-peak N_1–P_1 amplitude in response to the first individual click and a progressive decrease in the amplitude of the responses to the following clicks in the train. Such a decrease of the response amplitude to subsequent pulses in the train reflects the phenomenon of adaptation. The aCNP latencies determined for the peaks N_1 and N_2 are presented in figure 4A and exhibited a concurrent decrease (constant interwave latencies) as a function of intensity from 10 to 50 dB SPL. Similar to the records obtained with acoustic stimuli, the responses to individual electric biphasic square pulses (fig. 3B) exhibited successive negative peaks (N_1 and N_2), separated by a peak of opposite polarity (P_1). In contrast, the recordings showed large residual artifacts (at most 5 times of the N_1–P_1 height) 300 μs after the onset of the stimulus and a substantially smaller N_2 peak than N_1. At the rate of 100 pps (fig. 3B), eCNP magnitudes varied largely as a function of stimulation intensity but remained remarkably constant along the train irrespective of the stimulation intensity tested (30, 50 or 70 μA). Thus, in contrast to acoustic clicks (fig. 3A), repetitive electric pulses delivered at low rates (100 pps) did not result in a significant adaptation of the response (fig. 3B). The eCNP latencies determined for the peaks N_1 and N_2 are presented in figure 4B and appeared to be independent of the intensity.

The effect of stimulation rate and intensity is illustrated quantitatively in figure 5, where amplitudes of individual CNPs to consecutive clicks (in the left column) or electric pulses (in the right column) were plotted for 1 representative animal. Generally, the evoked CNP showed a progressive decrease (adaptation) of the normalized amplitudes as a function of the position of the pulse along the train. On the one hand, the adaptive pattern of acoustic responses is a decay which became more pronounced for increasing rates and when stimulation intensity was increased. Similarly to our previous results [Loquet and Rouiller, 2002], we found that each adaptive

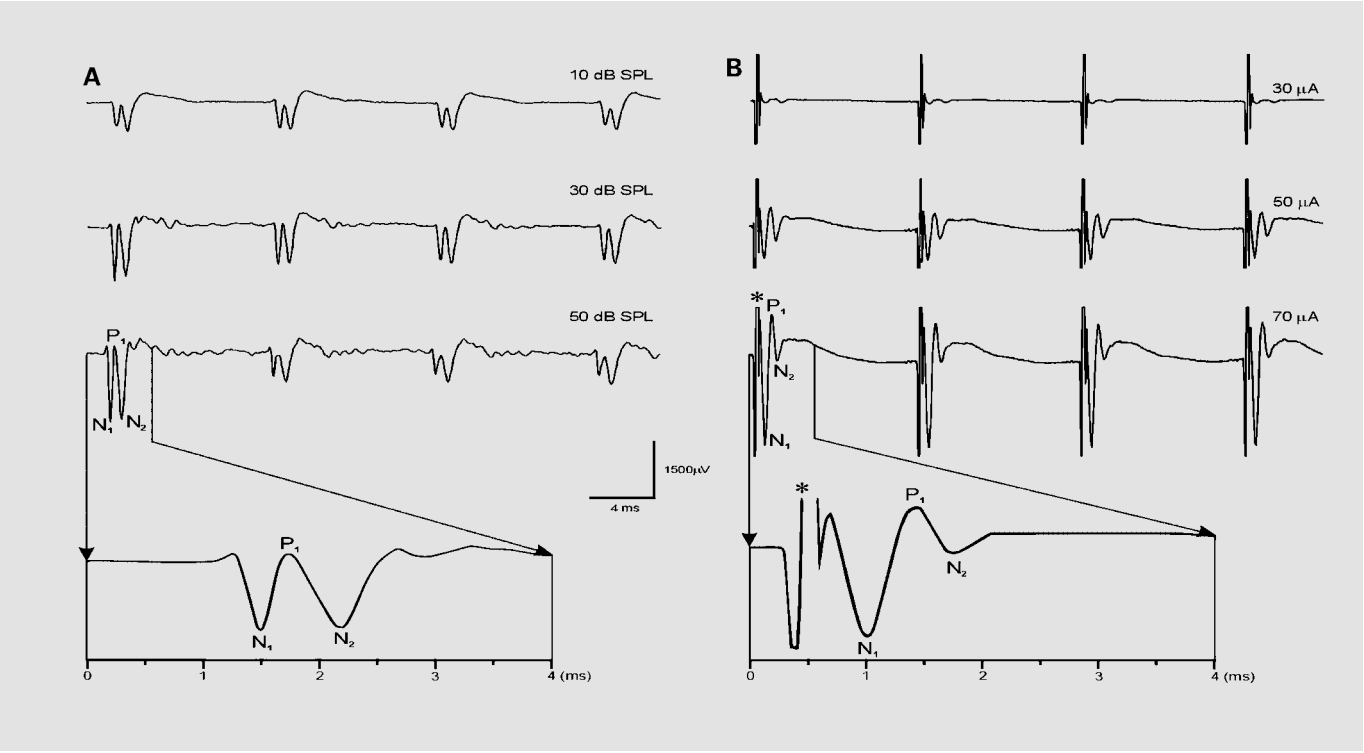


Fig. 3. Typical VCN near-field evoked potentials recorded from the same chronic monopolar intracranial electrode in 1 animal (rat No. 3). **A** Auditory evoked potentials (aCNP) elicited by 250-ms trains of repetitive rectangular acoustic clicks (100 μs) presented at a rate of 100 pps. **B** Electrically evoked potentials (eCNP) elicited by 250-ms trains of repetitive biphasic square pulses (50 μs/phase, anodic-first) applied to a monopolar intracochlear electrode at a rate of 100 pps. No stimulus artifact cancellation procedure was used, and the artifacts (asterisk) were at most 5 times larger than the compound action potential height. For clarity's sake, artifacts were graphically reduced. In both panels, only the first 35 ms of the train are shown. In addition, the trace at the highest intensity exhibits an expanded time scale (bottom graph) which demonstrates the response to an individual pulse. N_1, P_1 and N_2 deflections are easily recognizable (negativity polarity is downward). Stimulus intensity is given on the upper right of each response curve.

