

Research Article

Concurrent Acoustic Activation of the Medial Olivocochlear System Modifies the After-Effects of Intense Low-Frequency Sound on the Human Inner Ear

KATHRIN KUGLER,^{1,2,3} LUTZ WIEGREBE,^{1,3} ROBERT GÜRKOV,^{1,2} EIKE KRAUSE,^{1,2} AND MARKUS DREXL^{1,2,3}

¹German Center for Vertigo and Balance Disorders (IFB), Grosshadern Medical Centre, University of Munich, 81377, Munich, Germany

²Department of Otorhinolaryngology, Head and Neck Surgery, Grosshadern Medical Centre, University of Munich, 81377, Munich, Germany

³Division of Neurobiology, Department Biology II, University of Munich, 82152, Martinsried, Germany

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ABSTRACT

>Human hearing is rather insensitive for very low frequencies (i.e. below 100 Hz). Despite this insensitivity, low-frequency sound can cause oscillating changes of cochlear gain in inner ear regions processing even much higher frequencies. These alterations outlast the duration of the low-frequency stimulation by several minutes, for which the term ‘bounce phenomenon’ has been coined. Previously, we have shown that the bounce can be traced by monitoring frequency and level changes of spontaneous otoacoustic emissions (SOAEs) over time. It has been suggested elsewhere that large receptor potentials elicited by low-frequency stimulation produce a net Ca^{2+} influx and associated gain decrease in outer hair cells. The bounce presumably reflects an underdamped, homeostatic readjustment of increased Ca^{2+} concentrations and related gain changes after low-frequency sound offset. Here, we test this hypothesis by activating the medial olivocochlear efferent system during presentation of the bounce-evoking low-frequency (LF) sound. The efferent system is known to modulate outer hair cell Ca^{2+} concentrations and receptor potentials, and therefore, it should modulate the characteristics of the bounce phenomenon. We show that simultaneous

presentation of contralateral broadband noise (100 Hz–8 kHz, 65 and 70 dB SPL, 90 s, activating the efferent system) and ipsilateral low-frequency sound (30 Hz, 120 dB SPL, 90 s, inducing the bounce) affects the characteristics of bouncing SOAEs recorded after low-frequency sound offset. Specifically, the decay time constant of the SOAE level changes is shorter, and the transient SOAE suppression is less pronounced. Moreover, the number of new, transient SOAEs as they are seen during the bounce, are reduced. Taken together, activation of the medial olivocochlear system during induction of the bounce phenomenon with low-frequency sound results in changed characteristics of the bounce phenomenon. Thus, our data provide experimental support for the hypothesis that outer hair cell calcium homeostasis is the source of the bounce phenomenon.

Keywords: cochlea, spontaneous otoacoustic emissions, low-frequency sound, medial olivocochlear system

INTRODUCTION

The human hearing threshold, a psychoacoustic measure and essentially a read-out of inner hair cell (IHC) activity, is poor for very low frequencies (i.e. below 100 Hz). At least three mechanisms attenuate the response of IHCs to low-frequency (LF) sound in

Correspondence to: Markus Drexler · Department of Otorhinolaryngology, Head and Neck Surgery, Grosshadern Medical Centre · University of Munich · 81377, Munich, Germany. Telephone: +49 (0)89 / 2180 74340; email: markus.drexler@med.uni-muenchen.de

humans: First, the transfer characteristics of the middle ear display high-pass filter characteristics (Dallos 1973). Second, the fluid-filled spaces of scala vestibuli and the scala tympani of the inner ear are connected via a small opening, the helicotrema. In order for the organ of corti to detect pressure differences between the two scalae, the impedance of the helicotrema must be high enough to prevent shunting of acoustic energy between the two compartments. The impedance decreases for sounds with decreasing frequencies, resulting in increased shunting and thus decreased sensitivity for LF sound (Cheatham and Dallos 2001). Last, the stereocilia of IHCs, in contrast to outer hair cells (OHCs), are not connected to the overlying tectorial membrane. Therefore, IHC stereocilia are coupled to fluid velocity, not to basilar-membrane displacement. As fluid velocity decreases with decreasing frequency, IHC sensitivity also decreases (Nowotny and Gummer 2006; Guinan 2012). Together, these three mechanisms serve to attenuate LF sound by about 18 dB/octave below 100 Hz (Cheatham and Dallos 2001). While the first two mechanisms reduce the signal level for both IHCs and OHCs, the third mechanism only applies to IHCs. Thus, OHC stereocilia displacement is not reduced at low frequencies as strongly as for the IHCs, which relay sound perception to the auditory pathway. This results in large cochlear potentials even at very low frequencies (Salt et al. 2013).

Despite the poor perception by humans, LF sound has been used extensively to study the properties of OHCs during slow and large movements of the cochlear partition (Scholz et al. 1999; Bian and Scherrer 2007; Hensel et al. 2007; Drexel et al. 2012), but LF sound can also cause alterations of inner ear properties which outlast the duration of the LF stimulation. This has been found more than half a century ago in experiments with human subjects where the hearing threshold was tracked after presentation of intense, LF sound. Hirsh and Ward (1952) found that the time course of hearing thresholds after LF stimulation shows oscillatory, rather than monotonic characteristics. Specifically, the initial hearing threshold depression was often followed by hypersensitivity at about 1 min post-exposure before the thresholds returned to baseline values. The term 'bounce' phenomenon was coined for this hypersensitivity. Later, Patuzzi and Wareing (2002) showed that after presentation of an intense LF sound, human subjects experienced a tinnitus-like sensation during a period comparable to the changes of the hearing threshold (Drexel et al. 2014). The detection of the bounce phenomenon is not limited to perceptual measures; it can also be

seen in measures of cochlear physiology, such as cochlear microphonics (Kirk et al. 1997; Kirk and Patuzzi 1997), changes of the endolymphatic potential (EP, Kirk and Patuzzi 1997), evoked (Kemp 1986; Kirk et al. 1997; Kirk and Patuzzi 1997; Kevanishvili et al. 2006; Kemp and Brill 2009; Drexel et al. 2014) and spontaneous otoacoustic emissions (Kemp 1986; Kugler et al. 2014). Since the phenomenon is not affected by blocking the auditory nerve activity (Kirk and Patuzzi 1997), it is generally believed to be of cochlear, rather than neural, origin. Patuzzi (2011) suggested that the bounce phenomenon is a direct result of OHC activation by LF sound, rather than a secondary effect caused by modulation of stria vascularis activity, as the LF sound-induced EP modulation during the bounce (Kirk and Patuzzi 1997) could suggest. Patuzzi (2011) hypothesised that large, LF-induced receptor potentials in OHCs are the underlying cause of cochlear sensitivity oscillations associated with the bounce phenomenon. Note that the bounce phenomenon is usually explored with probe tones at much higher frequencies than the LF sound, indicating that OHCs at virtually all tonotopic locations are stimulated by intense LF sound and generate LF receptor potentials. These LF OHC receptor potentials are not subject to filtering due to the electrical properties of the OHC membrane. Patuzzi (2011) postulated that large, LF receptor potentials activate voltage-sensitive, L-type Ca^{2+} channels in the OHC membrane, finally resulting in an increase of intracellular Ca^{2+} in OHCs. Ca^{2+} -induced Ca^{2+} release and uptake mechanisms, with different time courses, can then cause slow, underdamped oscillations of OHC cytosolic Ca^{2+} concentrations, modulating the gain of the cochlear amplifier (Patuzzi 2011).

