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RESEARCH ARTICLE

Development of vocalization and hearing in American mink (*Neovison vison*)

Christian Brandt[1,*], Jens Malmkvist[2], Rasmus L. Nielsen[1], Nanna Brande-Lavridsen[1] and Annemarie Surlykke[1]

[1]Institute of Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark and [2]Department of Animal Science, Aarhus University, PO Box 50, DK-8830 Tjele, Denmark

*Author for correspondence at present address: Institute of Neuroscience, Henry Wellcome Building, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK (chrbrandt@gmail.com)

SUMMARY

American mink (*Neovison vison*) kits are born altricial and fully dependent on maternal care, for which the kits' vocalizations appear essential. We used auditory brainstem responses (ABRs) to determine: (1) hearing sensitivity of adult females from two breeding lines known to differ in maternal behaviour and (2) development of hearing in kits 8–52 days of age. We also studied sound production in 20 kits throughout postnatal days 1 to 44. Adult female mink had a broad hearing range from 1 kHz to above 70 kHz, with peak sensitivity (threshold of 20 dB SPL) at 8–10 kHz, and no difference in sensitivity between the two breeding lines ($P>0.22$) to explain the difference in maternal care. Mink kits showed no signs of hearing up to postnatal day 24. From day 30, all kits had ABRs indicative of hearing. Hearing sensitivity increased with age, but was still below the adult level at postnatal day 52. When separated from their mothers, kits vocalized loudly. Until the age of 22 days, 90% of all kits vocalized with no significant decline with age ($P=0.27$). From day 25, concurrent with the start of hearing, the number of vocalizing kits decreased with age ($P<0.001$), in particular in kits that were re-tested ($P=0.004$). Large numbers of mink are kept in fur industry farms, and our results are important to the understanding of sound communication, which is part of their natural behaviour. Our results also suggest mink as an interesting model for studying the development of mammalian hearing and its correlation to sound production.

Key words: auditory brainstem response, ABR, American mink, hearing, maternal behaviour, *Mustela vison* syn. *Neovison vison*, social communication, vocalization.

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INTRODUCTION

American mink [*Neovison vison* syn. *Mustela vison* (Schreber 1777)] is native to America, but because of mink farming, they have spread to many other countries, where they can have a significant effect on the native fauna (Bonesi and Palazon, 2007). Studies have found that mink can have a significant effect on ground-nesting birds, rodents, amphibians and mustelids. Hunting of living prey by mink involves several stages: searching, localization, recognition, chase, capture and killing, followed by eating before or after transport back to the home den (Dunstone, 1993). Mink are semiaquatic mammals that spend a significant part of their time diving if they have access to water, even during the coldest months of the year (Harrington et al., 2012).

Powell and Zielinski (Powell and Zielinski, 1989) have found that American mink can react to high-frequency sounds of up to 40 kHz, but only two animals of unknown age and sex were tested in this study so little is known about the true hearing thresholds. Apart from hunting, hearing is important for auditory communication between young kits and their mothers. The mink kits are born altricial, fully dependent on maternal care, until they gradually start to replace suckling with eating (on farms from 4 weeks of age) and water drinking (around 6 weeks). Weaning, by removal of the mother from the litter, typically takes place at 7–8 weeks of age in farmed mink. Vocalizations of kits are suggested to play a role in the maternal care and kit survival (Clausen et al., 2008). When a young mink kit is isolated from its mother, it starts vocalizing loudly and almost continuously. These 'isolation calls' consist of multi-harmonic pulses with the first harmonic around 2.4 kHz and an average duration around 500 ms. The so-called 'kit-retrieval test' makes use of this behavioural response to measure how long it takes a mother to retrieve her vocalizing kit. Clausen et al. (Clausen et al., 2008) reported that young mink kits (3–7 days postpartum) vocalize with pronounced energy in the frequency area of 40–50 kHz. However, knowledge about their sound production and hearing ability is sparse, despite the importance of kit survival in the production of farmed mink. It is, for example, unknown what part of the kits' vocalizations the mothers actually can hear and thus which part of the signal is important for communication. To unravel the acoustic communication between kit and mother, we therefore studied: (1) the hearing sensitivity of adult females combined with (2) the development in hearing and sound production in kits before weaning.

Clausen et al. (Clausen et al., 2008) reported a significant delay in maternal kit retrieval in females of the Palomino colour type. Compared with females from the black breeding line, it took them on average three times longer to react and retrieve their kit, when placed away from the nest (Clausen et al., 2008). Therefore, we studied hearing sensitivity in adult breeding females from these two breeding lines, to test whether Palomino females have reduced hearing, explaining their delayed reactions towards their kits.

For this study we used auditory brainstem responses (ABRs), which measure the electrical response from the auditory nerve when

the animal is exposed to sound. This gives us a very fast way of measuring the auditory thresholds even though the measured thresholds are 10–20 dB less sensitive than behavioural audiograms, i.e. 20 dB in humans (Gorga et al., 1988), 12 dB in killer whales (Szymanski et al., 1999) and around 16 dB in mice (Heffner and Heffner, 2003). The ABR method is very useful in this case because it does not depend on training the animals and can therefore be used on newborn kits. The method is also relatively non-invasive so we can measure repeatedly on the same animals.

MATERIALS AND METHODS

Animals

We tested the hearing of 12 adult female mink (six of the Palomino variant and six of the black variant), transferred from the research farm at Aarhus University (Tjele, Denmark) to the Institute of Biology, University of Southern Denmark. The animals were kept in conditions similar to the research farm, individually in outdoor wire cages (30×45×91 cm, width × height × length) connected to a straw-filled wooden nest box (28×20×23 cm, width × height × length; cage system from Hedensted Group, Hedensted, Denmark), with *ad libitum* access to water and food. All animals were the same age (1.5–1.6 years old), unrelated at least two generations back, and had delivered one litter during their first birth season 5–6 months earlier. The black mink were slightly larger than the Palomino (1.5±0.2g *versus* 1.2±0.2 kg), which is a normal result of the commercial breeding programme. During testing, the animals were anaesthetized by an intramuscular injection of medetomidin [Dormitor vet., Orion Corporation, Turku, Finland, 0.2 mg kg^{-1} body mass (BM)] and ketamine (Ketaminol vet., Intervet International BV, Boxmeer, The Netherlands, 10 mg kg^{-1} BM). The level of anaesthetization was checked every 15 min by pinching the skin between their toes, and an extra dose of ketamine was given when necessary. After the experiment, the animals were euthanized with pentobarbital-sodium (200 mg kg^{-1}, injected in the heart) as the animals could not be returned because of the risk of introducing pathogens to the farm.

