Research paper

Comparison of noise-induced changes of auditory brainstem and middle latency response amplitudes in rats

Jiri Popelar *, Jolana Grecova, Natalia Rybalko, Josef Syka

Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, v.v.i., Videnska 1083, 142 20 Prague 4, Czech Republic

ARTICLE INFO

Article history:
Received 10 March 2008
Received in revised form 3 September 2008
Accepted 5 September 2008
Available online 11 September 2008

Keywords:
Noise exposure
Long evans rats
Middle latency responses
Auditory brainstem responses
Amplitude-intensity functions

ABSTRACT

Auditory brainstem responses (ABRs) and middle latency responses (MLRs) were compared after noise exposure to elucidate the specific effects of a loud sound on the central auditory system in rats. Rats were exposed twice for 1 h to broad-band noise (BBN) of 118 dB SPL (first exposure) and 122 dB SPL (second exposure) with an interval between the exposures of three weeks. The first noise exposure produced threshold shifts (TSs) amounting to 5–45 dB, and the second exposure resulted in 40–70 dB TSs. The slope of MLR amplitude-intensity functions (AIFs) increased significantly in correlation with the TS, resembling loudness recruitment. However, maximal MLR amplitudes measured at 8 kHz increased after the first and second noise exposures to almost equal values in individual animals regardless of the TS. In addition, maximum MLR amplitude enhancement was dependent on pre-exposure MLR voltage, probably reflecting the level of metabolic activity or neurotransmitter processes in individual animals. In contrast to MLR amplitudes, ABR amplitudes were suppressed after noise exposure without changing the slope of ABR AIFs. The MLR changes reflect the specific effects of noise exposure on the central auditory system.

© 2008 Elsevier B.V. All rights reserved.

1. Introduction

It is generally accepted that noise exposure affects not only the peripheral, but also the central part of the auditory system. Injury of the periphery is reflected in a hearing threshold shift and in weaker responses to sound at many levels of the central auditory system, from the cochlear nucleus to the auditory cortex (see Syka, 1984; Salvi et al., 1990; Kaltenbach et al., 1998; Wang et al., 2007) as well as in humans (Jastreboff, 1990; Jastreboff and Afman, 2000; Bauer and Brozoski, 2001; Brozoski et al., 2002; Yang et al., 2007) as well as in humans (Jastreboff, 1990; Jastreboff and Hazell, 1993; Gerken, 1996; Jastreboff and Jastreboff, 2000). An increase in pathological neuronal excitability following acoustic trauma or produced by salicylate treatment can lead to phantom auditory sensations (tinnitus), as has been reported in rodents (Kaltenbach and Aman, 2000; Bauer and Brozoski, 2001; Brozoski et al., 2002; Yang et al., 2007) as well as in humans (Jastreboff, 1990; Jastreboff and Hazell, 1993; Gerken, 1996; Jastreboff and Jastreboff, 2000). Increased amplitudes of auditory evoked potentials in response to simple auditory stimuli were also detected in patients with the neurological disorder focal dystonia (Lim et al., 2005). The changes in electrophysiological response parameters are most frequently interpreted as resulting from an increase in excitation and/or a lack of inhibition. However, the mechanisms leading to such an excitatory – inhibitory imbalance are still poorly understood.

Previously, we recorded MLRs in guinea pigs and rats from electrodes implanted on the surface of the auditory cortex. After noise exposure, MLR amplitudes in response to high-intensity stimuli were enhanced in most animals in comparison with pre-exposure amplitudes (Popelar et al., 1987; Syka et al., 1994; Syka and Rybalko, 2000). However, the noise-evoked amplitude enhancement observed in our experiments varied enormously between individual animals, and the MLR changes were only weakly correlated with noise exposure intensity or TS.
The aim of the present work was to investigate the specific effects of noise exposure on the central part of the auditory pathway by comparing noise-induced ABR and MLR changes. Whereas noise exposure produced a suppression of ABR amplitudes, the MLR amplitudes were enhanced and the slopes of the MLR amplitude-intensity functions were increased after noise exposure. The extent of MLR amplitude enhancement was not dependent on noise exposure intensity, but was related to pre-exposure MLR voltage, suggesting a limitation in terms of capacity of the brain to evoke larger responses after noise exposure.

