

Auditory steady-state responses to click trains from the rat temporal cortex

Guido Conti^{a,*}, Rosamaria Santarelli^b, Claudio Grassi^b, Fabrizio Ottaviani^a, Gian Battista Azzena^b

^a*Institute of ENT Clinic, Università Cattolica del S. Cuore, Largo F. Vito 1, I-00168 Rome, Italy*

^b*Institute of Human Physiology, Università Cattolica del S. Cuore, Largo F. Vito 1, I-00168 Rome, Italy; accepted for publication: 2 June 1998*

Abstract

In order to investigate the mechanisms underlying the generation of steady-state responses (SSRs), auditory evoked potentials elicited by click trains presented at several stimulation rates (30, 40, 50, 60 Hz) were recorded in 7 awake rats by means of epidural electrodes placed over the temporal cortex. Mean amplitude-rate function calculated on the recorded responses appeared almost flat and showed the maximum value at 50 Hz, while mean phases showed a linear increase when increasing the stimulation rate. In each rat, predictions of the recorded responses at 30, 40, 50 and 60 Hz were synthesized by superimposing middle-latency auditory evoked potentials (MAEPs) at suitable time intervals at each rate. Mean amplitudes calculated on the predicted curves decreased linearly when increasing the stimulation rate and appeared higher in comparison to those obtained from the recorded SSRs. Predicted phases showed a linear increase when increasing the stimulation rate and were leading with respect to corresponding phase values calculated for recorded SSRs. Our findings indicate that the MAEP superimposition mechanism does not adequately predict the generation of temporal recorded SSRs in rats. This was explained by admitting that phenomena related to the recovery cycle and, to a lesser extent, to rate-dependent facilitating effects come into play. © 1999 Elsevier Science Ireland Ltd. All rights reserved

Keywords: Auditory evoked potential; Middle-latency auditory evoked potential; Steady-state response; Repetition rate; Adaptation; Rat

1. Introduction

According to Regan (1989), steady-state evoked potentials can be defined as repetitive responses whose discrete frequency components remain constant in amplitude and phase over a prolonged time period. Among the auditory steady-state responses (SSRs) the 40 Hz SSR gained interest as a helpful clinical tool in the detection of hearing loss in the low frequency range (Galambos et al., 1981; Stapells et al., 1984; Sturzebecher et al., 1985; Lenarz et al., 1986; Picton et al., 1987), in the assessment of central auditory dysfunctions (Spydell et al., 1985; Firsching et al., 1987), and in monitoring the effects of general anesthetics on the brain (Plourde and Picton, 1990; Plourde, 1993). Recently, several studies renewed the interest in the 40 Hz SSR, since this response may share several features with high frequency oscillations (Llinás et al., 1991) that may be related to basic physiological mechanisms underlying perceptive and attentive processes (Tiitinen et al., 1993; Joliot et al.,

1994; Pöppel, 1994).

At present, there is no general agreement with respect to the mechanisms as well as to the neural sources underlying SSR genesis. As the generation mechanisms are concerned, the most favored hypothesis states that auditory SSRs result from the linear addition of middle-latency auditory evoked potentials (MAEPs) evoked by individual stimuli during repetitive stimulation (Galambos et al., 1981; Stapells et al., 1984; Hari et al., 1989; Plourde et al., 1991; Pantev et al., 1993). According to this hypothesis, differences in latency and amplitude of MAEP components may explain differences in the optimal stimulation rate utilized to obtain SSRs in humans (Galambos et al., 1981; Stapells et al., 1984; Hari et al., 1989), as well as in several animal species (Yoshida et al., 1984; Mäkelä et al., 1990; Ottaviani et al., 1990). However, it has been suggested that, in addition to MAEP superimposition, other mechanisms interact in a complex way to generate SSRs (Mäkelä et al., 1990; Ottaviani et al., 1990; Franowicz and Barth, 1995; Pantev et al., 1996). Our recent studies show that non-linearities related to the recovery cycle can determine parameters of individual responses which summate to generate SSRs in humans (Azzena et al., 1995). Furthermore, phenomena dealing

* Corresponding author. Clinica ORL, Università Cattolica del S. Cuore, Largo Gemelli 8, I-00168 Roma, Italy. Tel.: +39 6 30154439; fax: +39 6 3051194.

with the resonant frequency of the activated system can enhance the contribution of individual responses within the 40 Hz SSR (Santarelli et al., 1995). The above reported mechanisms have been shown for the surface-recorded SSRs which mainly reflect the activation of cortical generators (Yoshida et al., 1984; Mäkelä and Hari, 1987; Hari et al., 1989; Mäkelä et al., 1990; Forss et al., 1993; Liégeois-Chauvel et al., 1994), although the contribution of subcortical sources cannot be ruled out (Galambos, 1982; Yoshida et al., 1984; Spydell et al., 1985; Firsching et al., 1987). In particular, surface recordings in humans do not allow us to differentiate the cortical versus subcortical contribution since the potential fields eventually generated by multiple neural structures are superimposed (Simpson and Knight, 1993a). Several reports (Borbély, 1970; Simpson and Knight, 1993a,b) have shown that auditory evoked potentials obtained by epidural recordings from the temporal cortex of the rat mainly result from the potential fields arising within the auditory cortex. Moreover, Franowicz and Barth (1995) have recently shown that 40 Hz SSRs obtained by epipial recordings in the lightly anesthetized rat are mainly generated at the level of the primary auditory cortex. Therefore, the present study was undertaken to clarify whether the mechanisms involved in the SSR generation which we detected in human surface recordings were effective at the level of the rat auditory cortex, i.e. in the absence of a possible subcortical contribution. Towards this aim, auditory evoked potentials were recorded from the temporal cortex of the awake rat utilizing a click train paradigm. The latter allowed us to attain the steady-state, as well as to evaluate the properties of individual responses within the SSRs, by analyzing the response-segment taking place after the train offset.

