

Peripheral auditory processing in the bobtail lizard *Tiliqua rugosa*

V. Seasonal effects of anaesthesia

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Summary. Several parameters of the peripheral auditory physiology of the bobtail lizard have been examined with respect to the time of the year. These include gross-potential and neural thresholds across the entire hearing range as well as a standard measure of gross-potential size and an estimate of the success in recording primary auditory afferents.

1. The results do not indicate any pronounced changes in hearing ability across the year. This contrasts with earlier reports by Johnstone and Johnstone (1969a, b) and Holmes and Johnstone (1984a, b) who found gross potential size, gross potential thresholds and recordability of auditory-nerve fibres to change markedly with the seasons.

2. An additional examination of the anaesthetic regimes used in the present report, however, revealed seasonal changes in the animals' anaesthetic requirements. The time course of this phenomenon closely matches the previously reported time course of changes in hearing over the year.

4. It is therefore suggested that the previous results represent an artefactual variation in hearing ability due to the use of standard anaesthetic doses causing a variable physiological state of the experimental animals. Evidence supporting the idea of a gradual impairment of hearing by a varying degree of anaesthetic overdose is discussed.

Key words: Lizard – Hearing – Anaesthesia – Seasonality

Introduction

In 1969, Johnstone and Johnstone first reported seasonal variation in the hearing of the Australian bobtail lizard. Measuring gross potentials within scala tympani, they found a roughly ten-fold increase in the size of the summing potential during October (Johnstone and Johnstone 1969a), when the animals are naturally most active. This was accompanied by a sharp rise in the number of active, recordable auditory nerve fibres, whereas dur-

ing the rest of the year, recordings could only be obtained with great difficulty (Johnstone and Johnstone 1969b). Holmes and Johnstone (1984a, b) followed this up with a more extensive study of changes in gross potentials over the course of the year. They confirmed the earlier results of Johnstone and Johnstone and, in addition, reported an enhanced auditory sensitivity of the bobtail lizard from September to November.

In an unrelated study on the discharge properties of primary auditory nerve fibres in the bobtail lizard (Köppl and Manley 1990; Manley et al. 1990) experiments were performed between September and January and, in a second series, during March and April. The absence of any obvious seasonal change in hearing in our data lead us to investigate other possibilities of explaining previously-observed changes.

Material and methods

The present report is based on two experimental series conducted from September 1985 up to the first days of January 1986 and from the end of March through April 1987, respectively. Australian bobtail lizards (*Tiliqua rugosa*) of both sexes were wild-caught in the vicinity of Perth, Western Australia, and held in an outside enclosure subject to seasonal variations in light and weather conditions. For maximally 10 days (mostly only 1–2 days) prior to the experiment, the lizards were transferred to an inside animal room with natural daylight but kept at constant temperature.

The anaesthesia was initiated by intraperitoneal administration of 25–30 mg/kg (September to December) or 15–20 mg/kg (March/April) Na-pentobarbital (Nembotal), respectively. Depending on the animal's reaction and the speed with which any sedative effects set in, successive doses of approximately a third to a half of the initial dose were given at half-hour intervals until a surgical level of anaesthesia was achieved, i.e. when no reaction other than local reflexes occurred during surgery. At that stage, all animals received a single subcutaneous injection of about 0.15 mg/kg Atropin sulphate. The upper jaw was firmly clamped and additionally secured with dental cement to a custom-built head-holder. The animals were artificially respired with air via a tube inserted through the glottis into the trachea and warmed by a heating blanket to a constant body temperature (as measured in the rectum) of 30 ± 1 °C. During the experiments, the depth of anaesthesia was judged by periodically checking leg-withdrawal reflexes and supplementa-

ry Na-pentobarbital was given when reflexes could be elicited in all four legs. Care was taken to adjust the amount and frequency of supplementary doses to the individual animal. Visual inspection of the carotid arteries (which were easily visible after the initial stage of surgery, see below) as an indication of frequency and strength of the heartbeat also helped sometimes to assess the relative depth of anaesthesia during the experiment.

