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Revision articles

Animal models for hearing evaluations: a literature review

Modelos animais para avaliação auditiva: revisão de literatura

Aléxia dos Reis[(1)]
Suelen Pizzolatto Dalmolin[(1)]
Eliane Dallegrave[(1)]

[(1)] Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Rio Grande do Sul, Brasil.

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ABSTRACT

This review aims to outline which animal models are viable for preclinical hearing research, considering their anatomical and physiological characteristics, and their advantages and disadvantages of use. PubMed, Scielo, and Portal Periodicos Capes were consulted, using descriptors concerning hearing, hearing tests and animal species, individually and crossed with each other. The abstracts of the articles found in the databases were read, with subsequent selection based on the following criteria: free articles, use of animal models in audiological procedures that included the description of the evaluation methods, the advantages and/or disadvantages of using the species, and published between 1995 and 2016. Despite the existence of alternative models, mammals are still widely used in research. It has been found that rats, mice and guinea pigs are frequently used, and, in addition to these, sheep, rabbits and chinchillas. The methods for auditory evaluation mainly comprise distortion product otoacoustic emissions, brainstem auditory evoked potential and histological evaluation, especially in rodents. Choosing the experimental animal to evaluate the auditory system depends on anatomical, physiological, economic, spatial and psychosocial factors, and on the evaluation's objective.

Keywords: Models, Animal; Hearing; Speech, Language and Hearing Sciences

RESUMO

O objetivo dessa revisão é delinear os modelos animais viáveis para a pesquisa pré-clínica auditiva, considerando suas características anatômicas, fisiológicas, vantagens e desvantagens. Foram consultadas as bases de dados Scielo, Pubmed e Periódicos Capes, utilizando descritores envolvendo audição, testes auditivos e espécies animais, individualmente e cruzados entre si. Foram lidos os resumos dos artigos encontrados nas bases de dados, com posterior seleção baseada nos critérios: artigos disponíveis em sua integridade, uso de modelos animais em procedimentos audiológicos que incluísse a descrição dos métodos de avaliação, as vantagens e/ou desvantagens do uso da espécie, publicados entre 1995 e 2016. Apesar da existência de modelos alternativos, os mamíferos são ainda amplamente utilizados em pesquisa. Constatou-se que os ratos, camundongos e cobaios são frequentemente utilizados e, além destes, ovelhas, coelhos e chinchilas. Os métodos para avaliação auditiva contemplam principalmente emissões otoacústicas por produto de distorção, potencial evocado auditivo de tronco encefálico e avaliação histológica, principalmente em roedores. A escolha do animal de experimentação para avaliação do sistema auditivo depende de fatores anatômicos, fisiológicos, econômicos, espaciais, psicossociais e do objetivo da avaliação.

Descritores: Modelos Animais; Audição; Fonoaudiologia

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Mailing adress:
Eliane Dallegrave
Rua Sarmento Leite, 245 - Porto Alegre
Rio Grande do Sul, Brasil
CEP: 90050-170
E-mail: elianedal@ufcspa.edu.br

INTRODUCTION

In the present scenario, many scientific tests are carried out by *in vitro* methods in a controlled laboratory environment, or *in silico*, mimicking biological processes with computer assistance. Neither of them use animals, but they have restrictions, as some research can only be performed *in vivo*.

The importance of animal use in research for scientific advancement and improvement of the knowledge of the physiological mechanisms of diseases is highlighted in several studies, showing the importance of *in vivo* evaluation techniques that can be applied in humans in the future[1].

The basis of the use of animal models permeates many aspects for its justification. To be able to use living beings in research, it is essential to know the characteristics of their anatomy and physiology, which tests are appropriate for the correct interpretation of results, and the advantages and disadvantages of using each species.

Thus, this review aims to delineate viable animal models for preclinical hearing research – that is, performed on animals to predict possible effects in humans –, considering their anatomical characteristics, and advantages and disadvantages of use.

METHODS

Scielo, PubMed and Portal Periodicos Capes were consulted, using descriptors in English concerning hearing, hearing tests and animal species, individually and crossed with each other. The descriptors used for hearing were *hearing, ear, auditory, hair cell, ear anatomy, anatomy hearing* and *hearing advantages,* while those related to hearing tests were *distortion product* and *streams processing auditory cortex*. As for the animals, the descriptors used to cross the terms were *animal, animal model, cat, dog, chinchilla*, *Rhesus*, *zebrafish* and *rabbits.*

The abstracts of the articles found in the databases were read, with subsequent selection based on the following inclusion criteria: free articles, use of animal models in audiological procedures that included the description of the evaluation methods, the advantages and/or disadvantages of use of the species, and published between 1995 and 2016.