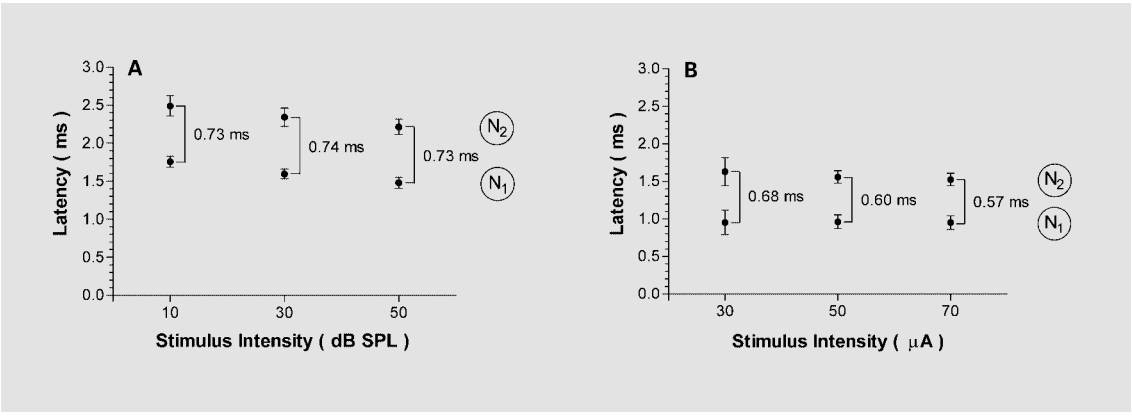
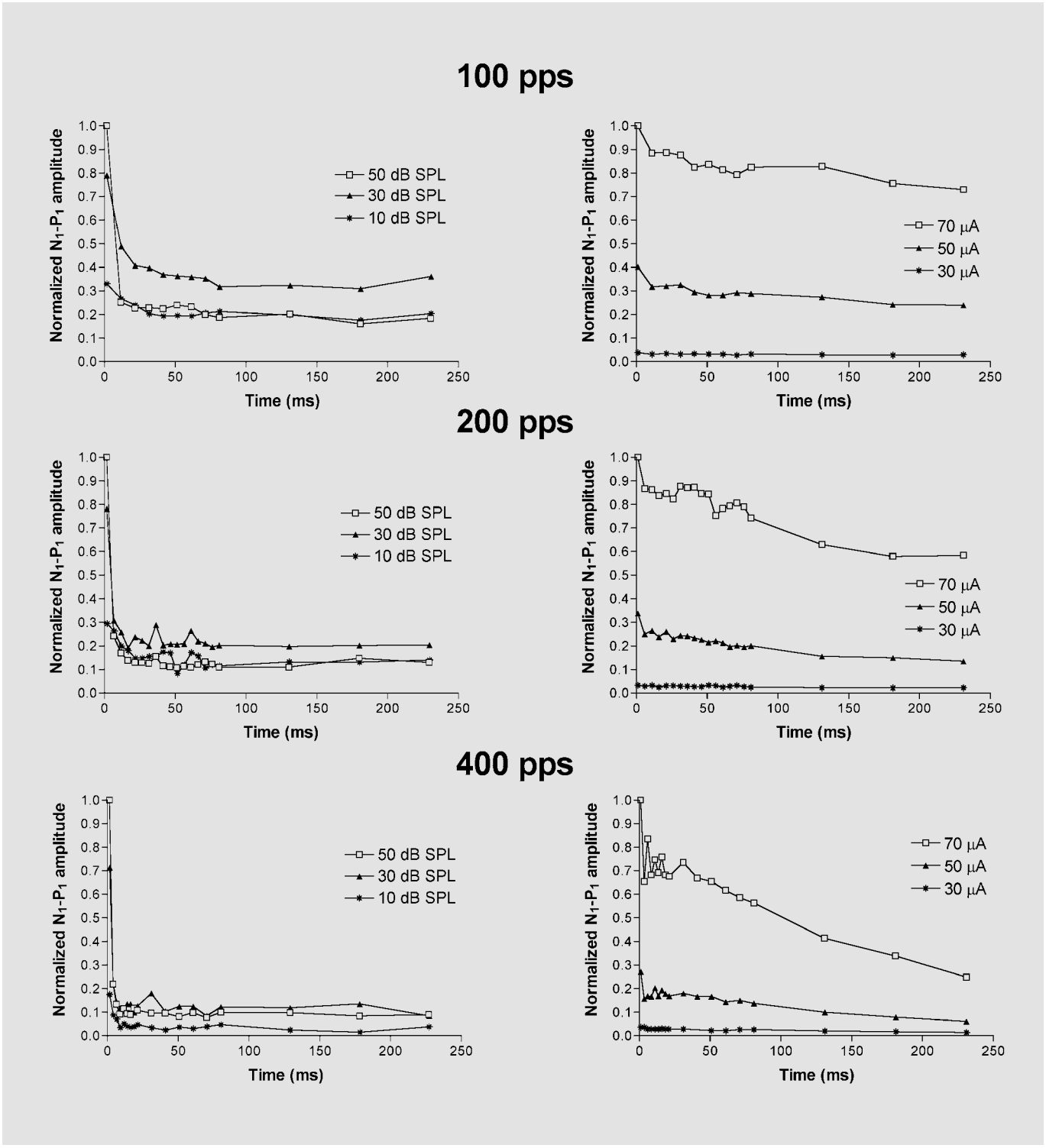


Fig. 4. Summary of wave N_1 and wave N_2 latencies (measured at the peak) derived from the 4 rats (6 measures per animal) in response to acoustic (**A**) or electric (**B**) stimulation. Interwave latencies were calculated from N_1 and N_2 latency mean values and are indicated on the right of the vertical bars. The error bars represent the standard deviation from the mean latency value.


100 pps
Normalized N1-P1 amplitude
Time (ms)
50 dB SPL
30 dB SPL
10 dB SPL
70 µA
50 µA
30 µA
200 pps
400 pps

