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Research paper

Spontaneous otoacoustic emissions from free-standing stereovillar bundles of ten species of lizard with small papillae

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Abstract

Spontaneous otoacoustic emissions (SOAE) were measured in 10 lizard species from the families Iguanidae, Agamidae and Anguidae. The typical feature of these papillae is that the hair cells in the higher-frequency papillar regions that produce SOAE are not covered by a tectorial structure. The number of hair cells in the species used here was between 58 and 292 per ear. SOAE could be measured from all species, but some of their characteristics varied with papillar anatomy. Thus very small papillae produced fewer and smaller SOAE than larger papillae.

Keywords: Otoacoustic emission; Lizard; Suppression tuning

1. Introduction

Spontaneous otoacoustic emissions (SOAE) have been reported from a variety of species of mammals, birds, lizards and frogs and are regarded as the most direct evidence that the inner ear of vertebrates actively produces mechanical energy (review in Köppl, 1995). Since their discovery, SOAE and the various kinds of evoked otoacoustic emissions have become an important noninvasive tool to study the function of the hearing organ. In lizards, SOAE occur as broad spectral peaks with center frequencies always above about 1 kHz and peak sound-pressure levels between −10 to +27 dB SPL (review in Manley and van Dijk, 2006).

Although the inner ear of mammals is morphologically quite distinct from the inner ears of non-mammals (Manley, 2000a; Manley and Köppl, 1998), the SOAE in both groups of vertebrates show very similar behavior in most respects. In lizards, however, inner ear morphology differs remarkably between families, including a tenfold variation in papillar length (Manley, 1990; Miller, 1992; Wever, 1978). In addition, the number of SOAE per ear and their amplitude correlate at least roughly with this variety of papillar structure, especially with the structure of the tectorial membrane (Manley, 1997). A comparison of the SOAE characteristics can thus help in gaining insight into which morphological parameters play a role in determining the patterns of SOAE. SOAE in mammals and non-mammals are so remarkably similar in many respects that it is logical to assume that the basic generation mechanism is evolutionarily old (Manley and van Dijk, 2006).

SOAE in all vertebrates can be suppressed by externally-applied tones, the degree of suppression depending on the tonal frequency relative to that of the emission. Iso-suppression-tuning curves (STC) strongly resemble neurophysiological rate-threshold curves for single primary auditory neurons and thus this non-invasive approach can be used to examine the frequency selectivity of ears of species that have not been studied electrophysiologically and/or in species that are rare. In addition, body temperature affects

Abbreviations: SOAE, spontaneous otoacoustic emissions; STC, iso-suppression-tuning curves; SPL, sound pressure level; LF, slope of low-frequency flank of STC; HF, slope of high-frequency flank of STC; CF, characteristic frequency

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SOAE frequencies in nonmammals and in lizards, and the degree of shift observed also correlates with papillar structure (Manley, 1997).

The lizard species in which SOAE have so far been studied in detail had some kind of tectorial covering on top of the hair cells (Köppl and Manley, 1993, 1994; Manley, 2004b; Manley et al., 1996). Since tectorial structures play a role in coupling hair cells involved in producing SOAE (Manley, 1997; Manley and van Dijk, 2006), it is interesting to study the emissions of lizard species that have many so-called free-standing hair cells i.e. hair bundles in which the bundles protrude into the endolymph without a covering structure. Thus lizards of these species lack one of the mechanical components found in all other vertebrate ears where SOAE have been measured. The tectorial membrane has been suggested to be critically important for normal sensitivity and frequency selectivity in lizards (Authier and Manley, 1995).