We tested the hearing in 36 mink kits (18 males, 18 females from 18 litters of a black breeding line) born in April/May 2008 at the research farm. We tested the hearing of four mink kits at each of the ages of postnatal day 8, 16, 20, 24, 28, 32, 36, 42–43 and 52 with day 0 as date of birth. We randomly selected one male and one female from each litter, containing three to nine kits (mean 7.1±1.39) and each litter were only used once. The mean mass was 43±1.7 g for the 8-day-old kits, increasing to a mean of 438±32.2 g for the 52-day-old kits. After the ABR measurements the kits were kept warm and allowed to wake up before they were returned to their mothers. The anaesthesia (same procedure and doses as for adults, but kits were given a wake-up injection of Atipamezol; Antisedan vet., Orion Corporation, Espoo, Finland) was successful, no animals woke up during the experiments and no animals died. All kits were accepted back by the mothers after the recording, observed 0, 1 and 24 h after the return to the nest box. At the time of weaning there was no surplus mortality in litters included in the study.

The development of sound production was studied in mink kits from postnatal day 1 to day 44 (days 1, 2, 4, 6, 9, 11, 13, 16, 19, 22, 25, 28, 31, 37 and 44), with day 0 as the day of birth. Housing and testing took place at the research farm. We tested 20 kits at each age, resulting in 300 observations in total. Half of these kits (N=10 per test age, one male and one female from five different litters) were used repeatedly throughout the study, whereas the other half (N=10 per test day, one male and one female from five different litters) was selected randomly from previously untested litters in order to control for potential effects of habituation or sensitization induced by the testing procedure. All experimental kits were selected from litters of seven to nine kits from the same breeding line. We marked the individual kits with ink or fur bleaching for identification; to avoid different maternal attention or rejection towards repeatedly marked and tested kits, all litter mates within a retested litter received a similarly sized mark.

Testing hearing in adults and kits

We used ABR to determine the hearing sensitivity of adult and young mink. The electrical response from the auditory nerve and the brainstem was recorded as sound was played back to the mink. We adopted the ABR method to test sensitivity over a very broad range of frequencies, by modifying the method of Berlin et al. (Berlin et al., 1991). The principle was to use the masking effect of a narrow band noise to determine the auditory threshold. Each measurement consisted of an average of 400 recordings with broadband click (500 Hz–90 kHz, 94 dB peak-to-peak) stimuli (unmasked ABR) and 400 recordings where the same click was masked by continuous low intensity band limited (centre frequency ±1/12 octave, second-order Butterworth bandpass filter) noise (masked ABR). By subtracting the masked ABR from the unmasked ABR we achieved a derived ABR, which was normalized by dividing the signal by the peak-peak intensity of the unmasked ABR. This method is useful for testing both high and low frequencies, which normally can be a problem when recording ABRs to pure tone pulses, because the same number of oscillations has vastly different durations and energy over a frequency range of 1–80 kHz. Additionally, the unmasked ABR to the broadband click allows for easy continuous control of changes in the state of the animal or level of anaesthetization. This is different from ABRs to stimuli changing in one or more acoustic features (e.g. frequency), where a change in response can be caused by either a change in stimuli or a change in animal state (Berlin et al., 1991).

The hardware was controlled by custom-made software (QuickABR, University of Southern Denmark, Denmark). The ABR system consisted of an RM2 digital signal processor (DSP), an RX6 high-speed DSP and an RA4PA Medusa preamplifier with an RA4LI headstage (all Tucker Davis Technologies, Alachua, FL, USA). The preamplifier was connected to the RM2 by a 5 m optical connection, and ran on battery power. The RX6 processor was connected to an Avisoft portable ultrasonic power amplifier, driving a ScanSpeak ultrasound loudspeaker (Avisoft Bioacoustics, Berlin, Germany). The sound levels were measured at the animal's ear with a ¼ inch microphone (G.R.A.S. 40BF, G.R.A.S. Sound & Vibration, Holte, Denmark) connected to a G.R.A.S. power module 12AA microphone supply and calibrated with a B&K 4231 acoustical calibrator (Brüel & Kjær, Nærum, Denmark). The sample rate of the playback was limited by the RX6 to 200 kHz, limiting the maximum stimulus frequency to 70 kHz.

Anaesthetized adult animals were placed in a holder in the centre of an anechoic chamber. All equipment was placed outside the chamber except the preamplifier and the loudspeaker. The loudspeaker was placed 100 cm from the animal's ear, on its left side. Responses were recorded using three subdermal needle electrodes (Rochester Electro-Medical, Lutz, FL, USA). One electrode (ground) was placed under the loose skin between the shoulder blades, another (vertex) was placed between the eyes and the third (mastoid) was placed behind the left ear. The impedance was adjusted to 3 kΩ or less between all electrodes. The setup for measuring hearing of kits was the same as described above except that we moved the setup to a room next to the stable,

to minimize the time the kits needed to be away from their nests/mothers. To eliminate noise and echoes, we built an improvised anechoic chamber around the setup using mattresses and blankets. Each test session took up to 1.5 h per individual for adults and the oldest kits (13 frequencies: 1, 2, 4, 8, 10, 12, 16, 20, 30, 40, 50, 60 and 70 kHz) and less time for the younger kits due to their limited hearing range.

For each individual, we first stimulated with the unmasked click stimulus and increased the intensity to find the threshold for response, i.e. the click threshold. If the click threshold was low enough (<80 dB SPL), i.e. for the adults and for the older kits, we continued with an ordinary audiogram based on masked thresholds. If the threshold was above 80 dB SPL, the click threshold was noted and no masked threshold measurement was conducted. This means that we have no audiograms for the youngest kits.

Sound production in kits

The male and female kit from each litter were placed on nest material in two separate test boxes (10×35×10 cm) and the kits' temperature was measured by infrared thermometry. We started the recordings 5 min after removing the kit from the mother and recorded continuously for 15 min at room temperatures between 19 and 20°C. After recording, we again measured the kit's temperature, weighed it and returned it to the litter. The vocalizations were recorded using two 0.25 inch free-field G.R.A.S. microphones (Type 40BF) and G.R.A.S. preamplifiers (type 26AC and Power Module type 12AA; G.R.A.S. Sound & Vibration). The signals were digitized with a 250 kHz sampling rate on two channels of an Avisoft UltraSoundGate 1216H, one for each kit. The UltraSoundGate was controlled by a laptop computer (HP Compaq nc8430). The sounds were analysed using commercial sound analysis software (BatSound version 4.03, Pettersson Elektronik AB, Uppsala, Sweden). For each recording we measured the number of pulses and their combined duration and calculated the duty cycle (% time with sound production). We chose 10 pulses, the pulse with the maximum amplitude and the nine subsequent pulses, from each recording for thorough analysis. This was to avoid calls where the kit was looking away from the microphone. We determined duration from oscillograms and the peak frequency from the power spectra. Sound pressures are given in dB SPL re. 2×10^{-5} Pa r.m.s. The distance from the mouth to the microphone was measured and if it was different from 1 m the value was corrected to the sound pressure it would have been at a distance of 1 m.