2. Materials and methods

Seven adult male Wistar rats (250–350 g) (R7, R8, R9, R10, R13, R15, R30) were anesthetized with an intramuscular injection of ketamine hydrochloride (53 mg/kg) and xylazine (11 mg/kg). After a midline incision, the scalp and the left temporal muscle were reflected to expose the left frontal, parietal and temporal bones of the skull. Epidural recordings were obtained by means of a silver-silver chloride ball electrode mounted through a burr hole drilled in the temporal bone and gently placed on the dura over the left auditory cortex. The latter was identified by anatomical landmarks: 5.5 mm posterior to Bregma and 4 mm ventral to the temporal muscle insertion line (Paxinos and Watson, 1982). A silver-silver chloride ball electrode placed epidurally in the left frontal bone served as the reference. A small stainless-steel screw was placed in the right parietal bone 2 mm lateral to the midline, midway between Bregma and Lambda. Teflon-coated wires from active and reference electrodes and from the screw (which also served as ground) were led to an electrical plug connector, which was secured

by means of dental cement to the small screw fixed to the skull.

The animals were allowed to recover for at least 7 days before the recording session.

Before performing the test, a female plug connector with long and very flexible insulated wires was connected to the male socket which had previously been secured to the skull, in order to get the connection between the skull electrodes and the pre-amplifier. During the recording session, each rat was unrestrained and placed in a small cage (12 × 12 × 12 cm) which was located in a sound-proof chamber. The stimulation was performed in free-field by two TDH 49P earphones which were located above the cage at about 10 cm from the animal's head. Stimuli consisted of 0.1 ms compression clicks presented at 105–107 dB p.e. SPL (sound level meter Brüel and Kjaer 2231).

In the first part of the recording session, clicks were presented at the repetition rate of 3.33 Hz in order to obtain MAEPs. Subsequently, stimuli consisted of trains of 23 clicks which were presented at 30, 40, 50 and 60 Hz, and the inter-stimulus interval between the last click of each train and the first click of the following one (inter-train interval) lasted as long as 300 ms (i.e. the same interval utilized to obtain MAEPs at 3.33 Hz). In 3 animals (R13, R15, R30), repetition rates of 120, 240 and 480 Hz with train duration of 366 ms (the same as 60 Hz trains) were also used.

Signals were amplified (5000) and filtered (3–1000 Hz) (P511 Grass preamplifier). Analogue-to-digital conversion was performed at sampling intervals from 72 to 128 μ s (sampling interval 72 μ s and 4000 point-curve for MAEPs; 8000 point-curve for all other recordings with sampling intervals of 128 μ s for 30 Hz, 102 μ s for 40 Hz, 90 μ s for 50 Hz and 80 μ s for 60, 120, 240 and 480 Hz) (ISC-16-E, Computerscope EGA-A System, Electronic Inc., USA). Artifact rejection was active in order to avoid the recording of high-voltage movement artifacts. Alertness was monitored by recording EEG throughout the whole recording session and by observing animal behavior.

Three hundred epochs were averaged at each repetition rate and each recording was replicated at least twice in order to test reproducibility. Replicated waveforms obtained at each rate were averaged together for the subsequent analysis.

MAEP components were labeled in accordance with previous reports (Barth and Di, 1990). The amplitude was measured from one peak to the following one of opposite polarity.

SSRs were obtained from whole train responses by extracting the activity taking place in the 175–300 ms time window. In each animal, prediction curves at 30, 40, 50 and 60 Hz were synthesized by linear addition of MAEP traces shifted by suitable time intervals (33.3, 25, 20, and 16.6 ms at 30, 40, 50 and 60 Hz, respectively). To obtain the predicted 30 Hz response, the 4000 point-MAEP waveform was cut to obtain a 1856 point-curve. This curve was

divided into 4 consecutive 464 point-segments, each corresponding to 33.39 ms. Then, 4 consecutive 1856 point-waveforms were added in such a way that each curve was shifted by 33.39 ms with respect to the previous one. To synthesize the predicted 40 Hz response, the 4000 point-MAEP curve was cut to obtain a 1740 point-curve. The latter was divided into 5 consecutive 348 point-segments, each corresponding to 25.04 ms. Then, 5 consecutive 1740 point-curves were added in such a way that each curve was shifted by 25.04 ms with respect to the previous one. To obtain the predicted 50 Hz response, the 4000 point-MAEP waveform was cut to obtain a 1674 point-curve. This curve was divided into 6 consecutive 279 point-segments, each corresponding to 20.08 ms. Then, 6 consecutive 1674 point-waveforms were added in such a way that each curve was shifted by 20.08 ms with respect to the previous one. To synthesize the predicted 30 Hz response, the 4000 point-MAEP waveform was cut to obtain a 1624 point-curve. This curve was divided into 7 consecutive 232 point-segments, each corresponding to 16.69 ms. Then, 7 consecutive 1624 point-waveforms were added in such a way that each curve was shifted by 16.69 ms with respect to the previous one.

To evaluate the amplitude and phase of the fundamental frequency, the Fourier series technique was applied to steady-state responses (175–300 ms time window), as well as to synthesized curves utilizing dedicated software which yielded amplitude, phase, a_n and b_n Fourier coefficients and a DC term a_0 at the requested frequencies. In order to keep the phase monotonically increasing with the repetition rate, we added 360° to phase values when needed (Hari et al., 1989; Azzena et al., 1995).

3. Results

Representative MAEPs from 3 animals are illustrated in Fig. 1. A constant pattern consisting of P_1 (positive, 9.07 ± 1.20 ms), N_1 (negative, 15.52 ± 2.76 ms), P_2 (positive, 27.87 ± 3.93 ms) and N_2 (negative, 61.35 ± 25.30 ms) deflections was observed in all rats. One animal showed a prominent N_1 peak which was much higher with respect to the positive deflections, so that the MAEP appeared to be triphasic. Mean amplitude values of MAEP components were as follows: P_1N_1 30.40 ± 30.33 μV ; N_1P_2 42.39 ± 35.09 μV ; P_2N_2 56.19 ± 16.36 μV .