5

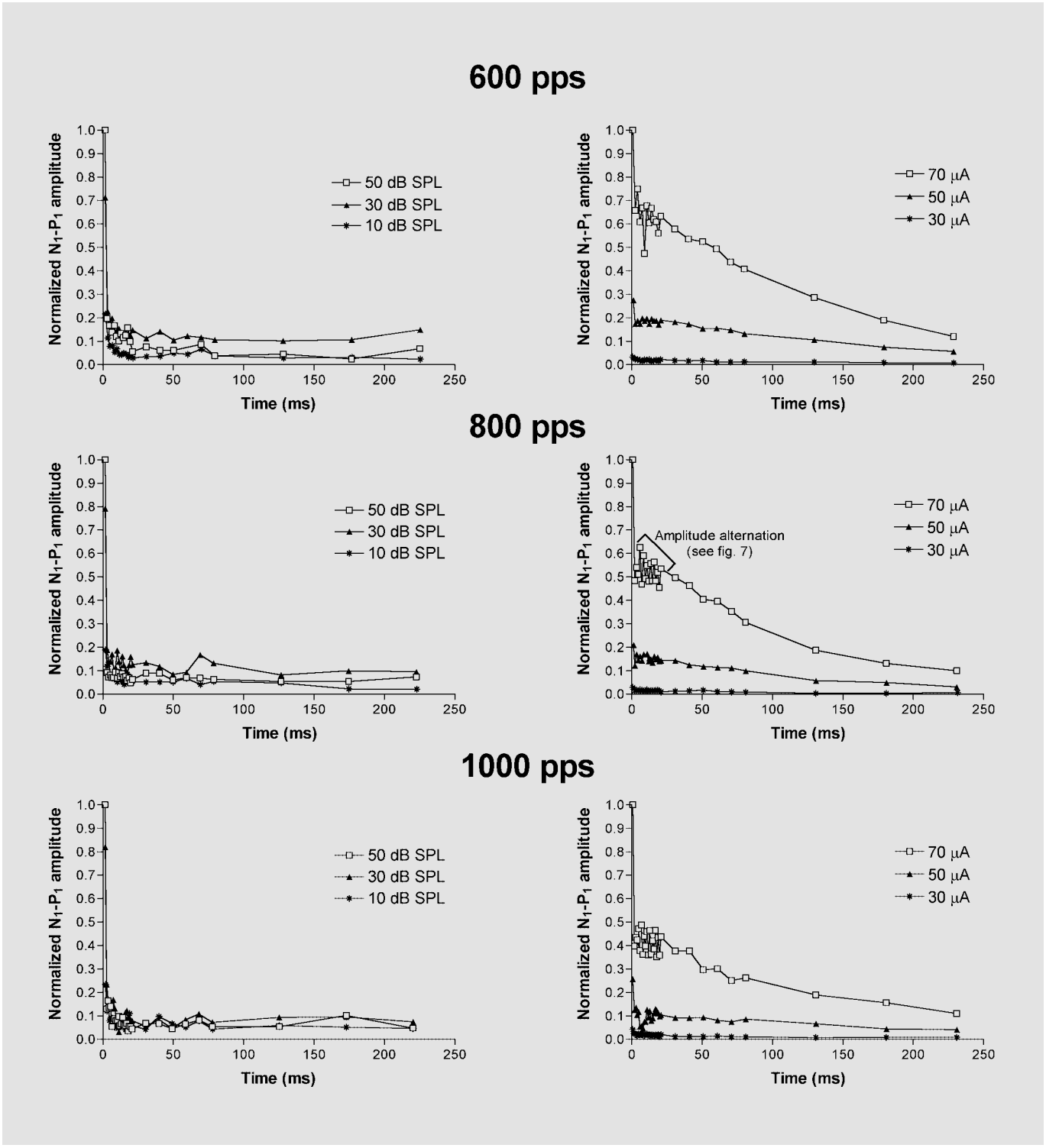


Fig. 5. Amplitudes of VCN near-field potentials normalized relative to the largest potential observed (usually the potential to the first pulse, at the highest stimulus level) evoked by acoustic stimulation (in the left column) or electric stimulation (in the right column). The recordings were obtained from the same chronic monopolar intracranial electrode in 1 animal (rat No. 4), and the potential amplitudes were displayed as a function of the position of the individual stimulating pulses along the 250-ms train. For each of the pulse rates ranging from 100 to 1000 pps, 3 intensities are presented: 50, 30, 10 dB SPL and 70, 50, 30 μA, for acoustic and electric stimulation, respectively.

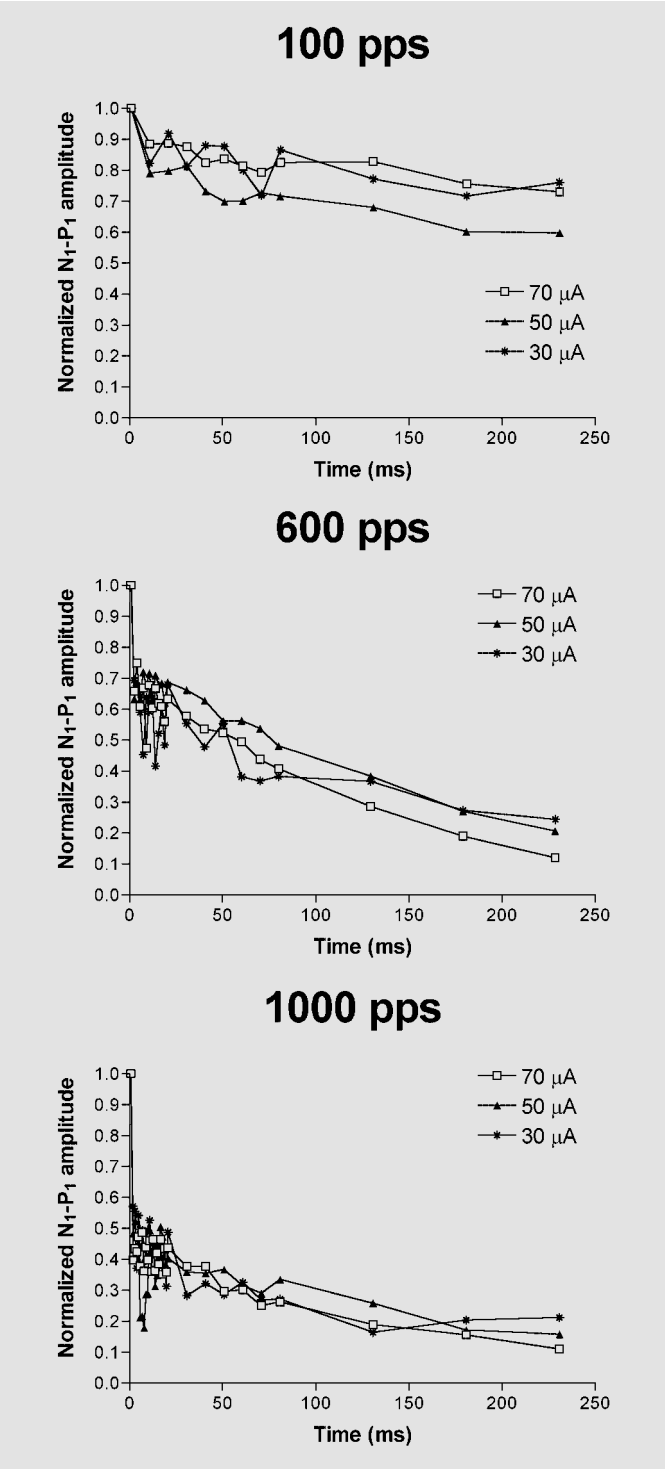


Fig. 6. Same data as in figure 5 but with N_1–P_1 amplitudes normalized relative to the largest potential observed at each intensity tested (usually the potential to the first pulse). As an example, responses evoked by electric stimulation are presented at repetition rates of 100, 600 and 1000 pps and at intensities of 70, 50 and 30 μA. The potential amplitudes were displayed as a function of the position of the individual stimulating pulses along the 250-ms train.

curve was best described by an exponential decreasing equation with 2 time constants, $y(t) = y_1e^{-t/K_1} + y_2e^{-t/K_2} +$ plateau, where y_1 and y_2 are the y intercepts of the rapid and short-term adaptive components, respectively, K_1 and K_2 their corresponding decay time constants and plateau equals the N_1–P_1 amplitudes during the steady-state response (results were obtained with GraphPad Prism® 3.02 software but are not shown in the present report). The adaptive pattern of electric responses was different (fig. 5, right panels). There was very little adaptation at low rates (100 and 200 pps), whereas at 400 pps and above, the decrement of amplitude became progressively greater, but still less pronounced than that observed for acoustic stimuli. The electric adaptive curves exhibited an initial rapid phase followed by a slower phase, without plateau. This pattern was hardly or not at all influenced by the intensity of stimulation (fig. 6), whereas individual eCNP magnitudes were largely dependent on the intensity of stimulation (fig. 5, right panels). In addition, one can note that during the first 20 ms of the train, where CNPs were collected for all consecutive clicks or pulses, the N_1–P_1 amplitudes of both aCNP and eCNP showed a sequential up and down alternating sequence, corresponding to an oscillation of the CNP amplitude along the train. This alternation or oscillation phenomenon was most pronounced at repetition rates of 400 pps and above, when electric stimuli were used. In contrast, oscillations were less prominent when using acoustic stimuli and only observed at high stimulation rates (fig. 7).