The experiments comply with the permit for performing animal experimentation (Danish Animal Inspectorate, journal no. 2004/561-855, Danish Ministry of Justice).

Statistical analysis

The hearing threshold in adult mink was tested across frequencies (1–70 kHz), taking repeated measures into account using linear normal models (Littell et al., 1996), with the colour type (Palomino, black) as a fixed factor. We divided the analysis into two ranges of frequencies, 1–10 kHz and 12–70 kHz, based upon the graphical appearance of the data (Fig. 1).

The change in click ABR threshold in mink kits was tested using ANOVA, including age (8–52 days), sex and their interaction as fixed factors, and body mass as a covariate. The hearing threshold in mink kits aged 32–52 days was tested across frequencies as well. Different ranges of test frequencies were used: 1–10 kHz at age 32 days, 2–40 kHz at age 36 days, and 2–70 kHz at 42 and 52 days. Each age class (day 36, 42 and 52) was modelled separately, taking repeated measures into account using linear normal models (Littell et al., 1996), with the sex as fixed factor and body mass as covariate. The model for day 36 did not converge.

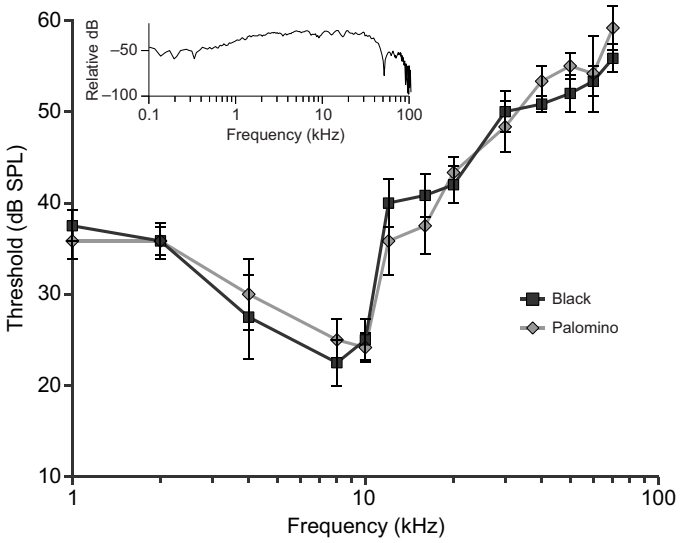

Fig. 1. Auditory brainstem response (ABR) audiograms (mean ± s.e.m.) of adult black and Palomino female mink. The best frequency for hearing capacity is around 8–10 kHz for both colour variants. There was no significant difference in hearing thresholds between black and Palomino lines (P>0.22). Inset shows the power spectrum for the click used in the ABR.

The occurrence of vocalization in mink kits during isolation from mother and nest was analysed as a binomial distribution (vocalization present or absent; analysed for days 1–22 and days 25–44 separately based upon the graphical appearance of data) in a generalized linear model (Dobson, 1997). The development in mean duration of emitted pulses, duty cycle and peak frequency (mean of 10 pulses per kit) with age were analysed in a normal linear model (Littell et al., 1996) using data from vocalizing kits only (i.e. days 1–37). The duty cycle was analysed for days 1–13 and days 16–37 separately based upon the graphical appearance of data. Data on pulse duration were log transformed and data on duty cycle days 16–37 were square root transformed, as this resulted in better residuals. The models included age, sex, previous experience (tested before or naïve), change in body temperature during the test session (–14.5 to +1.5°C) and body mass (7–354 g) as explanatory variables, including the interactions between both sex and previous experience with age. The development in number of pulses with age was not subjected to additional statistical analysis, as the number of pulses and the mean pulse duration are combined in the duty cycle. All models were analysed using SAS statistical software (SAS Institute, Cary, NC, USA), and the P-values from the linear models are based on the Satterthwaithe approximation for the denominator degrees of freedom. The requirement for dispersion and variance homogeneity was tested, and the validity of the final model was judged by the appearance of the residuals. A probability level (P) of 0.05 was chosen as a limit for statistical significance in all tests. P-values between 0.05 and 0.1 are reported as trends in the results.

RESULTS

Hearing in adult mink females

We recorded the audiograms of six adult Palomino and six adult black mink females. The results demonstrated that mink have a broad hearing range from 1 kHz and way into the ultrasonic range up to at least 70 kHz. The best threshold of 22 dB SPL was at 8–10 kHz

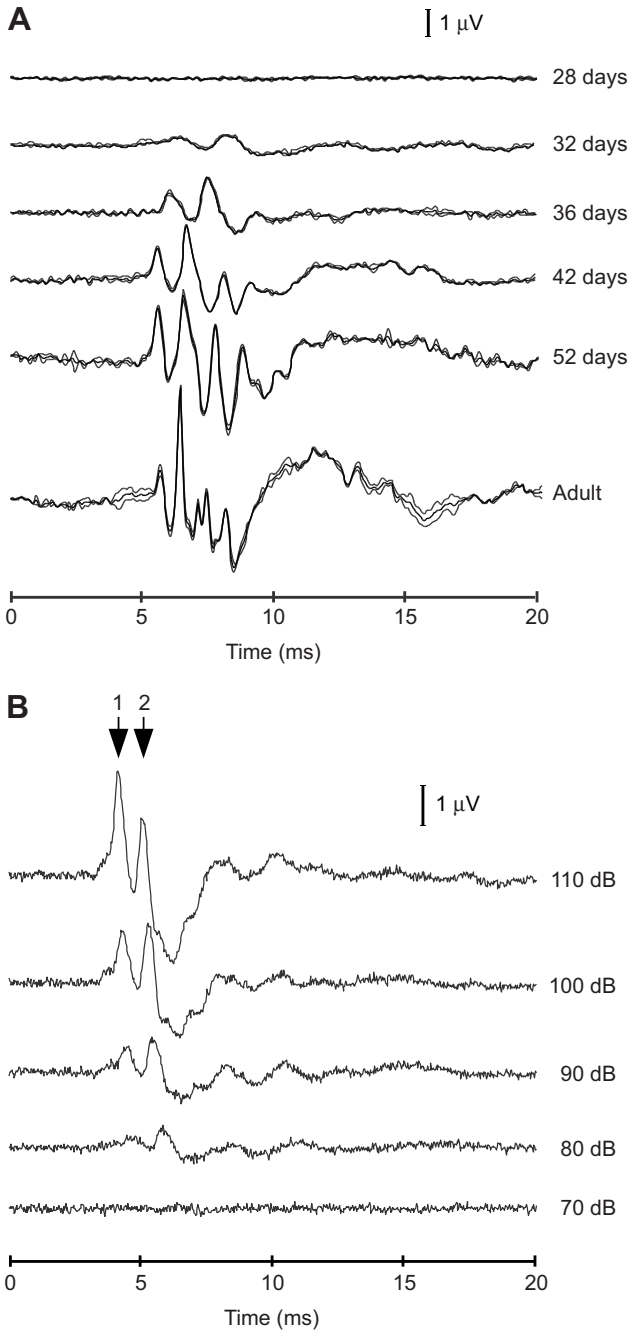

Fig. 2. (A) ABR elicited by a click at 94 dB SPL develops with age of mink kits. At 28 days there was no response. At 32 days it was weak and consisted of only two peaks. The response became stronger and at 42 days we could clearly distinguish four peaks, but even at 52 days it had not developed the fifth peak of the adult ABR. The latency of the peaks was also reduced with age. (B) ABR in one 36-day-old mink kit elicited by clicks with intensities from 70 to 110 dB SPL. The ABR shows two clear peaks (black arrows 1 and 2) and two smaller peaks. Higher intensities gave a shorter latency and a stronger response, but the overall shape of the ABR changes very little.

