Twin study of neonatal transient-evoked otoacoustic emissions

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Our knowledge of which physiological mechanisms shape transient evoked otoacoustic emissions (TEOAEs) is incomplete, although thousands of TEOAEs are recorded each day as part of universal newborn hearing-screening (UNHS). TEOAE heritability may explain some of the large TEOAE variability observed in neonates, and give insights into the TEOAE generators and modulators, and why TEOAEs are generally larger in females and right ears.

The aim was to estimate TEOAE heritability and describe ear and sex effects in a consecutive subset of all twins that passed UNHS at the same occasion at two hospitals during a six-year period (more than 30 000 neonates screened in total). TEOAEs were studied and TEOAE level correlations compared in twin sets of same-sex (SS, 302 individual twins, 151 twin pairs) and opposite-sex (OS, 152 individual twins, 76 twin pairs). A mathematical model was used to estimate and compare monozygotic (MZ) and dizygotic (DZ) intra-twin pair TEOAE level correlations, based on the data from the SS and OS twin sets.

For both SS and OS twin pairs TEOAE levels were significantly higher in right ears and females, compared to left ears and males, as previously demonstrated in young adult twins and large groups of neonates. Neonatal females in OS twin pairs did not demonstrate masculinized TEOAEs, as has been demonstrated for OAEs in young adult females in OS twin pairs. The within-twin pair TEOAE level correlations were higher for SS twin pairs than for OS twin pairs, whereas the within-pair correlation coefficients could not be distinguished from zero when twins were randomly paired. These results reflect heredity as a key factor in TEOAE level variability. Additionally, the estimated MZ within-twin pair TEOAE level correlations were higher than those for DZ twin pairs. The heritability estimates reached up to 100% TEOAE heritability, which is numerically larger than previous estimates of about 75% in young adult twins.

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1. Introduction

Otoacoustic emissions (OAEs) are sounds in the ear canal, caused by processes in the cochlea, directly associated with the hearing process (Kemp, 1978). OAEs comprise evoked and spontaneous OAEs (SOAE). The OAEs can be evoked by clicks or transients (transient-evoked OAE, TEOAE), one tone (stimulus-frequency OAE, SFOAE), or two nearby tones (the distortion product OAE, DPOAE). Furthermore, DPOAEs arise from nonlinear distortion, whereas low level TEOAEs, SFOAEs and SOAEs arise from linear reflection, that is, two different mechanisms in the organ of Corti (Shera and Guinan, 1999). Distortion emissions originate primarily from prestin-based mechanoelectrical transducer channels in outer hair cells (OHCs), and the distortion product is injected into the organ of Corti motion by OHC electromotility (Guinan, 2018; Ren and He, 2020; Shera and Guinan, 1999). Reflection emissions originate from the reflection of traveling-wave energy by minute irregularities along the cochlea, where the peak of the travelling wave contributes most to the reflection (Guinan, 2018; Shera and Guinan, 1999). Even so, our understanding of the global system involved in shaping the OAEs is incomplete.

The OAE offers an objective, non-invasive measure of peripheral hearing sensitivity, where the TEOAE is widely used in clinical practice, partly due to its high intra-individual stability (Franklin et al., 1992; Harris et al., 1991; Johnsen and Elberling, 1982a; Marshall and Heller, 1996), and sensitivity in identi-
flying minor cochlear hearing losses, e.g. Berninger et al. (1995). Thousands of TEOAEs are recorded each day worldwide as part of universal newborn hearing-screening (UNHS) programs.

While the intra-individual variability in TEOAE level in neonates is low, the neonatal inter-individual variability in TEOAE level is large (Berninger, 2007; Bray and Kemp, 1987). The causes for the large variability have only partly been explained. Sex differences (female > male) and ear asymmetries (right > left) exist in TEOAEs (Aidan et al., 1997; Berninger, 2007; Kei et al., 1997; Thornton et al., 2003), although these effects may not be observed in samples smaller than approximately 500 neonates (Cassidy and Ditty, 2001; Johnsen et al., 1988; Khalfa et al., 1997).

The sex differences in OAEs have been attributed to the sex differences in exposure to androgens during prenatal development (McFadden, 1993b; McFadden, 2009). Support for this theory can be found in studies of hyenas (McFadden, 2008; McFadden et al., 2006b), sheep (McFadden, et al., 2009), and rhesus monkeys (McFadden et al., 2006a), where prenatal hormone levels were manipulated. An explanation suggested for the ear difference in OAEs is that efferent inhibition may be relatively less in right ears (McFadden, 1993b), as the major efferent neural pathway synapse on OHCs. That the olivocochlear systems are involved in modulating the OAEs has been demonstrated (Guinan, 2006; Guinan, 2018; Veulliet et al., 1991). However, it is yet unknown where in the auditory system ear and sex differences stem from, although the main effects presumably do not arise in the outer and middle ear. McFadden (1993b) argued that ear and sex differences have been demonstrated at the level of the cochlea, but not in the outer and middle ear, also supported by results of no ear and sex effect in electrically constant TEOAE peak stimulus levels in the outer ear canal (Berninger, 2007), in a study where ear and sex effects were demonstrated for TEOAEs.

The effect of inheritance of TEOAE levels could contribute to our understanding of which structures are involved in shaping TEOAEs and the large inter-individual TEOAE variability. One previous study exists of TEOAE heredity, where it was estimated that about 75% of the variation in TEOAE level could be attributed to heredity, by comparing monozygotic (MZ) and dizygotic (DZ) young adult twins (McFadden et al., 1996). The percentage not explained by heredity was suggested to be caused by differential exposure to sex hormones prenatally (McFadden et al., 1996). In support of a prenatal masculinization effect a twin testosterone transfer effect was indicated for SOAEs (McFadden, 1993a; McFadden and Loehlin, 1995), and a similar effect was observed for TEOAEs (McFadden et al., 1996), although not statistically significant. Young adult female twins in opposite-sex (OS) twin pairs had half as many SOAEs, compared to female twins in same-sex (SS) twin pairs, corresponding to the prevalence in young adult males (McFadden, 1993a; McFadden and Loehlin, 1995). An influence of sex hormones on the cochlear amplifier in humans is not unlikely, as drugs like quinine and salicylate influence the presence and magnitude of OAEs (Alvan et al., 2017; Johnsen and Elberling, 1982b), including TEOAEs (Berninger et al., 1995; Karlsson et al., 1991), and presumably block the cochlear amplifier and the motor protein prestin (Alvan et al., 2017). However, more research is needed in order to link the effect of androgens on the cochlear amplifier to a prenatal period in humans. The twin testosterone transfer effect was observed in twins that were students at the university, and different hormones can influence TEOAEs in young adults (see e.g. Burke et al. (2020)). Additionally, a postnatal surge, sometimes referred to as “mini puberty” (Hines et al., 2015), may also have effects on TEOAE levels. During this period (peak 1–2 months after birth) urine testosterone levels are notably higher in males than in females, while no testosterone sex difference exists in newborns (7 days of age) and in 6-month-old infants (Hines et al., 2015; Lamminmäki et al., 2012).