CNP to Modified Electric Pulse Trains

Since VCN adaptive curves in response to steady electric pulse trains were very different from those obtained with click trains (fig. 5), our goal was to adjust the parameters of electric stimulation in order to restore natural adaptation phenomena. Therefore, acoustic adaptation was tentatively mimicked by building modified electric pulse trains from acoustic adaptive curves (fig. 8). To achieve this purpose, acoustic data were reported on an electric growth function (established for each rat) in order to determine the electric stimulus intensity required to elicit a similar CNP amplitude. The resulting transformed intensity values were then plotted as a function of its position in the 250-ms train, and the curve was fitted using the equation $y(t) = y_1e^{-t/K_1} + y_2e^{-t/K_2} +$ plateau. As previously, the curve fitting was obtained using the Levenberg-Marquardt method with GraphPad Prism 3.02 software, and the deviation from model was assessed by considering the correlation coefficient ($R^2 \geq 0.70$) and by testing the Gaussian distribution of the residuals around the curve

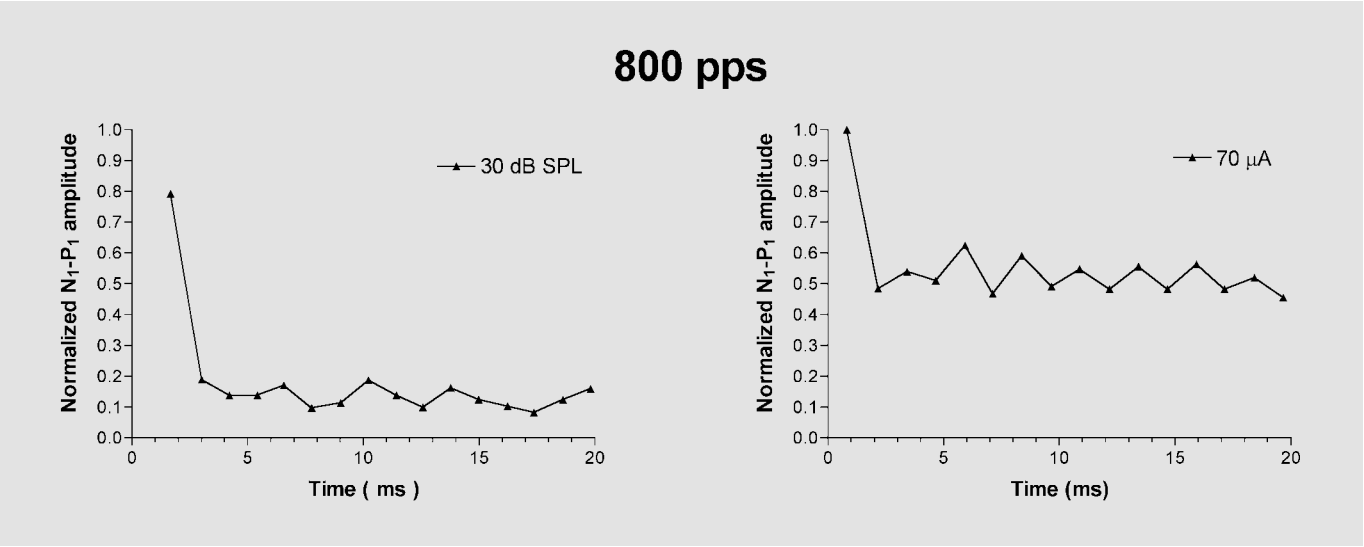


Fig. 7. Phenomenon of alternation (oscillation) of the N_1–P_1 amplitudes of VCN near-field potentials evoked by acoustic stimulation (left side) or electric stimulation (right side). Data are derived from rat No. 4 at the rate of 800 pps and presented with an expanded time scale where only the first 20 ms are shown.

($p > 0.1$). The 5 parameters (y_1, y_2, K_1, K_2 and plateau) were extracted and fed into an interactive MATLAB subroutine to produce the 250-ms exponentially decreasing electrical stimuli. In this way, new electrical adaptive patterns were obtained with the modified train of pulses (fig. 9). These data (blue curves) were obtained before the 5th postimplantation day and showed exponential decays more comparable to those obtained with click trains (red curves), namely a 3-phased adaptation exhibiting an initial rapid phase followed by a slower phase and a plateau. Concurrently, the exponential decrease became more pronounced for increasing rates. In contrast, the pattern of the curve obtained with steady electric pulse trains (black curves) appeared different for rates of 200–1000 pps. Such curves (fig. 9) were compared statistically for the 4 rats using a two-way analysis of variance performed by GraphPad Prism 3.02 software. The results are summarized in table 1. It can be concluded that the two curves in figure 9 obtained with constant amplitude acoustic (red) and electric (black) pulse trains are significantly different, reflecting a clearly less dramatic adaptation for steady electric stimulation. In contrast, the lower curves (blue) obtained in figure 9 with modified electric pulse amplitudes were not significantly different from the corresponding acoustic curves (red) at rates ranging from 200 to 1000 pps. These data thus support the notion that the proposed modification of the electric pulse trains was successful in mimicking the natural adaptation pattern. Moreover, a further consequence of the modified electric pulse train towards a better replication of the acoustic adaptive pattern was to reduce the occurrence and amplitude of alternating patterns.

Table 1. Statistical comparison of adaptation curves elicited by acoustic and electric or modified electric pulse trains as a function of stimulus rate

Rate pps	$Acoustic_{30\ dB\ SPL}$ – $electric_{50\ \mu A}$			$Acoustic_{30\ dB\ SPL}$ – modified electric		
	F	d.f.	p value	F	d.f.	p value
100	2.191	36	0.0002	2.271	36	0.0068
200	2.235	60	<0.0001	1.536	60	0.2737[1]
400	2.712	54	<0.0001	1.546	54	0.3059[1]
600	1.470	66	<0.0001	1.613	66	0.0365[1]
800	3.095	78	<0.0001	1.872	78	1.0000[1]
1000	1.422	90	0.0190	3.476	90	0.9595[1]

Two-way analysis of variance tests the null hypothesis that there is no interaction between the two groups.

[1] The two curves are not significantly different (confidence level set to 99%).

