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Differential actions of isoflurane and ketamine-based anaesthetics on cochlear function in the mouse

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Abstract

Isoflurane is a volatile inhaled anaesthetic widely used in animal research, with particular utility for hearing research. Isoflurane has been shown to blunt hearing sensitivity compared with the awake state, but little is known about how isoflurane compares with other anaesthetics with regard to hair cell transduction and auditory neurotransmission. The current study was undertaken in C57Bl/6J and C129/SvEv strains of mice to determine whether isoflurane anaesthesia affects hearing function relative to ketamine-based anaesthesia. Cochlear function and central auditory transmission were assessed using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE), comparing thresholds and input/output functions over time, for isoflurane vs. ketamine/xylazine/acepromazine anaesthesia. ABR thresholds at the most sensitive region of hearing (16 kHz) were initially higher under isoflurane anaesthesia. This reduced hearing sensitivity worsened over the 1 h study period, and also became evident with broadband click stimulus. Ketamine anaesthesia provided stable ABR thresholds. Although the growth functions were unchanged over time for both anaesthetics, the slopes under isoflurane anaesthesia were significantly less. Cubic ($2f_1-f_2$) DPOAE thresholds and growth functions were initially similar for both anaesthetics. After 60 min, DPOAE thresholds increased for both groups, but this effect was significantly greater with ketamine anaesthesia.

The isoflurane-mediated increase in ABR thresholds over time is attributable to action on cochlear nerve activation, evident as a right-shift in the P1-N1 input/output function compared to K/X/A. The ketamine-based anaesthetic produced stable ABR thresholds and gain over time, despite a right-shift in the outer hair cell – mediated DPOAE input/output function.

1. Introduction

General anaesthetics have been in use for over a century and a half, with chloroform and ether being initial agents of choice. Halothane became popular in the 1970's and subsequently the less toxic isoflurane has been adopted. Despite the long-use of general anaesthetics, the exact molecular mechanisms of their action are still largely unknown. In 1984, Franks and Lieb (1984) showed that general anaesthetics bind to specific non-lipid

receptors in the cell. Since then, several studies have shown that general anaesthetics affect neuronal function (Dilger et al., 1994; Hall et al., 1994; Martin et al., 1995; Moody et al., 1993; Yamashita et al., 2005), via action on ligand-gated ion channels (LGICs), voltage-gated ion channels, enzymes and carrier proteins (Chau, 2010; Yamakura et al., 2001b).

Isoflurane is a volatile inhaled anaesthetic and has become popular in animal research for both ethical and experimental reasons. This is largely due to ease of administration, consistency in the level of anaesthesia and the relatively fast induction and better recovery. Several studies have investigated the effect of isoflurane and other anaesthetic agents on hearing sensitivity (Chung et al., 2007; Drexler et al., 2004; Kim et al., 2005; Santarelli et al., 2003; Stronks et al., 2010); however, comparison has largely been between anaesthetised animals and awake animals, rather than considering different anaesthetics. Moreover, these studies analysed ABR amplitudes and latencies while disregarding ABR thresholds. Recently, Ruebhausen and colleagues published data comparing the effects of isoflurane and ketamine/xylazine anaesthesia on ABR thresholds in rats (Ruebhausen et al., 2012). They found that isoflurane anaesthesia substantially increases ABR hearing thresholds compared to ketamine/xylazine, reflecting a selective isoflurane-induced decrease in hearing sensitivity.

Ketamine anaesthesia is also widely used in animal research and is usually administered along with xylazine. An advantage of using ketamine as an anaesthetic for hearing research is that it confers low ABR thresholds (retained hearing sensitivity) that is stable over time (Smith and Mills, 1989). However, the anaesthetic action is short-lived and the animal orientation may therefore be disturbed with repeated intraperitoneal (i.p.) injections. Therefore, for prolonged analysis of auditory function in small rodent models, inhalational anaesthesia is desirable.

In a previous, as yet unpublished, study of hearing in mice, we observed that C57Bl/6J mice showed a significant increase in their ABR thresholds over a period of several hours whilst under isoflurane anaesthesia. We eliminated factors such as hypoxia, dehydration and altered temperature using pulse oxymetry, normal saline administration and temperature monitoring. Therefore, the loss of hearing sensitivity over time was most likely due to the action of isoflurane anaesthesia itself. Review of the literature suggests that investigation of the action of isoflurane anaesthesia on ABR thresholds is limited to the report by Ruebhausen et al. (2012), which showed that in rats, ABR thresholds under isoflurane anaesthesia were on average more than 27 dB greater across a broad frequency range, compared to ketamine/xylazine anaesthesia.