We are not aware of any studies of TEOAE inheritance in neonatal twins, where the environmental factors between two twins in a pair is minimal. In previous studies of OAE inheritance in young adult twins, differences in environmental exposure needed to be considered (McFadden and Loehlin, 1995; McFadden et al., 1996), and heritability models taking environment into account have limitations. More research in large groups of neonates is needed, due to the incomplete understanding of the large inter-subject variability in TEOAE strength, as well as ear and sex differences. Specifically, the study of heredity in neonates, in combination with advancements in cochlear mechanics, can improve our understanding of OAE generation and modulation.

The aim was to study ear and sex differences in a consecutive subset of all twins that passed the UNHS program in both ears at the same test occasion at two hospitals during a six-year period (more than 30 000 newborns screened). The purpose was to estimate TEOAE heredity, and to study ear and sex differences in TEOAE level in twins and non-twins. We demonstrate the presence of ear and sex differences in TEOAE from the neonatal period, and that the individual differences in neonatal TEOAEs may be fully inherited. How these results may influence the current understanding of the auditory system involved in shaping OAEs is discussed.

2. Material and methods

2.1. Study design

TEOAEs were recorded from more than 30 000 neonates (98% of all newborns) at Karolinska University Hospital, Huddinge and Södertälje Hospital, Södertälje, Sweden, from November 1998 until the end of 2004 (Berninger, 2007; Berninger and Westling, 2011). The bedside UNHS was based on multiple TEOAE recordings to enhance the specificity of the screening program (Berninger, 2014). Off-line analysis was performed on the entire TEOAE levels of all twin sets that passed UNHS in all four ears at the same test occasion, and had valid data on ear tested, sex, name, and test date.

To estimate heredity, intra-twin pair TEOAE level relationships were studied. Specifically, the TEOAE intra-twin pair relationships were compared for SS pairs and OS pairs, and estimates of MZ and DZ relationships were compared. TEOAE relationships also were studied for twins that were randomly paired, as a non-heredity and sex-effect control. Finally, broad heritability was estimated with the Falconer’s formula based on the difference between the estimated MZ and DZ correlations (Falconer and Mackay, 1996). All non-twins that passed the UNHS in both ears, with valid data on ear tested, sex, name, and date of test, were used as a non-twin control group (n = 21 199 individuals).

This study was approved by the Swedish Ethical Review Authority (no: 2019-03826).

2.2. Transient-evoked otoacoustic emissions (TEOAEs)

TEOAEs were recorded in the non-linear quickscreen default mode with ILO 288 (Otodynamics Ltd., program version 5.6 years, time window: 3.0–12.8 ms, click rate 78 Hz, for details on the non-linear stimulus paradigm see Kemp et al. (1990)). All the TEOAE stimulus and response levels were recorded at the probe-tip in the outer ear canal using an electrically constant stimulus, corresponding to a typical stimulus level of 81.8 dB SPL peak (Berninger, 2007).

TEOAE pass criteria included: ≥ 70% whole wave reproducibility, signal-to-noise ratio (S/N) ≥ 3 dB in at least three of the upper four wide-frequency bands provided by the ILO instrument (center frequencies from about 1500 to 4000 Hz, ± 400 Hz bandwidth), and ≥ 50 sweeps.
The entire TEOAE level was calculated as the root mean square (RMS) value of the time signal. A sweep consisted of two sets of non-linear responses in response to four 80-125 click stimulations. Whole-wave reproducibility was calculated as the correlation coefficient of interleaved non-linear responses.

TEOAEs from all except three twin pairs (i.e., 99%) were obtained at the first TEOAE test occasion, which was typically performed at postnatal day 3 (Berliner and Westling, 2011), during a period when it was typical for the mothers to stay at least three days at the hospital after delivery. The left ear was tested first in 58% of all neonates that were screened during the six-year period (Berliner and Westling, 2011). The twins were included in a consecutive manner, which should be important, as TEOAEs have been demonstrated to be larger in the ear tested first (Thornton et al., 2003).

2.3. Data files and data analysis

A Matlab program was developed for retrieving data from the TEOAE files generated by ILO 288 (file extension: .dta) and cross-checking pass criteria. It was also used for computing response levels in non-overlapping half-octave frequency bands, geometrically centred at 707, 1000, 1414, 2000, 2828 and 4000 Hz, where the four upper frequency bands were used for the S/N pass criteria.

2.4. Subjects

In total, 642 individual twins (321 pairs) were identified in a clinical database of all newborns that had passed UNHS at Karolinska University Hospital, Huddinge and Södertälje Hospital, Södertälje, Sweden, during the six-year period. Out of the identified twins, the custom-made Matlab program identified 454 individual twins (227 pairs) that passed with the strict inclusion criteria, i.e., passed beside UNHS in all four ears at the same test, and had data files that included information on ear tested, sex, name and test date.

In all, 227 twin pairs were included, 151 SS twin pairs (66.5%) and 76 OS twin pairs (33.5%). Of all the 454 individual twins, 51.1% were male. Of the SS twin pairs, 78 twin pairs were male (51.6%). The first-born and second-born twins are henceforth denoted twin 1 and twin 2.

The non-twin comparison group consisted of 21,199 neonates (50.3% males), derived from the material of all >30 000 screened neonates Berninger (2007). The inclusion criteria comprised data on ear tested, sex, name and test date, whereafter the twins in the material were excluded based on the file name. The sex inclusion criterion was somewhat different for the twin group and the control group, due to the sample size. The sex information was manually typed into the ILO-program, and for the large non-twin group inclusion was strictly based on checking that of all but the last typed letter corresponded to the Swedish word for boy or girl. In the smaller twin group, the sex of the individual twins (n = 454) could be verified manually, so that the twins with, e.g., a “p” for “pojk” (i.e., Swedish word for boy) or the Swedish word for “twin” instead of the sex information could be included in the analysis. Typing errors of information on sex were a main reason for exclusion of TEOAEs in the non-twin group. Other main reasons for exclusion were were less than 70% whole-wave reproducibility and less than 3 dB S/N in three of the four upper half-octave frequency bands (i.e., 1414, 2000, 2828 and 4000 Hz), especially in the twin group, as all four ears were excluded if one ear did not fully correspond to the inclusion criteria.

