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Auditory Steady-State Responses in the Rabbit

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Abstract. The authors have studied auditory brainstem (ABRs), middle latency (MLRs) and steady-state potentials (SSRs) in 15 adult male rabbits weighing between 2.5 and 3 kg in order to verify if SSRs are due to a mere superimposition of ABRs and MLRs or to a resonance phenomenon. Ten of them were awake while 5 were studied under urethane anesthesia. Acoustic stimuli consisted in 0.1-ms square-wave pulses delivered at presentation rates ranging between 1 and 80/s at a stimulus intensity of 80 dB p.e. SPL. Our data show that reliable auditory SSRs can be obtained in the rabbit at a presentation rate of 30 stimuli/s, probably due to the superimposition of ABRs and MLR P_b waves which show an interwave interval of about 35 ms. The nonlinear aspects which can be detected are probably due to the effect of decreasing interstimulus intervals on the duration and amplitude of the P_b wave. It can then be concluded that SSRs in the rabbit are due more to a superimposition of ABR and MLR waves than to a resonance phenomenon.

Introduction

Many data are available in the literature concerning auditory brainstem response (ABRs) and middle latency response (MLRs) normative data in human adults, children, newborns and in experimental animals, while reports on the auditory steady-state potentials (SSRs), firstly described by Galambos et al. [1] in humans, are fewer.

It has been suggested that in the experimental animal, fast waves (I–VII) reflect the activation of the primary auditory system, from the acoustic nerve to the auditory cortex, while the longer latency potentials (middle, 17–75 ms latency) seem to be generated through other forebrain systems which receive auditory information in parallel from the brainstem [2]. The MLRs, which are probably due to different generator sources [3], showed marked amplitude fluc-

tuations and were affected by click rate and state of alertness.

Still questionable are the nature and site of origin of the SSRs. According to some authors [1, 4, 8], the auditory SSRs would result from a superimposition of the ABR and MLR components, while others [9] think that they are due to a 40-Hz resonance phenomenon, already described in the cat auditory cortex [10] and hippocampus [11], related to a 40-Hz rhythm of the mammalian brain [1].

In order to define the true nature and origin of such potentials, we have studied auditory-evoked potentials (ABR, MLR 40-Hz SSR) in rabbits. This study was justified by the opportunity to correlate the frequency at which the auditory-evoked SSRs occur with the latencies of the acoustic-evoked potentials ABR and MLR which, in rabbits, are different from humans. Furthermore, the animal model is more suitable to vary the amplitude of MLR potentials through different levels of arousal and to correlate them with the characteristics of SSRs.

Materials and Methods

Auditory-evoked potentials have been studied in 15 adult male rabbits weighing between 2.5 and 3 kg. All rabbits reacted behaviorally to acoustic stimuli and their ear canals appeared normal under otoscopic examination.

The skull was exposed under pentobarbital anesthesia (30 mg/kg b.w., i.v.) and the active electrode (brass screw, 2.2 mm diameter) implanted into the bone, laterally to the midline, 4.0 cm posteriorly to the bregma line, without touching the dura. It was referenced to a needle electrode inserted into the nose and grounded to a needle electrode at the mastoid on the side not stimulated. The interelectrode impedance was always below 2 kΩ. The recordings were performed in 10 awake animals, secured by means of a restraining frame, and in 5 under different levels of urethane anaesthesia (1 g/kg b.w., i.v.). The MLRs and SSRs were tested at different times after the anesthetic injection (between 0 and 6 min).

Acoustic stimuli consisted of 0.1-ms square pulses delivered through an earmould inserted into the rabbit's ear canal, at rates ranging from 1 to 80/s in 10-Hz steps, and at a stimulus intensity of 80 dB p.e. SPL. Analysis time was 100 ms. Also 35/s and 45/s presentation rates were performed. The signals obtained were preamplified, filtered through a 3- to 3000-Hz bandpass filter, stored on a magnetic tape recorder (store 4 DS) and processed by a Hewlett-Packard 5480 averager. The overall number of averaged sweeps was 1024 for each presentation rate, and at least two recordings were made to test intraindividual reproducibility.