The exclusion criterion was use of animal models in audiological procedures without the description of the evaluation method.

Other sources, such as the Alternative Methods Network (RENAMA)[2] and the National Council for Animal Experimentation Control[3] (CONCEA), were consulted in order to standardize the concept of the conscious use of animals and examine other sources, duly cited, outside the described databases.

LITERATURE REVIEW – RESULTS

Table 1 shows the research results, according to the descriptors and consulted databases.

The search for alternative methods for auditory evaluation in animal models that are adequate to predict the possible effects in humans of the exposure to ototoxic agents reveals that, among the specific methods, distortion products and auditory evoked potential were the most used (Table 2).

Regarding animal models in hearing research, rodents are the most commonly used animals, often in the investigation of the pathophysiological mechanisms of hearing damage and their possible reversal. In addition to these animals, fish, sheep, dogs, cats, monkeys and other alternative models were also cited.

After evaluating the abstracts of the studies selected by the inclusion criteria, the anatomical, histological and audiological characteristics, which will base the classification of the models regarding the advantages and disadvantages, will be presented and discussed.

Anatomical studies

The external ear of domestic animals, such as dogs and cats, is composed of pinna, external ear canal and tympanic membrane, and is responsible for the transmission and direction of sound waves to the middle ear[4]. The external ear canal does not lead directly to the tympanic membrane; it makes a turn (important for proper otoscope insertion and cleaning)[5].

Table 1. Research results according to the databases consulted, descriptors, evaluated articles and referenced articles

Database	Descriptor	Evaluated	Referenced
Periódicos CAPES	Animal hearing	3	1
Scielo	Auditory and dogs	10	2
	Animal model anatomy	2	1
Pubmed	Chinchilla distorction product	10	6
	Rabbit model hearing	13	5
	Hair cell zebrafish	178	3
	Alternative methods animal ear	72	2
	Alternative methods animal hearing	27	2
	Hearing mice advantages	13	2
	Rabbit ear anatomy hearing	10	2
	Streams processing auditory cortex	82	2
	Auditory evaluation methods in animal models	18	1
	Alternative methods model insects hearing	2	1
	Cat eat anatomy	101	1
	Dogs ear anatomy	87	1
	Rabbit distorction product	12	1
	Rhesus hearing	34	1

Table 2. Studies containing hearing evaluations in animal models

Evaluation	Species	Number of studies
Distortion Product Otoacoustic Emissions (DPOAEs)	Paca[12], Chinchilla[18,31,32,33], Guinea Pig[26], Rabbit[28,29,34,40], Rabbit/Rat/Chinchilla[30]	11
Brainstem Auditory Evoked Potential (BAEP)	Paca[12], Rhesus Monkey (*Macaca mulata*)[15], Chinchilla[18,32], Rabbit[25], Guinea Pig[26], Mouse[27], Dog[36,37]	9
Tympanometry	Chinchilla[18], Mouse[27], Rabbit[28,29]	4
Cochlear microphonic	Chinchilla[32,33]	2
Summating potentials; Compound action potentials	Chinchilla[32,33]	2
Auditory Evoked Potential (AEPs)	Zebrafish[19], Turtle (*Caretta caretta*)[38]	2
Evoked Potentials from a Inferior Colliculus	Chinchilla[31,33]	2
Compound action potentials; Optically evoked compound action potentials	Cat[35]	1
Transient otoacoustic emissions	Paca[12]	1
Pure Tone Responses	Locust (*Locusta migratoria*)[39]	1
Behavioral methods	Turtle (Caretta caretta)[38]	1
Otoscopy	Mouse[27]	1
Otologic surgery	Pig[8]	1
Histology by optical microscopy	Cat[6,35], Chinchilla[7,31,32], Guinea Pig[9], Sheep[11], Rhesus Monkey (*Macaca mulata*)[15], Guinea Pig/Rat[17], Zebrafish[19], Mouse[27], Rabbit[28]	12
Histology by electron microscopy	Paca[12], Guinea Pig/Rat[17], Rabbit[25], Guinea Pig[26]	4
Cytocochleogram/cochleogram	Chinchilla[31,32]	2
Time-Lapse Imaging	Zebrafish[22]	1
Computed tomography	Chinchilla[7]	1