2.5. Estimated monozygotic (MZ) and dizygotic (DZ) intra-twin pair correlations

A mathematical model was used to estimate and compare MZ and DZ intra-twin pair TEOAE level correlations, based on the data from the SS and OS twin pairs. The model uses Weinberg’s differential rule (WDR) (Fellman and Eriksson, 2006), which was created for estimation of MZ and DZ twinning rates in twin studies. WDR has been demonstrated to be robust despite its simplicity and gives reliable results when official birth registers are analysed (Fellman and Eriksson, 2006). WDR is implicitly based on the assumptions that the probability of a male equals the probability of a female in the entire twin material (our material 51.1% males), and that there is independence between the sexes in DZ twin pairs, which is true for all OS twin pairs (same number of males and females) (Fellman and Eriksson, 2006). For DZ twin pairs of SS we based our assumption on independence between the sexes on the SS twin pairs (our twin material: 51.6 % male SS twin pairs, close to 50%).

The OS twin pairs are DZ. Hence, the TEOAE variance and correlation coefficient for the DZ twins was calculated from the OS twin pairs.

According to WDR the rate of DZ twinning is twice the rate of twin maternities in which the twins are of OS (nDZ = 2nOS; our set: nDZ estimated = 2nOS = 152). The MZ twinning rate is the difference between the rates of SS and OS twin pairs (nMZ = nSS - nOS; our set: nMZ estimated = nSS - nOS = 151–76 = 75). Accordingly, the number of DZ pairs in the SS set should then approximately be equal to the estimated number of MZ twin pairs (our set: nDZ estimated in SS group = nSS - nMZ estimated = 151–75 = 76 ≈ nMZ estimated). Additionally, based on the 50% probability of a MZ twin set (75/151 = 0.50), and 50% probability of a DZ twin set (76/151 = 0.50), for a random twin pair in the SS twin sets, MZ twin’s TEOAE variance was estimated as:

$$\sigma^2_{SS} = P(MZ)\sigma^2_{MZ} + P(DZ)\sigma^2_{DZ} = \frac{1}{2}(\sigma^2_{MZ} + \sigma^2_{DZ}) \tag{1}$$

where \(P\) is probability, and \(\sigma_{SS}\) are estimated from the TEOAE levels of the SS set, and \(\sigma_{DZ}\) from the TEOAE levels of the OS set.

Then, the correlation coefficient \(\rho_{SS}\) was calculated according to:

$$\rho_{SS} = \frac{\sum^n_{i=1}[(X_i - \bar{X})(Y_i - \bar{Y})]}{\sqrt{\sum^n_{i=1}(X_i - \bar{X})^2}\sqrt{\sum^n_{i=1}(Y_i - \bar{Y})^2}} \tag{2}$$

$$= \frac{P(MZ)\rho_{MZ}\sigma_{MZ}\sigma_{YMZ} + P(DZ)\rho_{DZ}\sigma_{DZ}\sigma_{YDZ}}{\sigma_{SS}\sigma_{SS}}$$

where \(P\) is probability, and \(X\) is for twin 1 and \(Y\) for twin 2 in each twin set. It is assumed that the standard deviation is equal for twin 1 (\(X\)) and twin 2 (\(Y\)) in the population. Thus:

$$\rho_{SS}/\sigma_{SS}^2 = \frac{1}{2}(\rho_{MZ}\sigma^2_{MZ} + \rho_{DZ}\sigma^2_{DZ}) \tag{3}$$

From Eqs. (1) and (3) the correlation coefficient for the MZ twins is estimated as:

$$\rho_{MZ} = \frac{\rho_{SS}/\sigma_{SS}^2 - \rho_{DZ}\sigma^2_{DZ}}{2\sigma^2_{SS} - \sigma^2_{DZ}} \tag{4}$$

where the variance for the SS twins was estimated as 0.5 \(\times\) (variance for twin 1 + variance for twin 2) from the SS set, and the variance for the DZ twins was estimated as 0.5 \(\times\) (variance for twin 1 + variance for twin 2) from the OS set.
2.6. Estimate of heritability

Falconer’s formula was used to calculate broad heritability (Falconer and Mackay, 1996):

\[ H^2 = 2(r_{MZ} - r_{DZ}). \]  

(5)

where the MZ and DZ intra-twin pair correlation coefficients (\(r_{MZ}\) and \(r_{DZ}\)) are estimated from Eqs. (1)–(4). Falconer’s formula is twice the difference in correlation between MZ and DZ twins, which originate from MZ twins sharing all the same genes, whereas DZ twins normally share half their genes, so MZ twins are on average twice as genetically similar as DZ twins (Falconer and Mackay, 1996).

2.7. Twins in randomized pairs

The TEOAE levels for randomly paired twins were compared, as a control that differences in correlations were driven by heredity (between OS and SS pairs, and estimated DZ and MZ correlations). In the dataset of the 227 twin pairs, the TEOAE level for twin 1 in twin pair \(n\) was compared to that of twin 2 in twin pair \(n+1\) (twin 2 from pair 1 was paired with twin 1 in the final pair).

Additionally, correlations were studied for twins that were randomly paired under condition that their randomly assigned co-twin was of the same sex as their biological co-twin (i.e., randomization with stratification for sex). The analysis was performed to control if sex may be a confounding variable when comparing the SS and OS twin groups. Accordingly, the dataset was divided into four subsets: (1) male SS-pairs, (2) female SS-pairs, (3) OS pairs with a male twin 1, and (4) OS pairs with a female twin 1. Within each subset twin 1 from pair \(n\) was paired with twin 2 from pair \(n+1\) (twin 2 from twin pair 1 was paired with twin 1 in the final pair in each subset). Thereafter subsets 1 and 2 were combined into an SS group and subsets 3 and 4 were combined into an OS group for analysis.

2.8. Statistical analysis

All the statistical analyses were performed with Statistica version 13 (Statsoft Inc., USA).

One-way ANOVA was used when comparing the TEOAE level for the SS, OS and non-twin group. Factorial ANOVA was used for ear and sex effects on TEOAE level. Moreover, paired and unpaired \(t\)-tests were used for the study of sex and ear differences on data that reflected a Gaussian distribution. The average TEOAE level for each twin pair and ear was used, to avoid violation of independent observations (same method as used by McFadden and Loehlin (1995)).