An incision was made through the skin, muscles and mucous membrane of the floor of the mouth to expose the columella and the ventral bony ridges of the inner ear and brain capsule. Gross potentials were then recorded by placing the cut end of an enamelled silver wire onto the mucous covering of the round window, using a similar wire on the muscles surrounding the incision as a reference. Signals were amplified, band-pass filtered, averaged over 128 stimulus cycles and fed to an oscilloscope. Details of the acoustic stimulation and calibration are described in Manley et al. (1990). Briefly, sound stimuli were generated by a frequency synthesizer, attenuated and gated to produce short tone bursts with, for gross-potential measurements, a 10 ms plateau and 1 or 2 ms rise/fall times presented at a rate of 10/s. The stimuli were delivered by a Beyer DT48 earphone through a closed system sealed into the external ear canal with vaseline. The sound pressure for the whole range of frequencies used was measured after each experiment via a calibrated probe tube near the eardrum and used to calculate the absolute sound pressures (in dB re. 2×10^{-5} Pa).

The tone bursts had a random phase onset; thus we assume that cochlear microphonic responses (CM) were averaged out. Judging from the form of the measured gross response, the filtering effect of the round window (Holmes et al. 1984) together with the bandpass filtering used also removed most of the summing potential (SP) such that the gross potential measured was essentially a compound action potential (CAP). Thresholds of this gross response were defined visually as the sound pressure level where a potential was subjectively just distinguishable in the averaged signal trace. Thresholds were routinely determined at 5–7 frequencies across the hearing range of the bobtail lizard (0.5, 1, 1.5, 2 and 4 kHz, in most cases also 0.2 and 6 kHz). As a standard measure of gross potential size, the amplitude of the first positive deflection was measured for stimulation with 1.5 kHz at about 80 dB SPL (± 5 dB).

All animals were subsequently used in different kinds of auditory experiments not of detailed importance to the present report. Neural thresholds used below for comparison were recorded from VIIIth-nerve fibres at their point of entrance into the brain capsule. This technique has been described in Manley et al. (1990). All animals were killed and studied anatomically after the experiment and thus sequences of data presented below over the course of the year are from different animals rather than from the same individuals monitored over time.

Results

Our standard estimate of gross potential size, the response to a 1.5 kHz-tone at about 80 dB SPL, varied considerably between individual lizards (Fig. 1a). The values mostly fell between 2 and 10 μ V, but in a few exceptional individuals they reached up to 28 μ V. Although there is a concentration of high gross potential values in October, these are not accompanied by consistently lower threshold values (Fig. 1b). There is no tendency for the thresholds at any frequency to be lowest around the Australian springtime; if anything, thresholds seem to be slightly higher in November and December.

Recordings from the VIIIth nerve were carried out in about half of the animals, permitting an assessment of auditory thresholds at the single-unit level. It was

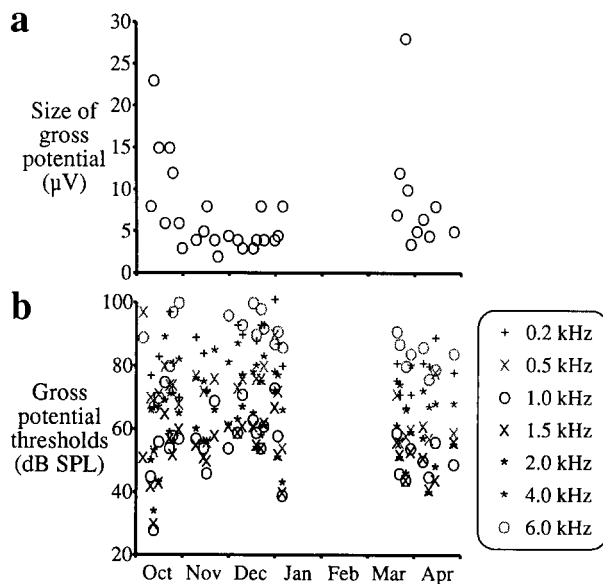


Fig. 1a. Size of the gross potential during stimulation with 1.5 kHz at about 80 dB SPL for animals measured at different times of the year. **b** Thresholds of the gross potential response for 7 different stimulation frequencies across the hearing range of the bobtail lizard