The tympanic membrane separates the external from the middle ear, being supported by a tympanic ring, which is dorsally interrupted by a notch. The tympanic ring has the configuration of an inclined semi-transparent blade, being oval in dogs, pointed in cats, circular in swine, and oval in equines and bovines[4]. In cats, it spans the width of the fibrocartilaginous rings that form the entrance to the external ear canal, and is fine, semitransluscent, and of white-gray color[6].

The middle ear consists of tympanic cavity, hearing ossicles and auditory tube. The latter connects the tympanic cavity to the nasal pharynx and has the function of equalizing the air pressure on both sides of the tympanic membrane[4]. The tensor tympani muscle provides greater sensitivity to the transmission system, and the stapes muscle has a mitigating effect on the transmission[4].

The inner ear consists of membranous chambers and ducts filled with endolymph – the membranous labyrinth –, and the endolymph movement stimulates the inner ear's sensory cells. The membranous labyrinth comprises the vestibular labyrinth, including sacculus, utricle, cochlear labyrinth and union duct, where the spiral organ of Corti and the cochlea are situated[4].

The most important differences in relation to human anatomy include, in rats, the facial nerve, which emerges more superficially and in the antero-rostral temporal bone, the thickness of the ossicles in the middle ear, which are almost entirely hidden on the epitympanum, and the carotid artery, which passes between the crura of the stapes[1]. Among the differences identified in chinchillas, we highlight the fusion of the malleus and the incus ossicles[7]; and, in pigs, analysis revealed that the external appearance of the temporal bone shows discrepancy when compared to humans[8].

Anatomically, there is a difference between the VIII nerve of guinea pigs and men, due to the fact that guinea pigs have the cochlear component involved by the vestibular component until both fascicles join so it is not possible to distinguish the cochlear vestibular component[9]. In monkeys, the Eustachian tube is shorter and flexible, especially in the first years of life, and the physiological function is lower due to the paratubal muscles' anatomy[10].

In sheep, the temporal bone has no defined den – as there is in the human mastoid –, and the mastoid cells are not filled by adipocytes and hematopoiesis's precursor cells[11]. There is an anatomical bulla, the mastoid is not aerated, and the hypotympanum opens inferiorly to the bulla[11]. Moreover, the otoacoustic emissions in animals are different from the results found in humans, and these can be correlated with the differences already known in hair cell patterns[12].

The use of monkeys as animal models for auditory evaluation is especially recommended in the analysis of cerebral cortex function in central processing deficits, because this area finds more similarities between monkeys and humans than between humans and rodents[13-15]. Besides that, there are similarities between monkeys and humans in progressive hearing damage, which increase in severity with aging[15].

The function of the Eustachian tube in humans is smaller when compared to monkeys, probably due to differences between the two species in the anatomy of the tensor veli palatini muscles and the levator veli palatini muscles. In addition, it is highlighted that the occurrence of otitis media in humans, especially in children, is remarkably higher than in laboratory monkeys[10].

There are similarities between the anatomy of human and sheep ears, and the access paths used for surgery are preserved in the procedure. An important similarity between the auditory system of humans and sheep is the size relationship between the structures[16].

Rats have a fragile junction of the tympanic bulla and two and a half turns of the cochlea. They are not as easy to handle as guinea pigs and often present otitis media, because the tympanic membrane does not seal around the external auditory canal and the Eustachian tube is inherently horizontal in the anatomy of rats[1].

Guinea pigs have full bullas, fused malleus and incus, and three and a half turns of the cochlea[17]. In Guinea pigs, the anatomy of the temporal bone, the cochlea and its components, and the vestibulocochlear nerve resembles the humans', which makes them excellent models for comparative studies to the human ear. This animal does not have internal auditory meatus, only external, and the Eustachian tube is cartilaginous[9].

Authors claim that, in research on drugs that have an effect on the cochlea, guinea pigs are a better model than rats due to the greater number of cochlear turns[17]. Furthermore, guinea pigs were easily handled in surgical experiments concerning stapes, the tympanic membrane and the oval window, as well as in micro-dissections, by reason of the size and strength of the temporal bone[17].