Non-parametric tests were used for data that reflected a non-Gaussian distribution, e.g., the stimulus levels. Wilcoxon’s matched-pairs test was used for dependent samples, and the Mann–Whitney \(U\) test for independent samples. Moreover, for data that reflected non-Gaussian distributions, medians are presented instead of means, and in Table 1 medians and means.

Pearson’s correlation coefficient was calculated for co-twins’ TEOAEs to quantify twin resemblance in TEOAE. The differences between correlation coefficients were computed using Fisher’s \(r\)-to-\(z\) transform with an unpaired two-tailed \(t\)-test.

Additional effect sizes were calculated for the main TEOAE sex and ear differences, as well as the difference between estimated MZ and DZ intra-twin pair correlations. The effect sizes for sex and ear effects were calculated as the difference between two means divided by the square root of the weighted mean of the two variances (Cohen’s \(d\)), where effect sizes of 0.2, 0.5, and 0.8 are viewed as small, medium and large, respectively (Cohen, 1992). The effect size for the difference between correlations was calculated as the difference between two \(z\)-values after using the Fisher’s \(r\)-to-\(z\) transform, where effect sizes of 0.1, 0.3, and 0.5 are viewed as small, medium and large, respectively (Cohen, 1992).

Bootstrapping was used to estimate the variance of the heritability estimates. The input data were the ear-average TEOAEs of the included 227 twin pairs, divided into the SS and OS groups, as in the main heritability analysis. Then, the number of twin pairs found before we applied our strict inclusion criteria, i.e., 321 pairs, was used as the number of slots available for resampling from the 227 twin pairs’ data. The resampling was performed 10,000 times with different random samples of twin pair data. Variances and correlations were calculated for each resample Eqs. (1)–(4)), and the 10,000 calculations of \(H^2\) were used to estimate the broad heritability variance.

3. Results

3.1. TEOAE stimulus and response parameters

No lateral asymmetries or sex differences existed in stimulus levels. The median interaural peak stimulus level difference was 0.0 dB (\(n = 454\) and 0.0 dB (\(n = 21\) 199) for the individual twins and non-twins, respectively. The sex difference (males–females) in median peak stimulus level was 0.0 dB (\(n = 454\) for the individual twins, and –0.1 dB (\(n = 21\) 199) for the non-twins. Moreover, twins and non-twins displayed no significant difference in stimulus levels (\(U = 2234506, p = 0.06\), with a median of 81.9 dB SPL peak for the twins (\(n = 227\); twin pair average), and 81.5 dB SPL peak for the non-twins (\(n = 21\) 199). No significant correlation was found for TEOAE level as a function of the (electrically constant) stimulus level in twins (\(r = -0.02, p = 0.71, n = 454\) individual twins), indicating that the variability in TEOAE levels could not be explained by the small variability in stimulus levels (3.1 dB inter-quartile range).

The median TEOAE levels were 19.4 dB SPL (\(n = 227\); twin pair average) for the twins and 19.0 dB SPL (\(n = 21\) 199) for the non-twins, with a significant difference between the groups (\(U = 2221052, p = 0.045\) (see Table 1 for TEOAE level by group, ear and sex). A median of 56 sweeps were used (\(n = 908\) twin ears), and the median S/N was 9.6 dB (non-outlier range: 4.5–25.2 dB, \(n = 908\) twin ears). Moreover, the whole-wave reproducibility was high (median = 94%, \(n = 908\) twin ears).

3.2. TEOAE ear and sex differences

The twins showed statistically significant effects of ear (\(F(1, 904) = 171, p < .0001\)) and sex (\(F(1, 904) = 175, p < .0001\)) on TEOAE level, with larger TEOAEs in right ears and females (Table 1).

The mean TEOAE levels were 1.5 dB larger in right compared to left ears in twins (\(p < .0001, n = 227\); twin pair average), as compared to the average 1.1 dB difference for non-twins (\(p < .0001, n = 21\) 199). Likewise, significant TEOAE interaural differences (right > left) existed in the subgroups of OS twin pairs (\(p = .0005, n = 76\); twin pair average) and SS-twin pairs (\(p < .0001, n = 151\); twin pair average). For further ear comparisons see Table 1, although the TEOAE levels are shown for each neonate, not the twin pair averages used for statistical purposes. No significant difference was found when comparing the interaural difference between twins in SS pairs (mean = 1.6 dB), twins in OS pairs (mean = 1.2 dB), and non-twins (mean = 1.1 dB) (\(F(2, 21\) 650) = 2.8, \(p = .055\) (Table 1).

Female twins (\(n = 222\) individuals) showed 1.5 dB larger mean TEOAE levels (ear-average) compared to male twins (\(n = 232\) individuals) (\(p = .001\), similar to the 1.2 dB sex difference for non-twins (\(p < .0001, n = 21\) 199) (Table 1). In the subgroups, females in OS twin pairs showed 2.1 dB larger mean TEOAE levels (ear-average) compared to their male co-twins (\(p = .002, n = 76\) (Table 1).
twin pairs, 152 individual twins) (Table 1). Moreover, female SS-pairs (n = 73 pairs, 146 individual twins) showed 2.1 dB larger median TEOAE levels (ear-average) compared to male SS-pairs (n = 78 pairs, 156 individual twins) (U = 2273, p = 0.03).

Cohen’s d for the effect of sex on ear average TEOAE was 0.29, 0.23, 0.42, and 0.25 for the groups of all twins, SS, OS, and non-twins, respectively (see Table 1). Corresponding figure for the effect of ear was 0.27, 0.29, 0.22, and 0.21, respectively.

To examine whether a twin testosterone effect existed, the subgroups were analyzed for males and females separately. A significant difference between the OS, SS and the non-twin group was found for females in mean TEOAE level (F(2, 10745) = 3.6, p = .03), but not for males (F(2, 10902) = 1.5, p = .22) (Fig. 1). However, no significant ear-average TEOAE level difference was found between female twins in OS-pairs (20.7 dB SPL, n = 76 females), and female twins in SS-pairs (20.2 dB SPL, n = 146 females) (p = 0.41), indicating no twin testosterone effect. The statistically significant difference for females revealed by ANOVA was most likely driven by female twins in OS-pairs (n = 76 females) having 0.9 dB larger median TEOAEs than female non-twins (n = 10526 females) (U = 346743, p = .045) (Fig. 1).

Post-hoc analysis of ear and sex effects in a random sample of non-twin neonates with the same sample size as the twins (n = 454 individuals, 2.1% of the non-twin group) also was performed due to the statistically significant effects in the twin group. A significant effect of ear (p < .0001, n = 454 non-twins) and sex (p = 0.02, n = 454 non-twins) existed in TEOAE levels (50% males in the random sample chosen by Statistica).