Since isoflurane anaesthesia is widely used in hearing research (Kim et al., 2005; Santarelli et al., 2003), it is important to confirm the extent to which isoflurane affects hearing sensitivity. We investigated this in a mouse model, comparing ABR and DPOAE indices of hearing function over time against i.p. administered ketamine/xylazine/acepromazine anaesthesia.

2. Methods

2.1. Animals

Measurements were performed on two different strains of mice: C57Bl/6J and C129/SvEv (8–16 weeks) in a sound attenuating chamber (Sonora Technology, Japan). ABR and DPOAE measurements in response to sound stimuli were taken as soon as the mouse was anaesthetised (t_0) and then approximately 1 h later (t_{60}). Animals used in this study and experimental procedures, were approved by the UNSW Animal Care and Ethics Committee.

Mice were anaesthetised with either isoflurane (4% induction, then 1–1.5% maintenance with O₂), or i.p. injections of a K/X/A anaesthetic cocktail containing ketamine (40 mg/kg), xylazine (8 mg/kg) and acepromazine (0.5 mg/kg) (Barkdull et al., 2007). Supplemental doses of the K/X/A cocktail were given as necessary throughout the experiments (typically after 30 min). Mice under K/X/A anaesthesia were also on inhalational O₂ to control for the O₂ delivered with the isoflurane anaesthesia, and to provide optimum O₂ saturation. All mice were routinely hydrated with 0.9% saline during the experiments (approximately every 20 min) to prevent dehydration. Body temperature was maintained using a heating pad, and monitored using a rectal probe. Once anaesthetised, ophthalmic ointment was applied to the eyes of the animal to prevent corneal drying. For ABR recording, sub-dermal needle electrodes were inserted at head vertex and under the pinna, with the reference electrode inserted close to the right hind limb. The cardio-pulmonary status of the animals, and the depth of anaesthesia, was monitored using an oxymeter (MouseOx[®], STARR Life Sciences). Experiments were alternated between isoflurane and K/X/A anaesthetics.

2.2. Auditory brainstem response

ABR potentials were evoked with digitally produced 5 ms tone pips (0.5 ms rise/fall time, delivered at 10/sec) using an electrostatic speaker (EC-1, Tucker-Davis-Technologies, Ft Lauderdale, FL, USA (TDT)). Signal recordings (10 ms duration) in response to broadband click (100 μ s) or tone pip (8, 16 and 24 kHz) sound stimuli were amplified, filtered and averaged 512 times using the TDT systems III evoked potential workstation. For each sound stimulus, sound pressure levels (SPL) were decreased in 5 dB steps, starting from 70 dB, down to 10 dB below the threshold level. The intensity where an ABR waveform still could be visually detected above the noise floor (100 nV) was defined as the ABR threshold – typically around 200 nV. All response values (thresholds, peak-to-peak amplitudes and latencies) were determined by off-line analysis. Speaker calibration was carried out using a calibrating microphone (Aco Pacific, Belmont, CA, USA), and SigCalRP and RPDvsEx software (TDT).

2.3. Distortion product otoacoustic emissions

The cubic distortion product otoacoustic emissions (DPOAE at $2f_1-f_2$) were recorded using a custom-made ear canal probe containing a microphone (ER-B10+, Etymotic Research, IL, USA). Two EC1 speakers delivered the primary tones (f_1 and f_2 ; f_2/f_1 ratio = 1.25) about 8, 12, 16 and 24 kHz (168 ms duration, 6/sec from 0 to 70 dB in 5 dB increments). The f_1 and f_2 amplitudes were equivalent. The auditory canal signals were analysed by fast Fourier transformation with 50 traces per sound level being averaged. DPOAE threshold was defined as the lowest sound level producing a cubic DPOAE with an amplitude of 5 dB above the noise floor.

2.4. Data analysis

Results are presented as the mean $\pm$ S.E.M., and statistical analysis was performed using two-way repeated measures Analysis of Variance on Ranks (Holm–Sidak method) (Sigmaplot[®], Systat Software Inc.). Significance was determined as $p < 0.05$.