Whole responses to stimulus trains recorded in one representative animal at various repetition rates are reported in Fig. 2. At 40, 50 and 60 Hz, the addition of the responses to consecutive stimuli takes place after the second click of the train and the steady-state is achieved within the first 80–100 ms. At 30 Hz, the responses to individual stimuli appear to overlap but some components of MAEPs evoked by individual clicks (P_1 , N_1) can still be recognized. Furthermore, the periodic response to train stimulation appears superimposed in the first 100 ms on a slow activity, consisting of an alter-

nating positive (at about 23–26 ms)-negative (at about 61–65 ms) deflection which resembles the corresponding MAEP pattern.

Recorded SSRs were extracted from the whole train responses as the segment taking place in the 175–300 ms time window. Grand averages of SSRs recorded across animals at various repetition rates are shown in the upper part of Fig. 3, together with the corresponding grand averages of the predicted SSRs which were synthesized utilizing the MAEP as the basic response. It can be seen that the amplitude of the synthetic curves was higher in comparison to that of the recorded curves at all stimulation rates. Mean and



Fig. 1. Middle-latency auditory evoked potentials from 3 animals. The response components are labeled according to their polarity and occurrence.

standard deviations of amplitude and phase values calculated across animals for recorded and synthetic SSRs at each repetition rate are plotted in the lower part of Fig. 3. Amplitude was normalized and was expressed in each rat as the percent of the maximum value; as a matter of fact the maximum amplitude was observed in the synthetic curve series in all animals. The amplitude-rate function calculated for the recorded responses appeared almost flat even though the mean amplitude value was highest at 50 Hz. In fact, the amplitude at 50 Hz resulted very close to that obtained at 40 Hz (1.08 times greater) and 60 Hz (1.18 times greater). Looking at individual values, two animals showed the highest amplitude at 30 Hz (R10, R13), two animals at 40 Hz (R8, R7) and the remaining 3 at 50 Hz (R9, R15, R30). Mean amplitude values calculated for the synthetic SSRs were higher than the corresponding ones obtained from the recorded responses at all repetition rates. Furthermore, predicted values showed a linear decrease when increasing the stimulation rate (linear regression coefficients: $a = 119.72$; $b = -1.086$; $r = -0.998$), so that the maximum amplitude was observed at 30 Hz. When considering individual values, the highest amplitude was found at 30 Hz in all but one animal which showed the greatest amplitude at 40 Hz.

Mean phase values calculated for both recorded and synthetic SSRs increased linearly when increasing the stimula-

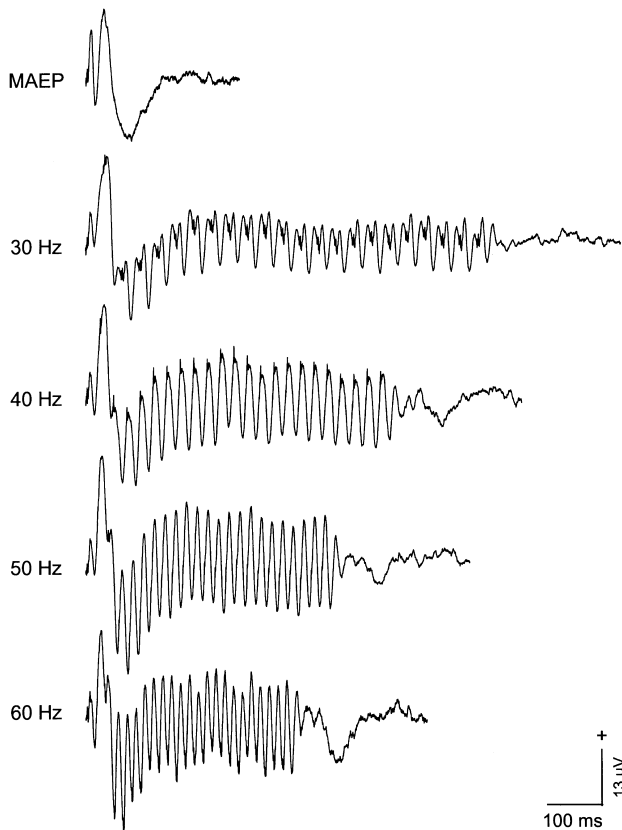


Fig. 2. Middle-latency auditory evoked potential and train responses recorded at several stimulation rates from a representative animal. Train responses achieved the steady state within the first 80–100 ms.

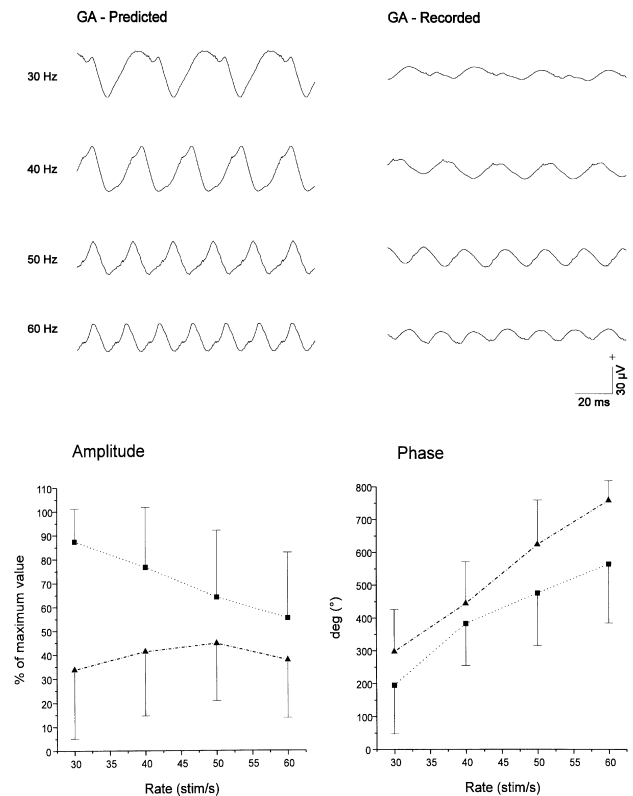


Fig. 3. Recorded and synthesized steady-state responses (SSRs). Grand averages (GAs) from all the tested animals of recorded (right) and synthesized (left) SSRs are shown at several repetition rates in the upper part. The lower part of the figure shows amplitude-rate and phase-rate functions for recorded (triangles) and predicted (squares) SSRs; mean and one standard deviation are reported. Amplitude was normalized and was calculated in each animal as the percent of the observed maximum value.

tion rate (linear regression coefficients for recorded values: $a = -173.3$; $b = 15.66$; $r = 0.999$; linear regression coefficients for predicted values: $a = -137.08$; $b = 12.039$; $r = 0.981$). Nevertheless, mean phases obtained from the recorded responses were lagging in comparison to those calculated for the predicted ones at all stimulation rates.