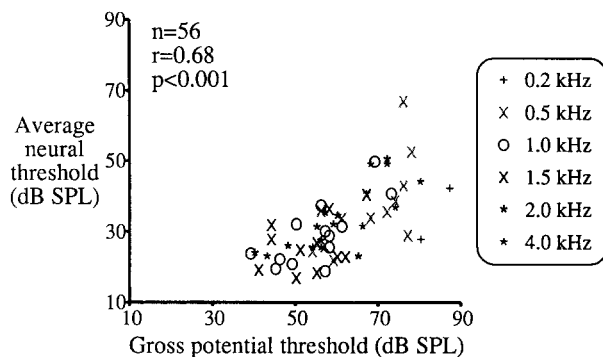


Fig. 2. Correlation in individual animals between the gross potential thresholds and the average neural thresholds for 6 different stimulation frequencies. Neural thresholds were averaged over the following ranges of characteristic frequencies: 0.2–0.3 kHz, 0.4–0.7 kHz, 0.8–1.2 kHz, 1.3–1.7 kHz, 1.8–2.5 kHz and 3.5–4.5 kHz. Only cases with 2 or more neural thresholds available for a given frequency range were used in this comparison

found that the gross-potential thresholds predict the neural thresholds reasonably well, although, of course, neural measurements lie 20–30 dB lower (Fig. 2). The range of neural thresholds does not show any consistent change over the seasons. Individual variation is an adequate explanation for the observed differences in neural sensitivity (Fig. 3). In addition, no exceptional difficulties were experienced in recording from auditory nerve fibres outside the month of October, contrary to the report of Johnstone and Johnstone (1969b). Figure 4 shows that the number of recorded auditory-nerve fibres does not differ systematically between experiments at different times of the year.

While we were thus unable to reproduce the previously-reported seasonal changes in the hearing ability

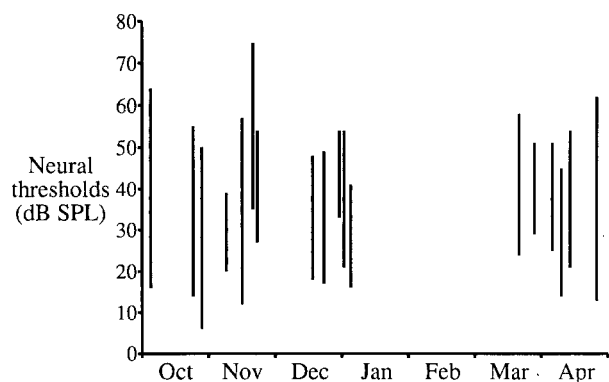


Fig. 3. Range of neural thresholds found in individual animals measured at different times of the year. Each line connects the lowest and highest threshold at characteristic frequency (CF) for 7–33 auditory nerve fibres representing most of the CF-range of the bobtail lizard

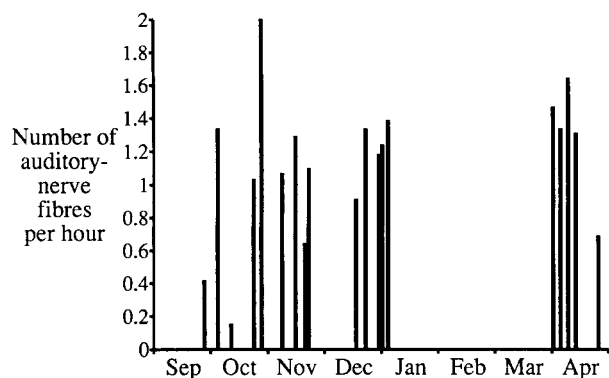


Fig. 4. Frequency of encountering single primary auditory nerve fibres in the VIIIth nerve at different times of the year. Since the experiments were of greatly varying duration, this has been normalized to the number of fibres per hour

of the bobtail lizard, we did notice a seasonal variation in the Nembutal doses necessary to produce a subjectively similar level of anaesthesia. During the Australian springtime, most animals required markedly more anaesthetic than animals used during the other months of the year (Fig. 6b). In a detailed investigation of the anaesthetic regimes used, this effect was most obvious in the dosage of supplementary injections and the time intervals between them. From about the middle of October to the end of November, animals needed supplementary injections more frequently (Fig. 5a). They often also received more anaesthetic with each injection (Fig. 5b). The cumulative initial dose required to reach a surgical level of anaesthesia (Fig. 5c) seemed to be more dependent on the volume of the first injection, which was lower in March/April.