An experimental modulation of OHC receptor potentials or Ca^{2+} concentrations during the presentation of LF sound would be suitable to test this hypothesis. Fortunately, a non-invasive option for modulating the OHC receptor potential or Ca^{2+} concentrations is available via acoustic activation of the medial olivocochlear efferent (MOC) system. An activation of the MOC system during the presentation of LF sound should thus diminish the manifestations of the bounce phenomenon, here assessed with spontaneous otoacoustic emission (SOAE) recordings after LF sound (and simultaneous efferent stimulation) cessation, and will serve to test the hypothesis that OHC Ca^{2+} oscillations are the trigger for the bounce phenomenon (O'Beirne and Patuzzi 2007; Patuzzi 2011). If this hypothesis is valid, the bounce phenomenon should be affected by contralateral acoustic stimulation (CAS) delivered simultaneously with the bounce-evoking LF sound.

METHODS

Signal Generation and Data Acquisition

The experimental setup used in this study for ipsilateral stimulation and SOAE recording was identical to a setup used in a previous study (Kugler et al. 2014). An ER-10C DPOAE probe system (Etymotic Research Inc., Elk Grove Village, IL, USA) was used for SOAE recordings. The LF tone to induce the bounce was supplied by a separate loudspeaker (NSW1-205-8A, Aura Sound Inc., Santa Fe Springs, CA, USA). This loudspeaker was connected to a 50-cm-long polyethylene tube (inner diameter 1 mm), the tip of which was fed through the foam ear tip of the ER-10C DPOAE probe so that it faced the tympanic membrane. The loudspeaker was driven by a RB-960BX power amplifier (Rotel, Worthing, UK). Stimulation to the contralateral ear was provided by an earphone (Etymotic Research ER-4PT, Etymotic Research, Elk Grove Village, IL, USA), which was sealed into the contralateral ear canal with dedicated foam ear tips. The earphone was driven by the headphone amplifier of the external sound card.

Signal generation and data acquisition was carried out with a RME Fireface UC 24-bit external sound card (RME, Audio AG, Haimhausen, Germany), operated at a sampling rate of 44.1 kHz. The LF tone to induce the bounce was a 30 Hz sine wave presented at 120 dB SPL for 90 s, including 0.1 s raised-cosine ramps. The CAS consisted of a 90 s, bandpass-filtered Gaussian noise (100–8 kHz) presented at 65 or 70 dB SPL to the ear contralateral to the ear emitting the SOAE of interest. The microphone signal of the ER-10C probe was amplified 30 dB by the preamplifier of the external soundcard. Scripts written in MatLab 7.5 (MathWorks, Natick, MA, USA) and run on an ASUS G60 VX laptop (ASUSTeK Computer Inc., Taipei, Taiwan) controlled the external sound card. The SoundMexPro application (HörTech, Oldenburg, Germany) was employed for synchronised stimulation and recording in the MatLab environment. The stimulation pathway was checked in an artificial ear (B&K 4157, Brüel & Kjær Sound & Vibration Measurement A/S, Denmark). No artefacts exceeding the noise floor of the system could be detected. The CAS was also calibrated on the same artificial ear for constant sound pressure level across the frequency range used.

Procedure

The experimental paradigm consisted of an initial control recording followed by a sequence of four experimental recordings. As each experimental recording lasted more than 8 min, during which the

subjects had to remain absolutely still, breaks were often made during sessions.

A control recording lasted for 120 s and tracked the time course of identified SOAE on the ipsilateral ear without any acoustic stimulation. Four different experimental recordings were used, each lasting 510 s in total (eight and a half minutes). The sequences of ipsilateral and contralateral acoustic stimulations in these experimental recordings are illustrated in Figure 1: Specifically, in the reference recording, the bounce was induced by the LF tone which lasted for 90 s. SOAEs were recorded subsequently for 420 s. In the LF + CAS 65 recording, the ipsilateral procedure was identical but a 65 dB SPL CAS was presented simultaneously with the ipsilateral LF tone. Likewise, in the LF + CAS 70 recording, the CAS was presented at 70 dB SPL. Finally, in the CAS 70 control recording, only the CAS at 70 dB SPL and no ipsilateral LF tone was presented before the SOAE recording period.

Each recording was preceded and concluded by a probe-fit-check procedure: Band-stop noise consisting of a low- and a high-frequency band was presented via one of the speakers in the ER10C probe and recorded via the ER10C microphone. The spectrum of the recorded signal was checked in terms of the sound level of the low-frequency and the high-frequency noise bands: A drop in the low-frequency noise-band level would indicate a loss of the seal of the probe in the meatus; a drop in the high-frequency noise-band level would indicate blocking of the microphone tube. If probe-fit-checks after the experimental recording indicated one of these, the experimental recording was rejected and repeated after probe re-positioning.

Analysis

The ipsilaterally recorded time domain signal was divided into consecutive segments of 5-s duration. The magnitude spectrum in each segment was calculated with MatLab's *pwelch* function, implemented with a 0.5-s Hann window, 25 % overlap and a zero-padded FFT size of 1 s, resulting in a frequency bin width of 1 Hz.