In order to analyze the activity following the last click of the stimulus train, the 300 ms time window which takes place after the last click was extracted from the whole train response (Fig. 4) and was named post-stimulus response (PSR) for the sake of brevity. From 30 to 60 Hz, PSRs in the first 40 ms showed several deflections which had the same polarity as MAEP components at corresponding latencies. Thus, we labeled these waves as the corresponding MAEP components. Table 1 shows latencies and amplitudes of MAEP and PSR components. Amplitude was normalized and was expressed in each rat as the percent of the maximum value that corresponded to the P_2N_2 MAEP component in all but one animal (R10) which showed the maximum amplitude for N_1P_2 of MAEP. Regardless of the stimulation rate, PSR amplitudes were lower in comparison to MAEP amplitudes (Table 1). The difference was significant (t test, paired) for P_1-N_1 at 30 Hz ($P < 0.05$), for N_1-P_2 at 30 ($P < 0.01$), 40 ($P < 0.05$), 50 ($P < 0.05$) and 60

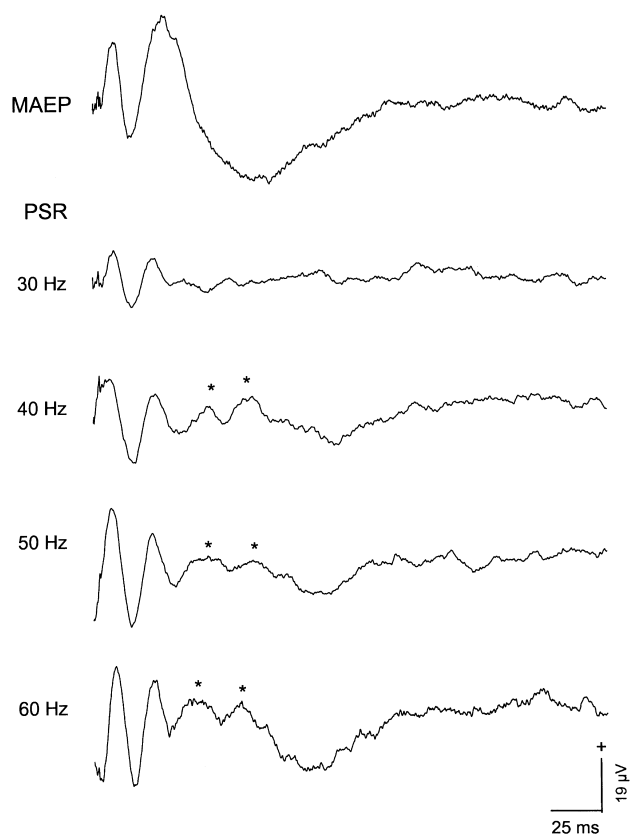


Fig. 4. Middle-latency auditory evoked potential (MAEP) and post-stimulus responses (PSR) at several repetition rates are shown from a representative animal (the same one as in Fig. 2). Asterisks indicate additional positive components seen in PSRs in comparison to MAEP.

($P < 0.05$) Hz and for P_2 - N_2 at 30 ($P < 0.001$), 40 ($P < 0.0001$), 50 ($P < 0.0001$) and 60 ($P < 0.0001$) Hz.

From 30 to 60 Hz, P_1 - N_1 and N_1 - P_2 showed the highest amplitude at 50 Hz (Table 1).

A further feature of PSRs with respect to MAEPs was the appearance in the 40–100 ms latency range of one or two additional components (Fig. 4). Furthermore, additional waves appeared to be superimposed over a slow deflection of alternating polarity. This activity was observed in 5 animals out of 7 at 30 Hz, while it was found in all rats at 40, 50 and 60 Hz. With the aim of evaluating the relationship between PSR additional components and off-responses possibly evoked by the stimulus offset, thereafter we recorded the responses to click trains at 120, 240 and 480 Hz in 3 rats. These stimulus rates were believed to be high enough to prevent the synchronization of MAEPs elicited by individual clicks within the train. The results are shown in Fig. 5 (left column) for a representative animal (R30). At frequencies higher than 60 Hz, train responses were mainly represented by two large deflections at the train onset (on-response) and at the train offset (off-response) respectively and by a fast, low amplitude activity in between.

Thereafter, whole train responses were digitally low-pass filtered (30 Hz). The obtained low frequency traces (Fig. 5,

middle column) allowed us to identify on- and off-responses at all repetition rates except for off-responses at 30 and 40 Hz which were not unequivocally identifiable. When subtracting the low frequency traces from the recorded potentials (Fig. 5, right column), on- and off-responses appeared to also contain a double-peaked high frequency component which is clearly identifiable at 120, 240 and 480 Hz, whereas it was indistinguishable from the steady-state activity at low repetition rates. On- and off-deflections on low frequency traces were labeled as shown in Fig. 5. Latency and amplitude values were calculated in all animals at each rate. Latency of the off-response components was measured with respect to the last click of the train. Amplitudes were normalized with respect to the individual maximum value.