The mean ± 1 SD latency values for ABR, MLR and SSR peaks were analyzed, as well as the amplitude-rate functions for the auditory SSRs. The relationships between MLR P_b and the SSR waves were also studied at the different repetition rates we have tested. The single 10-Hz responses recorded on tape were also employed to predict the higher repetition rate evoked potentials. In order to obtain a prediction for a 30-Hz response, three averaged samples of the 10-Hz response were added. The second response was delayed by 33 ms while the third one was delayed by 66 ms with respect to the first one. The 10-Hz presentation rate was chosen as it represents the fastest one able to evoke a transient response using an analysis time of 100 ms.

The obtained averaged responses were divided into 4 consecutive segments of equal length. A DFT was performed on the observed SSR and on the synthetized ones.

Results

ABR tracings were characterized by five main vertex-positive peaks (I, II, III, IV, V), the fourth one (IV) being the largest, while the fifth was often represented by a notch on the tail of wave IV, thus being different from the human ABRs, in which wave V is the largest and the most stable. The ABR peaks latency values remained unchanged when testing under different conditions of arousal during the increase of anesthesia level. The

Table 1. Latency values (ms, mean ± 1 SD) of ABR, MLR and SSR at 80 dB p.e. SPL intensity level

ABR	Latency	MLR	Latency	SSR	Latency
I	1.4±0.3	P_0	5.4±0.4	P_1	5.5±0.8
II	2.3±0.3	N_a	11.6±1.4	N_1	11.0±1.2
III	3.1±0.3	P_a	32.9±3.1		
IV	4.2±0.4	N_b	33.9±3.7		
V	5.4±0.5	P_b	49.4±3.3		

mean ± 1 SD of peak (I, II, III, IV, V) latency values are represented in table 1.

The MLRs were represented in all the tested awake animals by 2 vertex-positive (P_0, P_b) and one vertex-negative (N_a) wave (fig. 1). P_a and N_b waves, which were present at 1 and 10 stimuli/s, were less stable and showed a reduced amplitude and tended to markedly decrease at presentation rates exceeding 10/s. The P_b wave was higher and larger than P_a and its amplitude was only slightly affected by increasing stimulation rates. Conversely, P_b wave became narrower at higher stimulation rates because of the lead of its descending branch. The rabbit MLR is then different from the human one, where the P_a wave is the most robust. Also the latencies were different as they were reduced compared to humans. The mean ±1 SD of peak latency values are represented in table 1. P_0 latency values were superimposable to the ABR V wave, while P_a ranged between 21.3 and 25.2, and P_b between 46.7 and 52.2 ms.

An SSR, characterized by the repetition of a positive (P_1) and a negative (N_1) wave, was clearly detectable at 80 dB p.e. SPL in all rabbits (fig. 1), the best repetition rate being around 30/s, while in humans the best results are obtained at 40 stimuli/s. At higher repetition rates (50, 60, 70 and 80/s),

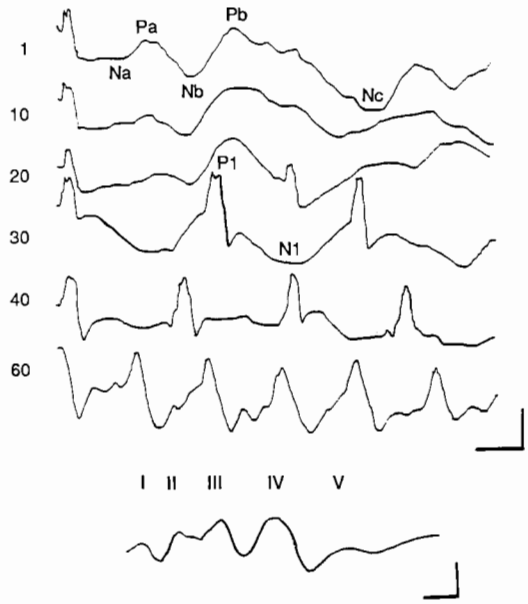

Fig. 1. MLR response was clearly detectable at presentation rates of 1 and 10 stimuli/s. In the first portion of the tracing, the ABR is clearly detectable. It is characterized by five main vertex-positive peaks (I, II, III, IV, V), the fourth one being the largest, while the fifth one was often represented by a notch on the tail of wave IV. Note the decreasing of P_b amplitude and duration which is detectable when the presentation rate is increased from 1 to 10 stimuli/s. An SSR characterized by the repetition of a positive (P_1) and a negative (N_1) wave is clearly detectable in all rabbits, the best presentation rate being around 30 stimuli/s (calibration bars are 10 ms and 500 nV). Due to the employed filter settings, selection criteria in order to define the best repetition rate were based on the amplitude increase, while in order to obtain a smooth sinusoidal waveform, a lower low-pass filter should be used (in this and in the subsequent figures, upward deflection indicates vertex positivity).

the tracing was made up by a mere repetition of ABR complexes (fig.1).