(Fig. 1). The low frequency roll-off was shallow, with threshold increasing to around 37 dB SPL at 1 kHz. The high-frequency roll-off was somewhat steeper, but even at 70 kHz (the maximum frequency we could test) the threshold was still below 60 dB SPL. The difference between the audiograms of Palomino and black mink

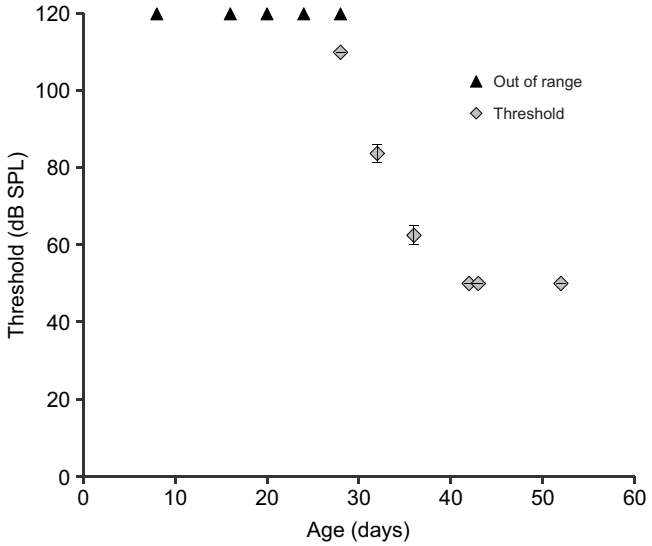

Fig. 3. Change in click ABR thresholds with age in mink kits given as means ± s.e.m. in dB SPL. The triangles indicate that the threshold was outside the range of the equipment. The first response was detected in two kits at 28 days (this day is marked with both a triangle and a diamond). After that all the kits responded and the threshold decreased quickly until it stabilized at day 42.

was always less than 5 dB and on average the difference was only 0.33 dB. There was no significant difference between the two colour variants (1–10 kHz: $F_{1,19.9}$=0.5, P=0.49; 12–70 kHz: $F_{1,20.8}$=1.6, P=0.22). The ABR of the adults was large and complicated with one major peak and three to four distinct minor narrow peaks between 5 and 10 ms post stimulus, as well as a broader peak around 12 ms post stimulus (Fig. 2A).

Development of hearing in mink kits with age

The hearing in kits changed dramatically with age ($F_{4,13}$=163.1, P<0.001). The mink kits showed no indication of ABRs to click stimulation at ages of 24 days or younger, even at the maximum click amplitude the equipment could deliver (120 dB). The first reactions to clicks were recorded in two of the four mink kits tested at 28 days of age (Fig. 3). There was no significant sex difference in the development of ABR threshold with age ($F_{3,8}$=0.4, P=0.75) or in mean thresholds between male and female kits ($F_{1,12}$=1.3, P=0.28). The body mass strongly correlated with age of kits (Pearson correlation coefficient=0.94, P<0.001); therefore, we cannot separate the effects of age and body mass in this study. From 30 days of age, ABRs could be elicited in all kits tested and from that age the ABR quickly developed (Fig. 2A). Both amplitude and number of peaks increased with age. At 42 days the ABR had up to four peaks (Fig. 2A), but even at 52 days the ABR was neither as large nor as complex as that of the adults. At any given age the ABR delay decreased and the amplitude of the peaks increased with stimulus amplitude, whereas the number of peaks did not depend on the stimulus strength (Fig. 2B). Thresholds for all frequencies decreased considerably with age (Fig. 4). When we tested the 32-day-old kits, we could only measure thresholds at the lowest frequencies, 1 and 2 kHz, and only around the maximum intensity of the system. At 2 kHz, 52- to 56-day-old kits had thresholds around 20 dB lower than 32-day-old kits. The best frequency (the frequency with the lowest threshold) was 8–10 kHz at all ages, where a full audiogram could be measured. Even though 52-day-old kits had

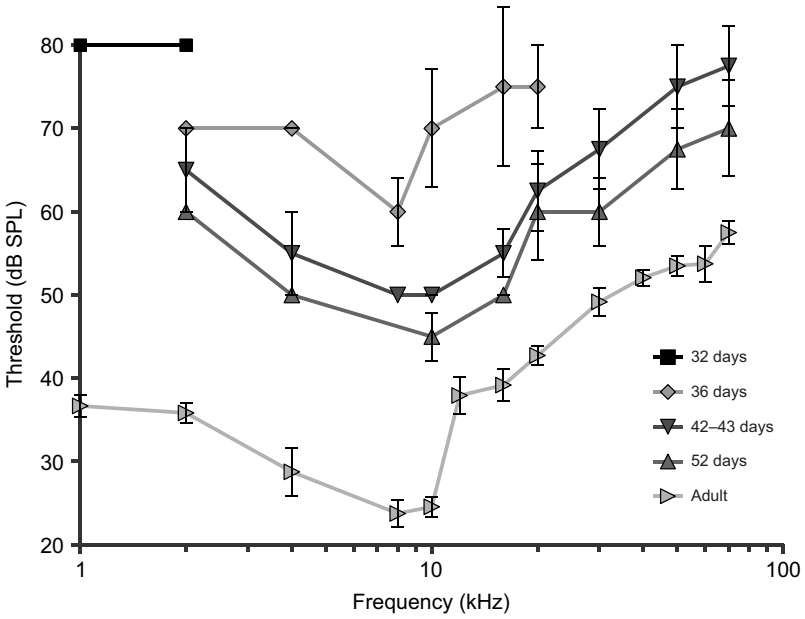

Fig. 4. Audiograms (mean ± s.e.m.) for mink kits of different ages. Each audiogram is the average of four animals, except day 32, where only three animals were used. The audiogram for the adults is the average of 12 animals. Half of the kits were tested repeatedly for the kit audiograms, but the adult animals were only used once.

reached a developmental stage where they can be weaned and removed from their mother, their hearing threshold was not yet as low as in adults. At frequencies above the best frequency, kits were ~10 dB less sensitive than adults. At lower frequencies the difference was much larger, around 25 dB (Fig. 4).

