Isoflurane anaesthesia impacts on mouse hearing thresholds

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Isoflurane has become more popular as an anaesthetic of choice 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 faster induction and recovery times. There have been previous studies investigating the effect of isoflurane and other anaesthetics on hearing thresholds (Chung *et al.*, 2007; Kim *et al.*, 2005; Stronks *et al.*, 2010); however these have mainly compared anaesthetised animals with awake animals rather than between different anaesthetics.

We therefore decided to compare mouse auditory brainstem response (ABR) hearing thresholds using isoflurane *versus* ketamine/xylazine/acepromazine (k/x/a) in two mouse strains (C129/SvEv and C57Bl/6J; 8 - 16 weeks old). Click ABR as well as pure tonepip 8, 16 and 24 kHz ABRs (5 ms duration; 10/s, averaged up to 512 times; 5 dB decrements) were carried out in mice anaesthetized by inspiration of isoflurane/O_2 (0.8 - 1.2%) or i.p ketamine(40mg/kg)/xylazine(8mg/kg)/acepromazine(0.5mg/kg). Animals were anaesthetized for 1 - 2 h and heart rate and O_2 saturation were measured using a pulsed oxymeter (MouseOx, STARR Life Sciences Corp.). All statistics were performed using a two-way ANOVA test with Holm-Sidak pair wise multiple comparisons. All experiments were approved by the UNSW Animal Care and Ethics Committee. Study one used C129/SvEv mice and compared baseline ABR thresholds between isoflurane and k/x/a anaesthetics immediately after induction. We found significantly higher baseline ABR thresholds in isoflurane anaesthetized mice compared to k/x/a anaesthetized mice (P<0.001). This anaesthetic effect was particularly due to differentials in the thresholds seen with pure tonepip ABR. Under isoflurane anaesthesia 16.1 ± 2.5 dB and 16.4 ± 2.4 dB (n = 9) thresholds were observed at 8 and 16 kHz compared to lower baseline thresholds of 6.5 ± 5.0 dB and 0.0 ± 2.2 dB (n = 5) for k/x/a at these frequencies.

Having seen a significant difference between anaesthetics on baseline thresholds, we went on to assess ABR threshold stability under isoflurane and k/x/a anaesthesia over time in C57Bl/6J mice. Analysis of baseline data replicated the observed elevation in tonepip thresholds with isoflurane anaesthesia (16 kHz, P = 0.02). Retesting after approximately 1hr of anaesthesia, showed significantly increased thresholds with isoflurane compared with k/x/a (P<0.001) (e.g. click threshold increased from 26 ± 2.9 dB to 43 ± 4.2 dB, n = 5, cf 20.6 ± 6.5 dB to 21.8 ± 6.0 dB for k/x/a, n = 4).

In summary, while isoflurane gave a very stable level of anaesthesia, baseline hearing sensitivity was reduced and underwent progressive loss over time, whereas hearing was stable with k/x/a anaesthesia for the period of assessment. One potential mode of action for isoflurane may be to reduce spiral ganglion neuron excitability *via* inhibition of sodium conductance (Shiraishi & Harris, 2004). As such, this may underlie the reported protection by isoflurane against noise-induced hearing loss (Kim *et al.*, 2005).

Chung J.W., Ahn J.H., Kim J.Y., Lee H.J., Kang H.H., Lee Y.K., Kim J.U., Koo S.W. (2007). *Anesthesia and Analgesia* **104(6):** 1404-8.

Kim J.U., Lee H.J., Kang H.H., Shin J.W., Ku S.W., Ahn J.H., Kim Y.J., Chung J.W. (2005). *The Laryngoscope* **115:** 1996-99.

Shiraishi M., Harris R.A. (2004). *The Journal of Pharmacology and Experimental Therapeutics* **309:** 987-994.

Stronks H.C., Aarts M.C.J., Klis S.F.L. (2010). *Hearing Research* **260:** 20-9.