Table 1

Latency and amplitude of middle-latency auditory evoked potentials (MAEP) and corresponding components seen in post-stimulus responses from 30 to 60 Hz

	MAEP	PSR			
		30 Hz	40 Hz	50 Hz	60 Hz
Latency (ms)					
P_1					
<i>n</i>	7	7	6	7	7
Mean	9.07	8.44	7.84	7.92	9.96
SD	1.20	1.72	0.72	1.16	2.54
N_1					
<i>n</i>	7	7	7	7	7
Mean	15.52	13.91	16.06	16.16	17.68
SD	2.76	2.87	2.81	3.09	4.88
P_2					
<i>n</i>	7	7	7	7	7
Mean	27.87	23.13	24.48	25.88	26.48
SD	3.93	4.22	4.69	4.03	4.95
N_2					
<i>n</i>	7	6	6	6	6
Mean	61.35	36.69	36.15	35.63	33.55
SD	25.30	5.06	6.17	6.02	4.67
Amplitude					
P_1 - N_1					
<i>n</i>	7	6	6	7	7
Mean	38.7	16.2	22.2	27.3	22.7
SD	22.4	12.8	9.1	14.7	16.4
N_1 - P_2					
<i>n</i>	7	6	7	7	7
Mean	56.9	22.5	19.9	22.7	20
SD	22.7	6.9	9.6	11.5	12.3
P_2 - N_2					
<i>n</i>	7	6	7	6	6
Mean	90.6	26.2	22	20.2	14.5
SD	24.9	16.2	9.8	6.7	12.9

Number of observations (*n*), mean and standard deviation (SD) are reported. Amplitude was normalized and was calculated in each animal as the percent of the observed maximum value.

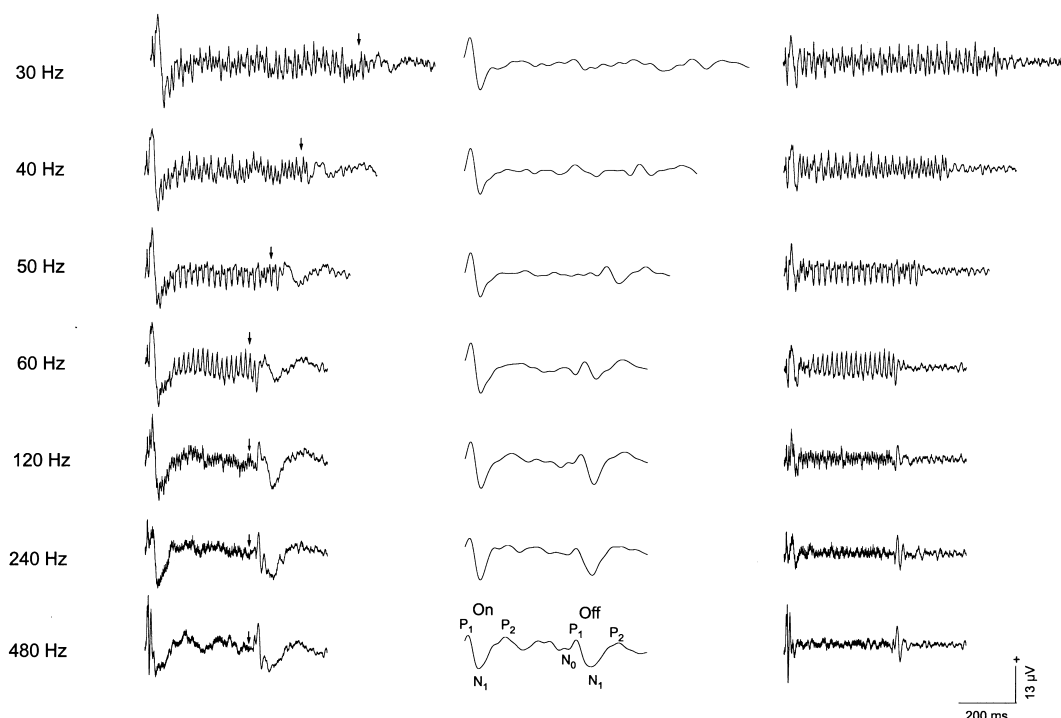


Fig. 5. Whole responses to click trains at 30, 40, 50, 60, 120, 240 and 480 Hz from a representative animal. The recorded responses (left column) were digitally low-pass filtered (30 Hz) to obtain low-frequency responses (middle column). The latter were subtracted from the recorded responses to obtain high-frequency responses (right column). Arrows indicate the train cessation at each rate. On- and off-responses are clearly identifiable at the beginning and at the cessation of the stimulus train.

Mean latencies of $N_{0\text{off}}$, $P_{1\text{off}}$ and $N_{1\text{off}}$ tended to decrease with an increasing repetition rate: $N_{0\text{off}}$ from 38.8 ± 14.7 ms at 30 Hz to 14.3 ± 9.1 ms at 60 Hz and 0 ms at 480 Hz; $P_{1\text{off}}$ from 71.6 ± 21.3 ms at 30 Hz to 48.3 ± 17.9 ms at 60 Hz and 37.0 ± 18.5 ms at 480 Hz; $N_{1\text{off}}$ from 115.7 ± 27.9 ms at 30 Hz to 96.2 ± 24.7 ms at 60 Hz and 99.5 ± 32.5 ms at 480 Hz. Mean amplitudes of $P_{1\text{off}}-N_{1\text{off}}$ and $N_{1\text{off}}-P_{2\text{off}}$ tended to increase with an increasing repetition rate: $P_{1\text{off}}-N_{1\text{off}}$ from $23.0 \pm 13.2\%$ at 30 Hz to $34.4 \pm 15.0\%$ at 60 Hz and $65.5 \pm 20.5\%$ at 480 Hz; $N_{1\text{off}}-P_{2\text{off}}$ from $20.5 \pm 11.2\%$ at 30 Hz to $32.1 \pm 10.0\%$ at 60 Hz and 46.0% at 480 Hz.

On-response components were not greatly modified in latency or amplitude when increasing repetition rate, except for $N_{1\text{on}}-P_{2\text{on}}$ which increased from $45.8 \pm 26.5\%$ at 30 Hz to $65.2 \pm 25.3\%$ at 60 Hz and $73.0 \pm 38.2\%$ at 480 Hz.

4. Discussion

We recorded auditory evoked potentials at several stimulation rates in the awake, unrestrained rat by means of epidural electrodes placed over the auditory cortex.