If lizard papillae lack a tectorial structure, they do so only over the hair cells that belong in the group that responds to higher frequencies, i.e. above 1 kHz. Based on the species studied so far, this is always the region of hair cells that produce SOAE. Papillae lacking a tectorial membrane over these hair cells are found only in members of the three families Iguanidae, Agamidae and Anguidae. Whereas iguanids and agamids are considered to be closely related, the anguids belong to a quite different lineage and have evolved this structural constellation independently (Manley, 2004a; Miller, 1992). Based on more subtle differences of papillar structure, both Wever (1978) and Miller (1985) distinguished various sub-families of the Iguanidae. In this paper, these groups will not be distinguished, since the essential feature for the purposes of this study – the lack of a tectorial covering over the high-frequency hair cells – is found (with possible but rare exceptions) in all species of these families. Of the 10 species studied here, seven, the Bahamas brown anole *Anolis sagrei*, the four-striped basilisk lizard *Basiliscus vittatus*, the leopard lizard *Crotaphytus wislizenii*, the curly-tailed lizard *Leiocephalus carinatus*, the central american spiny-tailed iguana *Ctenosaura similis*, the green iguana *Iguana iguana* and the Madagaskar spiny-tailed lizard *Oplurus cyclurus* all belong in the family Iguanidae. Two species, the butterfly agama *Leiolepis belliana* and the water dragon *Physignathus concincinus* belong in the family Agamidae and one, the Texas alligator lizard *Gerrhonotus leiocephalus*, in the family Anguidae. In this group of 10 species, *Leiolepis belliana* may be an exception to the rule concerning the missing tectorial structure (and apparently unique in these families), as Wever (1978) reports that the high-frequency hair cells are covered by a form of sallet.

The type of lizard basilar papilla that is considered to be ancestral consists of three areas of hair cells, one central area that responds to frequencies below 1 kHz and two flanking groups of hair cells at the apical and basal ends of the papilla that respond to frequencies above 1 kHz (Manley, 2002, 2004a). In these papillae, all the high-frequency hair cells lack a tectorial covering of any kind. In the ancestral state, the two high-frequency hair-cell areas are mirror images both structurally and physiologically (Manley, 1990). In the granite spiny lizard *Sceloporus orcutti*, it has been demonstrated that the primary auditory nerve fibers innervating these two high-frequency areas really do show physiological mirror-image responses (Turner, 1987).

The species used in this study mostly show this pattern of three hair-cell areas, with the exceptions of *Anolis* and *Gerrhonotus* (that are assumed to have – independently – lost the apical high-frequency area), and *Leiolepis*, that lost the basal high-frequency area. Even more exceptionally, *Basiliscus* has established a divider across the papilla that widely separates the apical, high-frequency area from the rest of the papilla, thus creating two unequally-sized subpapillae. This situation also occurs in species not studied here, in monitor lizards and lacertid lizards (again independently evolved), and in the latter case it has been demonstrated that this separation can remove the constraints that produce mirror-imagery of the apical and basal hair-cell areas (Manley, 1990, 2002). The main question to be answered in the present study was: Do lizard hair-cell areas that lack a tectorial membrane produce SOAE and, if so, are their characteristics the same as those of hair cells that have a covering?

2. Materials and methods

The present study was carried out using animals of both sexes: nine *Anolis sagrei* with body weights from 3.4 to 8.2 g, three *Basiliscus vittatus* (63–108 g), four *Crotaphytus wislizenii* (22–35 g), two *Ctenosaura similis* (130–280 g), two *Iguana iguana* (70–101 g), four *Oplurus cyclurus* (50–125 g), one *Leiocephalus carinatus* (11 g), two *Leiolepis belliana* (24–29 g), two *Physignathus concincinus* (13.5–26.3 g), and two *Gerrhonotus leiocephalus* (25 and 29 g). Since many of these animals were held for several months and grew, the weights given are the extremes in each case.

The care and use of these animals was in accordance with German law. The animals were held in terraria with normal daylight but with additional flourescent lighting switched on and off in a 14:10 hour cycle. Heating lamps were used for 2 intervals of 2 h each day and UV-enhanced illumination for 3 h per day. All terraria contained one or more plants and provided shelter and climbing facilities. The air was humidified and the temperature during the day was between 20 and 30 °C, the substrate temperature directly beneath the heating lamps between 30 and 40 °C. Water was available ad libidum, and most animals were fed twice a week with mealworms, fly maggots or small crickets with regular fruit or vegetable supplements; these were dusted with vitamins twice a month. The green iguanas were fed with bean sprouts, grated apple or carrot and lettuce, also with mineral and vitamin additives. The iguanas, the basilisks and the water dragons also had larger water containers for bathing.

For the measurements of SOAE, which were carried out in a sound-attenuating chamber, the animals were lightly anaesthetized using i.p. doses of Narcoren® with initial doses of between 25 and 30 mg/kg. This anesthetic dose was effective for between one and three hours. For longer experimental periods, animals showing signs of waking up (small movements, ejection of the temperature sensor from the mouth) were given additional half doses of Narcoren. The animals breathed unaided and recovered completely within a few hours following the measurements.