Pacas are also animals used in hearing research. Anatomically, their cochlea has a spiral structure constituted by three and a half turns, called: basal turn

(1 turn), turn 2 (1 turn), turn 3 (1 turn) and apical turn (1/2 turn)[12].

One advantage of using chinchillas in auditory system research is easy access to surgery of middle ear structures, as these animals have large tympanic bullas[18]. Their ear has similar structures to the human ear, such as stapes, cochlea, distribution of hair cells and vestibular system[7]. However, it has anatomical differences when compared to the human ear, such as a fusion of the malleus and the incus ossicles, which has also been identified in guinea pigs[7].

Zebrafish (*Danio rerio*) do not have an auditory organ, such as the cochlea, but they have vestibular otolith organs similar to those of mammals, such as the saccule and the utricle[19]. In addition, these fish have an accessible set of hair cells in the lateral line, neuromasts, similarly to other cold-blooded vertebrates, such as salamanders, newts and tadpoles[20]. They also have small organs, which require fewer cells to perform body functions[21], and the access to hair cells in the zebrafish's body surface is a factor that allows the precise determination of the time of exposure to an agent, in this study, the cisplatin[22].

Yet, despite the advantages of the zebrafish model over the mammal model as to the high throughput and easy access to the sensory hair cells, some data on fish cannot be applied to mammals, by virtue of the cell differences, molecular characteristics of the teleost and auditory cells of mammals[23].

The pig is an alternative model for otologic surgery because, anatomically, the temporal bone is in the same position as in humans, and the tympanic membrane, middle ear and ossicular chain have similarities regarding structure dimensions. The temporal line, the spina suprameatum, the external auditory canal and the mastoid cells are considered classic landmarks found in humans; however, these structures were not identified in pigs. In addition, it has advantages over stapes surgery, such as easy vision and manipulation of the incudostapedial joint[8]. The disadvantage of using them as a model is the access difficulty to the middle ear as the temporal bone is covered with soft tissue. The pneumatized cell system is located inferiorly to the anterior tympanic cavity and not posteriorly – as in humans –, does not have a den, and, for viewing the side channel, it is necessary to remove part of the external auditory canal[8].

An evaluation compared the development, organization, structure and function of a specific neuronal circuit in chickens (*Gallus gallus domesticus*) with transgenic quails, focusing on two brain regions fundamental for the sound localization circuit in the auditory brainstem. The results demonstrated that there are structural and functional similarities between the neurons of the regions analyzed in transgenic quails and chickens; quails can be a great model[24].

Histological studies

The use of histological methods allows the evaluation of the pathophysiology of hearing loss. In this sense, one study analyzed cochlear lesion induced by experimental bacterial meningitis in rabbits, observing structures such as the organ of Corti, hair cells, support cells and *stria vascularis*, scala tympani, basilar membrane, scala media, spiral ligament and tectorial membrane through electronic microscopy[25].

Scanning electron microscopy was used in guinea pigs to evaluate the acute organophosphorus toxicity in the auditory system[26]. The presence, in this animal, of three and a half turns of the cochlea, Hensen's cells, tectonic membrane, Reissner's membrane and organ of Corti was evidenced by the same method[17]. In rats, it was possible to demonstrate the presence of tectonic membrane, Reissner's membrane and organ of Corti[1]. This analysis also allowed the electrophysiological, functional and ultrastructural characterization of the paca's inner ear[12].

Another possibility is the histological evaluation by optical microscopy, which evaluated the operation of the middle ear of genetic strains of 61 mice[27]. The external, middle and inner ears of cats were also analyzed by this method[6], as well as the cochlea of rabbits exposed to vibration[28]. In addition, characteristics of the sheep's temporal bone allow the visualization, through optical microscopy, of cellular aspects, ear architecture, intracavitary spaces and anatomy.[11]

One less-used analysis is time-lapse imaging. A study with zebrafish analyzed, using this imaging test, the death of ciliated cells in the lateral line induced by cisplatin[22]. It should be emphasized that this animal is considered a good model for an evaluation of hair cell loss[19].

Studies on audiological evaluation

Distortion product otoacoustic emissions (DPOAE) are widely cited in the literature in studies with animal models. Pre- and post-noise exposure analyses in rabbits have shown that DPOAE can be used to assess and diagnose initial noise-induced hearing loss, even when the result of tonal audiometry is normal[29].