Table 1

|               | Mean | SD  | Median | 25-75% | Min-Max | n  |
|---------------|------|-----|--------|--------|---------|----|
| **All twins** |      |     |        |        |         |    |
| Ear-average   |      |     |        |        |         |    |
| All           | 19.6 | 5.1 | 19.6   | 16.1–23.1 | 7.1–32.4 | 454 |
|♀             | 20.4 | 4.6 | 20.0   | 17.2–23.7 | 8.5–32.4 | 222 |
|♂             | 18.9 | 5.6 | 18.7   | 14.4–22.7 | 7.1–32.1 | 232 |
| Left          |      |     |        |        |         |    |
| All           | 18.9 | 5.6 | 18.8   | 14.9–22.5 | 5.8–32.4 | 454 |
|♀             | 19.7 | 5.0 | 19.6   | 16.5–22.9 | 6.6–32.4 | 222 |
|♂             | 18.0 | 6.0 | 17.6   | 12.8–22.3 | 5.8–32.1 | 232 |
| Right         |      |     |        |        |         |    |
| All           | 20.4 | 5.6 | 20.8   | 16.5–24.3 | 6.2–34.5 | 454 |
|♀             | 21.1 | 5.0 | 21.2   | 17.4–24.6 | 8.0–32.4 | 222 |
|♂             | 19.7 | 6.0 | 20.2   | 15.3–24.2 | 6.2–34.5 | 232 |
| **SS**        |      |     |        |        |         |    |
| Ear-average   |      |     |        |        |         |    |
| All           | 19.6 | 5.2 | 19.5   | 15.9–23.3 | 8.5–32.4 | 302 |
|♀             | 20.2 | 4.6 | 19.8   | 17.2–23.8 | 8.5–32.4 | 146 |
|♂             | 19.0 | 5.6 | 18.6   | 14.3–22.7 | 8.6–32.1 | 156 |
| Left          |      |     |        |        |         |    |
| All           | 18.8 | 5.7 | 18.8   | 14.6–22.5 | 5.8–32.4 | 302 |
|♀             | 19.5 | 5.1 | 19.4   | 15.8–22.5 | 6.6–32.4 | 146 |
|♂             | 18.1 | 6.1 | 17.5   | 12.6–22.6 | 5.8–32.1 | 156 |
| Right         |      |     |        |        |         |    |
| All           | 20.4 | 5.5 | 20.9   | 16.5–24.1 | 6.5–34.5 | 302 |
|♀             | 20.9 | 5.2 | 21.3   | 17.3–24.5 | 8.0–32.4 | 146 |
|♂             | 19.9 | 5.8 | 20.1   | 15.7–23.8 | 6.5–34.5 | 156 |
| **OS**        |      |     |        |        |         |    |
| Ear-average   |      |     |        |        |         |    |
| All           | 19.7 | 5.1 | 19.7   | 16.5–22.9 | 7.1–31.2 | 152 |
|♀             | 20.7 | 5.6 | 20.5   | 17.7–23.5 | 10.8–31.2 | 76 |
|♂             | 18.6 | 4.4 | 19.3   | 14.6–22.0 | 7.1–29.3 | 76 |
| Left          |      |     |        |        |         |    |
| All           | 19.1 | 5.3 | 18.8   | 15.4–22.3 | 7.1–32.1 | 152 |
|♀             | 20.2 | 5.6 | 19.6   | 17.1–23.4 | 11.1–32.1 | 76 |
|♂             | 17.9 | 4.7 | 17.7   | 14.2–21.9 | 7.1–31.7 | 76 |
| Right         |      |     |        |        |         |    |
| All           | 20.3 | 5.6 | 20.7   | 16.4–24.7 | 6.2–31.4 | 152 |
|♀             | 21.3 | 6.3 | 21.0   | 17.7–24.6 | 10.5–31.4 | 76 |
|♂             | 19.3 | 4.7 | 20.2   | 14.7–24.8 | 6.2–29.7 | 76 |
| **Non-twin**  |      |     |        |        |         |    |
| Ear-average   |      |     |        |        |         |    |
| All           | 18.9 | 4.9 | 19.0   | 15.5–22.3 | 5.4–32.3 | 21 199 |
|♀             | 19.5 | 4.9 | 19.6   | 16.2–22.9 | 6.1–33.0 | 10 526 |
|♂             | 18.3 | 4.8 | 18.4   | 15.0–21.6 | 5.4–31.5 | 10 673 |
| Left          |      |     |        |        |         |    |
| All           | 18.4 | 5.3 | 18.4   | 14.7–22.1 | 3.7–33.1 | 21 199 |
|♀             | 19.0 | 5.3 | 19.1   | 15.3–22.8 | 4.3–33.7 | 10 526 |
|♂             | 17.7 | 5.2 | 17.8   | 14.1–21.3 | 3.4–32.0 | 10 673 |
| Right         |      |     |        |        |         |    |
| All           | 19.5 | 5.3 | 19.5   | 15.8–23.1 | 4.9–34.0 | 21 199 |
|♀             | 20.0 | 5.3 | 20.1   | 16.4–23.7 | 5.4–34.6 | 10 526 |
|♂             | 18.9 | 5.2 | 19.0   | 15.3–22.5 | 4.6–33.3 | 10 673 |

SS = Same-sex twin group. OS = Opposite-sex twin group.
3.3. TEOAE correlations for opposite-sex (OS) and same-sex (SS) twin pairs

The twins in SS pairs showed a significantly larger mean TEOAE intra-twin pair correlation coefficient of \( r = .52 \) (n = 151 pairs, 302 individual twins), compared to the \( r = .27 \) (n = 76 pairs, 152 individual twins) for the twins in OS pairs (p = 0.04) (Table 2). Depending on ear under comparison, the TEOAE correlation coefficients varied between \( r = .37-.48 \) (n = 151 pairs, 302 individual twins) in SS-pairs, and \( r = .19-.30 \) (n = 76 pairs, 152 individual twins) in OS-pairs (Table 2), thereby reflecting the genetic contribution to the TEOAE levels, as \( \approx 50\% \) of SS twin pairs are MZ. The intra-twin pair TEOAE correlation between the left ear for twin 1 and the right ear for twin 2 (L1–R2) and the right ear for twin 1 and the left ear for twin 2 (R1–L2) were combined into a single opposite-ear comparison in Table 2 (L1–R2 + R1–L2). The opposite-ear comparison demonstrated larger intra-twin pair resemblance for SS twins as compared to OS twins (Table 2). It should be noted that both ears of both twins (dependent observations) were included in the L1–R2 + R1–L2 comparison, and if studied separately the L1–R2 condition also showed a statistical difference between the groups (SS twins: \( r = .43 \), n = 151 pairs, 302 individual twins vs OS twins: \( r = .16 \), n = 76 pairs, 152 individual twins; difference \( p = .04 \), although the R1–L2 condition did not reach statistical significance (SS twins: \( r = .47 \), n = 151 pairs, 302 individual twins vs OS twins: \( r = .24 \), n = 76 pairs, 152 individual twins; difference \( p = .06 \)).