For kits tested at 42 days of age, the mean ABR threshold tested across 2–70 kHz was higher in male (71±3.4 dB) than in female (59±2.5 dB) kits ($F_{1,4.12}$=64.8, P=0.001). However, this indication of better hearing in female kits was not evident at other age classes tested (age 36 days, male: 73±4.1 *versus* female: 70±2.8 dB; age 52 days, male: 58±3.3 *versus* female: 60±3.7 dB).

Sound production in mink kits

The mink kits were very vocal in their first weeks of life (Fig. 5). On the first two days (days 1 and 2), 100% of kits vocalized when isolated from their mothers. Vocalizing decreased dramatically to only 15% on day 37. From day 44 onwards, none of the kits produced any sound when isolated from the mothers. Half the observations were from 10 kits tested repeatedly, in total 15 times during the period from 1 to 44 days after birth, whereas the other half of the observations originated from naïve kits tested only once. Testing including being away from the nest for 15 min, during which the kit for the first 32 days experienced a drop in body temperature (Fig. 5); however, there was no negative effect of the repeated testing on the kits' body mass at the end of the experimental period (kits tested 15 times: 299±14.6 g *versus* kits tested once: 257±17.2 g; $F_{1,15}$=0.4, P=0.53) nor on mortality (being zero) during and 4 weeks after the test period.

During the first 22 days, the occurrence of vocalization did not change significantly with kit age ($F_{1,198}$=0.8, P=0.27); on average,

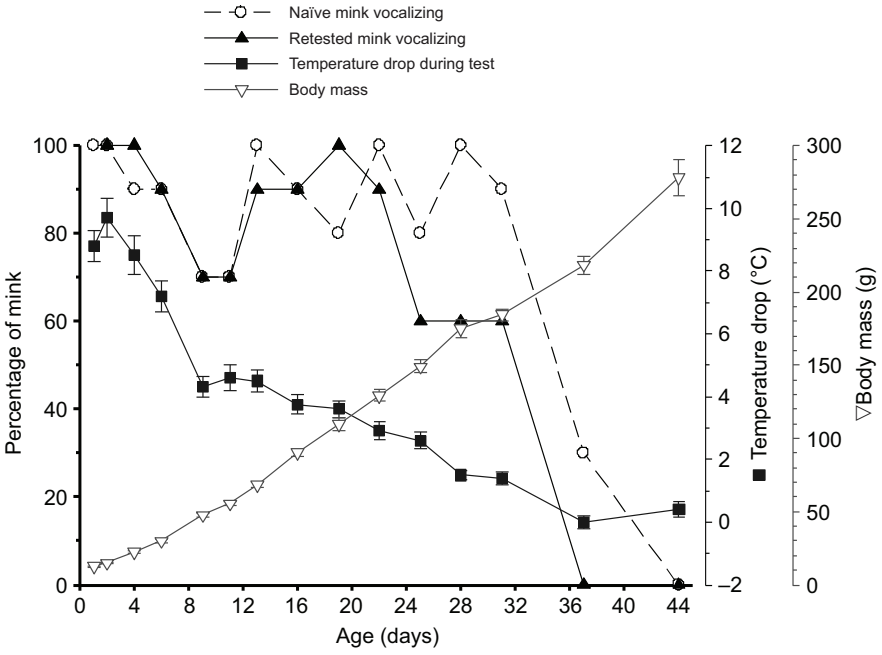

Fig. 5. Percentage of mink kits (%) vocalizing as a function of age, with one group tested repeatedly (N=10) and another group (N=150) tested only once (10 naïve kits per test day). The drop in surface temperature during the 15 min test and the body mass of tested kits (mean ± s.e.m.) are given on the right-hand axis. The occurrence of vocalization decreased with age (P<0.001), but not for the first 22 days. The re-tested kits were less vocal than the naïve kits (P=0.004) from the age of 25 days (see Results for details).