3. Results

3.1. Baseline ABR thresholds are significantly higher with isoflurane anaesthesia

Experiments employed application of a single anaesthetic condition (isoflurane or K/X/A) to separate groups of mice. We tested the ABR baseline threshold of C129/SvEv mice using broadband click as well as 8, 16 and 24 kHz pure tone sound stimuli. The baseline hearing thresholds at 16 kHz, corresponding to the most sensitive region of hearing, were

significantly greater ($p = 0.003$) for the group anaesthetised with isoflurane (16.4 ± 2.5 dB, $n = 9$) than for the group anaesthetised with K/X/A (0.0 ± 1.7 dB, $n = 5$) (Fig. 1A; Table 1).

A similar result was noted for the C57Bl/6J mice (21.0 ± 3.0 dB for isoflurane, $n = 7$, vs. 6.7 ± 1.8 dB for K/X/A anaesthesia, $n = 6$, at 16 kHz; $p < 0.001$) (Fig. 1B; Table 1). In later experiments, where the effect of isoflurane and K/X/A anaesthetics on the DPOAE was investigated, we confirmed this differential (13.9 ± 1.3 dB for isoflurane, $n = 7$, vs. 7.8 ± 1.3 dB for K/X/A, $n = 9$, at 16 kHz; $p = 0.002$) (data not shown). An unpaired t -test demonstrated comparable ABR thresholds for the two replicates of isoflurane and K/X/A anaesthesia ($p = 0.095$ and $p = 0.612$, respectively).

3.2. Hearing sensitivity decreases over time with isoflurane

After initial testing of baseline ABR thresholds (t_0) we kept the C57Bl/6J mice anaesthetised for a total of 60 min from induction (t_{60}), with either isoflurane or K/X/A, and repeated the hearing threshold measurements. With isoflurane, we observed a significant increase in threshold over time (Fig. 2A; Table 1); ABR threshold shifts were 15.3 ± 2.3 dB ($p < 0.001$) and 11.9 ± 1.6 dB ($p = 0.007$) for click and 16 kHz, respectively (Fig. 2B; Table 1). Mice anaesthetised with K/X/A had very stable ABR thresholds over this time period. There was no significant difference between ABR thresholds measured at t_0 versus t_{60} (Fig. 2; Table 1).

These results confirm that isoflurane anaesthesia causes an increase in hearing threshold, and hence a reduction in hearing sensitivity, over time.

3.3. Nerve fibre recruitment is affected by isoflurane

By analysing the peak amplitudes and latencies of the ABR waveforms, effects on the recruitment and conduction of nerve fibres can be determined (Legatt, 2002). This allowed us to consider the site of action of isoflurane along the auditory pathway.

Measuring the amplitudes of ABR waveforms P1-N1, N1-P2 and P2-N2 (from 30 dB to 50 dB SPL; click and 16 kHz stimuli) revealed that there were no significant reductions in the growth function (measuring the increased cochlear nerve recruitment with increasing stimulating sound intensity) between t_0 and t_{60} in response to a click stimulus for either anaesthetic (Fig. 3A, C and E; Table 2). However, significant differences were observed in the slope of the input/output functions between isoflurane and K/X/A anaesthetics ($p < 0.05$). In contrast, at 16 kHz, the slopes of the growth functions were comparable for isoflurane and K/X/A anaesthesia; however, a right-shift was evident in the t_{60} isoflurane data due to the increased threshold ($p < 0.001$; Fig. 3B, D and F; Table 3).

Peak latencies did not change over time, for either isoflurane or K/X/A (data not shown). This suggests that the cochlear nerve fibre conduction rate is similar in animals anaesthetised with either K/X/A or isoflurane. However, the right-shift in the P1-N1 input/output function for isoflurane over time implies a decrease in nerve fibre recruitment at the level of the cochlea.

3.4. DPOAE is affected by both isoflurane and ketamine in C57Bl/6J mice

DPOAE growth function analysis confirmed anaesthesia action on outer hair cell function over time. This was more evident for K/X/A anaesthesia than isoflurane.