3.4. TEOAE correlations for randomly paired twins

The correlation coefficients of TEOAE levels between ears in randomized pairs were close to zero; \( r = .03 \) for left ears (L–L), \( r = .00 \) for right ears (R–R), and \( r = .06 \) (L1–R2) and \( r = -.04 \) (R1–L2) for opposite ears (n = 227 non-biological pairs, 454 individual twins) (L1–R2 + R1–L2: \( r = -.01 \), n = 454 individual twins, n = 908 individual ears).

3.5. Within-subject TEOAE correlations

The interaural TEOAE correlation coefficients were similar for twins (\( r = .71 \), p < .0001, n = 454 individual twins), non-twins (\( r = .70 \), p < .0001, n = 21 199), twins in SS-pairs (\( r = .69 \), p < .0001, n = 302 individual twins), and twins in OS-pairs (\( r = .77 \), p < .0001, n = 152 individual twins) (Table 4).

3.6. Estimated TEOAE heritability

The estimated intra-twin pair MZ correlation based on ear-average TEOAE level (\( r = .77 \), p < .0001, n = 75 pairs, 150 twins) was significantly higher (p < .0001) than the estimated intra-twin pair correlation for DZ twins (\( r = .27 \), p = .03, n = 76 pairs, 152 twins) (Table 5). Depending on ear under comparison, the estimated intra-twin pair DZ correlations varied between \( r = .19-.30 \) (n = 76 pairs, 152 twins), compared to \( r = .51-.68 \) for the estimated MZ correlations (n = 75 pairs, 150 twins) (Table 5). Separate opposite-ear comparisons also demonstrated statistical significance between estimated MZ and DZ correlations, i.e., the L1–R2 comparison (estimated MZ: \( r = .67 \), n = 75 pairs, 150 twins vs DZ: \( r = .16 \), n = 76 pairs, 152 twins; difference p = .0001) and R1–L2 comparison (estimated MZ: \( r = .68 \), n = 75 pairs, 150 twins vs DZ: \( r = .24 \), n = 76 pairs, 152 twins; difference p = .0006).

The effect size for the difference between the estimated intra-twin pair MZ and DZ correlations was 0.74, indicating a large effect (0.5 = large).

The estimated broad heritability based on the average TEOAE level of each twin was \( H^2 = 1.0 \), i.e. 100% heredity. When comparing specific ears, the estimated heredity of TEOAE level was at maximum for opposite ears, \( H^2 = .95 \), while the corresponding heritability estimate for same ears was \( H^2 = .59 \) (Table 5).

The bootstrapping analysis based on ear-average TEOAEs demonstrated a median of approximately 1 and variance of 0.33 for \( H^2 \). Of all \( H^2 \) estimates, 75% exceeded 0.69.

When the randomized pairs for the SS and OS groups were compared (randomization with stratification for sex within each subgroup), the correlation coefficients of TEOAE levels were likewise close to zero, with no significant difference between the groups (Table 3). The results support the interpretation that the comparatively high intra-twin pair correlation coefficients for the SS group in Table 2 were due to heredity, not because the twins were of SS (as compared to OS). This was also true for separate L1–R2 comparisons (SS: \( r = .06 \), n = 151 pairs, 302 individual twins vs OS: \( r = -.08 \), n = 76 pairs, 152 individual twins; difference: \( p = .33 \)) and R1–L2 comparisons (SS: \( r = .08 \), n = 151 pairs, 302 individual twins vs OS: \( r = .05 \), n = 76 pairs, 152 individual twins; difference: \( p = .83 \)).

4. Discussion

The individual differences in neonatal TEOAE levels are largely inherited. Our estimates of heredity based on the TEOAEs of neonatal twins that passed UNHS suggest that typical TEOAE levels may be up to 100% inherited, in comparison to the previous heredity estimate in young adults of about 75% (McFadden et al., 1996).

The heritability results are consistent, i.e., estimated MZ within-pair TEOAE level relationships were significantly closer than estimated TEOAE level relationships for DZ pairs (Table 5), twins in SS-pairs showed significantly closer within-pair TEOAE level resemblance than twins in OS-pairs (Table 2), and randomly paired twins showed no TEOAE level resemblance (Table 3). Further supporting a large influence of heredity, the estimated MZ within-pair TEOAE level relationships (Table 5), were as close as the within-subject TEOAE level relationships for twins and non-twins (Table 4). The heredity results show that neonatal TEOAEs are nonrandom events. Moreover, that TEOAE levels are very similar within-twin pairs is noteworthy in light of the generally large neonatal variability.

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Table 2: TEOAE intra-twin pair correlations for twin pairs of same-sex (SS, n = 151 pairs, 302 individual twins) and opposite-sex (OS, n = 76 pairs, 152 individual twins). The analysis included both left and right ears for the L1–R2 + R1–L2 comparison (SS, n = 302 ears; OS, n = 152 ears). The differences between correlation coefficients were calculated using Fisher’s r-to-z transform with a two-tailed t-test.

| Twin 1 vs. Twin 2 | SS     | OS     | \( r_{ss} \) vs. \( r_{os} \) |
|-------------------|--------|--------|-----------------------------|
| L–L               | .48    | < .0001| .30                         |
| R–R               | .37    | < .0001| .24                         |
| L1–R2 + R1–L2     | .42    | < .0001| .19                         |
| Ear-average TEOAE | .52    | < .0001| .27                         |

L=left ear, R=right ear. Twin 1 was born before Twin 2.
with a median TEOAE S/N of 9.6 dB, and that we included all subjects that were born during a six-year period that corresponded to the inclusion criteria. Significant effects of ear and sex in a random sample from the non-twin group, as large as the twin group \( n = 454 \) individuals, 2.1% of the non-twin group; post hoc analysis, support this hypothesis.

Our results on TEOAEs did not support the twin testosterone transfer hypothesis (McFadden, 1993a; McFadden and Loehlin, 1995; McFadden et al., 1996), as the females in OS twin pairs showed no indication of masculinization of their auditory systems a few days after birth (Fig. 1), in a larger subject group than that of young adult twins studied by McFadden et al. (1996).