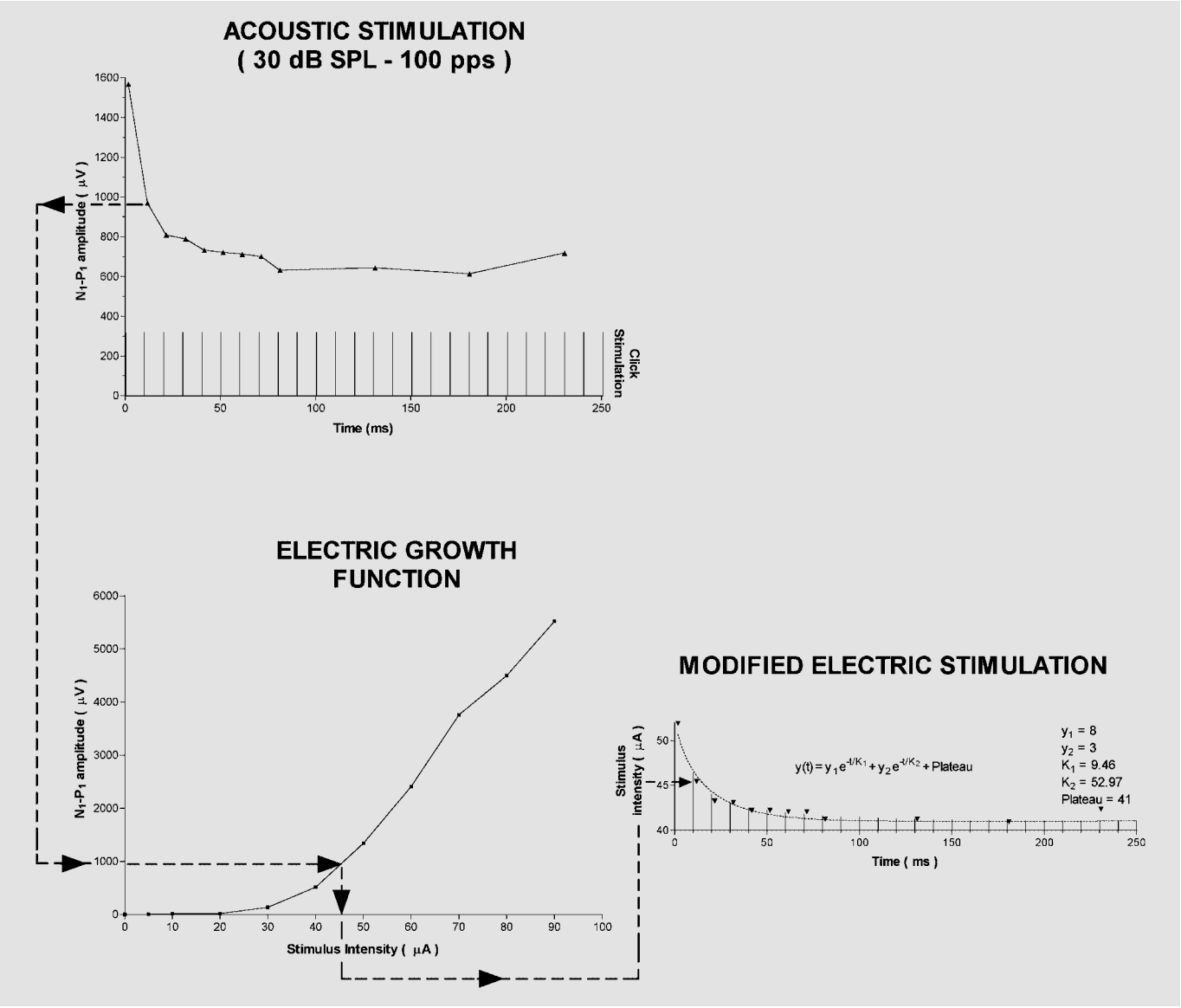


Fig. 8. Schematic representation of construction of the modified electric stimulus. The upper left panel shows N_1–P_1 amplitudes of VCN near-field potentials evoked, for instance, by 30 dB SPL repetitive clicks delivered at a rate of 100 pps (the acoustic click train is represented diagrammatically along the x-axis). Each amplitude point was first reported on the electric growth function established before for the same animal (lower left panel) in order to determine the electric stimulus intensity required to elicit the same response amplitude. The resulting transformation was displayed as a function of the position of the point along the 250-ms train and is depicted in the right panel. The mathematical description of the curve allowed to determine 2 time constants (K_1 and K_2), 2 y intercepts (y_1 and y_2) and 1 plateau which were fed into an application development computer system in order to produce the corresponding exponentially decaying electric stimulus.

Discussion

Origin of the Compound Action Potentials

Although the results of the present study are based on CNPs obtained from recording electrodes located in the VCN, the nature of the neural populations generating these potentials is a matter of debate. At first glance, it seems reasonable to hypothesize that these responses most likely reflect the behavior of VCN neurons plus possible contributions from neurons of the dorsal CN, as well as ascending branches of incoming AN fibers. One could argue that, when advancing the electrode on a dorsal-to-

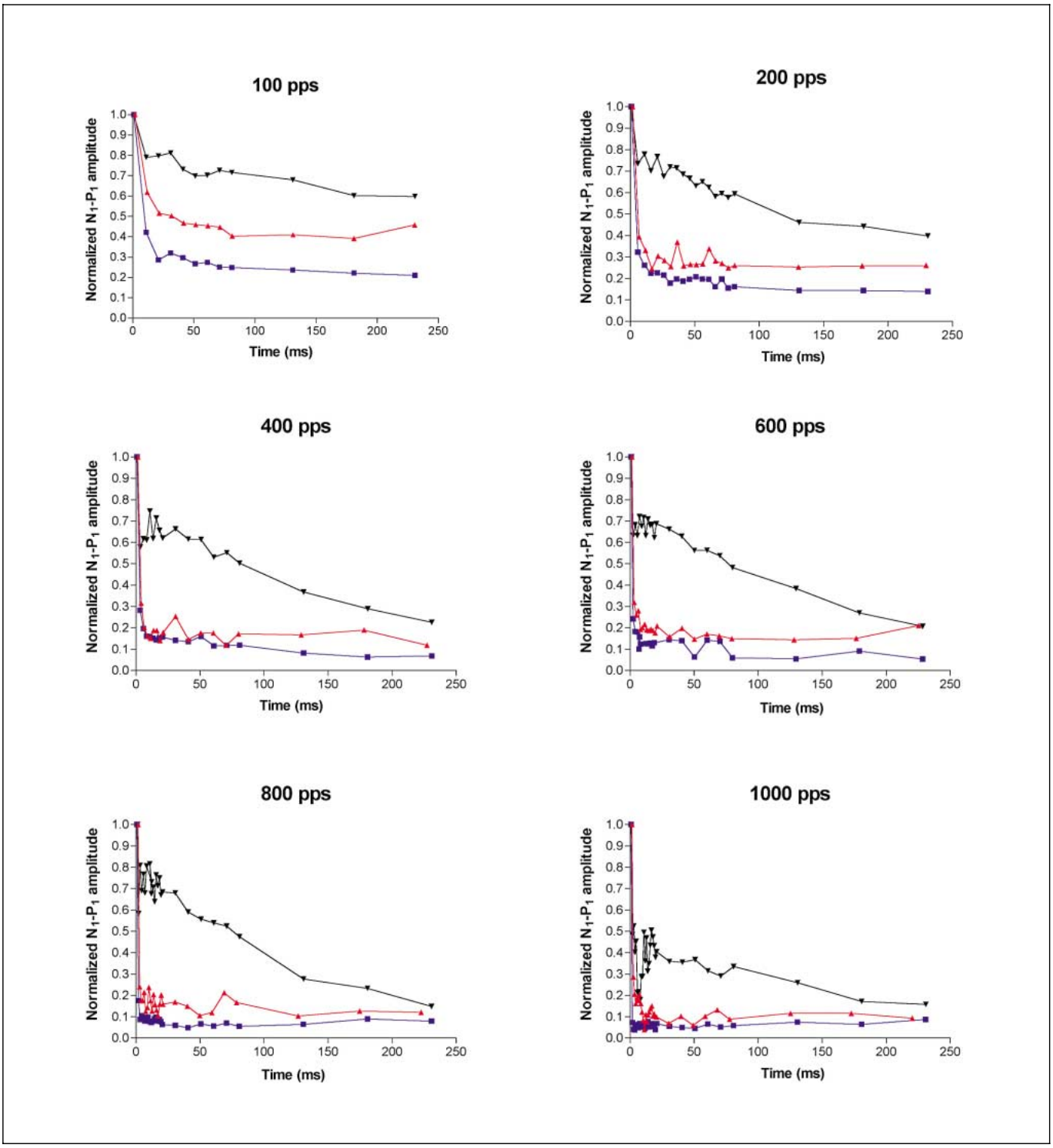


Fig. 9. Amplitudes of VCN near-field potentials normalized relative to the largest potential observed in the train (usually the response to the first pulse) evoked by 30 dB SPL acoustic clicks (red curve), 50-μA steady electric pulses (black curve) and modified electric pulses (blue curve) in 1 animal (rat No. 4). For each of the pulse rates ranging from 100 to 1000 pps, amplitudes were displayed as a function of the position of the stimulating pulse in the 250-ms train.