MAEPs showed a constant pattern consisting of two positive waves (P_1 , P_2), each followed by a negative deflection (N_1 , N_2). This pattern is similar to that described in other reports (Borbély, 1970; Shaw, 1988; Barth and Di, 1990; Franowicz and Barth, 1995). Latency values corresponded to those obtained by Borbély (1970) from the temporal cor-

tex of lightly anesthetized rats. Furthermore, mean latencies of P_1 and N_1 were comparable with P_a and N_{15} components obtained by Miyazato et al. (1995) for temporal recorded MAEPs in the awake rat. These authors have not reported a P_2 component in recordings obtained from the temporal cortex; nevertheless, it seems to us that a P_2 wave with a latency corresponding to our responses is identifiable in their temporal recordings even though this was not mentioned in the text. Mean latencies of MAEP components obtained in our study are shorter in comparison to the corresponding ones calculated by Barth and Di (1990) for epileptical recordings obtained from the auditory cortex of the anesthetized rat. We believe that this difference should be mainly attributed to the state of the animal which was awake in our study and lightly anesthetized with ketamine and xylazine in the study of Barth and Di (1990). As a matter of fact, it has been found that in guinea pigs (Crowther et al., 1990) and in rats (personal unpublished data) the administration of ketamine and xylazine induces an increase in latency and a reduction in amplitude of MAEP components recorded over the temporal cortex. More consistent differences were found between our data and temporal MAEPs recorded by Simpson and Knight (1993b) in the awake rat. These authors reported a double-peaked P_1 component (P_7 , P_{11}) followed by an inconstant P_2 wave. These differences could be explained, at least partially, as resulting from differences in stimulation parameters with regard to the P_1 component (Borbély, 1970), and even from minimal discre-

pancies in electrode placement with regard to the identification of the P₂ wave.

Electric or magnetic steady-state responses have been recorded utilizing a click train paradigm in humans (Mäkelä and Hari, 1987; Forss et al., 1993; Santarelli et al., 1995), cats (Mäkelä et al., 1990) and rats (Barth and Di, 1990; Franowicz and Barth, 1995). These studies report that train responses are represented by a steady-state activity which is superimposed on initial large amplitude potentials. The latter can be regarded as an on-response to the burst of clicks (Franowicz and Barth, 1995). Our results differ from data obtained by Franowicz and Barth (1995) in the rat, due to the very high ratio of the SSR amplitude to the on-response amplitude. We believe that this difference should be attributed to the state of the animal since we obtained train responses quite similar to those reported by these authors under ketamine and xylazine anesthesia (personal unpublished data).

Rate depending properties of SSRs were evaluated at several stimulation rates. Both recorded and predicted curves show a linear increase of mean phase values when increasing the repetition rate; however, predicted phases appear leading with respect to phases obtained from the recorded curves. When considering the amplitude-rate function, it can be seen that predicted values are higher than amplitudes calculated for the recorded curves at all stimulus rates. The above reported discrepancies between predicted and recorded curves indicate that the generation of temporal recorded SSRs in the rat cannot be considered as resulting from the simple superimposition of MAEPs. Two possible hypotheses can be proposed. Assuming that auditory evoked responses to stimulation rates from 3.3 Hz to 60 Hz arise from the temporal cortex, it is reasonable to admit that phenomena related to the recovery cycle of cortical neurons take place in SSR generation when increasing the stimulation rate. In this view, the amplitude reduction and the phase lag of the recorded SSRs with respect to the predicted ones would reflect the decrease in amplitude and the increase in latency of individual MAEPs when increasing the repetition rate. Alternatively, one can hypothesize that when increasing the repetition rate, the generator location of SSRs is shifted from the temporal cortex to other sites, possibly subcortical structures. The first hypothesis critically depends on the ability of auditory cortical neurons to respond to fast stimulation rates. Several studies performed in cats (Creutzfeldt et al., 1980; Eggermont, 1991; Schreiner and Raggio, 1996) and in rats (Gaese and Ostwald, 1995) under anesthesia have reported that neurons of the auditory cortex entrain well up to a 20 Hz repetition rate. This means that SSRs recorded beyond this rate are likely to be generated elsewhere than in the auditory cortex. However, data obtained in the cat under light anesthesia (Schreiner and Urbas, 1988) and in awake state (De Ribaupierre et al., 1972) indicate that neurons of the primary auditory cortex are able to respond at rates over 20 Hz and up to 100–200 Hz. Since our recordings were obtained from the awake

animal, it seems reasonable to us that, apart from species differences, SSRs recorded from 30 to 60 Hz originate at the level of the temporal cortex. This hypothesis is in agreement with data obtained by Franowicz and Barth (1995), which indicate that 40 Hz SSRs recorded by epipial electrodes in the lightly anesthetized rat originate from the auditory cortex. In this view, the above reported differences between predicted and recorded responses can be attributed to phenomena dealing with the recovery cycle of cortical generators coming into play when increasing the repetition rate.

The amplitude-rate function obtained from the recorded curves appears almost flat while predicted amplitudes show a linear decrease when increasing the stimulation rate. This decrease should be attributed to the phase relationship of individual MAEP components which summate at a given rate. Assuming that SSRs originate from MAEP linear addition, the lack of a decreasing trend in the amplitude-rate function calculated on the recorded curves can be explained by hypothesizing that facilitating phenomena come into play at 50–60 Hz, thus preventing the amplitude reduction expected on the basis of phase relationships of MAEP components.

When comparing the rate functions calculated for rats with the corresponding ones previously obtained from surface recordings in humans (Galambos et al., 1981; Stapells et al., 1984; Hari et al., 1989; Azzena et al., 1995), remarkable differences are found. The amplitude-rate function appears almost flat in rats while in humans it was peaked due to the high amplitude observed at 40 Hz. Secondly, in rats, MAEP amplitude was higher than SSR amplitude at all rates, while for humans the converse is true (Azzena et al., 1995). With regard to the MAEP linear addition which is an effective model in predicting vertex recorded 40 Hz SSRs in man (Galambos et al., 1981; Stapells et al., 1984; Hari et al., 1989; Plourde et al., 1991; Azzena et al., 1995), it fails to reproduce amplitude and phase of temporal SSRs in rats at all repetition rates. Furthermore, in rats the amplitude discrepancies between predicted and recorded curves are more consistent than in humans and predicted amplitudes are higher than the corresponding values calculated on the recorded curves at all rates.