SOAE candidates were automatically detected in the magnitude spectrum by finding local maxima (minimum level 15 dB SPL, minimal spacing between two maxima 10 Hz) within a frequency range between 0.5 and 5 kHz. This procedure was repeated for all 5-s segments of the recording, thereby building a list of SOAE candidates that could appear at any point in time. SOAEs in each segment were classified as valid when the power of the SOAE differed significantly from the power of the noise floor (F test, critical value 5.39, see Dobie and Wilson (1996)). The noise power was calculated as the averaged power in two, 8-Hz wide frequency bands located 10 Hz above and below

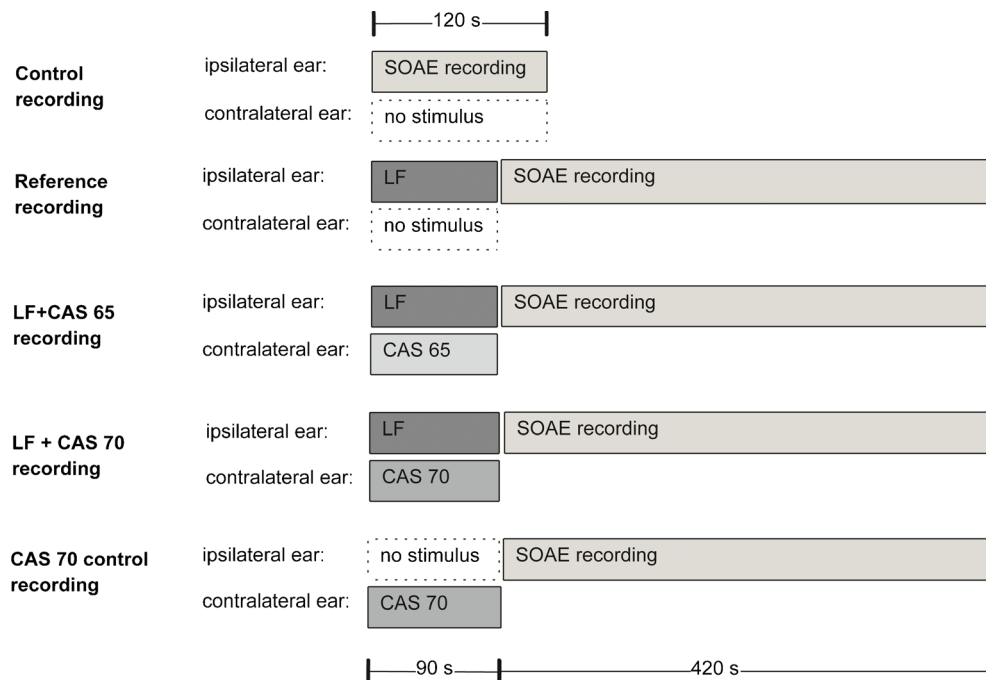


FIG. 1. Schematic of the experimental paradigm. Five different recordings were used in the present study. The ear where SOAEs were recorded, and LF sound was delivered, is referred to as the ipsilateral ear. Consequently, CAS was delivered to the contralateral ear.

the SOAE candidate. Frequency and level of SOAEs satisfying our signal-to-noise criterion (as determined in the F test) were extracted over the entire length of the recording for consecutive segments with a temporal resolution of 5 s. This analysis resulted in a time series for SOAE frequency and level (as determined by the frequency bin containing the highest power within a ± 30 Hz frequency band around the candidate frequency). For an estimate of the SOAE change duration, the time course of the level of permanent SOAEs was fitted with an underdamped, sinusoidal oscillation. Parameters fitted were carrier frequency, carrier magnitude, carrier phase and envelope decay time constant. The duration of transiently occurring SOAEs was determined by the first and last valid data point, as the duration was not sufficient for fitting.

Statistical procedures were carried out in the MatLab environment. Please note that P values were not corrected for multiple comparisons.

Subjects

Nineteen subjects (17 female, 2 male) were screened for SOAEs. SOAEs could be detected in at least one ear of 13 subjects (all female, aged between 19 and 28 years, mean age 23 years). Data from one ear of each subject (five left ears, eight right ears) contributed to the data set. Ears were chosen pseudo-

randomly, and only if no valid SOAEs could be detected in the initially tested ear, the other ear was also screened for SOAEs. Experiments were conducted in a double-walled, sound-attenuated booth, the subjects were seated in a comfortable recliner and were advised to remain still and quiet during the experiment.

All subjects underwent hearing screening (Békésy tracking, 250 Hz–8 kHz) before the SOAE screening. Hearing thresholds in all subjects were normal and did not exceed 20 dB HL at all tested frequencies. Experiments were conducted in a double-walled, sound-attenuated booth; the subjects were seated in a comfortable recliner and were advised to remain still and quiet during each recording.

The ethics committee of the University Hospital of the Ludwig-Maximilians University Munich, Germany, in agreement with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans, approved the procedures (approval number 186–14), and all subjects gave their written informed consent. This included a statement that we cannot exclude potential short- and long-term harm to the inner ear caused by the sound levels used in this study. We also stated that we considered the risk not greater than the one caused by the sound level one is routinely exposed to in daily life (e.g. with personal sound systems). The subjects were advised to immediately remove the ear probe

from the ear canal in case of any unpleasant sensations. All tested subjects tolerated the LF tone and rated it as intense, but not unpleasant. The sound level of the 30-Hz LF tone was 120 dB SPL, corresponding to an A-weighted level of 80 dB. In a piloting experiment with two additional female subjects, a series of LF sounds was presented at sound levels randomly chosen between 99 and 120 dB SPL in 3 dB increments. The accumulated daily LF sound exposure was monitored and controlled to be within the exposure limits for normal working conditions as regulated by the European Commission Noise at Work Directive 2003/10/EC.

RESULTS

Effect of LF Stimulation

As shown before (Burns 2009; Kugler et al. 2014), SOAEs recorded in quiet (as in the control recording in this study) were very stable over time. Specifically, SOAEs varied on average by no more than 0.8 dB in amplitude and by no more than 1 Hz. The spectrogram of an SOAE in a control recording is shown in Figure 2A (note the strongly magnified frequency axis). The same SOAE, recorded after the 90 s LF sound exposure (reference recording) is shown in Figure 2B. Here, the spectrogram reveals a pronounced biphasic bouncing of both SOAE frequency and sound level. The characteristics of the bouncing are very similar to those described in Kugler et al. (2014) and Kemp (1986). In total, 24 of the 26 permanent SOAEs measured in the control recording

(ranging from one to five per ear) could be retrieved in the reference recording. The remaining two SOAEs did not satisfy our signal-to-noise criterion (see “Methods”) in the reference recording. Changes of SOAE level were computed relative to the median values of SOAE level and frequency in the corresponding control recording and are stated in the following as median (first quartile, three quartile). In the reference recordings, SOAE levels slowly oscillated after LF sound offset with a decay time constant of 124 s (83, 162 s) and reached a maximum at 45 s after LF sound offset (24, 60 s) with +3.7 dB (+3.3, +6.5 dB) followed by a minimum at 165 s after LF sound offset (139, 226 s) with -3.2 dB (-5.2, -1.9 dB).

As also shown in Kugler et al. (2014) and Kemp (1986), the LF tone can induce the generation of new, transient SOAEs which are not detectable in recordings without LF sound exposure (cf. Fig. 2C). This exemplary SOAE rose above the measurement noise floor for only about 90 s shortly after the LF tone offset.