ventral path through the VCN, AN fibers tend to be encountered at greater depths (i.e. more ventrally) than VCN units [Paolini et al., 2001]. However, in vivo, a determination of the recording location can be inferred only by advancing the electrode through the CN and by checking for a match with the known orderly tonotopic organization of the CN. Unfortunately, such an approach was not performed during the implantation of the recording electrode (the optimal location was assessed by only identifying the two N_1 and N_2 deflections), and therefore one cannot elaborate further on the precise neural origin of the responses. The response latencies may also provide a basis useful to infer the neural origin of the responses, by comparing them to values drawn from previous reports conducted in the rat. For example, the latency values obtained by FitzGerald et al. [2001] in the response of single units to 90 dB SPL clicks ranged from 1.5 to 3 ms in the AN (depending on their characteristic frequency) and from 2 to 4 ms in the VCN. Latency values obtained by Paolini et al. [2001] for single units in response to 100 dB SPL clicks ranged from 1.4 to 2.7 ms in the AN and from 1.8 to 4 ms in the VCN. Using tone bursts and recording intra-axonally from the ventral acoustic stria, Friauf and Ostwald [1988] obtained latencies ranging from 2.2 to 2.6 ms for identified VCN neurons. When comparing these data to our results (acoustic latency values: 1.48 ms $< N_1 <$ 1.76 ms and 2.21 ms $< N_2 <$ 2.49 ms, depending on stimulus intensity), it is tempting to conclude, in line with Møller's data [1983], that N_1 mainly reflects the activity in the AN whereas N_2 is predominantly generated by CN neurons, mainly those of the VCN. Indeed, a contribution of units located in the dorsal CN is less likely because of their low ability to follow repetitive stimulation [Rhode and Smith, 1986]. However, the interpretation of the origin of the N_1 and N_2 waves is most likely not so schematic [Sellick et al., 2003]. Indeed, one may doubt that a single wave is generated by only one cell population. It is more realistic to consider the N_1–P_1–N_2 components as a continuum of activity reflecting a sum of superimposed activities generated by multiple neural subpopulations, both in the AN and CN.

Comparison of Acoustic and Electric Compound Action Potentials

Comparing the waveform of the acoustic and electric CNPs, it appears that they both exhibit two negative peaks N_1–N_2 (fig. 3), with a comparable interwave latency (fig. 4). This is in agreement with previous animal and human data [van den Honert and Stypulkowski, 1986; Pelizzone et al., 1989], suggesting that the aCNPs and eCNPs were produced by similar underlying events in the auditory periphery and brainstem nuclei. However, as previously established by others [Prijs and Eggermont, 1975], acoustic and electric responses differ not only by their latencies, but also by their detailed waveform pattern, especially when comparing N_1 and N_2 magnitudes (fig. 3, 4). In our experiments, the recording electrode was the same for both conditions and, therefore, the reason for such differences more likely lies in the stimulation techniques used. Nevertheless, the region of the cochlea driven by acoustic and electric stimuli is probably irrelevant. Indeed, when considering first the 100-μs acoustic click characterized by a frequency spectrum limited to below 10 kHz [Burkard, 1984], it follows that the acoustic stimulation influenced mainly the upper 40% of the rat's cochlear partition (~3.2 mm from the apex), where are represented the frequencies ranging from 0.25 to 10 kHz, according to Greenwood [1996]. The electric stimulation was delivered via an electrode inserted on a distance of 4 mm from the round window, and thus the ball at the tip was located at the middle of the basilar membrane (~4.0 mm). The stimulus current is likely to spread as much towards the base than towards the apex of the cochlea because the ground electrode was placed far from the active electrode. It can be concluded that both modes of stimulation influenced overlapping regions of the cochlea, in particular within the 1- to 8-kHz frequency range, which is a domain of good hearing sensitivity in the rat [Heffner et al., 1994]. A more likely interpretation for the differences observed between acoustic and electric responses is the absence of traveling wave and transduction apparatus in the cochlea as a result of insertion of the stimulating electrode. Indeed, this interpretation is supported by an observation that an acoustic stimulation delivered after cochlear implantation failed to induce any aCNP response in the VCN [pers. unpubl. data]. Although the extent of the damage to the cochlea due to electrode insertion is not known (histology of the cochleas was not performed), we assume, in line with previous data [Kiang and Moxon, 1972], that the electric stimulation directly affects the primary auditory neurons, thus bypassing the transduction elements, resulting in a shorter eCNP latency (0.95 ms $< N_1 <$ 0.96 ms and 1.52 ms $< N_2 <$ 1.63 ms, depending on stimulus intensity) than aCNP latency (1.48 ms $< N_1 <$ 1.76 ms and 2.21 ms $< N_2 <$ 2.49 ms). Concerning the magnitude of N_1 in comparison to that of N_2 (fig. 3), the larger N_1 wave observed in response to electric than acoustic stimuli may result from the stronger synchronized activities of auditory nerve fibers elicited by electric pulses than by clicks [Hartmann et al., 1984].