The idea of twin testosterone transfer originates from research on male and female traits in litter-bearing rodents (Clemens et al., 1978; Kinsley et al., 1986; vom Saal, 1989; vom Saal and Bronson, 1980). For example, female rats positioned between two males during gestation have demonstrated atypically frequent mounting behavior, that was significantly reduced in response to prenatal anti-androgen treatment (Clemens et al., 1978). Thus, it is not unreasonable to expect that females in OS-pairs would be born with weaker TEOAEs than females in SS twin pairs, as well as show similar TEOAE levels as males in OS twin pairs (masculinized TEOAEs). That expectation was not confirmed here. Contrary, female twins in OS-pairs showed 2.1 dB larger mean TEOAE levels compared to their male co-twins \( p = 0.002, \ n = 76 \) females, and female twins in OS twin pairs showed numerically larger TEOAEs than females in SS twin pairs, although no statistically significant difference existed (Fig. 1). After the publication of the heredity studies (McFadden and Loehlin, 1995; McFadden et al., 1996), McFadden (2000) has reported that oral contraceptives may produce a weak masculinizing effect on OAEs. Thus, the use of oral contraceptives may have affected the female twins’ OAEs differently, as the use cannot be excluded in the group of university students (McFadden and Loehlin, 1995; McFadden et al., 1996). Moreover, it has been found that TEOAEs can be modulated postnatally by sex hormones, in the form of hormonal treatment, indicating that hormones do influence TEOAEs, although TEOAEs “may not be used as an unequivocal measure of prenatal androgen exposure” (Burke et al., 2020).

Moreover, the neonatal twins only received the prenatal surge of testosterone. The absence of a second surge may have contributed to the lack of masculinization of females in OS twin pairs’ TEOAEs, although it is yet unclear why such a twin testosterone transfer effect would be attributed to postnatal hormone exposure.

Cohen’s effect sizes for ear and sex differences in TEOAE were similar around 0.2–0.4 for neonatal twins and non-twins, indicating small effects. Similarly, the effect of ear on TEOAEs was 0.24 in young adult twins (McFadden, 2009; McFadden et al., 1996). In contrast, the effect of sex on TEOAEs in young adult twins was 0.76 (McFadden, 2009; McFadden et al., 1996), indicating a large effect \( 0.7 = large \). Recordings of auditory evoked poten-
Estimates intra-twin pair correlation coefficients for monozygotic (MZ, estimated n = 75) and dizygotic (DZ, n = 76) twin pairs. The analysis included both left and right ears for the L1–R2 + R1–L2 comparison (MZ, estimated n = 150 ears; DZ, n = 152 ears). The differences between correlation coefficients was calculated using Fisher’s r-to-z transformation with a two-tailed t-test.

| Twin 1 vs. Twin 2 | MZ      | DZ      | rMZ vs. rDZ |
|-------------------|---------|---------|-------------|
| L–L               | .62     | .30     | .01         |
| R–R               | .51     | .24     | .06         |
| L1–R2 + R1–L2     | .68     | .19     | .0001       |
| Ear-average TEOAE | .77     | .27     | <.0001      |

L=left, R=right, Twin 1 was born before Twin 2.

Intra-twin pair correlations (Pearson’s r) were used in this study, and by McFadden et al. (1996) to estimate heredity. McFadden et al. (1996) used same-ear comparisons to estimate heredity, and not opposite ears. Unexpectedly, our results indicate that the estimated heredity may be larger for opposite ears than for same ears. Interestingly, the largest intra-twin pair correlation displayed by McFadden et al. (1996) was for opposite ears for MZ male twin pairs (r = 0.83), although the overall difference between same ears and opposite ears were less evident than in the present study. The difference may be explained by mirror-image twinning, i.e., that a right-side feature in one MZ twin appear asymmetrically, on the left side, of the other MZ twin. Mirror-image twinning occurs in about 10–15% of MZ twins that are otherwise healthy, a result of late zygotic splitting (Hall 2003; McNamara et al., 2016). Mirror-image features can even exist in as many as 25% of MZ twins, as reflected by eye and ear defects (McNamara et al., 2016; Springer and Searleman, 1978). The young adult MZ group also revealed features of mirror-imaging, as four pairs were reported to have opposite handedness (and footedness) (McFadden and Loehlin, 1995; McFadden et al., 1996). McFadden and Loehlin (1995) also considered the possibility of similar SOAE frequencies being inherited by MZ co-twins, which is another form of mirroring, that may be reflected in our high heritability estimates. The intra-twin pair correlations did not differ between same-ear (L–L and R–R) and opposite-ear (L1–R2 + R1–L2) comparisons for OS and SS twin pairs (Table 2), or for random pairs (Table 3), and not significantly for estimated DZ or estimated MZ correlation coefficients (Table 5). The mirror-image effect, reflected in comparably large opposite-ear correlations for MZ twins, is particularly noticeable in the heredity estimates, when the estimated MZ correlation coefficients are compared to the correlation coefficients of DZ twins (Table 5). Consequently, we believe that the intra-twin pair correlations based on the ear-average TEOAE (Table 5) is the most appropriate to interpret in the estimation of heredity, as the risk that the mirror-image effect becoming a confounding variable is removed in the ear-average.

Intra-twin pair correlations for same ears of neonatal and young adult DZ twins used to estimate heritability were similar in size: r = .24, and r = .30 for right and left ears respectively (estimated n = 76 pairs), compared to r = .13, and r = .31 for right and left ears, respectively (n = 28 pairs; Table 10 by McFadden et al. (1996)). It should be noted that we estimated heritability based on OS DZ twin correlations, whereas McFadden et al. (1996) used SS DZ twin correlations, but the OS DZ correlations were also similar: r = .10, and r = .47 for right and left ears respectively (n = 17 pairs; Table 4 by McFadden et al. (1996)). Likewise, the estimated within-pair MZ correlations were also similar to that of MZ young adult twins-pairs: r = .62, and r = .51 for right and left ears respectively (estimated n = 75 pairs), compared to r = .76, and r = .69 for right and left ears respectively (n = 38 pairs; Table 10 by McFadden et al. (1996)). The MZ young adult twin pairs displayed numerically larger correlation coefficients for left and right ears as compared to the estimates for neonates, but no statistical difference existed (p > .17, Fisher’s r to z transform). Unfortunately the ear-average TEOAE intra-twin pair correlations, used primarily in the heritability estimation due to potential mirroring, could not be compared to previous studies as these type of correlations were not presented (McFadden et al., 1996), nor the variability in the heritability estimate. Furthermore, TEOAE intra-twin pair comparisons by ear and sex for MZ twins could not be compared, nor data for a SS DZ group, as the estimated MZ cor-
relations were based on the data from the SS and OS twin sets in the present study.