As in Kugler et al. (2014), significant bouncing of permanent SOAEs was evaluated with a combination of a change detection test and the goodness-of-fit of an underdamped oscillation fitted to the data. According to these criteria, 21 of the 24 SOAEs (88 %) bounced in response to the LF tone (cf. Fig. 3A).

In the reference recordings, we could record 26 transient SOAEs in 10 ears. Twenty of these SOAEs could be classified as transient and bouncing (cf. Fig. 3B), appearing at 10 s (5, 28 s) after LF sound offset, rose to a maximum level of +11.5 dB re. noise floor (+9.7, +13.6 dB) at 45 s (38, 55 s) after LF sound offset and lasted for 60 s (55, 85 s).

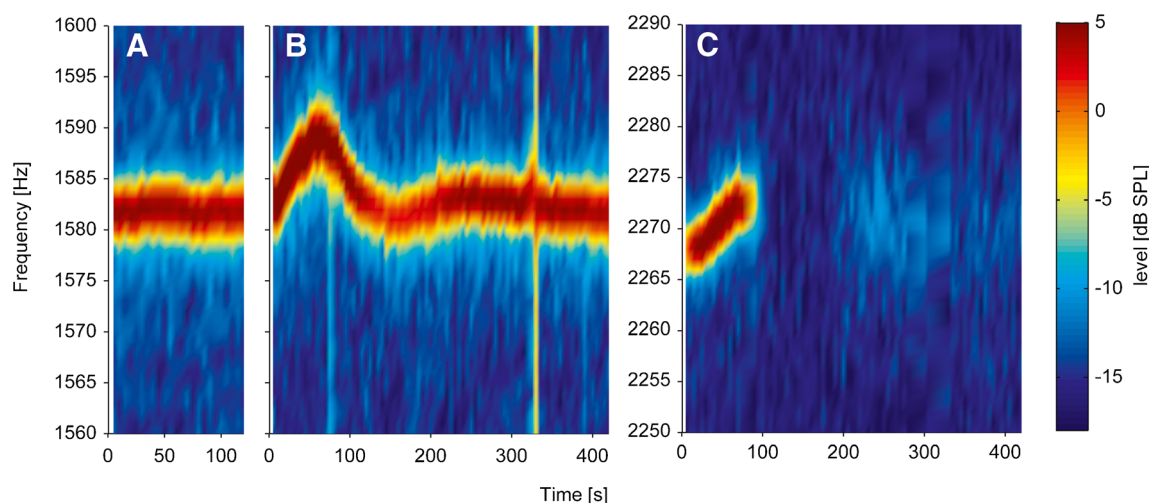


FIG. 2. Representative example of SOAEs in the control condition without preceding LF sound exposure (A) and of the same permanent SOAE (B) and a new SOAE (C) after 90-s presentation of LF sound in the reference condition. Please note that SOAEs in the control

condition (A) are very stable in both level and frequency, whereas after LF exposure, permanent and new SOAEs (B, C) undergo slow changes of level and frequency.

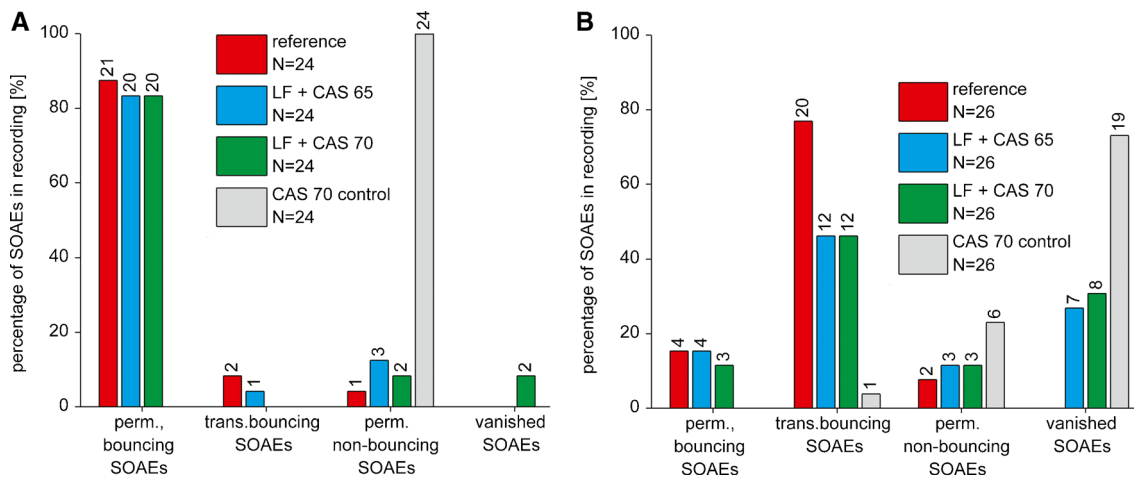


FIG. 3. Distribution of SOAE classes in the four recordings, **A** SOAEs present in control recordings. No significant differences were found in the distribution of cases in the LF + CAS 65 and LF + CAS 70 recordings relative to the reference recording (Fisher's exact test, $P=0.69$ and $P=0.36$, respectively). **B** New SOAEs not present in control recordings. Significant differences were found in the

distribution of cases in the LF + CAS 65 and LF + CAS 70 recordings relative to the reference recording (Fisher's exact test, $P=0.015$ and $P=0.0081$, respectively). The total number of SOAEs in the four recordings, respectively, is given in the legend; the absolute number of SOAEs is given at the top of the corresponding bin.

The remaining six SOAEs were classified as transient SOAEs in the control recording because they just failed to reach significance. In the reference recording, they were enhanced by the LF tone but did not diminish after the LF tone offset beneath the noise floor within the 420 s of data acquisition (Fig. 3B).

Effect of Contralateral Acoustic Stimulation

CAS-Induced Changes of Permanent and Bouncing SOAEs. CAS is well known to suppress ipsilaterally recorded SOAEs during presentation and SOAEs quickly recover after CAS offset within less than 1 s (Zhao and Dhar 2010). Here, however, we examine SOAEs within long time intervals well after CAS offset. The CAS control recordings (with CAS, but without LF tone) were performed with CAS 70 dB SPL. Analyses of these recordings show that the CAS never affected the ipsilaterally recorded SOAEs after the CAS offset (cf. Fig. 4, grey lines). Due to the duration of our analysis segments (5 s), the SOAEs already fully recovered from the CAS exposure within the first analysis segment. Consequently, we found no SOAEs fulfilling our criteria for the bounce or indeed any other significant changes in the CAS control recordings, and all SOAEs in this recording are therefore classified as non-bouncing (Fig. 3A).