DPOAEs about 8, 12, 16, and 24 kHz sound stimuli were measured. Initial DPOAE thresholds were indistinguishable between isoflurane and K/X/A (Fig. 4A). Unlike the findings for ABR, significant increases in DPOAE thresholds were observed at t_{60} for both isoflurane and K/X/A anaesthesia at 8, 12, and 16 kHz (Fig. 4A; Table 4). However, K/X/A

anaesthesia produced a significantly greater threshold shift (8–24 kHz; $p = 0.047$; ranked two-way ANOVA) than isoflurane (Fig. 4B). With regard to the input/output functions for DPOAE, the slopes for the two anaesthetics (threshold to 70 dB sound stimulus) remained stable and indistinguishable over time, but the threshold shifts were reflected in right-shifts at t_{60} , particularly for K/X/A anaesthesia ($p < 0.001$; 8, 12, 16 and 24 kHz); lesser shifts, were seen for isoflurane anaesthesia (8 and 12 kHz; $p < 0.001$; Fig. 5; Table 4).

4. Discussion

In this study we have shown that the baseline hearing thresholds obtained under isoflurane anaesthesia are greater compared to those measured under ketamine/xylazine/acepromazine anaesthesia in two mouse strains. The effect was primarily seen in the most sensitive region of mouse hearing (16 kHz). We further show that this decreased hearing sensitivity is more pronounced with longer anaesthesia. Our results demonstrate some resemblance to recently published work on Long-Evans rats, where Ruebhausen et al. (2012) show data indicating a broad elevation in ABR thresholds of 18–48 dB (SPL) between 8 and 32 kHz with isoflurane, with loss of differential hearing sensitivity in the 8–16 kHz range evident in these rats with ketamine anaesthesia. In addition, Alvarado et al. (2012) show ABR data from adult Wistar rats under isoflurane anaesthesia that also lacks the frequency-specific reduction in thresholds around 16 kHz. In the latter case, the thresholds were considerably lower than those shown in the Ruebhausen et al. (2012) study, associated with threshold discrimination voltages similar to those in our study (~200 nV).

In the current study in mice, the overall difference across the frequency range for ABR thresholds recorded under isoflurane vs. K/X/A is considerably less than the differential reported for rats by Ruebhausen et al. (2012) (maximum of 15 dB (average) at 16 kHz). This is likely to be a species difference rather than age-related, or due to differences in levels of anaesthesia. The mice in our study were 8–16 weeks old, compared to the 5–6 months old rats in the study by Ruebhausen et al. (2012) and “adult” rats used by Alvarado et al. (2012). Both mice and rats in this age band should have nominal hearing. We used a higher concentration of ketamine (40 mg/ml vs. 25 mg/ml) and xylazine (8 mg/ml vs. 3 mg/ml) compared with the Ruebhausen et al. (2012) study, but the baseline thresholds we report for the two strains of mice are lower across the frequency range tested. All three studies used comparable isoflurane levels.

Our study in mice has also shown, for the first time, that K/X/A anaesthesia has a greater effect on DPOAE sensitivity over time than isoflurane. Our analysis of the ABR waveform demonstrated that conduction in the cochlear nerve and auditory brainstem pathways was unaffected (i.e. no change in peak latencies) in mice under either isoflurane or K/X/A anaesthesia. Nerve recruitment was also unaffected, as demonstrated by the comparable slopes of the input/output functions at t_0 and t_{60} for both anaesthetics. Taken with the shift in threshold over time with isoflurane, this suggests that isoflurane may hyperpolarize the cochlear spiral ganglion neurons, thereby increasing the level of neurotransmission from the inner hair cells required to elicit action potentials.

DPOAE measurements are a very useful tool to quantify the contribution of the ‘cochlear amplifier’, mediated by outer hair cell electromotility (Ashmore, 2008; Kemp, 2002), which is a principal factor driving inner hair cell sound transduction. Mice under K/X/A anaesthesia showed a much greater increase in DPOAE thresholds over time than the isoflurane group. This direct action of K/X/A on the ‘cochlear amplifier’ must apparently be compensated, since the output from the cochlea was stable over time (i.e. ABR thresholds were unchanged) for K/X/A. This disconnect between DPOAE and ABR with K/X/A anaesthesia may reflect an alteration in synchrony or some other aspect of neural/synaptic

function. In contrast, the effect of isoflurane anaesthesia on the ‘cochlear amplifier’ is minor, but there is pronounced attenuation of cochlear neural output.