2. Materials and methods

2.1. Animal preparation

Experiments were performed on nine adult female pigmented rats (strain Long-Evans) weighing 250–300 g. Auditory evoked responses were recorded with chronically implanted electrodes. During implantation, the animals were anesthetized with an intramuscular injection of 35 mg/kg of ketamine (Narkamon 5%, Spofa) and 0.6 mg/kg of xylazine (Sedazine, Fort Dodge Inc.). MLRs were recorded with a teflon-coated platinum iridium ball electrode (ball diameter 0.5 mm) fixed on the surface of the primary auditory cortex. For ABR recordings, a miniature stainless steel screw was fixed in the skull at the vertex, touching the brain surface. The reference electrode was placed in the neck muscles. All electrodes were soldered to pins of a connector socket mounted on the skull with stainless steel screws and acrylic resin. Recordings of auditory evoked responses were performed in animals lightly sedated with an intramuscular injection of 0.03 mg/kg of medetomidin hydrochloride (Domitor, Farmos). The pre-exposure recording of auditory evoked responses was performed one day before noise exposure, while post-exposure recordings were made periodically during the next 2–3 months.

2.2. Recording of auditory evoked responses

The recording of auditory evoked responses was carried out in a sound-attenuation anechoic chamber. The walls and ceiling inside the chamber were covered by cones from sound-absorbent material reliably suppressing echo from 400 Hz up. A TDT (Tucker Davis Technologies, Florida) SystemIII setup was used for acoustical stimulation and signal acquisition. Evoked potentials were amplified and digitized with a Medusa RA16PA preamplifier and RA4LI headstage (A/D sample rate 25 kHz); the amplified digital signal was sent to a Pentusa RX5-2 base station via optic fibers. The signal was filtered (3–300 Hz for MLR recording and 300–3000 Hz for ABR recording) and averaged with the aid of TDT BioSig software. Acoustical stimuli were tone bursts (duration 5 ms with a 2 ms rise-fall times) of decreasing intensity (5 dB decrements) from 110 dB SPL to 0 dB SPL over a frequency range of 1–40 kHz. Tone stimuli were presented in free-field conditions from a two-way loudspeaker system (Jamo woofee and SEAS T25CF 002 tweeter) that was placed 70 cm in front of the animal’s head. The stimulus repetition rate was 1 Hz for MLR recording and 10 Hz for ABR recording. The acoustic system was calibrated with a B&K 4939 microphone, a ZC0020 preamplifier and a B&K 2231 Sound Level Meter. The microphone was placed in the position of the animal’s head during the experiment and facing the speakers.

2.3. ABR audiogram

The ABR audiograms were assessed from averaged ABRs to tone burst stimulation at 1, 4, 8, 12, 16, 24, 32 and 40 kHz of decreasing intensity in 5 dB steps. ABR thresholds were determined using the criterion of a just noticeable deflection of the averaged electrical activity around the baseline at the predicted time window.

2.4. ABR and MLR amplitudes

The ABR amplitudes were measured as the peak-to-peak amplitude of the positive value of the most dominant wave, usually wave III, and the negative value of the next trough. The peak-to-peak MLR amplitudes of the first positive wave P1 and the first negative wave N1 were measured and analyzed. The peak-to-peak N1–P2 MLR amplitudes were measured as well; however, the values were more variable than the P1–N1 amplitudes. Therefore, these results are not presented in this paper.

To compare the effect of noise exposure on auditory evoked response amplitudes in individual animals, the relative response amplitudes evoked by 8 kHz stimuli were computed. The relative post-exposure ABR amplitude was taken as a percentage of the pre-exposure ABR amplitude in response to stimuli with an intensity of 100 dB SPL. An intensity of 100 dB SPL was chosen because in most rats under pre-exposure conditions, ABR AIFs were monotonic at the tested stimulus intensities up to 100 dB SPL, and stimuli of this intensity evoked ABRs with the highest amplitude. Because the MLR AIFs were usually non-monotonic or saturated, the maximal pre-exposure MLR amplitude of the MLR AIF (regardless of stimulus intensity) was taken as 100% and relative post-exposure MLR amplitudes were computed as a percentage of this maximal pre-exposure MLR amplitude.

To quantitatively characterize the shape of the AIFs, the slope “k” of pre-exposure and post-exposure AIFs in individual animals was determined. The amplitude values were fit by linear regression, and the parameter “k”, as the slope of the linear regression line (formula \( y = kx + b \), units are mV/dB), was computed for the monotonically increasing part of the AIF by GraphPad Prism 5 software.