When the CAS was presented simultaneously with the ipsilateral LF tone, however, bouncing of permanent SOAEs changed significantly. Exemplary time courses of SOAE level changes are shown in Figure 4 (left column). While in the reference recording (red),

the SOAE showed a pronounced biphasic bounce, presentation of a 65 dB SPL (cyan) or 70 dB SPL (green) CAS together with the ipsilateral LF tone clearly affected the magnitude and phase of the bounce. Specifically, we found that with 70 dB SPL CAS, the subsequent SOAE level reduction was significantly smaller than without CAS (Wilcoxon signed rank test, $P=0.007$, Fig. 5A, right). With a 65 dB CAS, a trend in the same direction was observed ($P=0.085$, Fig. 5A, left). The SOAE level increases remained fairly unchanged (Wilcoxon signed rank test, $P=0.59$ and 0.21 , respectively, Fig. 5B). This resulted in a less symmetrical shape of the SOAE bounce compared to the reference recording. Consequently, the decay time constant of the fitted function shortened at both presented CAS levels (Fig. 5C, Wilcoxon signed rank test; LF + CAS 65 dB SPL: $P=0.016$ and LF + CAS 70 dB SPL, $P=0.036$). The carrier phase of the fitted function also decreased significantly for a CAS level of 70 dB SPL (Wilcoxon signed rank test, $P=0.014$, Fig. 5D, right). Again, this effect was not significant anymore when the CAS level was reduced to 65 dB SPL ($P=0.11$, Fig. 5D, left). However, the presentation of CAS was not sufficient to completely suppress the bouncing of SOAEs, and consequently, almost all SOAEs could still be classified as bouncing in the LF + CAS 65 dB SPL and LF + CAS 70 dB SPL conditions (Fig. 3A).

Overall, the CAS showed an asymmetric effect on permanent, bouncing SOAEs, especially with respect to the changes in SOAE level.

To explore how the bounce is affected by increasing LF tone level and whether variations of this

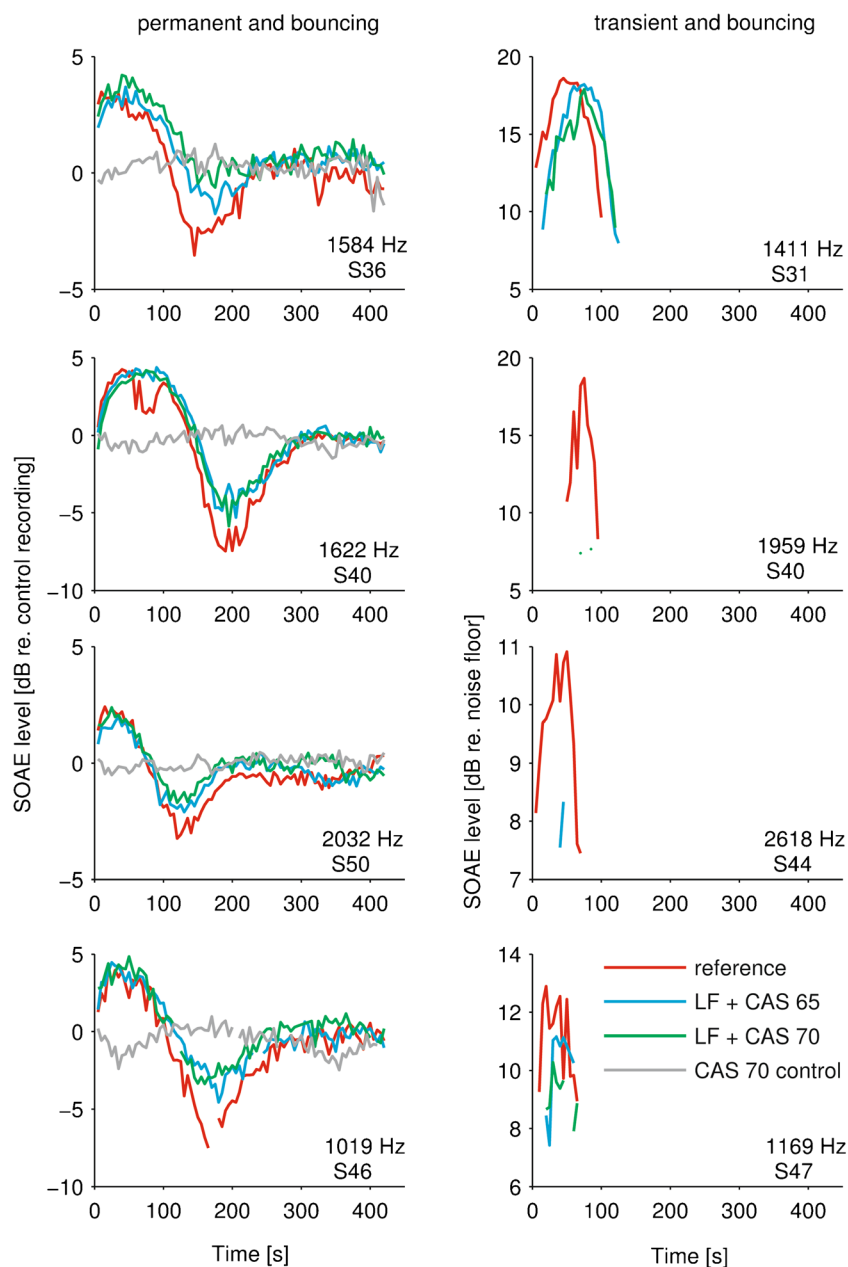


FIG. 4. Representative examples of permanent and bouncing SOAE (left column) and transient and bouncing SOAEs (right column) in the four experimental conditions (see legend) from seven subjects. Please note that the time course of LF-induced level changes of permanent SOAEs show a less pronounced minimum in LF + CAS 65

and LF + CAS 70 recordings. Transient SOAEs are suppressed and their onset often delayed in the LF + CAS 65 and LF + CAS 70 recordings.

parameter result in an equally asymmetric bounce manipulation, we obtained bounce parameters as a function of LF tone level, but without CAS. Specifically, the bounce was measured from 13 permanent SOAEs of two additional female subjects (aged 19 and 21). Figure 6A shows a typical example of level changes of a permanent, bouncing SOAE after LF tone offset for different LF sound levels. Decreasing the LF sound level clearly decreases the magnitude of both bounce minima and

maxima to a comparable extent, as summarised in Figure 6B for all 13 SOAEs. Thus, decreasing the LF tone sound level had different effects on SOAE bouncing than CAS.