What could the actions of these anaesthetics on cochlear function involve? It has been known for a long time that general anaesthetics have different effects on neuronal proteins. *In vitro* studies have shown that isoflurane potentiates glycine receptor (GlyR) currents (Downie et al., 1996; Harrison et al., 1993; Mascia et al., 1996), the GABA receptor (GABAR) (Harrison et al., 1993; Lin et al., 1993; Mihic et al., 1994) and the glutamate-selective kainate receptor (Dildy-Mayfield et al., 1996; Minami et al., 1998), while inhibiting the nicotinic acetylcholine receptor (nAChR) (Forman and Raines, 1998). Studies have also demonstrated that isoflurane has an effect on the N-methyl-D-aspartate (NMDA) receptor (de Sousa et al., 2000; Franks, 2008; Ishizaki et al., 1995; Kuroda et al., 1993; Martin et al., 1995). On the other hand, ketamine does not affect the GlyR or the GABAR (Yamakura et al., 2000, 2001a) and only has a small inhibitory effect on the nAChR (Wachtel and Wegrzynowicz, 1992). Ketamine’s greatest effect is on the NMDA receptor, by acting as a non-competitive antagonist (Hirota and Lambert, 1996, 2011; Yamakura et al., 1993). All these neurotransmitter systems are present in the mammalian cochlea and are important in hearing function. There is also more recent evidence that isoflurane inhibits neurotransmitter release (Herring et al., 2009) and interacts with voltage-gated Na^+ and Ca^{2+} channels, as well as presynaptic SNARE proteins (Hemmings, 2009; Westphalen et al., 2011). As discussed by Ruebhausen et al. (2012), the effects of isoflurane and ketamine on blood flow could also be a contributing factor. While isoflurane increases blood flow to the brainstem (Boarini et al., 1984), ketamine decreases blood flow to the inferior colliculus (Lo et al., 1991). With regard to the K/X/A anaesthetic, we cannot rule out the potential contribution of xylazine, an α_2 adrenergic receptor agonist with sedative, anaesthetic, muscle relaxing and analgesic effects (Greene and Thurmon, 1988); and acepromazine, a sedative and neuroleptic agent (Baldwin, 1959). The isoflurane-mediated inhibition of cochlear neurotransmission may be associated with altered GABAergic pre-synaptic inhibition via lateral olivary complex (LOC) neuronal efferent feedback, but other transmitter and voltage-gated ion channel mechanisms are also candidates, as suggested above. Anaesthetic interactions can be significant. For example, in the gerbil, evoked metabolic activity in the central auditory pathway was virtually unaffected by ketamine anaesthesia when compared to no anaesthesia, but ketamine enhanced an increase in evoked activity produced by pentobarbital (Wang et al., 1987). Furthermore, gaseous anaesthesia has been shown to result in immediate and gradually increasing positive pressure in the middle-ear cavity in humans (Acar et al., 2010; Karabiyik et al., 1996). This could lead to a change of the middle-ear transfer function, and thus contribute to the reported shift with isoflurane anaesthesia. However, this is unlikely in our study since there were minimal shifts in DPOAE in animals under isoflurane anaesthesia.

Despite these considerations, the observation that there was a frequency-selective differential isoflurane inhibition of cochlear function (e.g. compare the disproportionately greater effect on 16 kHz vs. 24 kHz ABR thresholds; Fig. 1A) warrants comment. The factors conferring greatest sensitivity of the mouse cochlea within the 8–16 kHz range (Zheng et al., 1999), likely also suffer impact from the isoflurane. These targets may be related to the coupling of outer hair cell electromotility to inner hair cell transduction in this mid-cochlea region. It is unlikely that outer hair cell electromotility is itself differentially affected because the growth functions for the DPOAE at 8–24 kHz for isoflurane anaesthesia both immediately, and after 1 h, are consistently better than those for ketamine anaesthesia. The differential isoflurane action may therefore lie with the inner hair cell – type I spiral ganglion synapses, which in this mid-cochlea region, have broader sensitivity in association with larger sized modiolar/basal CtBP2-labelled pre-synaptic ribbons, relative to

the pillar/apical ribbons; corresponding to variance in low – medium and high spontaneous firing rate fibres (Liberman et al., 2011).