2.5. Noise exposure

Each rat was exposed twice to broad-band noise (BBN) for 1 h at two different intensities (with a three-week interval between the two exposures):

1st exposure: 118 dB SPL
2nd exposure: 122 dB SPL

Awake rats were exposed to noise in a specially constructed anechoic box (inner diameters 24 × 24 × 34 cm) supplied with a loudspeaker (B&K Speakers DE700) and coupled to a horn. The broad-band noise was generated with a RFT 03 004 white noise generator and amplified with a custom-made power amplifier. The sound field within the cage was measured with a B&K 4939 microphone, a ZC0020 preamplifier and a B&K 2231 Sound Level Meter. The RMS value of the sound pressure level (SPL), averaged over one-second intervals, was measured. The frequency spectrum measured in the exposure box was flat (less than ±5 dB) over the frequency range 0.8–20 kHz. Measurements of sound intensity obtained at four points within the cage were found to vary by less than 1.5 dB SPL. During the exposure to noise, the animal was placed in a round wire mesh cage (diameter 17 cm, height 10 cm) that prevented the animal from resting its head against the wall of the exposure box and thus occluding the ear canal. The round wire mesh cage with an animal was placed in the center of the exposure box. Because the measurements of sound intensity obtained at four points within the cage were found to vary by less than 1.5 dB SPL and the rats often changed their position during...
noise exposure, it can be concluded that both ears were exposed almost equally.

In two rats, audiogenic seizures appeared at the beginning of the second noise exposure, and the head connector for the implanted MLR electrode was damaged during the initial stage of the seizures (wild running). A third rat lost the head connector while manipulating the animal after the second noise exposure. In these three rats the MLR amplitudes were not measured after the second noise exposure, and their hearing thresholds after the second noise exposure were assessed from ABR recordings using a subdermal needle electrode fixed at the location of the previously recorded ABRs with a miniature stainless steel screw.

2.6. Statistical procedures

Graph Pad Prism 4 software was used for computing linear regression line parameters and for statistical analysis using a paired t-test.

The care and use of animals reported on in this study were approved by the Ethics Committee of the Institute of Experimental Medicine, AS CR, and followed the guidelines of the Declaration of Helsinki.

3. Results

3.1. Hearing thresholds

The average ABR audiograms obtained before and after BBN exposure and the resulting thresholds shifts are presented in Fig. 1. One day after the first noise exposure (118 dB SPL, panel A), hearing thresholds increased across the whole frequency range with the maximal threshold elevation at high frequencies (above 8 kHz). Thresholds in most animals recovered during the next three weeks to pre-exposure levels. The second noise exposure (122 dB SPL, panel B) produced a significantly larger threshold elevation than did the first exposure (40–70 dB), and the thresholds almost did not recover during the next 30 days (except the threshold at 1 kHz) and stayed permanently elevated. The average TSs and their recovery are displayed in Fig. 1 panels C and D.

3.2. ABR and MLR amplitude changes after noise exposure

ABR and MLR amplitudes were evaluated at a stimulus frequency of 8 kHz. Typical ABR and MLR recordings elicited with 8 kHz stimuli at different intensities, before and after noise exposure, are shown in Fig. 2 (rat 11A). The maximal pre-exposure peak-to-peak ABR amplitude reached 12.5 µV (at 100 dB SPL stimulus intensity). One day after the first BBN exposure, ABR amplitudes were suppressed and the maximal ABR amplitude at 100 dB SPL amounted to 9.4 µV. The second noise exposure produced a significantly larger TS than did the first one, and the ABR amplitude at 100 dB SPL markedly decreased to 4.5 µV one day post-exposure (and this value dropped even further during a one-month recovery).

Noise exposure produced different changes in MLR amplitudes in comparison with ABR. The maximal peak-to-peak P1-N1 MLR amplitude measured before noise exposure amounted to 177 µV (at 60 dB SPL stimulus intensity). One day after the first BBN exposure, the maximal P1-N1 MLR amplitude increased almost two-fold in comparison with the pre-exposure value, reaching 350 µV.

![Fig. 1](image-url)
at 80 dB SPL. The value of the maximal MLR amplitude after the second noise exposure reached a similar level as measured after the first noise exposure (345 \, \mu V at 95 dB SPL) in spite of the significantly larger TS produced by the second BBN exposure.

3.3. ABR amplitude-intensity functions (ABR AIFs)

ABRs were recorded mainly for assessing hearing thresholds, but in four rats pre-exposure ABRs and post-exposure ABRs were recorded in response to a broad range of stimulus intensities, which allowed us to determine ABR AIFs. Two representative examples of ABR AIFs (rats 11A and 11C), constructed from pre-exposure and post-exposure recordings in response to 8 kHz tone stimuli, are shown in Fig. 3. In these two rats as well as in two other animals, ABR amplitudes were significantly suppressed after both noise exposures in correlation with the TSs, and the ABR AIFs were shifted almost in parallel to higher stimulus intensities after noise exposure. In all rats the maximal ABR amplitude suppression was observed immediately after noise exposure (measured one day post-exposure). Similarly as MLR amplitudes, the ABR amplitudes recovered to pre-exposure values only after the first noise exposure.