Comparison of Acoustic and Electric Adaptation

Increasing stimulus intensity induced, as expected [Møller, 1975; FitzGerald et al., 2001], a decrease in aCNP latencies (less marked in eCNP, fig. 4) and an increase in the N_1–P_1 amplitude in response to the first pulse in the train (fig. 3), due to AN fiber recruitment. However, responses to the following pulses in the train varied significantly for acoustic versus electric stimulation (fig. 3, 5). In response to trains of clicks at repetition rates ranging from 100 to 1000 pps, CNPs were synchronized to each individual click, but the amplitude of the CNPs decreased along the train in a 2-phase exponential pattern, reflecting the neural adaptation (fig. 5). This adaptive pattern varied as a function of stimulation rate and intensity with a faster decay as rate and intensity increased. These data for acoustic stimuli are fully in line with previous evoked potential studies in the VCN [Evans, 1975; Huang, 1981; Loquet and Rouiller, 2002; Loquet et al., 2003], and they are comparable to adaptive properties found for the AN [Peake et al., 1962; Eggermont and Spoor, 1973]. As a consequence, the acoustic adaptation in the VCN mainly reflects the adaptation present in the AN or more upstream, at the hair cell-nerve fiber synapse, where the major part of adaptation is thought to originate [Eggermont, 1975; Furukawa and Matsuura, 1978; Furukawa et al., 1978]. At this level, direct evidence for adaptive mechanisms is still lacking, and authors have therefore tempted to model adaptation. For example, Smith and Brachman [1982] suggested that adaptation is produced by the depletion in the cascade of neurotransmitter located in 3 presynaptic stores. This model is able to reproduce the 3-phased acoustic adaptation where increments and decrements in intensity are taken into account. However, there is clear evidence now that the events taking place at the hair cell-nerve fiber synapse do not account for the entire adaptation observed in the AN. Indeed, the present data derived from direct electric stimulation of the AN showed a weak adaptation at low stimulation rates (100–200 pps), which became larger at stimulation rates above 400 pps, although still less pronounced than the adaptation observed for acoustic stimuli (fig. 5). Furthermore, the electric adaptive decay appeared less sharp than that obtained with acoustic stimuli, especially within the initial 5 ms of the train whereas the last 200 ms led to a progressive additional adaptation until a quasi steady state was reached. These results regarding adaptation to electric stimulation of the cochlea are fully consistent with previous descriptions based on AN evoked potentials in animals [Haenggeli et al., 1998; Matsuoka et al., 2000] and intracochlear recordings in humans [Wilson et al., 1997], confirming that adaptive phenomena are still present even when the hair cells and the afferent synapses were bypassed. The remaining adaptation is probably mainly related to the refractoriness of AN fibers. Indeed, the eCNPs recorded in the present study represent the summed activity of several subpopulations of CN, which have different refractory properties. Therefore, in response to a train of pulses presented at high rate (for instance 800 pps), a subpopulation of CN neurons that responded to the first pulse will not be able to respond to the second pulse of the train, resulting in a strong decrease in the eCNP as compared to the eCNP to the first pulse. The units which did not respond to the second pulse recovered and can therefore respond to the third pulse, producing a larger eCNP than to the second pulse, thus corresponding to the initiation of the alternation phenomenon (fig. 7), which strongly depends on pulse rate, in line with the previous study of Matsuoka et al. [2000]. At low repetition rate (100 pps), the interpulse interval is sufficient for full recovery of all units since, according to Brown [1994], the relative refractory period of the AN may last not more than 5 ms. This absence of adaptation was previously established in the VCN by both extracellular [Glass, 1984; Maffi et al., 1988] and intracellular recordings [Paolini and Clark, 1998; Babalian et al., 2003]. Based on these results, one may therefore suggest that the adaptation curves obtained with repetitive acoustic clicks at 100 pps exhibited mainly an adaptation due to transmitter release by hair cells (fig. 5, left panels). Thus, when considering a mathematical model for adaptation, pre- and postsynaptic events as well as refractory mechanisms in the AN may be included [Eggermont, 1985; Meddis, 1988]. However, one must keep in mind that these mechanisms cannot completely explain the eCNP adaptation patterns and fatigue of the nerve is not excluded nor a survival of hair cells at regions apical to the cochlear implanted electrode [Hu et al., 2003].

CNP to Modified Electric Pulse Trains

In contrast to acoustic responses, the adaptive patterns obtained in response to electric stimulation in the 30- to 70-μA range were less influenced by the level of stimulation (fig. 6), whereas individual eCNP magnitudes were largely intensity dependent (fig. 5). One reason for such a difference is probably that the intensity of the electric stimulus in the chosen range is directly proportional to the number of highly synchronized responding fibers, whereas acoustic stimuli activate many units of different thresholds through a transduction apparatus which results in a weaker synchronization [Prijs and Eggermont, 1975]. Be-

cause less eCNP than aCNP adaptation is obtained when using an electric stimulation with a current burst of constant intensity, we tested therefore, in the second part of the present study, intensity-modified electric pulse trains in order to obtain a neural adaptation comparable to that observed with acoustic stimulation. The results illustrated in figure 9 show that, when stimulating the cochlea with a modified train of electric pulses of exponentially decreasing intensity, an adaptive pattern close to the natural phenomenon observed for acoustic stimulation can be obtained, in particular from 200 to 1000 pps. Therefore, the envelope of the response has mirrored the shape of the electric stimulus, and an abrupt decrease in the intensity during the first milliseconds of the stimulating train produced the desired adaptive pattern by diminishing mainly AN fiber recruitment. Nevertheless, it is noticeable that at low stimulation rate (100 pps), the curve established with the modified train of electric pulses revealed a more pronounced adaptation than that obtained with acoustic stimulation (fig. 9). This observation demonstrates that the equation used to modify the electric stimulus in the present study is not adapted to all repetition rates, especially when little or no neural refractoriness is involved as it is the case at low repetition rates. Based on these considerations, further studies are under way in the laboratory aimed at improving the current electric stimulation strategy.

Conclusion

In conclusion, although there is still uncertainty about the sound processing strategy which leads to the best discriminating abilities, the results of the present study emphasize the benefit which may be obtained from incorporating modified electric pulse trains in cochlear prosthesis processors to mimic the adaptive neural response patterns to natural acoustic stimulation. Indeed, because the adaptation process contributes largely to accentuate rapid changes in stimulus composition (speech transients) [Kiang et al., 1979], we propose that adding such a process to the human cochlear implanted prosthesis may substantially improve speech discrimination and intelligibility in noisy environments.

Acknowledgments

The authors wish to thank B. Aebischer (Unit of Physiology, University of Fribourg) and A. Sigrist (Cochlear Implants Center, Cantonal University Hospital of Geneva) for their technical assistance, J. Corpataux and B. Morandi for taking care of the rats in the animal room and F. Tinguely for histology. This research project was supported by the Swiss National Science Foundation (grant No. 32-56352.99; TANDEM) and the National Center for Competence in Research 'Neural Plasticity and Repair'.

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CONCLUSIONS

Le présent modèle mis au point chez le rat Long-Evans vigile avec une électrode implantée chroniquement dans le noyau cochléaire antéroventral prouve qu'il est possible d'enregistrer pendant une longue période de temps des potentiels de champ auditifs évoqués intracérébraux de façon stable d'une session à l'autre. Cette approche a le grand avantage de fournir des potentiels évoqués très comparables à ceux obtenus dans le nerf auditif puisque l'électrode d'enregistrement est placée à proximité de l'entrée de ce nerf dans le noyau cochléaire et ainsi d'assez bien en refléter ses propriétés d'adaptation. Ainsi nos résultats montrent qu'en réponse à des trains de clics on obtient une adaptation rapide durant les premières millisecondes suivie d'une adaptation à court terme pendant une dizaine de millisecondes avant d'atteindre un plateau. Cette adaptation devient plus marquée lorsque la fréquence de répétition ou l'intensité de la stimulation sont augmentées. Nous avons ensuite démontré qu'une modélisation de l'ensemble du processus d'adaptation en fonction de ces deux variables pouvait être effectuée à l'aide d'équations exponentiellement décroissantes à deux constantes de temps.

En additionnant au même modèle une électrode de stimulation chronique intracochléaire, nous avons enregistré également de façon stable des potentiels de champ auditifs évoqués intracérébraux en réponse à une stimulation électrique. Les résultats montrent qu'en utilisant un protocole de stimulation en tout point identique à celui précédemment mis en œuvre avec des clics nous obtenons, comme d'autres auteurs, des profils adaptatifs beaucoup moins marqués. Le développement d'un paradigme de stimulation électrique à partir de la modélisation des réponses acoustiques montre pour la première fois qu'il est possible de reproduire en partie les phénomènes adaptatifs perdus après l'implantation cochléaire. Ces résultats électrophysiologiques encourageants ne fournissent toutefois aucune information quant au bénéfice éventuel de cette nouvelle stratégie dans la perception auditive chez l'implanté. Pour en apporter la preuve, des données comportementales devront être recueillies et pourraient ensuite ouvrir la voie à un ajustement plus fin de cette stratégie de codage en vue d'une extension à des stimulations complexes.