The comparison between the present study and data obtained in human beings should be regarded with caution if one considers that data available about SSR neural sources in humans, as well as in animals, do not allow us to definitely identify which neural structures are involved. As a matter of fact, apart from species differences, the temporal recorded SSRs in rats are believed to be generated at the level of the auditory cortex (Yoshida et al., 1984; Mäkelä et al., 1990; Franowicz and Barth, 1995), whereas the surface recorded SSRs in man could reflect both cortical (Mäkelä and Hari, 1987; Hari et al., 1989; Liégeois-Chauvel et al., 1994) and/or subcortical (Galambos, 1982; Spydell et al., 1985; Firsching et al., 1987) contributions. In this view, one can speculate that the observed differences between

vertex recorded SSRs in man and temporal recorded SSRs in rats may be related, at least partially, to the contribution coming from sources other than the temporal cortex to the vertex recorded SSRs in humans.

As previously stated for human studies (Santarelli et al., 1995), we believe that the electrical activity following the stimulus train offset strictly reflects the properties of responses to individual stimuli within the steady-state potential. In human recordings the response taking place after the 40 Hz click train showed a higher amplitude in comparison with the corresponding middle-latency potential (Santarelli et al., 1995), and this finding was interpreted as coming from the superimposition of individual responses to the last clicks of the train under favorable phase relationships. On the contrary, in the rat PSR amplitude was lower than MAEP amplitude at all stimulation rates. This result is in agreement with the above reported discrepancy between predicted and recorded SSR amplitudes which we explained as resulting from phenomena related to the recovery cycle of auditory cortical neurons. On the other hand, since the highest PSR amplitude was found at 50 Hz, it does not seem unreasonable to hypothesize that facilitating phenomena come into play at this rate.

In comparison with MAEP, an additional feature of PSR was the appearance of an evoked activity in the latency range over 40 ms. A similar finding was previously reported in human SSR recordings utilizing a 40 Hz click train paradigm (Santarelli et al., 1995), and it was interpreted as related to the resonant properties of the auditory system. However, in the present study the utilization of 120–480 Hz click trains and the digital filtering of the whole train responses allowed us to isolate two potentials at the beginning and at the cessation of the stimulus train, which can be respectively regarded as on- and off-responses to the burst of clicks (Keidel, 1976; Franowicz and Barth, 1995). With regard to the slow component of the off-response, the decrease in latency and the increase in amplitude at increasing stimulation rates seem to be related to the increasing effective intensity of the burst of clicks, even if this hypothesis is not fully supported by auditory sensitivity data obtained in the albino rat (Kelly and Masterton, 1977). Taking into account latency changes of off-responses at low stimulation rates, it is possible that the additional activity seen in PSR is mainly, if not completely, identifiable with an off-response.

In conclusion, several mechanisms come into play to generate temporal recorded SSRs in rats. However, the weight which has to be attributed to each mechanism differentiates temporal recorded SSRs from vertex recorded SSRs previously studied in man, thus suggesting differences in neural sources which underlie the generation of these two kinds of auditory potentials.

Acknowledgements

The authors wish to thank J.L. Mente for editorial assistance in preparing the English text.

References

- Azzena, G.B., Conti, G., Santarelli, R., Ottaviani, F., Paludetti, G. and Maurizi, M. Generation of human auditory steady-state responses (SSRs): I: Stimulus rate effects. *Hear. Res.*, 1995, 83: 1–8.
- Barth, D.S. and Di, S. Three-dimensional analysis of auditory-evoked potentials in rat neocortex. *J. Neurophysiol.*, 1990, 64: 1527–1536.
- Borbély, A.A. Changes in click-evoked responses as a function of depth in auditory cortex of the rat. *Brain Res.*, 1970, 21: 217–247.
- Creutzfeldt, O.D., Hellweg, F.C. and Schreiner, C.E. Thalamocortical transformation of responses to complex auditory stimuli. *Exp. Brain Res.*, 1980, 3: 87–104.
- Crowther, J.A., Miller, J.M. and Kileny, P.R. Effect of anesthesia on acoustically evoked middle latency response in guinea pigs. *Hear. Res.*, 1990, 43: 115–120.
- De Ribaupierre, F., Goldstein, M.H. and Yeni-Komshian, G. Cortical coding of repetitive acoustic pulses. *Brain Res.*, 1972, 48: 205–225.
- Eggermont, J.J. Rate and synchronization measures of periodicity coding in cat primary auditory cortex. *Hear. Res.*, 1991, 5: 153–167.
- Firsching, R., Luther, J., Eidelberg, E., Brown, W.E., Story, J.L. and Boop, F. A 40 Hz middle latency auditory evoked response in comatose patients. *Electroenceph. clin. Neurophysiol.*, 1987, 67: 213–216.
- Forss, N., Mäkelä, J.P., McEvoy, L. and Hari, R. Temporal integration and oscillatory responses of the human auditory cortex revealed by evoked magnetic fields to click trains. *Hear. Res.*, 1993, 68: 89–96.
- Franowicz, M. and Barth, D.S. Comparison of evoked potentials and high-frequency (Gamma-band) oscillating potentials in rat auditory cortex. *J. Neurophysiol.*, 1995, 74: 96–112.
- Gaese, B.H. and Ostwald, J. Temporal coding of amplitude and frequency modulation in the rat auditory cortex. *Eur. J. Neurosci.*, 1995, 7: 438–450.
- Galambos, R. Tactile and auditory stimuli presented at high rates (30–50 per sec) produce similar event related potentials. *Ann. N.Y. Acad. Sci.*, 1982, 88: 722–728.
- Galambos, R., Makeig, S. and Talmachoff, P.J. A 40-Hz auditory potential recorded from the human scalp. *Proc. Natl. Acad. Sci. USA*, 1981, 78: 2643–2647.
- Hari, R., Hämäläinen, M. and Joutsiniemi, S.L. Neuromagnetic steady-state responses to auditory stimuli. *J. Acoust. Soc. Am.*, 1989, 86: 1033–1039.
- Joliot, M., Ribary, U. and Llinás, R. Human oscillatory activity near 40 Hz coexists with cognitive temporal binding. *Proc. Natl. Acad. Sci. USA*, 1994, 91: 11748–11751.
- Keidel, W.D. The physiological background of the electric response audiometry. In: W.D. Keidel and W.D. Neff (Eds.), *Handbook of Sensory Physiology*. Springer, Berlin, 1976, pp. 105–231.
- Kelly, J.B. and Masterton, B. Auditory sensitivity of the albino rat. *J. Comp. Physiol. Psychol.*, 1977, 91: 930–936.
- Lenarz, T., Güllow, J., Grözinger, M. and Hoth, S. Clinical evaluation of 40-Hz middle-latency responses in adults: frequency specific threshold estimation and suprathreshold amplitude characteristics. *Oto. Rhino. Laryngol.*, 1986, 48: 24–32.
- Liégeois-Chauvel, C., Musolino, A., Badier, J.M., Marquis, P. and Chauvel, P. Evoked potentials recorded from the auditory cortex in man: evaluation and topography of the middle latency components. *Electroenceph. clin. Neurophysiol.*, 1994, 92: 204–214.
- Llinás, R.R., Grace, A. and Yarom, Y. In vitro neurons in mammalian cortical layer 4 exhibit intrinsic oscillatory activity in the 10- to 50-Hz frequency range. *Proc. Natl. Acad. Sci. USA*, 1991, 88: 897–901.