A statistical hierarchical cluster analysis (Norusis 1986) was carried out using the cumulative initial anaesthetic dose, the cumulative supplementary dose, the average dose per supplemental injection and the average interval between supplementary doses, as well as gross potential size and the gross potential thresholds at 0.5, 1, 1.5, 2 and 4 kHz. The results indicated two groupings

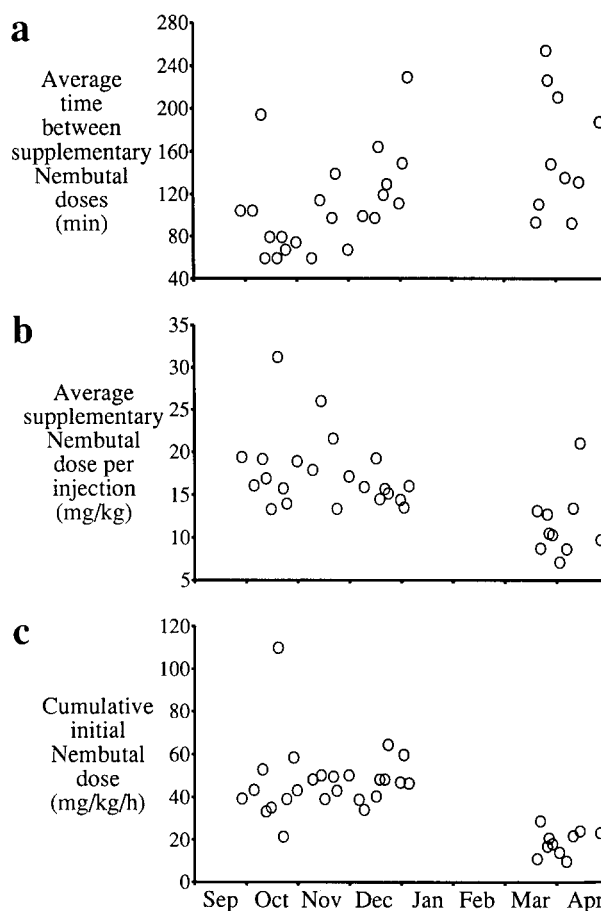


Fig. 5a–c. Variation of several anaesthetic parameters across different times of the year. Initial and supplementary doses were defined relative to the time when surgery was started

in the data: one group consists of experiments carried out in November/December and the other group (with one exception) of experiments from October/March/April. Subsequent *t*-tests revealed that the anaesthetic parameters (except the average interval between supplementary doses) as well as all gross potential parameters were significantly different between these groups. Both anaesthetic doses and gross potential thresholds tended to be higher in November/December. The average neural thresholds (see Fig. 2) which, due to their smaller sample size were not considered in the cluster analysis, proved, however, not to be significantly different between the two groups. When only the anaesthetic parameters were used for the cluster analysis, two groups were defined which are almost identical with our two experimental series from October to December and from March/April. Finally, using only the hearing parameters did not produce any homogeneous groups consistent with a certain time of the year, although all the higher-threshold animals were distributed between the end of October and the end of December.

These analyses confirm that in spring, animals received more anaesthetic to achieve a subjectively similar anaesthesia. Significantly, their gross potential thresholds tended to be higher at the same time, while the neural thresholds were not influenced. This seemingly

differential effect might be explained by the different times of day of the measurements. The gross potential thresholds were always determined after little surgery at the beginning of the experiment, whereas the neural recordings started considerably later. Judging from the anaesthetic data shown in Fig. 5, a stable, individually adjusted state of anaesthesia was probably only reached later during the experiment. If this interpretation is correct, it clearly points to an unfavourable influence of the anaesthetic on hearing. A possible seasonal effect on hearing that one might see in the gross potential data would have exactly the opposite time course to the previously reported improvement in sensitivity in spring (Holmes and Johnstone 1984b). We interpret this as another indication that seasonal changes in hearing are a secondary phenomenon caused by different states of anaesthesia.