3.4. MLR amplitude-intensity functions (MLR AIFs)

MLR AIFs were computed at individual stimulus frequencies and from individual pre-exposure and post-exposure recordings. Because the maximal noise-induced TS was observed at high frequencies (above 8 kHz) and the MLR amplitude changes were most pronounced at high frequencies, the MLR AIFs and ABR AIFs are demonstrated and analyzed for 8 kHz stimulus frequency. Thresholds and TSs based on ABR and MLR recordings were almost identical (within a 5 dB tolerance); their values can also be determined from ABR AIFs and MLR AIFs.

As a rule, large interindividual variability in TS and MLR amplitude enhancement was observed. Three examples of MLR AIFs in response to 8 kHz tone stimuli, constructed from pre-exposure and post-exposure recordings after the first and second noise exposures, are shown in Fig. 4. For better clarity, only the post-exposure recordings in which the MLR amplitude reached a maximal value are displayed. In the first example in Fig. 4, panels A and B (rat 11A), the first noise exposure produced only a minimal TS of 10 dB at 8 kHz, but the TS at this frequency after the second noise exposure amounted to 75 dB. However, the maximal MLR amplitude enhancement, observed on the first day after the first noise exposure (350 \, \mu V at 80 dB SPL) and on the seventh day after the second noise exposure (345 \, \mu V at 95 dB SPL), were almost identical. The MLR AIFs presented in panels C and D (rat 11F) are shifted after noise exposure by large TSs, but the MLR amplitude enhancement was negligible after both the first and second noise exposures. Panel E presents the MLR AIFs of an animal (11C) with a large threshold shift (45 dB) and a large MLR amplitude enhancement after the first noise exposure. The second noise exposure (panel F) elicited a more pronounced TS (70 dB) in this animal; however, the maximal MLR amplitude enhancement, reached on the seventh day post-exposure, was similar to that observed following the first noise exposure. During the recovery in this animal the threshold at 8 kHz decreased by 20 dB 60 days after noise exposure, but a MLR amplitude enhancement was still present at this time.

Fig. 4 shows examples of the most and least extreme combinations of TS and MLR amplitude enhancement; the results obtained in other animals ranged in between these extremes. Data obtained in all rats demonstrate that the maximal MLR amplitude enhancements reached after the first and second noise exposures were almost identical regardless of the TS. The MLR amplitudes usually reached their maximal values 1–4 days after the first noise exposure (in three rats first day, in two rats third day, in four rats fourth
day) and recovered to pre-exposure levels (at least partially) during the next three weeks. The MLR amplitude enhancement produced by the second noise exposure usually took several days (1–7 days) to reach maximal values (in two rats one day, in one rat two days, in three rats seven days), but this amplitude elevation was permanent and the MLR amplitudes did not recover to pre-exposure values even in animals with a partial threshold recovery (as demonstrated in Fig. 4F).

3.5. Maximal MLR and ABR amplitudes after noise exposure

The maximal relative ABR and MLR amplitudes (expressed as percentage of maximal pre-exposure amplitudes) as a function of the TS at 8 kHz produced by the 1st BBN exposure (filled triangles) and the 2nd BBN exposure (filled squares) are shown in Fig. 5. Dotted lines connect the symbols for individual animals.

Fig. 5A demonstrates the relationship between changes in maximal ABR relative amplitudes in percentage (100% equals the maximal ABR pre-exposure amplitude measured at 100 dB SPL) and TS at 8 kHz after the first and second noise exposures. Both noise exposures induced a suppression of maximal ABR amplitudes; the extent of ABR amplitude suppression markedly depended on the TS magnitude, and the linear regression line significantly declines from the horizontal ($p < 0.0001$).

Quite different results were obtained with MLRs (Fig. 5B). The MLR amplitude enhancements measured in individual animals (expressed as a percentage of the pre-exposure value) were almost equal after the first and second noise exposures without any correlation with the TS. The linear regression line is almost horizontal ($p < 0.99$).