An advantage of studying TEOAEs and heredity in newborn twins is minimal environmental influence, e.g., differences in noise exposure within a twin pair, which is a difference to previous heredity and OAE studies, wherein students were recruited at a university (McFadden and Loeblin, 1995; McFadden et al., 1996). Another factor that may have an effect later in development is sex hormones (e.g., Al-Mana et al., 2010; Burke et al., 2020), as described previously. Another factor that may have modulated adult twin's TEOAEs is cortical activity and selective attention. In animal models it has been found that cortical activity can influence otoocochlear efferents (Dragicevic et al., 2015), and DPOAEs (Jäger and Kössl, 2016). Specifically, in humans selective auditory and visual attention has been demonstrated to affect DPOAEs (Srinivasan et al., 2014; Wittekindt et al., 2014), and may affect a nonlinear version of SFOAEs in humans (Walsh et al., 2015), but has not been reproduced for SFOAEs (Beim et al., 2018; Beim et al., 2019). Specific studies of TEOAEs and selective attention is lacking, but the results represent another factor that may influence adults, whereas these higher functions have not yet developed in neonates.

Race/ethnicity may also influence TEOAE levels, and specifically sex differences (McFadden et al., 2018), possibly due to melanocytes in the cochlea (Lin et al., 2012). Thus, the pigmentation effect on TEOAEs may have differed between this study compared to previous studies, although comparisons are not possible as our ethical consent did not include the collection of this information.

Zygosity estimation represent another difference between this study and that of McFadden et al. (1996). The methods used are not fully comparable, as we used mathematical estimations of heredity based on the TEOAEs of the OS and SS group, whereas McFadden et al. (1996) used a questionnaire procedure (Nichols and Bilbro, 1966), with approximately 90% accuracy in identifying zygosity (McFadden and Loeblin, 1995). For future research of OAE heredity, it would be of value to use a different method for determining zygosity in neonatal twins to confirm a large heredity contribution, preferably by analyzing DNA samples. Another difference between the studies was the DZ group under comparison. Estimated MZ intra-twin pair correlations were compared to DZ twins of OS here, whereas McFadden et al. (1996) used exclusively DZ twins of SS. The DZ twins of OS were excluded by McFadden et al. (1996) due to the previous research showing weaker OAEs for the DZ females in OS-pairs (McFadden, 1993a), which we did not observe in our data (Fig. 1). A factor that could potentially influence our comparison of SS and OS twins, and perhaps that we use a DZ group consisting of solely OS-pairs, were the sex differences in TEOAE. Thus, we analyzed randomly paired twins under the condition that their randomly assigned co-twin was of the same sex and from the same group (SS or OS) as their biological co-twin. Although a trend can be perceived of a larger variability for the OS-group (than for the SS-group) when comparing different ear conditions, no significant difference was found when comparing the SS- and OS-group of randomly paired twins' TEOAE correlations (Table 3). Moreover, the correlation coefficients could not be distinguished from zero for any group or any ear comparison (Table 3), indicating that heredity and not sex differences are the cause for the larger correlation coefficients for SS twin pairs, as compared to OS twin pairs (Table 2), which is also reflected in the estimated MZ and DZ correlations (Table 5).

5. Conclusion

In a large consecutive sample of neonatal twins, born over a 6-year period, we demonstrate that individual differences in TEOAE levels are largely inherited. Furthermore, neonatal twins show similar TEOAE lateral asymmetry (right > left) and TEOAE sex differences (female > male) as neonatal non-twins and young adult twins.

The heritability estimates based on ear-average TEOAE levels indicate that up to 100% of the TEOAE level may be inherited, and 75% of our heritability estimates exceeded 69%. Thus, the heritability of the TEOAEs in neonates may be larger than the previous estimate of about 75% based on young adult twin's TEOAEs. Further study in neonates with DNA sampling for zygosity would be of value, to confirm whether TEOAEs are partially or fully inherited.

Author statement

All authors contributed to the study design, analysis, and interpretation of data, as well as drafting the article, and the approval of the final manuscript. Erik Berning and Åke Olofsson also contributed in the data collection. All data are sharable upon request.

Data statement

Data are sharable upon request.

Declaration of Competing Interest

None.

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References

Aidan, D., Lestang, P., Avan, P., Bonfils, P., 1997. Characteristics of transient-evoked otoacoustic emissions (TEOEs) in neonates. Acta Otolaryngol. 117, 25–30.
Al-Mana, D., Ceramic, B., Djanabakhch, O., Luxon, L.M., 2010. Alteration in auditory function during the ovarian cycle. Hear. Res. 268, 114–122.
Alvan, C., Berning, E., Gustafsson, L.L., Karlsson, K.K., Painaud, G., Walkelkamp, M., 2017. Concentration-response relationship of hearing impairment caused by quinine and salicylate: pharmacological similarities but different molecular mechanisms. Basic Clin. Pharmacol. Toxicol. 120, 5–12.
Beim, J.A., Oxenham, A.J., Wojtczak, M., 2018. Examining replicability of an otoacoustic measure of cochlear function during selective attention. J. Acoust. Soc. Am. 144, 2882–2895.
Beim, J.A., Oxenham, A.J., Wojtczak, M., 2019. No effects of attention or visual perceptual load on cochlear function, as measured with stimulus-frequency otoacoustic emissions. J. Acoust. Soc. Am. 146, 1475.
Berner, E., 2007. Characteristics of normal newborn transient-evoked otoacoustic emissions: ear asymmetries and sex effects. Int. J. Audiol. 46, 661–669.
Berner, E., 2014. Letter to the Editor regarding “Otoacoustic emissions in newborn hearing screening: a systematic review of the effects of different protocols on test outcomes”. Int. J. Pediatr. Otorhinolaryngol. 78, 2022.
Berner, E., Westling, B., 2011. Outcome of a universal newborn hearing-screening programme based on multiple transient-evoked otoacoustic emissions and clinical brainstem response audiometry. Acta Otolaryngol. 131, 728–739.
Berner, E., Karlsson, K.K., Alvan, G., 1998. Quinine reduces the dynamic range of the human auditory system. Acta Otolaryngol. 118, 46–51.
Berner, E., Karlsson, K.K., Hellgren, U., Eskilsson, G., 1995. Magnitude changes in transient evoked otoacoustic emissions and high-level 2f1–f2 distortion products in man during quinine administration. Scand. Audiol. 24, 27–32.