To study the relative role of P_a and P_b waves in determining the SSRs, we compared the MLR potentials with the SSRs at different levels of urethane anesthesia. A

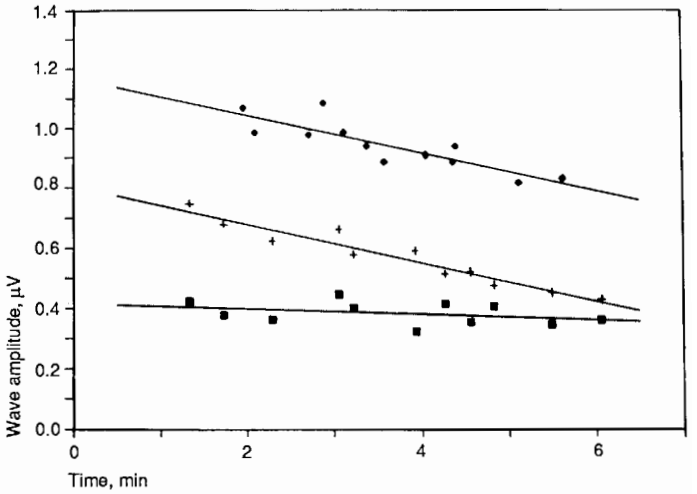

Fig. 2. Effect of urethane anesthesia upon amplitude values of P_a (◆; r=-0.85, a=1.19, b=-0.06), P_b (+; r=-0.97, a=0.81, b=-0.06) and P_1 (■; r=-0.35, a=0.42, b=-0.009) waves (y=bx+a). Note the amplitude decrease of P_b which parallels that of P_1, while P_a amplitude values remain unchanged, though reduced as absolute values.

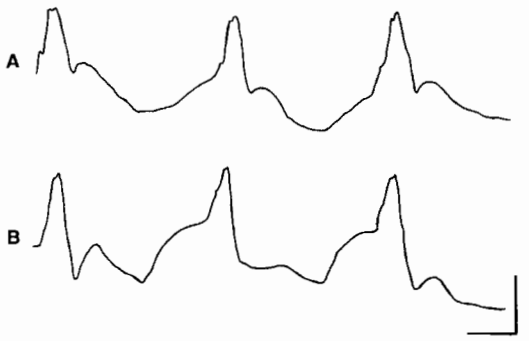

Fig. 3. SSR predicted response (A) and SSR observed response (B). Note that the observed SSR shows a leading of the slow component while the fast one (ABR) is not changed in latency, so that it corresponds to the tail of the slow component (calibration bars are 10 ms and 500 nV).

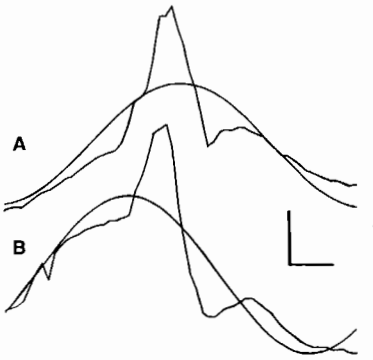

Fig. 4. The DFT analysis (Fourier series), performed on the predicted response (A) and on the observed one (B), shows a phase lead of the observed response with respect to the prediction of about 45° (calibration bars are 5 ms and 250 nV).

different effect was observed on the P_a and P_b waves. In fact, the P_b wave showed a sudden decay after urethane anesthesia. SSR amplitude also decreased and the reduction paralleled that of the P_b wave (fig. 2). The P_a amplitude remained almost unchanged at least at low and moderate levels of anesthesia.

By superimposing the averaged 10-Hz potentials delayed each other by 33 and

66 ms, the evoked 30-Hz responses could be predicted as a pure combination of single potentials. These potentials were compared with those resulting from 30-Hz stimulation. The most striking difference between the predicted response and the observed one consisted in the difference in phase of their first harmonic. In particular, the observed response showed a phase lead of 45° with respect to the predicted one (fig. 3, 4).