Overall, our data show that both isoflurane and ketamine anaesthetics affect cochlear transduction and transmission over time, but, the inhibitory effect on hearing sensitivity of ketamine is less pronounced and potentially more compartmentalized (viz outer hair cells) than the inhibitory effect of isoflurane. This effect may contribute to the reduction in noise-induced hearing loss that has been reported with isoflurane anaesthesia (Chung et al., 2007; Kim et al., 2005). This study has been undertaken in two quite genetically divergent strains of mice – C57Bl/6J and C129/SvEv. These two strains are widely used for knockout/transgenic studies. The C57Bl/6J strain is genetically distinct from other inbred strains: in contrast to 36 other inbred strains, C57 has a Y chromosome of Asian *Mus musculus* origin (Tucker et al., 1992) and a higher proportion of its genome from *Mus spretus* (Rikke et al., 1995). It is therefore highly likely that these findings will translate broadly across the more than 80 strains of mice evaluated for hearing function (Zheng et al., 1999).

5. Conclusion

Isoflurane anaesthesia increased ABR hearing thresholds in two mouse strains over time, reflecting diminution of hearing sensitivity. This inhibitory action was principally due to an effect on cochlear nerve activation. Ketamine anaesthesia resulted in maintained auditory nerve output sensitivity, but this masked an underlying increase in DPOAE thresholds, reflecting an effect on the outer hair cell – mediated ‘cochlear amplifier’.

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Fig. 1.

ABR baseline hearing thresholds. Isoflurane caused decreased hearing sensitivity (higher thresholds) in the most sensitive hearing region (16 kHz) in both C129/SvEv mice (A) and C57Bl/6J mice (B) compared to K/X/A, when assessed immediately after induction of anaesthesia. Data presented as mean $\pm$ S.E.M. (C57Bl/6J: $n = 6$ K/X/A, $n = 7$ isoflurane; C129/SvEv: $n = 5$ K/X/A, $n = 9$ isoflurane). ** $p < 0.01$, *** $p < 0.001$.

Fig. 2.

ABR thresholds in C57Bl/6J mice at baseline (t_0) vs. 60 min (t_{60}) of anaesthesia. (A) Isoflurane caused decreased hearing sensitivity (higher thresholds) over time for both clicks and 16 kHz tone pips. Data presented as box plots showing mean (dashed lines), median (solid lines), 95% confidence intervals (error bars) and individual data points (filled circles). (B) ABR threshold shifts for isoflurane and K/X/A (click and 16 kHz). Note the larger shifts for isoflurane anaesthesia. Data presented as mean $\pm$ S.E.M. ($n = 6$ K/X/A, $n = 7$ isoflurane). *** $p < 0.001$.

Fig. 3. ABR growth functions of P1-N1, N1-P2, P2-N2 amplitudes for click (A, C and E), and 16 kHz (B, D, and F) (see Tables 2 and 3 for regression data). Note the right-shift for isoflurane anaesthesia at t_{60} , and also the shallower slopes for the isoflurane data with click stimulus. Data presented as mean $\pm$ S.E.M. ($n = 6$ K/X/A, $n = 7$ isoflurane).

Fig. 4.

(A) DPOAE thresholds at baseline (t_0) vs. 60 min (t_{60}) of isoflurane and K/X/A anaesthetics for 8, 12, 16 and 24 kHz sound stimuli. Note there is no difference between isoflurane and K/X/A for baseline DPOAE thresholds (see Table 4 for threshold and regression data). Data presented as box plots showing mean (dashed lines), median (solid lines), 95% confidence intervals (error bars) and individual data points (filled circles). (B) DPOAE threshold shifts for isoflurane and K/X/A. Note the significantly larger shifts for K/X/A anaesthesia (ranked two-way ANOVA). Data presented as mean $\pm$ S.E.M. ($n = 9$ K/X/A, $n = 8$ isoflurane). ** $p < 0.01$, *** $p < 0.001$.

Fig. 5. DPOAE growth functions for isoflurane and K/X/A anaesthetics at t_0 and t_{60} (see Table 4 for regression data). Note the larger right-shifts for K/X/A anaesthesia at t_{60} . Data presented as mean $\pm$ S.E.M. ($n = 9$ K/X/A, $n = 8$ isoflurane).