CAS-Induced Changes of Transient SOAEs

Similar to the permanent SOAEs, several parameters of transient SOAEs generated by the LF tone were analysed. In the following, changes are expressed

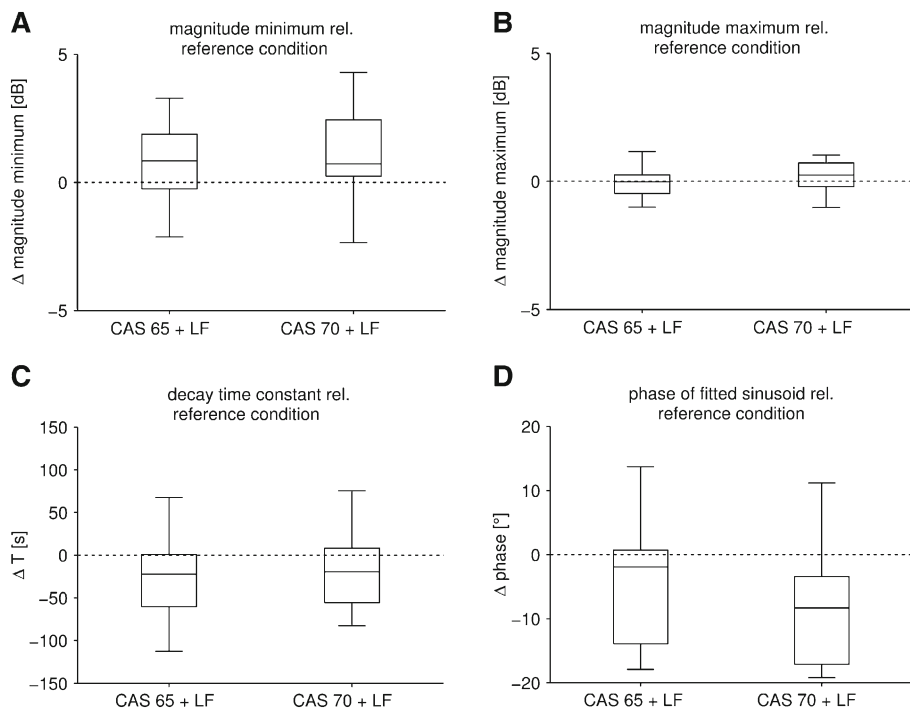


FIG. 5. Characteristics of the time course of permanent and bouncing SOAEs in the LF + CAS 65 ($N=20$) and LF + CAS 70 ($N=20$) relative to the reference condition. **A** Changes of magnitude minima, **B** changes of magnitude maxima, **C** changes of decay time constants and **D** changes of the phase of the underdamped sinusoid

fitted to the level oscillations. *Box plot borders* indicate 25th, 50th and 75th percentiles; *whiskers* indicate the most extreme values not considered as outliers.

relative to the reference recording without CAS. Exemplary cases of transient SOAEs in the various recordings are shown in Figure 4 (right column). Overall, the CAS suppressed the generation of transient SOAEs by the LF tone: Specifically, we observed 20 instances where we could induce a significant, transient SOAE with the LF tone alone (reference recording, Fig. 3B), but only 12 of these SOAEs could be induced with the LF tone paired with CAS (at both 65 dB SPL and 70 dB SPL, Fig. 3B).

Transient and bouncing SOAEs cannot be fitted with a damped sinusoid, because they are present only during a short period and they do not show sinusoidal oscillations. Thus, other parameters were used to describe their behaviour. The main effect of CAS on transient SOAEs were a later onset and smaller level maxima of the transient SOAEs after LF tone offset. Specifically, transient SOAEs tended to start about 10 s later when the LF tone was paired with 70 dB SPL CAS (Wilcoxon signed rank test, $P=0.063$, Fig. 7A,

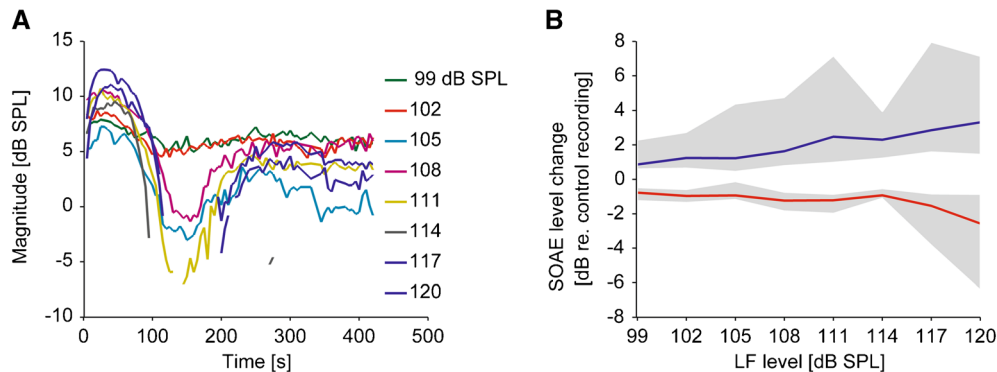


FIG. 6. Level changes of the bouncing SOAEs recorded after LF sound offset at various intensities. **A** Example of the time course of a permanent, bouncing SOAE after presentation of LF sound at various intensities (as indicated in the legend). **B** Bouncing permanent SOAE minima (red, median) and maxima (blue) relative to the correspond-

ing control recording from 13 SOAEs recorded from two ears. The lower and upper boundaries of the *grey shading* indicate the first and third quartiles. Note that minima and maxima of bouncing SOAE grow almost symmetrically with increasing LF sound level.