- Mäkelä, J.P. and Hari, R. Evidence for cortical origin of the 40 Hz auditory evoked response in man. *Electroenceph. clin. Neurophysiol.*, 1987, 66: 539–546.
- Mäkelä, J.P., Karmos, G., Molnár, M., Csépe, V. and Winkler, I. Steady-state responses from the cat auditory cortex. *Hear. Res.*, 1990, 45: 41–50.
- Miyazato, H., Skinner, R.D., Reese, N.B., Boop, F.A. and Garcia-Rill, E. A middle-latency auditory-evoked potential in the rat. *Brain Res. Bull.*, 1995, 37: 247–255.
- Ottaviani, F., Paludetti, G., Grassi, S., Draicchio, F., Santarelli, R., Serafini, G. and Pettorossi, V.E. Auditory steady-state responses in the rabbit. *Audiology*, 1990, 29: 212–218.
- Pantev, C., Elbert, T., Makeig, S., Hampson, S., Eulitz, C. and Hoke, M. Relationship of transient and steady-state auditory evoked fields. *Electroenceph. clin. Neurophysiol.*, 1993, 88: 389–396.
- Pantev, C., Roberts, L.E., Elbert, T., Ross, B. and Wienbruch, C. Tonic organization of the sources of human auditory steady-state responses. *Hear. Res.*, 1996, 101: 62–74.
- Paxinos, G. and Watson, C. *The rat brain in stereotaxic coordinates*. Academic Press, New York, 1982.
- Picton, T.W., Vajsar, J., Rodriguez, R. and Campbell, K.B. Reliability estimates for steady-state evoked potentials. *Electroenceph. clin. Neurophysiol.*, 1987, 68: 119–131.
- Plourde, G. and Picton, T.W. Human auditory steady-state response during general anesthesia. *Anesth. Analg.*, 1990, 71: 460–468.
- Plourde, G., Stapells, D.R. and Picton, T.W. The human auditory steady-state potential. *Acta Otolaryngol. (Stockh.)*, 1991, 491 (Suppl.): 153–160.
- Plourde, G. The clinical use of the 40-Hz auditory steady state response. *Int. Anesthesiol. Clin.*, 1993, 31: 107–120.
- Pöppel, E. Temporal mechanisms in perception. In: O. Sporns and G. Tononi (Eds.), *Selectionism and the Brain*. Academic Press, New York, 1994, pp. 185–202.
- Regan, D. *Human Brain Electrophysiology: Evoked Potentials and Evoked Magnetic Fields in Science and Medicine*. Elsevier, New York, 1989.
- Santarelli, R., Maurizi, M., Conti, G., Ottaviani, F., Paludetti, G. and Pettorossi, V.E. Generation of human auditory steady-state responses (SSRs): II: Addition of responses to individual stimuli. *Hear. Res.*, 1995, 83: 9–18.
- Schreiner, C.E. and Urbas, J.V. Representation of amplitude modulation in the auditory cortex of the cat: II. Comparison between cortical fields. *Hear. Res.*, 1988, 32: 49–64.
- Schreiner, C.E. and Raggio, M.W. Neuronal responses in cat primary auditory cortex to electrical cochlear stimulation: II. Repetition rate coding. *J. Neurophysiol.*, 1996, 75: 1283–1300.
- Shaw, N.A. The auditory evoked potential in rat—a review. *Prog. Neurobiol.*, 1988, 31: 19–45.
- Simpson, G.V. and Knight, R.T. Multiple brain systems generating the rat auditory evoked potential. I. Characterization of the auditory cortex response. *Brain Res.*, 1993a, 602: 240–250.
- Simpson, G.V. and Knight, R.T. Multiple brain systems generating the rat auditory evoked potential. II. Dissociation of auditory cortex and nonlemniscal generator systems. *Brain Res.*, 1993b, 602: 251–263.
- Spydell, J.D., Pattee, G. and Goldie, W.D. The 40 Hz auditory event-related potential: normal values and effects of lesions. *Electroenceph. clin. Neurophysiol.*, 1985, 62: 193–202.
- Stapells, D.R., Linden, D., Suffield, J.B., Hamel, G. and Picton, T.W. Human auditory steady-state potentials. *Ear. Hear.*, 1984, 5: 105–113.
- Sturzebecher, E., Kuhne, W. and Berndt, H. Detectability of acoustically evoked composite response (40 Hz potential) near threshold. *Scand. Audiol.*, 1985, 14: 214–219.
- Tiitinen, H., Sinkkonen, J., Reinikainen, K., Alho, K., Lavikainen, J. and Näätänen, R. Selective attention enhances the auditory 40-Hz transient response in humans. *Nature*, 1993, 364: 59–60.
- Yoshida, M., Lowry, L.D., Liu, J.J.C. and Kaga, K. Auditory 40-Hz responses in the guinea pig. *Am. J. Otolaryngol.*, 1984, 5: 404–410.