ABR hearing thresholds at click and 8, 16 and 24 kHz for C129/SvEv and C57Bl/6J mice under isoflurane (I) or ketamine-based (K) anaesthesia at baseline (t_0) and 60 min (t_{60}). Data presented as mean $\pm$ SEM (C57Bl/6J: $n = 6$ K/X/A, $n = 7$ isoflurane; C129/SvEv: $n = 5$ K/X/A, $n = 9$ isoflurane).

Table 1

Sound stimulus	Anaesthetic (C129/SvEv)		Anaesthetic (C57Bl/6J)		Δ I	Δ K			
	I t_0	K t_0	I t_0	K t_0					
Click	31.7 $\pm$ 1.9	23.5 $\pm$ 0.9	24.3 $\pm$ 2.0	19.2 $\pm$ 4.4	39.6 $\pm$ 2.1	21.2 $\pm$ 4.1	15.3 $\pm$ 2.3	***	2.0 $\pm$ 1.0 n.s.
8 kHz	16.1 $\pm$ 2.6	6.5 $\pm$ 3.7	—	—	—	—	—	—	—
16 kHz	16.4 $\pm$ 2.5	0.0 $\pm$ 1.7	21.0 $\pm$ 3.0	6.7 $\pm$ 1.8	32.9 $\pm$ 4.0	6.3 $\pm$ 1.9	11.9 $\pm$ 1.6	**	−0.4 $\pm$ 0.8 n.s.
24 kHz	22.2 $\pm$ 3.1	24.5 $\pm$ 2.8	—	—	—	—	—	—	—

*** $p < 0.01$,
*** $p < 0.001$, n.s. = not significant; Significance calculated on ABR thresholds using two-way repeated measures ANOVA on Ranks (Holm-Sidak method).

ABR growth functions (slope and correlation coefficient, R) at click for C57Bl/6J mice under isoflurane (I) and ketamine-based (K) anaesthesia at baseline (t_0) and 60 min (t_{60}). Data presented as mean $\pm$ SEM ($n = 6$ K/X/A; $n = 7$ isoflurane).

Table 2

Sound stimulus/peak Anaesthetic/time point	Click P1-N1			Click N1-P2			Click P2-N2		
	I t_0	I t_{60}	K t_0	K t_{60}	I t_0	I t_{60}	I t_0	I t_{60}	K t_{60}
Slope (μ V/dB)	0.065 $\pm$ 0.014	0.064 $\pm$ 0.014	0.121 $\pm$ 0.016	0.156 $\pm$ 0.015	0.067 $\pm$ 0.016	0.076 $\pm$ 0.016	0.122 $\pm$ 0.016	0.150 $\pm$ 0.017	0.088 $\pm$ 0.007
Δ slope	-0.010 $\pm$ 0.012 n.s.		0.035 $\pm$ 0.018 n.s.		0.009 $\pm$ 0.019 n.s.		0.028 $\pm$ 0.022 n.s.	0.010 $\pm$ 0.024 n.s.	0.014 $\pm$ 0.009 n.s.
R	0.944 $\pm$ 0.026	0.932 $\pm$ 0.037	0.949 $\pm$ 0.023	0.993 $\pm$ 0.002	0.867 $\pm$ 0.071	0.887 $\pm$ 0.049	0.929 $\pm$ 0.035	0.990 $\pm$ 0.005	0.906 $\pm$ 0.040

n.s. = not significant; Significance calculated on ABR growth function slopes using two-way ANOVA on Ranks (Holm–Sidak method).

ABR growth functions (slope and correlation coefficient, R) at 16 kHz for C57Bl/6J mice under isoflurane (I) and ketamine-based (K) anaesthesia at baseline (t_0) and 60 min (t_{60}). Data presented as mean $\pm$ SEM ($n = 6$ K/X/A; $n = 7$ isoflurane).