Discussion

Our findings clearly contradict earlier reports of a pronounced improvement in the hearing of the bobtail lizard in the Australian springtime. Our gross response measurements were originally only intended to serve as a relative measure of thresholds before and after surgery. Thus, unfortunately, no records were made concerning the precise filter setting or the threshold size of the potentials. The threshold sound pressure level values are therefore not directly comparable to those of Holmes and Johnstone (1984a, b), who used a standard criterion of 1 μ V summating potential. This does not explain, however, the rather homogeneous distribution of thresholds across the year in our data (Fig. 1b) compared to the relative threshold differences in the study of Holmes and Johnstone (1984b; Fig. 6a). The present study even showed a tendency towards higher gross potential thresholds in spring, which is exactly the opposite of earlier results. It might be argued that the interpretation of gross potential measurements from the surface of the round window is complicated by the fact that the round window changes its filtering characteristics during the year (Holmes et al. 1984). It is important to note therefore that the additional evidence from neural data presented here failed to indicate any change in hearing abilities with the seasons.

Hillery (1984) described seasonal variations in mid-brain auditory evoked potentials and multi-unit responses in a treefrog and concluded that, although the thresholds of individual neurones probably do not change, there is a reduced number of active midbrain neurones during the non-breeding season of the frogs. If a similar effect was present in the peripheral auditory system of the bobtail lizard, it should have been obvious in the success rate of recording from auditory fibres. Since there was no seasonal difference in this respect, our data do not support the suggestion of seasonal variation in the hearing of this lizard as observed previously. A possible alternative explanation is that the reported variations are secondary phenomena. According to our observations, the actual seasonal variation lies in the

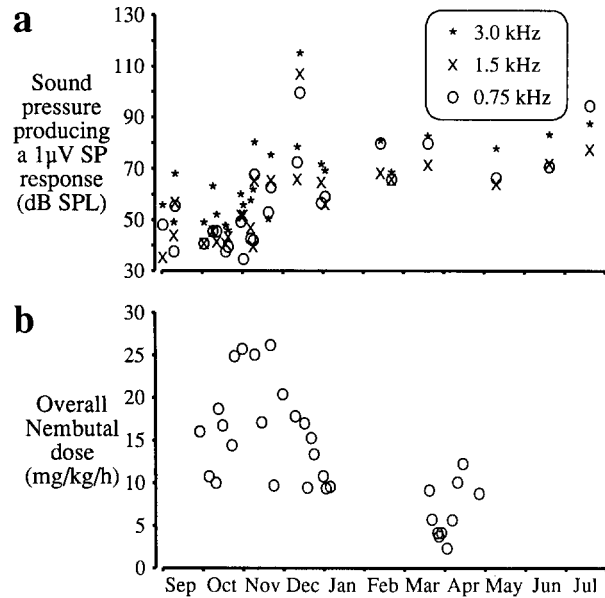


Fig. 6a, b. Comparison of the time courses over the year of a) the summating potential threshold values reported by Holmes and Johnstone (1984b) and b) the overall Nembutal dose (initial + supplementary) needed in our experiments to produce a subjectively similar level of anaesthesia

reaction to the anaesthetic agent (Na-pentobarbital) used in all studies on hearing in the bobtail lizard. Figure 6 illustrates the good temporal agreement of both phenomena, consistent with the idea of a causal relationship. Considering that the hearing thresholds reported by Holmes and Johnstone (1984b) and our data were collected in different years, the complementary time course is still obvious. Both sets of data show a great variation in springtime (September to November) following which they then settle to high or low values, respectively.

A varying sensitivity to anaesthetics can be easily explained by seasonal variations in the metabolism, since it is known that many lizards, being ectothermic animals, undergo cyclic changes in activity adapted to the environmental conditions of their habitat. For the Australian bobtail lizard, for example, the period of maximal activity is around September and October. This is their mating season and, correspondingly, the time when the size of the home range of individual lizards is greatest (Satrawaha and Bull 1981). It has been shown that there are associated changes in steroid biosynthesis (Bourne and Seamark 1978). Seasonal changes in plasma melatonin levels, a hormone which is implicated in the daily as well as seasonal control of reproduction and thermoregulation, were also observed (Firth et al. 1979).