3.6. The slopes of ABR and MLR AIFs

The slopes of individual ABR and MLR AIFs (computed for the monotonically increasing part of the AIF) were calculated with the aim of quantitatively characterizing the AIFs. The relationship between the slope of individual ABR and MLR AIFs and the TS at 8 kHz measured before noise exposure (diamonds), after the first noise exposure (triangles) and after the second noise exposure (squares) is shown in Fig. 6. Post-exposure slope values were calculated from AIFs in which the response amplitudes reached maximal values. The relationship between the slope of individual ABR AIFs and the TS at 8 kHz measured before noise exposure (diamonds), after the first noise exposure (triangles) and after the second noise exposure (squares) is shown in Fig. 6A. Dotted lines connect the symbols of individual animals. ABR AIF slopes did not change uniformly after noise exposure; ABR AIFs after noise exposure were shifted almost in parallel to higher stimulus intensities, keeping their slope more or less unchanged (average slope values were $0.099 \pm 0.047$ mV/dB pre-exposure, $0.104 \pm 0.037$ mV/dB after the first exposure and $0.106 \pm 0.041$ mV/dB after the second noise exposure). The linear regression line is not declined from the horizontal ($p < 0.99$). The pre-exposure MLR AIF slope (Fig. 6B) ranged between 1.2 and 4.3 mV/dB ($2.75 \pm 1.2$ mV/dB, mean ± SD) in individual animals, while the average slope increased after the first noise exposure to $7.4 \pm 3.7$ mV/dB and after the second noise exposure to $14.1 \pm 3.7$ mV/dB. Dotted lines connect the values measured in individual animals. The linear regression line of logarithmic values deviates significantly from the horizontal ($p < 0.0001$), and the MLR AIF slope strongly depends on the TS magnitude. Fig. 6A documents that the MLR AIF slope increased after noise exposure in all animals, whereas maximal MLR amplitude enhancement was much more variable (see Fig. 5).

3.7. MLR amplitude enhancement variability

Examples of the results presented in Fig. 4, as well as our data published previously (Syka et al., 1994; Syka and Rybalko, 2000), document a large interindividual variability in MLR amplitude enhancement after noise exposure. Statistical analysis of recently

---

**Fig. 3.** Representative examples of ABR AIFs recorded in two rats (C and A) in response to 8 kHz tone stimuli, constructed from pre-exposure, one-day and 18–30-days post-exposure recordings.
and previously obtained data revealed a close correlation between the voltage of the pre-exposure MLR and the resulting amplitude enhancement after noise exposure.

The dependence of the relative MLR post-exposure amplitude (in percentage) on pre-exposure MLR amplitude in mV, measured in the current experiments as well as in 40 rats tested in our previous experiments, is presented in Fig. 7. All rats were exposed for 1 h to BBN of 115–125 dB SPL; full diamonds represent data from current experiments, while open diamonds represent data obtained in rats in our previous experiments. The figure documents that rats with small original MLR amplitudes usually displayed a large MLR amplitude enhancement, whereas in animals with large pre-exposure MLR amplitudes exceeding 1 mV, the MLR amplitudes were either depressed or enhanced less than 150% after

Fig. 4. MLR AIFs recorded in three rats in response to 8 kHz tone stimuli, constructed from pre-exposure, post-exposure and recovery recordings measured after the first (panels A, C, and E) and second noise exposure (panels B, D, and F). The recording day is indicated in the legend in each panel.

Fig. 5. The maximal relative ABR and MLR amplitudes (expressed as percentage of the maximal pre-exposure amplitude) as a function of TS at 8 kHz produced by the first BBN exposure (filled triangles) and the second BBN exposure (filled squares). Interrupted lines connect the symbols of individual animals, solid lines represent linear regression lines.
noise exposure. This relationship is best fitted with the exponential curve: 

\[ y = \frac{1}{C_0} 51 \ln(x) + 473 \] (expressed as a line in the logarithmic scale in Fig. 7).  

4. Discussion  

The results demonstrate that noise exposure can specifically affect the function of individual parts of the central auditory pathways and differentially affect the function of the auditory brainstem and cortical and subcortical structures: whereas ABR amplitudes became reduced in correlation with the TS, predominantly reflecting noise-induced changes at the periphery, MLR AIFs became significantly steeper in noise-exposed rats, resembling an attribute of loudness recruitment. Repeated noise exposure revealed that the maximal MLR amplitudes exceeded pre-exposure values in most animals, but the MLR amplitudes measured in a particular rat after the first and second noise exposures were almost identical regardless of the TS. The MLR amplitude change was typical for an individual rat, was not related to the TS, but was significantly dependent on the pre-exposure MLR voltage. Noise exposure produced the largest MLR amplitude enhancement in animals with originally small MLR amplitudes, but in rats with large pre-exposure MLR amplitudes the MLR amplitude enhancement was small. This relationship can indicate a limitation in terms of capacity of the brain to evoke larger responses after noise exposure.  