DPOAE similarities between humans, rabbits, chinchillas and rats have been demonstrated[30], and this evaluation was used to determine if the application of buthionine sulfoximine by infusion directly into the cochlea improved the ototoxicity of carboplatin in the chinchilla cochlea[31]. The severity of ototoxicity caused by this drug was evaluated not only by DPOAE, but also by evoked potentials from the inferior colliculus and, anatomically, by cytochocleograms[31]. In a research whose objective was to differentiate the inhibitory mechanisms related to tympanic tensor muscles and stapes in chinchillas, the analyses occurred through real-time DPOAE, which allowed the comparison of components of the medial olivocochlear system and of the middle ear muscle reflex[18].

In an analysis, the carboplatin was applied in chinchillas' ears to verify if selective lesions in inner hair cells and in auditory nerve fibers would generate results of electrophysiological tests similar to those presented in cases of auditory neuropathy. The authors evaluated the animals by cochlear microphonic, DPOAE, summating potential, compound action potential and BAEP[32].

Another analysis with the application of carboplatin in chinchillas' ears aimed to investigate the effects of initial morphological damage on the cochlea and the auditory nerve in the central and peripheral auditory system. Cochlear microphonic and DPOAE were used for the evaluation of external ciliary cells, and the summating potentials, to evaluate the inner ciliary cells. The components of action potentials were measured to evaluate the function and integrity of the inner hair cells and the afferent synaptic fibers in the auditory nerve. The midbrain evoked potentials were measured in the inferior colliculus to evaluate the functioning of the central auditory system. The results indicated that the measures of thresholds and amplitudes failed to detect peripheral pathologies until relatively high damage was achieved[33].

DPOAE and tympanometry were used in rabbits in research on the effects of vibration on hearing. The protocol consisted of baseline audiometry, rest periods, exposure periods, rest periods[28]. This exam was also chosen in a study on the acute toxicity of organophosphorus in the auditory system of guinea pigs, together with BAEP[26].

Still using rabbits, an experiment performed topical application of Papaverine directly to the internal auditory artery and the cochleovestibular nerve, comparing cochlear blood flow and DPOAE between the control group and the treated group, showing the functional loss of cochlear activity[34].

In cats, the tests, found in the literature, to assess the researched cochlear function were composed of auditory evoked potentials and optically evoked action potentials recorded in the round window. The results demonstrated the effectiveness of pulsed infrared radiation stimulating auditory neurons without causing detectable injury, but a limitation on the effectiveness of the stimulation of spiral ganglion cells by pulsed infrared radiation may be the presence of a significant amount of bone in front of the optical fiber, which would cause light diffraction and scattering[35].

Brainstem auditory evoked potentials (BAEP) are also widely used in hearing research. Besides the advantage related to the functional assessment from the cochlea to the brainstem, it is a noninvasive test. In a study on dogs, the authors propose the test's wave latency values as a reference for comparison with Boxer dogs with different diseases, as well as for evaluation in dogs of different ages, in this case, without sedation[36]. However, one of the examination's disadvantages is the possible use of sedation in case of need for chemical restraint, which, in another study, was done through intramuscular administration of morphine and acepromazine. This did not affect the interpretation of the evoked potential, although it caused prolongation in latencies of waves II, III and intervals I-III and IV, without interfering with their identifications[37].

Brainstem auditory evoked potentials (BAEP) were used in Rhesus monkeys in a research that investigated presbycusis[15]. There was a connection between age, threshold increase in ABR and decrease in cochlear histopathology of this primate. The animals studied were between 10 years and three months to 35 years and three months-old, equivalent to 30 to 105 human years. Anesthetic ketamine and medetomidine were used to perform the functional test, in order to provide the appropriate position of the animals for the exam; this is one of the test's disadvantages, as there is evidence that their administration may increase wave latency.

An important difference in BAEPs performed in pacas is that the animal's IV-wave is equivalent to the human's V-wave, the first being used as a parameter for the analysis of the electrophysiological threshold in the evaluation[12].

The function of the middle ear of genetic strains of 61 mice was evaluated through tympanometry, otoscopy and analysis of brainstem auditory responses. The combination of these evaluations allows not only the morphological analysis of the middle ear, but also the evaluation of inflammation[27].