Given a seasonal variation in the metabolism and therefore in the effect of anaesthetics, the use of a standard initial and supplementary anaesthetic dose as in the previous studies on hearing in the bobtail lizard (Johnstone and Johnstone 1969a, b; Holmes and Johnstone 1984a), might have led to different depths of anaesthesia at different times of the year. Significantly, Holmes and Johnstone (1984b), measuring minimum ox-

oxygen consumption rate (OCR) as an indication for metabolic rate in their experimental animals over the course of the year, were unable to see a general trend in the pooled data. Separating the data according to the auditory gross potential thresholds measured within two days of the OCR assessment, however, revealed a significant difference. Animals with sensitive thresholds had a higher OCR than those with poor thresholds. It is quite conceivable that animals with a lower OCR and therefore different metabolic rate were influenced more strongly by the same anaesthetic dose than animals with a higher metabolic rate. It has also been emphasized by Holmes (1983) that the summating potential (SP) was the most reliably measurable gross potential and that the cochlear microphonic (CM) as well as the compound action potential (CAP) varied considerably between experiments, sometimes not even being detectable. Johnstone and Johnstone (1969b) describe essentially the same result, experiencing great difficulty in recording primary auditory fibres outside springtime. These findings point to a variable physiological state of the animals, since there is evidence in birds (Necker 1970) and in the bobtail lizard (Holmes 1983) that the SP is the most robust gross potential in response to anoxia.

In the long-term studies of Holmes and Johnstone (1984b), individual lizards were repeatedly anaesthetized and measured over the course of the year. About 70% of the animals survived for a complete annual cycle and thus recovered from each experiment. This, however, does not mean they had not been slightly overdosed. Lizards, as ectothermic animals, are adapted to tolerate large fluctuations in their physiology and can therefore recover from periods of suboptimal conditions where bodily functions might have already been impaired. This was shown directly, but by chance, in an unrelated series of experiments in which we measured acoustic distortion products in the external ear canal of the bobtail lizard (unpublished). Since no surgery is necessary for such measurements, the animals were only sedated with Nembutal and allowed to breathe by themselves. One individual, however, reacted unexpectedly strongly to the anaesthetic, received too much and subsequently stopped breathing sometime within about 1 h of the last anaesthetic injection. This was only noticed when the measured distortion products were already deteriorating due to anoxia. By the time the lizard was put on artificial respiration, the recorded values had reached the levels typical of a dead animal. Following artificial respiration, the distortion products returned almost back to normal, taking, however, another 3 h to do so. The animal was taken off the artificial respiration after 6 h and seemed to behave normally the next day. In a further test 2 days later, this individual showed completely normal, sensitive hearing. Although this is certainly an extreme example and it is not known whether the lizard would have survived without artificial respiration, it does show that in these animals an anaesthetic overdose does not necessarily cause a rapid deterioration followed by sudden death. It also emphasizes the potential advantage of using artificial respiration as soon as possible after anaesthesia, as we used in all our experiments involving

surgery. The dosage of anaesthetics is probably more critical if, as in Johnstone and Johnstone (1969a, b), artificial respiration is not used. Respiring the animal 'when necessary' (Holmes and Johnstone 1984a) may already be too late, as the above example shows, where the hearing was impaired for at least 3 h after the start of artificial respiration.

It is concluded that, depending on the metabolic state of the individual and the exact dosage of the anaesthetic, a varying degree of reversible impairment of auditory function can be obtained. Adverse effects of barbiturate anaesthesia have also been described on high-frequency CAP thresholds in the guinea pig (Cazals et al. 1980), on the spontaneous discharge rate of primary auditory afferents in the American toad (Capranica and Moffat 1980), on the discharge pattern of primary afferents of a semicircular canal in the guinea pig (Yagi and Ueno 1988) and pigeon (Anastasio et al. 1985). We suspect that such effects are more common than would be suspected from these few reports. The wide physiological tolerance of reptiles in combination with an often seasonally-varying metabolism make them especially difficult subjects for a well-balanced anaesthesia, maintaining optimal sensory function at least at the peripheral level. Thus, we conclude that the previously-reported pronounced seasonal changes in the hearing of the bobtail lizard are most probably an artefact of a varying physiological state under anaesthesia.

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