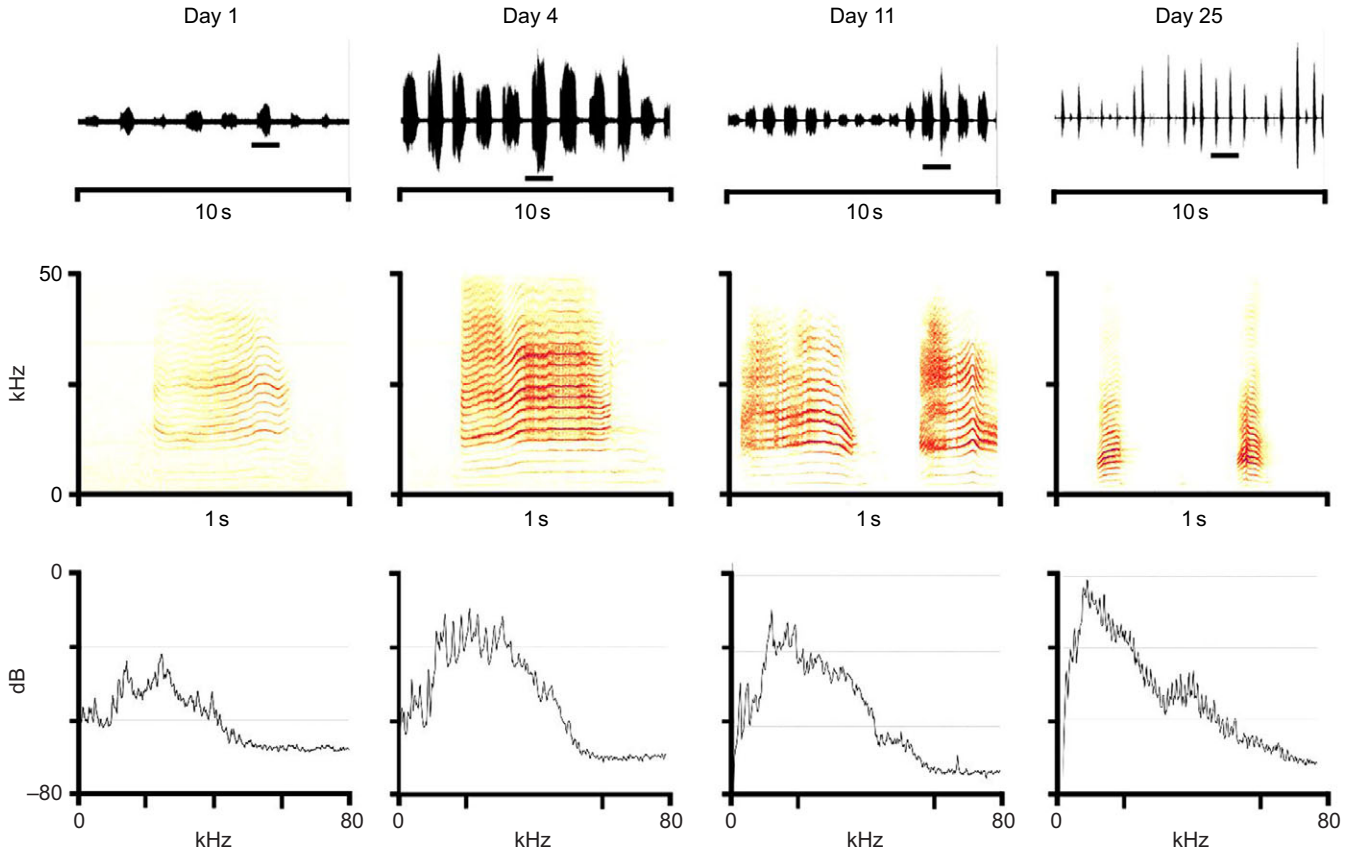

Fig. 6. Change in mink kit vocalization with age. Oscillograms, spectrograms and power spectra of the calls of a mink kit at 1, 4, 11 and 25 days of age. The 1-day-old kit has broad power spectra and the calls are quite long. With age, the calls become shorter and the peak frequency, maximum frequency and bandwidth decrease.

90% of all kits vocalized during this period, regardless of whether they had been tested before ($F_{1,197}$=0.0, P=0.91). From day 25, the number of vocalizing kits decreased with age ($F_{1,97}$=25.2, P<0.001) and fewer of the re-tested kits than the naïve kits were vocal ($F_{1,97}$=8.5, P=0.004). Thus habituation towards being tested is evident in older kits. The relative likelihood of vocalization in mink tested repeatedly *versus* mink tested once was estimated to be 0.2 (odds ratio, 95% confidence interval: 0.05–0.57). The age at which 50% of the population stopped vocalizing during the testing period was estimated to be 30 days for retested kits and 35 days for kits tested once. There was no difference between male and female kits in whether they vocalized (days 1–22: $F_{1,196}$=0.9, P=0.35; days 22–44: $F_{1,96}$=1.2, P=0.28).

Not only the occurrence, but also the acoustic features of the kits' sound pulses changed significantly with age: the calls became shorter and the peak frequency, maximum frequency and bandwidth decreased (Fig. 6). The number of pulses each kit emitted during the 15 min test period depended substantially on age, but did not develop monotonically. The number first decreased from around 1 pulse s^{-1} at days 1–2 to *ca.* 0.56 pulse s^{-1} at day 4. From then the number increased to 0.89–1.78 pulses s^{-1} from day 9 to day 25, after which the number decreased steeply (Fig. 7A). The average duration of each pulse, in contrast, decreased ($F_{1,226}$=199.9, P<0.001) monotonically from the first day after birth until vocalization ceased (Fig. 7B,E), with no influence of repeated testing (retested *versus* naïve mink: $F_{1,224}$=0.8, P=0.36) or sex ($F_{1,224}$=0.9, P=0.35). The duty cycle (Fig. 7C) did not change systematically with age before day 16 (however, a trend existed: $F_{1,122}$=3.8, P=0.055). Hereafter the duty cycle decreased significantly with age ($F_{1,100}$=43.2, P<0.001), on average with a 47% greater duty cycle in vocalizing naïve kits than in retested mink kits during days 16–37 ($F_{1,100}$=12.0, P<0.001). There was no difference between sexes in duty cycle (days 1–13: $F_{1,120}$=0.4, P=0.56; days 16–37: $F_{1,99}$=0.8, P=0.37). The power of testing for sex differences is generally low at day 37, with only one male and two females vocalizing, in contrast to at day 31 and earlier, with recordings from eight to 10 males and seven to 10 females per test day.

The emitted frequency decreased monotonically with age ($F_{1,231}$=205.6, P<0.001), with no difference between sexes ($F_{1,230}$=0.7, P=0.39). The mean peak frequency was around 17 kHz in newborns and 4 kHz in kits at 37 days of age (Fig. 7C–E). There was a non-significant trend of a marginally higher peak frequency (7.3%; 11.2 *versus* 10.4 kHz) in naïve mink averaged across the entire test period ($F_{1,231}$=3.1, P=0.079). The mean sound pressure of the vocalizations varied between 59 and 69 dB SPL (data not shown) recorded 100 cm from the kit. The sounds were recorded when the kit seemed to be vocalizing in the direction of the microphone, but both direction as well as distance varied a bit, and may cause some variation in sound pressure.

DISCUSSION

We report here for the first time the hearing thresholds of adult American mink, as well as the development of hearing of the kits.