4.1. ABR generation  

Numerous studies have used a variety of approaches to identify the brainstem regions involved in ABR generation (i.e. Starr and Hamilton, 1976; Huang and Buchwald, 1977; Møller and Burgess, 1986; Caird and Klinke, 1987; Fullerton and Kiang, 1990; Melcher et al., 1996; Kaga et al., 1997). The data suggest that each peak wave of the response (P1–P5) is composed of evoked potentials generated from multiple auditory brainstem nuclei and tracts, and each of the auditory nuclei elicits potentials that are reflected in several ABR peaks. The results obtained in animals indicate that the origin of the earliest peak P1 is attributable to the cells of the ipsilateral cochlear nerve and cochlear nucleus, while the P2 wave is mainly generated by potentials coming from the ipsilateral anteroventral cochlear nucleus (AVCN) and the posteroventral cochlear nucleus (PVCN). The major role in generating P3 is played by the AVCN, PVCN and the contralateral superior olivary complex (SOC). The AVCN is the main structure involved in P4 generation, although the contralateral and ipsilateral SOC and the lateral lemniscus nucleus are also producing P4. Concerning the generation of P5, the generally accepted idea is that the inferior colliculus is responsible for generating this wave.  

4.2. MLR generation  

MLRs are generated by a complex neuronal system involving contributions from, and interactions between, many centers in the auditory and extra-auditory pathways from the midbrain to the cortex. Intracranial recordings in cats (Buchwald et al., 1981), rats (Simpson and Knight, 1993a; Simpson and Knight, 1993b), and guinea pigs (Kraus et al., 1988) suggest that two parallel circuits contribute to MLR generation: (1) a midline generator system that reflects the activation of non-primary auditory projections, and (2) a lateral generator system that reflects the activation of primarylemniscal projections including the ventral cochlear nuclei, the lateral and medial superior olives, the central nucleus of the inferior colliculus (IC), the ventral division of the medial geniculate body (MGB), and the primary auditory cortex. In addition to the primary auditory nuclei, the mesencephalic reticular formation also contributes to MLR generation (Wood and Wolpaw, 1982; Hinman and Buchwald, 1983; Caird and Klinke, 1987; McGee et al., 1991; Kraus et al., 1992; Kraus and McGee, 1995). Similar sources of MLR origin, as those demonstrated in animals, were
identified in humans. A source localization model in patients with localized lesions in the thalamus or subcortical white matter demonstrated that the first major component of the MLR (P20), in both healthy control subjects and in patients, is generated subcortically, whereas subsequent MLR components are mainly generated cortically (Kaseda et al., 1991; Leavitt et al., 2007; Morita et al., 2007).

4.3. Noise-induced MLR amplitude changes

In most previous papers dealing with the effects of noise exposure on the central auditory system, enhanced responses and steeper input-output functions were usually considered together to be a common feature of noise-induced neural hyperactivity. The results following repeated noise exposure, as used in the present study, lead us to suggest that the noise-induced increase in the slope of the MLR AIFs and the MLR amplitude enhancement can have distinct mechanisms of generation.

4.3.1. Increased slope of MLR AIFs

A significantly increased MLR AIF slope was found in all exposed animals in the present study independently of MLR amplitude enhancement. The slope increase was detected immediately post-exposure, and the slope value was correlated with the TS. These results resemble the attributes of the psychophysical phenomenon loudness recruitment, which refers to an abnormally rapid rate of loudness increase with increasing stimulus intensity. Kiang et al. (1970) and Evans (1975) suggested that loudness recruitment can be attributed to cochlear pathology. Many data, however, do not support this view because the gross neural output of the cochlea is substantially reduced in damaged ears, suggesting that loudness recruitment depends on neural mechanisms located in the central auditory structures (Wang and Dallos, 1972; Eldredge et al., 1973; Salvi et al., 1979; Heinz et al., 2005). Clinical data have demonstrated that steeper MLR AIFs of auditory evoked potentials were also found in patients with a temporary threshold shift during the acute phase of inner-ear hearing loss (Morita et al., 2007) or under physiological conditions during a masking procedure (Radionova, 2003). The results of the present study confirm the above-mentioned data, because ABR amplitudes were suppressed after noise exposure without changing the slope of ABR AIFs, whereas MLR AIFs were significantly steeper. It seems possible that the central auditory system compensates for the diminished neural input from the periphery resulting from permanent or temporary injury. The central origin of loudness recruitment was also confirmed using PET imaging (Lockwood et al., 1998).

4.3.2. MLR amplitude enhancement – time pattern and the locus of origin

The most surprising and original result of our study is that the first noise exposure produced the same MLR amplitude enhancement as did the second, more intense noise exposure. MLR amplitude enhancement seems to result from a complex system of generation. Enhanced MLR amplitudes were detected almost immediately after the first, lower-intensity noise exposure and recovered to pre-exposure values (at least partially). In this case the MLR amplitude enhancement could be caused by temporary, probably more metabolic-dependent changes in neuronal activity. The MLR amplitude enhancement produced by the second noise exposure did not appear immediately after noise exposure, but usually needed several days to fully develop and reach the same degree of enhancement as seen immediately after the first noise exposure. This situation is demonstrated in Fig. 4, panels B and F, and similar data were found in many other rats (except animal 11F demonstrated in Fig. 4D in which relatively small MLR amplitude enhancement reached maximal value 1 day after the second noise exposure). The delayed MLR amplitude change produced by the second noise exposure may be caused by the evolution of structural changes in the neural networks, such as axonal pruning or trans-synaptic degeneration, as demonstrated previously (Morest and Bohne, 1983; Sie and Rubel, 1992; Bilak et al., 1997; Kim et al., 1997). The data obtained in the present study demonstrate that similar values of noise-induced MLR amplitude enhancement can be seen during a temporary threshold shift as well as after more severe permanent injury.