PERSPECTIVES ET PROGRAMME DE RECHERCHE

Bien qu'initialement testé chez l'homme[42], l'implant cochléaire doit une grande partie de son développement pendant les 40 dernières années à la recherche sur des modèles expérimentaux, principalement avec des rongeurs et des chats. Néanmoins, sur le plan cochléaire, ces modèles restent assez éloignés de l'homme tant au niveau morphologique que fonctionnel. Par ailleurs, ces animaux se prêtent assez difficilement aux approches comportementales en particulier lorsque l'on cherche à leur faire discriminer de subtiles modulations dans un signal acoustique. C'est pourquoi, avant de lancer des essais chez l'homme, nous proposons de finaliser notre nouvelle stratégie de codage chez le singe. À la vue de notre approche expérimentale, ce modèle apparaît prometteur pour combiner les données comportementales aux données électrophysiologiques. Par le passé, de nombreux travaux sur le système auditif ont déjà été effectués chez le singe comme par exemple la détermination des seuils auditifs comportementaux[43, 44, 45] ou l'enregistrement des potentiels évoqués auditifs[46, 47, 48] mais ce n'est que durant les 30 dernières années que les implants cochléaires ont commencés a être testés chez cet animal[49, 50, 51]. Il est intéressant de noter que ces rapports font état de peu de différences de performance entre ces animaux et l'homme[49, 52] ce qui laisse prévoir un transfert de connaissance relativement direct aux patients. Pour le moment, les seuils auditifs comportementaux en réponse à différentes fréquences de stimulation électrique ont été établis[53] tandis qu'une étude signale que les phénomènes adaptatifs caractéristiques ne sont plus observés au niveau unitaire lorsqu'une stimulation électrique est délivrée[54]. Nous proposons d'aller plus loin en essayant de restaurer les propriétés normales d'adaptation chez le singe adulte. Pour atteindre cet objectif, différentes données doivent être élaborées.

Dans un premier temps, il convient d'évaluer la fonction auditive de notre modèle et de tester sa capacité à répondre à une stimulation acoustique. Le moyen le plus direct est fourni par l'obtention de seuils auditifs comportementaux en conditionnant l'animal à presser un levier pendant un certain temps puis à le relâcher lorsqu'un signal acoustique est perçu. En cas de succès, l'intensité de stimulation est diminuée et l'animal reçoit une récompense (protocole de renforcement positif). Une automatisation complète de cette méthode au laboratoire a déjà été réalisée par mes soins et a permis d'obtenir des valeurs de seuils très proches de celles rapportées par d'autres auteurs.

Toujours sur le plan comportemental, nous proposons de déterminer la plus petite variation de fréquence que notre animal est capable de détecter. Pour cela, le singe est conditionné à presser un levier durant tout le temps où un stimulus acoustique est présent et à fréquence constante. Au cas où survient une soudaine augmentation de fréquence, l'animal doit relâcher le levier pour signifier que cette différence a bien été perçue. Un test préliminaire de ce protocole au laboratoire a fourni des résultats encourageants et permettra, une fois transposé sous forme électrique, de tester différentes stratégies de stimulation pour obtenir la meilleure discrimination possible.

Dans l'optique de retrouver les phénomènes neuronaux d'adaptation déjà enregistrés chez le rat (Chapitres 2 et 3), nous envisageons de recueillir des potentiels de champ auditifs évoqués au niveau du noyau cochléaire. Toutefois, compte tenu de la taille de cette structure chez le singe (2mm au maximum) et de sa profondeur (environ 30mm), l'utilisation de repères externes seuls (stéréotaxie) n'est pas envisageable. Une collaboration avec l'Institut de Neuroradiologie Diagnostique et Interventionnelle de l'Hôpital de l'île de Berne a donc été amorcée afin de déterminer, à l'aide de techniques d'imagerie (résonance magnétique et

tomodensitométrie), le point exact où devra se situer l'extrémité de l'électrode d'enregistrement. De cette manière, il semble possible de réduire la marge d'erreur de positionnement au-dessous du millimètre. Une fois en place, cette électrode chronique sera destinée à enregistrer les phénomènes d'adaptation en réponse à une stimulation acoustique (trains de clics) ou électriques (trains de pulses) de différentes fréquences et intensités.

En ce qui concerne les électrodes intracochléaires destinées à fournir la stimulation électrique, il semble raisonnable de penser à utiliser des dispositifs déjà développés pour l'homme puisque les dimensions cochléaires sont assez voisines. Nous avons donc sollicité une société spécialisée dans la recherche et le développement des implants cochléaires humains, MED-EL, pour participer à l'élaboration d'un implant cochléaire adapté aux singes. Ce dispositif est actuellement en cours de fabrication.

Si nous parvenons à confirmer chez le singe que l'adaptation est plus marquée en réponse à une stimulation acoustique qu'à une stimulation électrique, nous appliquerons alors la nouvelle stratégie de codage électrique développée chez le rat (Chapitre 4) pour restaurer la fonction normale d'adaptation. A ce stade, nous envisageons de perfectionner notre stratégie de codage en utilisant par exemple des fonctions mathématiques plus sophistiquées pour modéliser notre train de stimuli électriques ou, au sein du modèle lui-même, en éliminant la quantité d'adaptation commune aux réponses acoustique et électrique (adaptation neuronale) pour ne travailler que sur l'adaptation issue de la synapse entre les cellules ciliées internes et les fibres afférentes du nerf auditif. Le bénéfice de ces manipulations dans la perception auditive pourra être évalué directement en enregistrant les seuils auditifs différentiels électriques.

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2004-2005 En charge des colloques de physiologie de l'audition pour les étudiants de 2ème année de médecine de l'université de Fribourg.

2 Contrats de recherche

Grant No 32-56352.99 du Fonds National Suisse de la Recherche Scientifique, Programme TANDEM, Projet « Better speech reception in noise for hearing-impaired and profoundly deaf patients » (requérant principal : Pelizzone M, corequérants : Kompis M, Rouiller EM, François J), 2000-2003.

Grant No 32-56352.99 du Fonds National Suisse de la Recherche Scientifique, Programme TANDEM, prolongation du projet « Better speech reception in noise for hearing-impaired and profoundly deaf patients » (requérant principal : Pelizzone M, corequérants : Kompis M, Rouiller EM, François J), 2003-2005.

Grant No 31-61857.00 du Fonds National Suisse de la Recherche Scientifique, Projet « Neuronal mechanisms of acoustico-motor integration in primates » (requérant principal : Rouiller EM), 2003-2005.

Grant No 3100-110005 du Fonds National Suisse de la Recherche Scientifique, Projet « Recovery of motor control after cortical lesion and polysensory-motor integration in the primate » (requérant principal : Rouiller EM, corequérants : Belhaj-Saif A, Scheffold F, Loquet G), 2005-2008.

3 Liste de publications

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