Table 3

Sound stimulus/peak Anaesthetic/time point	16 kHz P1-N1				16 kHz N1-P2				16 kHz P2-N2			
	I t_0	I t_{60}	K t_0	K t_{60}	I t_0	I t_{60}	K t_0	K t_{60}	I t_0	I t_{60}	K t_0	K t_{60}
Slope (μ V/dB)	0.038 $\pm$ 0.007	0.026 $\pm$ 0.013	0.026 $\pm$ 0.003	0.034 $\pm$ 0.006	0.042 $\pm$ 0.008	0.041 $\pm$ 0.010	0.038 $\pm$ 0.004	0.048 $\pm$ 0.009	0.027 $\pm$ 0.007	0.039 $\pm$ 0.008	0.039 $\pm$ 0.002	0.034 $\pm$ 0.003
Δ slope	-0.012 $\pm$ 0.018 n.s.		0.008 $\pm$ 0.005 n.s.		-0.001 $\pm$ 0.014 n.s.		0.010 $\pm$ 0.007 n.s.		0.012 $\pm$ 0.006 n.s.		-0.005 $\pm$ 0.002 n.s.	
R	0.876 $\pm$ 0.039	0.880 $\pm$ 0.070	0.788 $\pm$ 0.086	0.848 $\pm$ 0.082	0.911 $\pm$ 0.029	0.993 $\pm$ 0.004	0.944 $\pm$ 0.030	0.914 $\pm$ 0.058	0.837 $\pm$ 0.047	0.955 $\pm$ 0.025	0.906 $\pm$ 0.014	0.943 $\pm$ 0.035

n.s. = not significant; Significance calculated on ABR growth function slopes using two-way ANOVA.

DPOAE thresholds and growth functions (slope and correlation coefficient, R) at 8, 12, 16 and 24 kHz for C57Bl/6J mice under isoflurane (I) and ketamine-based (K) anaesthesia at baseline (t_0) and 60 min (t_{60}). Data presented as mean $\pm$ SEM ($n = 9$ K/X/A; $n = 8$ isoflurane).

Table 4

Sound Intensity Anaesthetic/time point	8 kHz			12 kHz			16 kHz			24 kHz		
	I t_0	I t_{60}	K t_0	I t_0	I t_{60}	K t_0	I t_0	I t_{60}	K t_0	I t_0	I t_{60}	K t_{60}
Threshold (dB)	16.3 $\pm$ 1.8	24.4 $\pm$ 3.2	15.0 $\pm$ 1.9	16.3 $\pm$ 1.6	24.4 $\pm$ 2.6	17.8 $\pm$ 1.7	28.3 $\pm$ 4.1	20.6 $\pm$ 1.8	15.6 $\pm$ 1.8	38.1 $\pm$ 5.7	43.1 $\pm$ 5.1	38.9 $\pm$ 1.8
Δ threshold	8.1 $\pm$ 2.3 **		17.2 $\pm$ 4.9 ***	8.1 $\pm$ 1.9 ***		10.6 $\pm$ 3.3 **		5.6 $\pm$ 1.1 **	13.3 $\pm$ 3.5 ***	5.0 $\pm$ 1.6 n.s.		5.6 $\pm$ 2.6 n.s.
Slope (dB/dB)	0.921 $\pm$ 0.034	1.140 $\pm$ 0.051	0.944 $\pm$ 0.033	0.902 $\pm$ 0.032	0.903 $\pm$ 0.031	0.955 $\pm$ 0.033	0.828 $\pm$ 0.051	0.950 $\pm$ 0.028	0.934 $\pm$ 0.031	0.900 $\pm$ 0.054	0.889 $\pm$ 0.048	0.990 $\pm$ 0.065
Δ slope	0.219 $\pm$ 0.056 n.s.		0.361 $\pm$ 0.143 n.s.	0.001 $\pm$ 0.031 n.s.		-0.127 $\pm$ 0.078 n.s.		0.067 $\pm$ 0.034 n.s.	-0.112 $\pm$ 0.062 n.s.	-0.011 $\pm$ 0.078 n.s.		0.013 $\pm$ 0.145 n.s.
R	0.986 $\pm$ 0.003	0.948 $\pm$ 0.023	0.983 $\pm$ 0.002	0.981 $\pm$ 0.003	0.975 $\pm$ 0.009	0.976 $\pm$ 0.007	0.963 $\pm$ 0.011	0.966 $\pm$ 0.005	0.981 $\pm$ 0.004	0.943 $\pm$ 0.016	0.948 $\pm$ 0.013	0.964 $\pm$ 0.005

**
 $p < 0.01$,

 $p < 0.001$, n.s. = not significant; Significance calculated on DPOAE thresholds and growth function slopes using two-way repeated measures ANOVA on Ranks (Holm–Sidak method).