The locus of the origin of MLR amplitude enhancement can only be hypothesized from existing published data. Increased neuronal activity at individual levels of the auditory system induced by intense sound exposure has been previously reported in several studies. Salvi et al. (1990, 2000) demonstrated that whereas the post-exposure amplitude of the compound action potential (CAP), recorded from the auditory nerve, and the amplitude of evoked responses in the CN were reduced, the IC response amplitude measured after loud tone exposure at higher stimulus intensities increased at an abnormally rapid rate and exceeded the pre-exposure amplitude. Loud-tone exposure was shown to produce an increase in the spike rate of IC neurons with a characteristic frequency below the frequency of the traumatizing tone (Wang et al., 1996). In our previous paper (Popelar et al., 1987), the suppression of the CAP after BBN exposure was also observed, but extremely increased post-exposure IC evoked response amplitudes were never measured even though the IC AIFs were often steeper after noise exposure in comparison with the pre-exposure slopes (Popelar et al., 1987). The differences between the IC results obtained in our studies and the results reported by Salvi et al. (1990, 2000) and Wang et al. (1996) may depend on noise exposure conditions.

As to the AC, it has been reported previously that noise exposure can produce a tonotopic reorganization of the AC accompanied by an increase in neuronal spontaneous activity in those regions with reorganization (Komiya and Eggermont, 2000; Seki and Eggermont, 2003). Increased MLR amplitudes, recorded with implanted electrodes on the surface of the AC, were found in many noise-exposed rats in our previous experiments (Popelar et al., 1987; Syka et al., 1994; Syka and Rybalco, 2000). To identify the source of enhanced MLR amplitudes, in preliminary experiments performed in three rats we recorded local field potentials using chronically implanted electrodes in the AC. The slope of AIFs of the local field potential increased significantly after noise exposure, but the response amplitude did not exceed the pre-exposure values in any of these three animals (unpublished data). These data suggest that MLR amplitude enhancement can be observed using a microelectrode fixed on the surface of the brain, when recording a signal originating from several generators in cortical and subcortical areas. However, when the local field potential is recorded with a microelectrode inserted into a limited cortical region, local field amplitude enhancement does not occur. Thus, the MLR amplitude enhancement can originate in any structure involved in MLR generation. However, because the ABR amplitudes were reduced and the slopes of the ABR AIFs did not change significantly after noise exposure, it is highly probable that the origin of the MLR amplitude enhancement is located in structures above the brainstem. In addition, the data presented above indicate that not just a single generator of MLR amplitude enhancement exists, but that cooperation among several subcortical structures and cortical areas is necessary.

4.4. Mechanisms of MLR amplitude enhancement

It is generally accepted that the enhanced response amplitudes observed after noise exposure might be caused by the loss of lateral inhibition. Many neurons in the central auditory nuclei possess receptive fields with an excitatory core at the unit’s characteristic frequency, activated by discrete frequencies, surrounded by inhibitory sidebands (Evans and Nelson, 1973; Caspary...
et al., 1983; Shofner and Young, 1985; Evans and Zhao, 1993). The noise-induced imbalance between excitatory and inhibitory interactions in a given nucleus could reflect either local changes or changes in a more peripheral auditory structure.

An increase in MLR amplitude as a consequence of the loss of inhibition after cochlear damage could coincide with changes in neurochemical processes, mainly with alterations in gamma-aminobutyric acid (GABA) levels. The optical density of cells immunolabeled for glutamic acid decarboxylase (GAD), the synthesizing enzyme for GABA, measured in the rat IC, increased immediately after noise exposure, but 30 days post-exposure the level of GAD expression was significantly lower than that in unexposed controls (Abbott et al., 1999). Our preliminary experiments have demonstrated increased GAD immunoreactivity in the IC, but decreased GAD immunoreactivity in the AC in rats examined one to three days post-exposure (Ouda, unpublished results). Tan et al. (2007) found significantly elevated immediate early gene c-Fos expression in the IC after noise exposure, but the expression of brain-derived neurotrophic factor, activity-dependent cytoskeletal protein (Arg3.1/arc) and c-Fos in the AC was reduced after noise exposure. These results suggest a reduced synaptic efficacy in the AC in response to acoustic trauma. However, the basis of noise-evoked changes in neurochemical processes are largely unknown.