30-Hz SSR amplitude values were directly related to P_b wave amplitude values, as both varied accordingly.

The mean ± 1 SD latency values of P_1 and N_1 waves are represented in table 1.

Discussion

Our data show that in rabbits SSRs are characterized by the highest amplitude values in response to a 30/s stimulation rather than to a 40/s one, as observed in man [4, 7]. This observation is important as if one supposes that the SSRs are due to a resonance phenomenon, the resonant frequency is not constant in the central nervous system of mammals, as previously reported [10, 11]. Furthermore, it appears that the SSRs are dependent on the characteristics of the MLRs because one can obtain a good prediction of SSRs from the MLRs. In fact, in humans and in cats, P_a, which is the most reliable and consistent wave, shows latency values around 25 ms [1, 2, 12], being thus superimposable at presentation rates around 40/s. In rabbits, the most reliable and consistent waves are the ABR complex and P_b, the latter showing latency values around 35 ms. Hence P_b wave would superimpose to ABR at presentation rates around 30/s. Furthermore, in rabbits, after urethane anesthesia, a reduction of P_b wave duration and amplitude occurs without any appreciable changes of P_a. At the same time, one can observe a reduction of SSR amplitude that parallels that of the P_b wave. In humans, anesthesia induces significant decrease in amplitude of P_a and N_b waves [13, 14] and thus a reduction of SSR amplitude would be expected.

The prediction of SSR from MLR could be possible in the case of a resonant circuit only if the MLR could be considered a transient response of a single resonator. If one supposes that MLR are impulse responses, each determined in shape by the resonance characteristics of the system, the presentation of the impulses closer together will result in overlapping of single impulse responses. An SSR is obtained when the stimulus presentation rate is equal to the resonant frequency of the system. However, it is unlikely that the MLRs represent the output of a single resonator. In fact, one can modify only one wave of the MLRs without altering the remaining ones. Furthermore, many studies suggest different generator sources for P_a and P_b waves in man [7, 15] as well as in animals [2, 16]. Thus, it is more likely that the SSRs result from a simple superimposition of MLR waves originating from different generator sources.

If one compares the predicted SSRs with those experimentally obtained, a phase lead of the first harmonic of the observed one was evident. This phase difference was also observed by Stapells et al. [17], although reported as not significant, and may be explained taking into account a reduction of the late components of P_b wave in increasing the stimulus presentation rate as a result of an adaptation effect. The adaptation of

the MLR waves has already been observed in cats [2].

According to our data, it can be concluded that auditory evoked SSRs are probably due, in the rabbit, to a superimposition of ABR and P_b waves, as proposed in man by Kileny [4], Lynn et al. [5], Musiek et al. [6], Stapells et al. [7] and Kileny and Shea [8], rather than to a resonance phenomenon, as described by Galambos et al. [1] and Basar et al. [9].

Potentiels évoqués auditifs en régime permanent chez le lapin

Les auteurs ont étudié les potentiels évoqués auditifs du tronc cérébral, les potentiels de latence moyenne et les potentiels en régime permanent chez 15 lapins mâles, de poids compris entre 2,5 et 3,5 kg pour vérifier si les potentiels en régime permanent sont liés à une simple superposition des ondes ABR et MLR ou dus à un phénomène de résonance. Dix d'entre eux ont été étudiés en état de veille tandis que 5 ont été anesthésiés avec de l'uréthane. Les stimuli employés étaient des clics présentés à des cadences comprises entre 1 et 80 stimuli par seconde à l'intensité de 80 dB p.e. SPL. Nos résultats montrent que les potentiels en régime permanent peuvent être obtenus chez le lapin avec une cadence de présentation de 30 clics par seconde car il se produit, vraisemblablement, une superposition des potentiels du tronc cérébral et de ceux de latence moyenne, séparés par 35 ms environ. Les aspects de non-linéarité qu'il est possible d'observer sont liés à l'effet de la diminution de l'intervalle interstimulus sur la durée et sur l'amplitude de l'onde P_b. On peut conclure que les potentiels en régime permanent chez le lapin sont vraisemblablement liés à la superposition des ondes ABR et MLR, plutôt qu'à un phenomène de résonance.

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