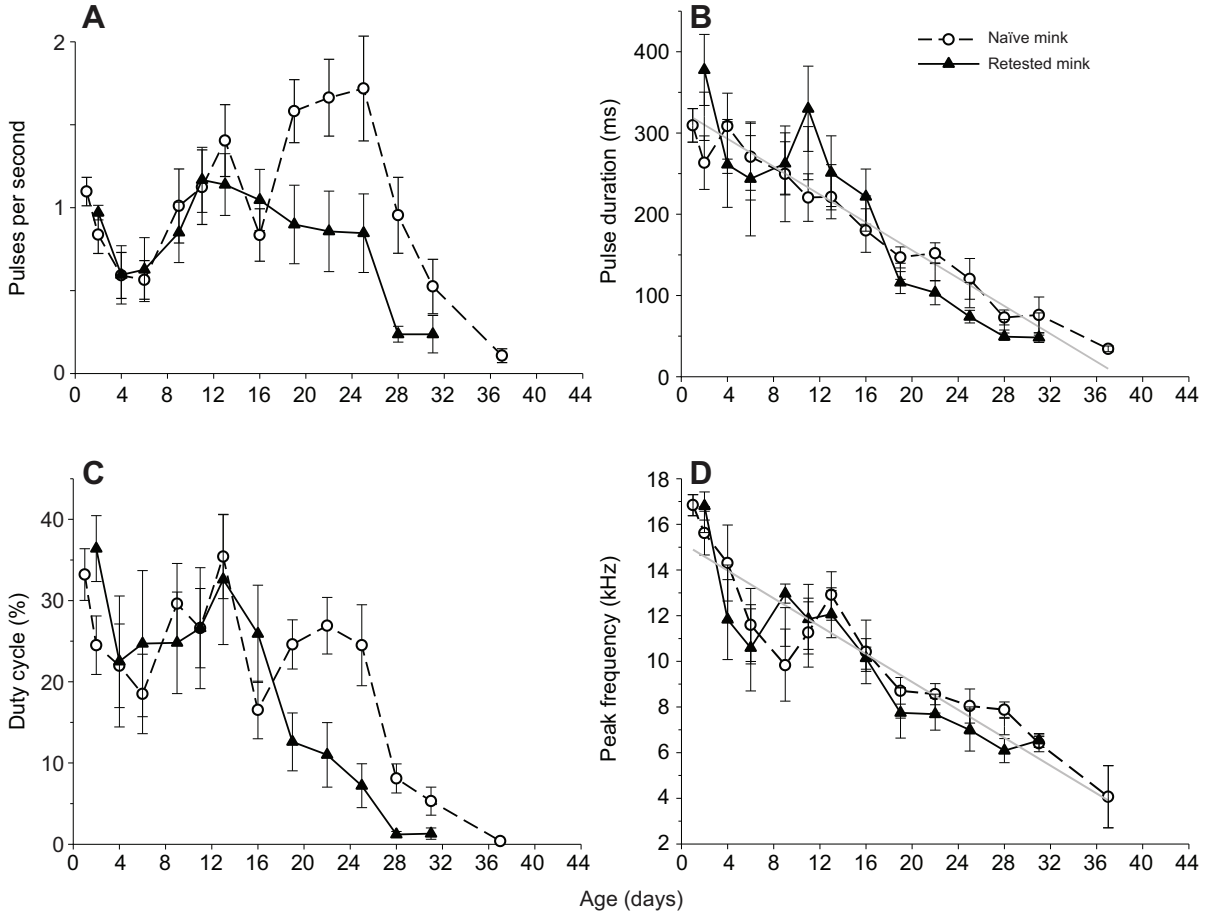

Fig. 7. Mink kit vocalization as (A) number of pulses per second, (B) pulse duration, (C) duty cycle and (D) peak frequency (all given as means ± s.e.m.) *versus* age, with one group of mink kits tested repeatedly and another group tested only once per test day. The average pulse duration and peak frequency decreased with age (P<0.001); the grey lines in B and D indicate the linear regression line. Retested kits vocalized with the same mean pulse duration (P=0.36) and peak frequency (P=0.079), but from the age of 19 days had fewer pulses per time unit, leading to a lower duty cycle than the naïve kits (P<0.001) after this age (see Results for details).

The results showed that kits are practically deaf until the age of 25–28 days; kits showed no signs of hearing until day 24, whereas 100% of mink had ABRs indicative of hearing at day 30 after birth. Any auditory function in mink at day 24 or younger would be very insensitive or absent, as we did not detect ABRs even to broadband (500 Hz–100 kHz) stimuli as loud as 120 dB SPL. Previous studies based on behavioural reactions (startle reflex, behaviour change), reported the first response to a range of sound stimuli at the age 29 days, with 100% of mink reacting at day 33 (Flottorp and Foss, 1979). Lassen (Lassen, 2007) tested kits' reaction to a tone (~800 Hz, 60 dB for 2 s, distance 5 cm) on postnatal days 27, 30 and 33. At day 27, between 12 and 47% (dependent on breeding line) of the mink kits showed retraction or a startle response. At day 33, all tested kits (N=166) reacted to the test tone. These findings are well in accordance with the more detailed ABR results in the present study. Conclusions based on behavioural testing, especially the startle response, may sometimes be ambiguous as an animal may not show a response even if it hears the sound. The ABR data, however, are much stronger proof of the non-occurrence of hearing at postnatal day 24 or earlier.

Delayed onset of hearing has been reported in other altricial mammals, such as cats (6 days), mice (12 days) and ferrets (32 days) (Moore, 1982), which, similarly to the mink, are born in an immature state. Our results in mink are comparable to findings in the ferret (*Mustela putorious*), in which the onset of hearing occurs on postnatal days 26–30 (Morey and Carlile, 1990; Moore and Hine, 1992). In comparison, the rat starts to hear after postnatal days 11–12 (Blatchley et al., 1987; Grécová et al., 2009), with an adult-like ABR developed within days 24–36 (Blatchley et al., 1987). In contrast, adult-like ABR was not acquired in mink within the study period; even at 52 days the ABR was neither as large nor as complex as that of the adults. Thus 52-day-old kits are not as sensitive as adult mink, in particular at the low frequency range below the best frequency. This result is also in contrast to data from ferrets, in which most achieved adult-like ABR threshold levels by days 34–36 (Moore and Hine, 1992). At present we have no explanation for why the mink with an almost equal age of onset have a slower development of hearing than what was observed in ferrets. In ferrets, the time of the opening of the ear canal and/or the clearance of fluid from the middle ear are suggested to explain the major and rapid change in hearing threshold after the time of hearing onset (Moore and Hine, 1992). From around day 30 the hearing in mink developed rapidly, but in a step-wise manner. The threshold improved very quickly between day 28 and day 42 and then slows down. It is difficult to say anything about the cause of this phenomenon, but we speculate that the improvement in threshold coincides with the

opening of the ear canal. However, the ABR results speak against ear canal opening as the only reason for the improvement in hearing. ABRs depends on synchronous activity in the auditory system. Fig. 2 shows that the ABR is not only increasing in amplitude with age, but also changing its shape. If the attenuation of a half-closed ear canal had been the only difference, an increase in stimulus intensity would compensate for that, such that, for example, the ABR at 110 dB for 36-day-old kits should resemble that at 94 dB for 42-day-old kits. By comparing Fig. 2A and 2B this is clearly not the case. Fig. 2B shows ABRs from a 36-day-old kit at increasing stimulus amplitudes. The resulting ABR simply increases in amplitude, but only changees in shape very slightly. At all intensities there are only two peaks, whereas at day 42 there are at least three peaks (Fig. 2A), and with increasing age the number of peaks increases even more. Also with age the peaks become sharper and better defined. It is likely that this is caused by increased synchronization in the brain areas processing sound, in parallel with the maturation of the auditory system, as has been demonstrated in pre-hearing postnatal ferrets (Brunso-Bechtold et al., 2006; Harper and Wallace, 1995).

The development of the auditory system cannot be isolated from the general development of senses and motor ability in growing kits. The onset of eye-opening begins gradually from postnatal day 27, with fully open eyes by day 36 in mink selected for production traits (Lassen, 2007). Thus the time window of eye-opening is concurrent with the onset of hearing in mink. In contrast, mink kits are already able to smell on postnatal day 1 [tested as behavioural reaction towards vinegar (Jonansen, 1987)]. After the onset of hearing and vision, increased motor abilities, such as gait and running, are observed initially between postnatal days 34 and 40 in production mink; during this period kits also begin to eat solid food followed later by water drinking (Brink and Jeppesen, 2005; Lassen, 2007). Thus weaning is gradually initiated during this period. On farms, separation of the kits from the mother typically happens around 6–8 weeks of age; however, in several countries (e.g. Denmark) this is not legal before the litter age of 56 days. Considering that the kit is not moving around much until 34 days after birth there is probably no significant selection pressure for the earlier onset of hearing in the mink kits.