4.5. Hyperacusis

The presence of loudness recruitment is often connected with hyperacusis, which is a decreased threshold to discomfort from sound. The possibility exists that the noise-evoked increased MLR amplitudes found in the present study can be related to hyperacusis. To our knowledge, only few data are available in the literature on the relationship between increased neural activity and the presence of hyperacusis. Eggermont (2007) speculated that in tinnitus patients with a reorganized auditory cortex in relationship to hearing loss, the large number of neurons with nearly identical tuning properties and the increased spontaneous neural synchrony could be the cause of hyperacusis and recruitment. Gerken et al. (2001) reported that two patients in his cohort had extremely large MLR amplitudes, significantly exceeding the normal hearing group mean. However, these two subjects had only mild, occasional tinnitus, but did not exhibit hyperacusis. On the other hand, Novikova and Rybalko (1982) recorded MLRs in children with sensorineural hearing loss and found large MLR amplitudes that increased with increasing click intensity up to 100 dB SPL. However, further increases in click intensity to 120 dB SPL resulted in decreasing MLR amplitudes and changes in MLR waveforms in many subjects. These children reported hyperacusis during the MLR amplitude decrease. Similar shapes of MLR AIFs (nonmonotonic or plateau types) were observed in the majority of our noise-exposed rats (e.g., 11A and 11C in Fig. 3). However, in animal experiments the presence of hyperacusis is usually hard to detect. It has been speculated previously that exaggerated startle reactions to low-frequency stimuli in aged C57BL/6j mice (Isom et al., 2007) or enhanced CAP amplitudes in guinea pigs after recovery from noise exposure (Sendowski et al., 2004) could be analogous to hyperacusis in humans. However, the data of Gerken et al. (2001) and Novikova and Rybalko (1982) suggest that hyperacusis is not connected with maximal evoked response amplitudes, but more probably with the amplitude decrease seen with further increasing stimulus intensity.

4.6. Interindividual variability in MLR amplitude enhancement

An important and original result of the present study was the finding that the maximum MLR amplitude enhancement in individual animals was dependent on their pre-exposure MLR voltage: the smaller the MLR pre-exposure amplitude, the larger the MLR amplitude enhancement after noise exposure. This relationship can explain the large variability in MLR amplitude enhancement found in noise-exposed rats in our previous experiments (Syka et al., 1994; Popelar et al., 1987; Syka and Rybalko, 2000) as well as in other studies (Salvi et al., 2000; Wang et al., 1996). We did not find any data in the literature explaining interindividual variability in auditory evoked response amplitudes. Because the MLR recording conditions in the present study were constant as much as possible in individual experiments (electrode impedance, electrode position, anesthesia depth), the differences in MLR pre-exposure amplitudes may be related to interindividual differences in the biochemical mechanisms that regulate brain metabolic activity, neurotransmitter release, reuptake and binding to the receptors on post-synaptic membranes in the auditory centers. Since the maximal MLR amplitude enhancements observed after the first (118 dB SPL) and second (122 dB SPL) noise exposures were almost identical, it seems possible that the brain metabolism in individual animals is limited to a certain capacity that cannot be exceeded even under excessive stimulation.

5. Conclusions

Repeated noise exposure as used in the present study enabled us to demonstrate different effects of noise exposure on ABR and MLR amplitudes. Whereas ABR amplitudes measured after noise exposure were reduced in correlation with TS and ABR AIFs were shifted in parallel to higher stimulus intensities while keeping the ABR AIF slope more or less unchanged, MLR changes were much more complex: (i) the MLR AIF slope increased in all exposed animals according to the TS, resembling loudness recruitment, and (ii) maximal MLR amplitudes reached equal values in individual animals after the first and second noise exposures regardless of the TS. In addition, animals with originally small MLR amplitudes usually showed a greater MLR amplitude enhancement following noise exposure than those animals with originally large MLR amplitudes, probably reflecting the different levels of metabolic activity or neurotransmitter processes in individual animals. The noise-induced increased slopes of the MLR AIFs and the MLR amplitude enhancement characterize the specific effect of noise exposure on the central part of the auditory system, whereas ABR amplitude suppression reflects noise-induced changes at the periphery.

Acknowledgements

The study was supported by Grants AV0Z50390512 and 309/07/1336 from the Grant Agency of the Czech Republic, NR 8113-4 from the Internal Grant Agency of the Czech Ministry of Health and LC 554 from the Center of Neuroscience.

References