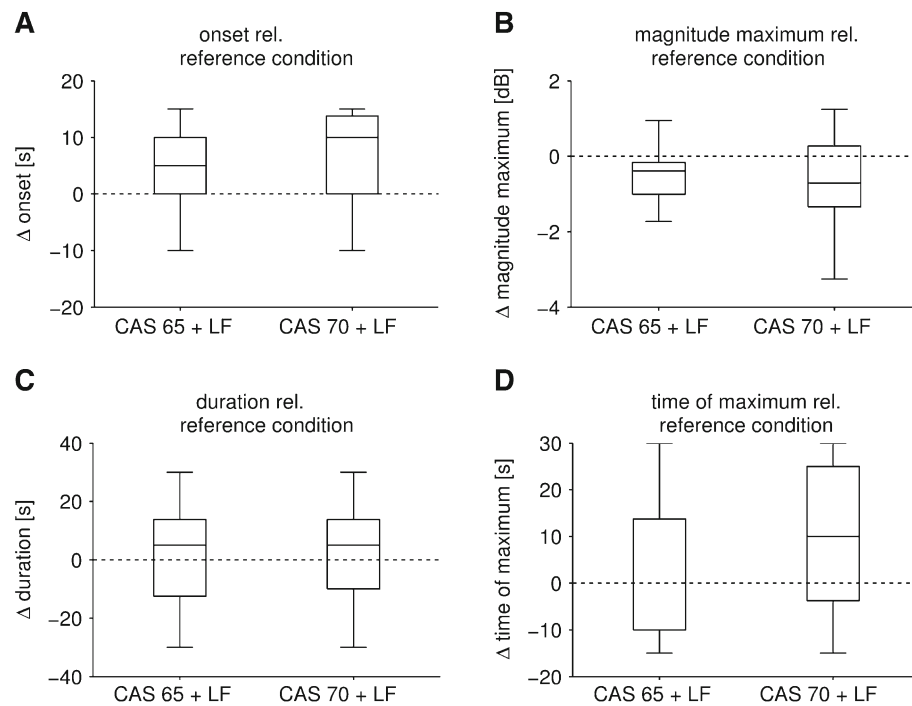


FIG. 7. Characteristics of the time course of transient, bouncing SOAEs in the LF + CAS 65 ($N=12$) and LF + CAS 70 ($N=12$) relative to the characteristics of the same SOAEs in the reference condition. **A** Changes of transient SOAE onset, **B** changes of magnitude maximum, **C** changes of SOAE duration and **D** changes of the timing of the SOAE maximum; box plot boundaries as in Figure 5.

right). This latency increase was not significant with 65 dB SPL CAS ($P=0.12$, Fig. 7A, left). Second, level maxima of transient SOAEs decreased when the LF exposure was paired with 65 dB SPL CAS (Wilcoxon signed rank test; $P=0.032$, Fig. 7B, left) while the duration of transient and bouncing SOAEs (Fig. 7C) did not differ significantly (Wilcoxon signed rank test, LF + CAS 65 dB SPL: $P=0.66$; LF + CAS 70 dB SPL: $P=0.68$). This level decrease was, however, not significant with 70 dB SPL CAS ($P=0.12$, Fig. 7B, right). Finally, we observed a significant shift in the latency of the transient SOAE level maximum when the LF exposure was paired with 70 dB SPL CAS ($P=0.043$; Fig. 7D, right).

DISCUSSION

In this study, we have used CAS during LF sound presentation to modulate the characteristics of the bounce phenomenon. In the following, we will discuss what these findings may contribute to our understanding of the bounce.

We have shown that CAS, delivered simultaneously with the bounce-inducing LF sound, leads to consistent changes of bounce phenomenon characteristics. As the initial hypothesis, we have assumed that the starting point for the bounce phenomenon are OHC

Ca^{2+} oscillations induced by the LF sound (O'Beirne and Patuzzi 2007; Patuzzi 2011) even though other explanations for the bounce phenomenon are feasible: LF sound has been shown to also induce endolymphatic hydrops (Salt 2004), a volume increase in the endolymph-containing scala media, which could contribute to changes of cochlear gain and associated phenomena such as otoacoustic emissions during the bounce. However, the buildup and decay takes more than 10 min and does not oscillate (Salt 2004), which makes endolymphatic hydrops unlikely to be the dominating mechanism for the bounce phenomenon. Changes of the endolymphatic potential can also affect the gain of the cochlear amplifier and have been observed during presentation of LF sound (Salt 2004). Since a direct effect of LF sound on stria vascularis activity seems unlikely, the observed endolymphatic potential changes are probably a consequence of LF sound-induced OHC mechano-electrical transducer operation point changes, probably mediated by Ca^{2+} -controlled slow motility.

As the LF stimuli used in this study are intense, mechanisms associated with acoustic overexposure and recovery thereof could also be responsible for the bounce phenomenon. Temporary or permanent damage to OHCs represent an unlikely cause, as recovery from acoustic overexposure with sounds in the sensitive range of hearing is typically much slower, monotonic, does not oscillate and no hypersensitivity

can be seen (Le Prell et al. 2012). It has been shown, however, that intense sound stimulation of the cochlea in the isolated temporal bone preparation increases intracellular Ca^{2+} level of hair and supporting cells (Fridberger et al. 1998; Jacob et al. 2013). Acoustic injury consists of a plethora of structural and metabolic changes to the cochlea, with structural damage possibly masking more subtle (and possibly oscillating) metabolic changes of cochlear sensitivity caused by the rise of intracellular Ca^{2+} levels.

Early work has shown that intense LF sound may not only stimulate the cochlea but also vibrotactile receptors, e.g. on the skin surrounding the ear (Boothroyd and Cawkwell 1970). Given the current mechanical detachment of the LF device from the subjects' skin and the clearly cochlear generation of the measured responses, it appears highly unlikely that the observed SOAE changes and effects thereupon by CAS are mediated via vibrotactile stimulation.

One of the hitherto unanswered key questions regarding the bounce phenomenon is why induction is most efficient at low frequencies (Kemp 1986; Kemp and Brill 2009). Patuzzi (2011) identified as a distinguishing element that OHC receptor potentials induced with LF sound are attenuated to a considerably lesser extent by the OHC membrane time constant than receptor potentials induced with medium- or high-frequency sound. However, recent data showed that this distinguishing feature might have a less prominent effect than previously thought, as resting OHCs, at least in the apical turn of the cochlea, might be more depolarised than it has been estimated in earlier studies (Johnson et al. 2011). As a result, K^+ conductances in the basolateral aspect of OHCs are larger and thus RC time constants shorter, resulting in higher cut-off frequencies of the OHC membrane. It is unlikely, however, even with revised values for OHC resting potentials, that OHC receptor potentials, evoked with equal-level stimuli, but very different frequencies, are identical. As suggested by O'Beirne and Patuzzi (2007) and Patuzzi (2011), only large OHC receptor potentials (which can presumably only be evoked with LF stimulation) will suffice to produce a net Ca^{2+} increase in OHCs. Subsequent Ca^{2+} -induced Ca^{2+} release and uptake mechanisms with different time courses could then lead to slow Ca^{2+} oscillations and the resulting slow changes of cochlear activity in the bounce.