Strikingly, the deaf kits begin to vocalize extensively from day 1. Recordings during four deliveries (data not presented) reveal that kits begin to vocalize even earlier, immediately after birth. Considerable energy is put into these early vocalizations (e.g. day 1: frequencies up to 69 kHz, average of 1.1 pulse per second continuously for 15 min with a mean pulse duration of 300 ms, sound pressure around 64 dB SPL at 1 m). This sound production represents a considerable cost for the neonate kit (average mass day 1: 12.6 g), having limited energy reserves with only 1% of body mass as fat (Tauson, 1994). The behaviour must therefore be adaptive, e.g. in the communication to the mother eliciting maternal care or attention in case of predators at the nest. After the period of eye opening and development of hearing, the occurrence of vocalizations drop, concurrent with the developing kit becoming less dependent on maternal nursing. This temporal development further indicates the function of kit vocalization in eliciting maternal care and/or nursing. The drop in vocalization is also concurrent with the first dramatic increase in hearing sensitivity, which could indicate that the ears are blocked to protect them and when vocalizations are reduced, hearing can start to develop. The drop in vocalization also coincides with the ability to maintain constant body temperature when away from the mother and this is likely the cause of the drop in call frequency.

Colour type affects both kit vocalization and maternal retrieval, as Palomino kits had a higher variation in their pulse duration than black kits, and Palomino mothers were less efficient in retrieving their kits compared with black mothers (Clausen et al., 2008). Adults of another breeding line of mink, the colour variant Hedlund white, have been shown to be deaf (Sugiura and Hilding, 1970; Flottorp and Foss, 1979). However, our results clearly show that impaired hearing does not explain the reduced maternal behaviour in the Palomino mothers, as adult black and Palomino females have the same hearing thresholds and identical ABR curves. Because the ABR method measures electrical signals in the auditory nerve, the results validate that both the cochlea and the auditory nerve are working at the same level in the two different colour variants. In addition, the similarity of later peaks in the ABR track corresponding to later centres in the auditory pathway indicates no obvious differences in the auditory processing in the two colour variants. For the kits, the risk of dying is highest in the early postnatal days, even in the protected environments at farms, and mothers of kits with high survival rates show more maternal behaviour during the early postpartum period (Malmkvist et al., 2007). Based on the present results, the difference in maternal kit-retrieval between Palomino and black mink breeding lines could not be explained by a general difference in adult hearing ability. Further studies of the social communication between kits and mothers are needed to fully understand the function and importance of, in particular, early mink vocalization. Other factors, for example the nesting environment, can also influence early maternal behaviour on farms, as females with access to the highest nest quality were more attentive and quicker to retrieve one of their 5-day-old progeny placed away from the safe nest (Malmkvist and Palme, 2008).

Before days 18–24, we found no evidence of differences in vocalization between mink kits tested once or several times; that is, before this age we have no evidence of mink being able to perceive and habituate to the repeated handling and the test situation. However, as early as at postnatal day 18, previously handled mink exhibited lower vocalization rates when handled. Thus mink at this age, without auditory and visual ability, already have the ability to perceive and change behaviour in response to repeated handling and testing. Therefore, care should be taken in the design of future studies and in the interpretation of previous studies of ontogeny and development of, for example, vocalization when using repeated handling, as the results may not reflect development over age alone. The reason for naïve minks to vocalize more (higher chance of calling and a higher duty cycle of calls) could be related to fear being expressed in the older mink kits. Other studies have reported increased vocalization in fearful mink during exposure to humans, but this was only studied in adults (Malmkvist and Hansen, 2002). Even though mink kits with different experience differ in vocalization rates, they do not differ in mean pulse duration or spectral frequency during calls.

Previously, the peak sensitivity of hearing of mink has been suggested to be within the 1–16 kHz range, based upon data collected from other carnivores (Powell and Zielinski, 1989). We document that adult female mink (1.5–1.6 years old) have a broad hearing range from 1 kHz into the ultrasonic range up to at least 70 kHz, with the peak sensitivity (threshold of 20 dB SPL) at 8–10 kHz. Thus the highest frequencies recorded in mink kit calls (69 kHz recorded day 1) can be detected by the adult female mink. Our results also agree with the results from ferrets: if we assume that the difference between the best behavioural sensitivity and the ABR thresholds are 20 dB (Gorga et al., 1988), then our results are virtually identical to those of Kelly et al. (Kelly et al., 1986), except

at the highest frequencies, where mink seems to be more sensitive. It has been suggested that hearing of high frequencies in least weasel and mink is evolved primarily as an adaption for locating and hunting rodents emitting ultrasonic vocalization (Heffner and Heffner, 1985; Powell and Zielinski, 1989). Based on our results, we suggest that adult female hearing also plays a role in vocal social communication related to maternal behaviour and kit survival in mink, but because the kits calls are far above the hearing threshold for the dam, it is more likely that the threshold is determined by the need to detect prey or predators. Reports have been made of both sex- and maternal-related differences in hearing in mammals. For example, female chinchilla (*Chinchilla lanigera*) have a slightly superior hearing than males at frequencies above 2 kHz (McFadden et al., 1999). Interestingly, hearing has also been described as more sensitive in the maternal (compared with virgin) female mouse as a function of them recognizing ultrasonic communication (distress calls) in their 2- to 6-day-old offspring (Ehret, 1987). At present we have no data on hearing range or sensitivity in adult male mink. However, there is no paternal behaviour in mink, and a considerable body size dimorphism exists, with males being larger and on average 1.7 times heavier than females; accordingly males take larger prey in nature [e.g. a higher proportion of rabbit and hares in diets (Birks and Dunstone, 1985)]. Therefore, following their different size and different lifestyles, we predict that the ability for detection of high frequencies is lower in male than in female mink.

We did find some effect of gender for kits tested at 42 days of age. However, this indication of better hearing in female kits was not evident at other age classes tested. Thus, this sex difference in hearing may only be transiently present around the age of 42 days or just be a result of random effects, as a low number of kits per sex (N=2 randomly selected from different litters) were tested at each class in the present study.

The understanding of the auditory system in mink, and thereby the limitations and possibilities for social communication, is important in order to facilitate a housing environment taking both production economics (e.g. a higher number of mink kits surviving) and animal welfare into consideration. The early kit vocalizations are loud and may interfere with communication of neighbouring mink in the commercial setting. Conspecific sounds as well as noise within the hearing range of the mink could result in disturbance of the mothers during the season of delivery, with negative consequences on survival of young and resulting in reduced mink welfare. Mink escaped from farms are very successful as invasive species in areas where they are not a native species (Northern Europe, for example). To evaluate their behaviour and ecological impact, fundamental knowledge of sensory input is also crucial, and the results we present here provide the basis for understanding their acoustic behaviour in relation to conspecifics as well as prey. The delayed development of hearing synchronized with the disappearance of loud vocalizations suggest that mink may prove to be an interesting model for future studies of mammalian hearing development.

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AUTHOR CONTRIBUTIONS

All authors contributed to the planning, design and execution of the experiments. All authors contributed to the analysis of data and the drafting of the paper, with A.M.S. and C.B. being responsible for most of the revising.

COMPETING INTERESTS

No competing interests declared.

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