Activation of the MOC system can modulate OHC Ca^{2+} concentrations and, as a result, receptor potentials. Therefore, MOC system activation could affect characteristics of the bounce if the bounce is mediated by Ca^{2+} oscillations. The MOC system innervates both the ipsilateral and the contralateral ear and exclusively contacts OHCs in most adult mammals

(Warr et al. 1986). The MOC system can be activated by sound and causes a binaural reduction of the cochlear amplifier gain (for review, see Guinan 2006, 2010). This is presumably the result of a shunting effect on OHC receptor currents and a consequent reduction of the receptor potential (Fex 1967; Rabbitt et al. 2009; Guinan 2010; Rabbitt and Brownell 2011). The MOC system innervates OHCs via cholinergic synapses. The release of acetylcholine from MOC terminals causes Ca^{2+} influx into OHCs. The increased Ca^{2+} levels (augmented by Ca^{2+} -induced Ca^{2+} release from internal Ca^{2+} stores, the synaptic cisternae) open Ca^{2+} -controlled SK channels, resulting in K^+ efflux and hyperpolarization. This has been termed the fast efferent effect (Sridhar et al. 1997), which can also undergo enhancement during prolonged stimulation periods (Larsen and Liberman 2009). During prolonged stimulation, as in this study, Ca^{2+} from additional stores (the lateral cisternae) will be released, causing a reduction in OHC axial stiffness and related gain changes. This has been termed the slow efferent effect (Sridhar et al. 1997). It is important to note that the activity of the MOC system does not seem to affect SOAEs after CAS offset, as seen in our CAS control recordings. This is because the fast efferent effect decays very shortly after CAS offset (Zhao and Dhar 2010) and is therefore unlikely to influence SOAEs over an observation interval of several minutes. The slow efferent effect, however, persists over tens of seconds and could therefore influence SOAEs after CAS offset. The findings of Zhao and Dhar (2011) and our results, however, also show that the slow efferent effect affects human SOAEs only to a very small extent. Therefore, we hypothesise that the MOC system activity in our experiments affects exclusively the OHC response to the LF sound during presentation and that the changes of the SOAE bounce we observed are a consequence of this and not of a direct action of the slow efferent effect on SOAEs. In the following, we will consider how CAS might modulate OHC response to LF sound and thus the SOAE bounce.

At low frequencies, the electrical impedance of OHCs is dominated by resistance, not capacitance. Opening of SK channels, induced by activity of the MOC system, should result in decreased resistance and consequently increased shunting of LF-induced receptor potentials. The consequence would be a reduced drive for the bounce phenomenon, which should result in changes of the bounce phenomenon similar to a reduction of LF sound levels, which is not what we observed. Rather, we saw a shortening of the decay time constant and an asymmetry in the time course of permanent and bouncing SOAEs and a reduced number of transient and bouncing SOAEs, which is not in line with a simple reduction of OHC

receptor potentials. The alternating hypersensitivity and hyposensitivity during the bounce has been related to an undershoot and overshoot, respectively, of OHC Ca^{2+} levels following LF sound offset (Patuzzi 2011). The increased damping (corresponding to decreased decay time constants) we observed manifested in a less pronounced SOAE level minimum, or hyposensitivity, while the maximum was almost unchanged. The maxima and minima of bouncing SOAEs can be related to modulation of cochlear amplifier gain and non-linear saturation during the gain-enhanced phase might represent a reason for the asymmetric effect of MOC system activation on bouncing SOAE minima and maxima. Alternatively, it might be an indication that processes re-adjusting the Ca^{2+} overshoot may accelerate due to MOC system activation. Even while the LF sound is on, the LF-induced Ca^{2+} concentration changes presumably also undergo oscillations (Patuzzi 2011). In contrast, the slow efferent effect can cause a constant and additional Ca^{2+} release from OHC internal stores, the lateral cisternae, while CAS is on. Since the speed of Ca^{2+} sequestration into Ca^{2+} stores is Ca^{2+} -controlled, we hypothesise that this constant and additional release of Ca^{2+} may help to accelerate Ca^{2+} -dependent Ca^{2+} -uptake and thus cause a quicker recovery of the system.

CAS, as opposed to additional ipsilateral stimulation, is usually the method of choice for non-invasive activation of the MOC system. This is because there is no risk that the moderate-level CAS, if low enough and thus no acoustic cross talk is to be expected, causes intracochlear suppression in the ipsilateral ear. Unfortunately, CAS not only activates the efferent system, but also, at higher sound levels, the acoustic middle ear reflex in both ears. The middle ear reflex in humans is mainly driven by activity of the stapedius muscle (reviewed in Møller 1984) which changes the middle ear impedance and, as a consequence, attenuates the sound levels reaching the inner ear. Thus, selective contributions of the MOC system and the middle ear reflex can be difficult to differentiate (Relkin et al. 2005). In our study, LF sound can be considered the probe tone, responses to which might be subject to attenuation by CAS-induced activity of either the MOC system or the middle ear reflex. Even though there is a theoretical possibility that the observed effects of CAS can also be caused by the middle ear reflex, it is unlikely that the middle ear reflex dominates our results for the following reasons: First, the highest CAS level employed in this study (70 dB SPL) is at or below typical human reflex thresholds induced with broad-band CAS reported in the literature, obtained with experimental, rather than clinical

measures (reviewed in Wilson et al. 1984). Note, however, that reports of individuals with lower reflex thresholds can also be found in the literature (Guinan 2006; Zhao and Dhar 2010). Second, we used long-duration, continuous CAS. During long-duration CAS, the middle ear reflex shows adaptation, which significantly reduces reflex-mediated probe attenuation. In the adapted state, the impedance change caused by broad-band CAS is reduced by up to 40 % at 30 s after CAS onset (reviewed in Wilson et al. 1984). Third, the attenuating effect of the middle ear reflex is stronger in the ear where the reflex-eliciting sound was presented (i.e. where CAS was presented, Møller 1984) than in the ipsilateral ear, where LF sound was delivered in this study. Fourth, there are indications that high-level probe tones (as the LF sound in this study) are less affected by CAS than probe tones at moderate sound levels (Day and Feeney 2008). Finally, the data from our pilot experiment showed that simply decreasing the level of the bounce-inducing LF sound led to an almost symmetrical reduction of the magnitude of both SOAE minima and maxima. This is in contrast to what we observed in our experiments with LF sound and CAS, where only SOAE minima were altered, and SOAE maxima remained almost unchanged. The changed characteristics of the bounce phenomenon observed in this study can thus not be explained by a simple reduction of the acoustic input to the inner ear, as it would be mediated by activity of the middle ear reflex. Taken together, the middle ear reflex is unlikely to dominate the effects we observed.

CONCLUSION

In our experiments, we have shown that CAS systematically affected the bounce, elicited by an ipsilateral LF tone: CAS led to a reduced number of transient SOAEs, a reduced decay time constant of level oscillations of permanent SOAEs, reduced level changes and a phase shift of the level changes of both transient and permanent SOAEs. We conclude that a modulation of Ca^{2+} oscillations by the MOC system activity during LF exposure is the underlying cause of the changes in bounce phenomenon characteristics we observed. This is in agreement with the elaborate model of the bounce phenomenon as suggested by O'Beirne and Patuzzi (2007) and indicates that the bounce phenomenon can be interpreted as the result of homeostatic processes re-adjusting the Ca^{2+} level after intense LF stimulation.

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