An alternative experimental model for hearing evaluation is the *Caretta caretta* turtle. Auditory evoked potentials and behavioral methods were chosen for measuring audiograms of a captive adult female turtle completely submerged. It was evidenced that the audiograms collected through the behavioral testing and auditory evoked potentials are similar, being the auditory evoked potentials advantageous as they can be conducted in a few hours and with untrained animals[38]. Another alternative model, the zebrafish, has already been evaluated by auditory evoked potentials to analyze the death of hair cells induced by the administration of aminoglycosides[19].

There is also research in the literature using *Locusta migratory* locusts for auditory analyses. These animals are characterized by the responses of the auditory receptor cells in pure tones and by having the tympanal auditory organ located in the first abdominal segment[39].

Besides these, rabbits were models in the testing of the effects of mannitol administered topically in the round window after the induction of episodes of repeated ischemia by compression of the internal auditory artery[40].

Other features

In the literature, there are auditory studies using alternative models. Birds, such as quails, are often used, since they have advantages such as small size, great egg production and early sexual maturity, being possible to develop transgenic lines of quails in the laboratory[24].

Birds and chickens are cited as a study model for the evaluation of ciliary cells, being the birds previously characterized as to the time of regeneration, identification of such precursor cells and cellular processes[20].

Zebrafish have been widely used as a model in biological research because of their tolerance to temperature variations, ease of reproduction, identification of genes through mutations, and excellent embryology. The embryos are large and transparent and can be seen through the chorion during the first twenty-four hours post-fertilization[21].

In a descriptive analysis of the ovine ear anatomy, which, among its purposes, was to identify a suitable animal for experimentation and training in otologic surgery, advantages were reported, such as docile behavior, and no need to keep the animal confined in the laboratory. Thus, in long observational periods, the sheep could be kept in farms, other than in the laboratory, increasing the animals' comfort and reducing the susceptibility to infection by diseases. In addition, sheep are widely available due to the economic activities related to meat consumption and wool use[16].

The easy manipulation of the guinea pig, by reason of its small size and for being a docile animal, has also been highlighted in the literature[9]. Animals such as cats, dogs and monkeys – in addition to having a different body size from humans – can be difficult to handle in the laboratory, because they are aggressive and susceptible to diseases, can be costly, be less available, and, for being pets, can cause a negative psychosocial effect and meet objection from animal rights agencies[16].

DISCUSSION

Table 3 shows the advantages and disadvantages of using animal models in audiological assessments, by species.

In relation to the anatomical characteristics of mammals as animal models, there are several advantages regarding their use in audiological evaluations, mainly with reference to anatomical similarities to humans, especially monkeys, although some structures are different or absent. However, in some cases, these animal models require sedation for the tests, bring a negative psychosocial reflex and are more expensive.

Economically, small animals are more advantageous because of the smaller volume of food ingested and less space required in the laboratory to maintain animal comfort. Animal size does not seem to be a completely dependent factor in the manipulation of the model, since sheep – although they have a significantly larger size than rats – are easily manipulated in audiological studies, unlike reports related to the possible difficulty in manipulating rats.

As for the anatomical differences between humans and animals, the difference with rats, whose tympanic membrane is semi-occluded, was highlighted. It should be noted that these animals, because of this characteristic, have a greater tendency to otitis media, being a good model for mimicking this pathology. However, in studies whose objective is impaired by the occurrence of otitis, other models should be considered.

Table 3. Advantages and disadvantages of the use of animal models, by species

Species	Advantages	Disadvantages
Chinchilla	Easy access for surgery of middle ear structures[18]. Ear structure similar to human's[7].	This animal's anatomy makes it difficult to access the auditory canal[7].
Rabbit	Similarity of DPOAE emission characteristics between humans, rabbits and rats[30].	There might be a lower availability of the animal.
Dog	Anatomical advantages due to similarities to humans.	High cost and lower availability; negative psychosocial effect[16]. Use of sedation in case of chemical containment[37].
Zebrafish	Tolerance to variations in temperature; ease of reproduction; identification of genes through mutations and excellent embryology[21]. Easy access to hair cells; presence of otolithic vestibular organs similar to mammals[19].	Absence of an auditory organ per se[19]. Some fish data cannot be applied to mammals[23].
Cat	Anatomical advantages due to similarities to humans.	High cost and lower availability; negative psychosocial effect[16].
Rhesus Monkey (*Macaca mulatta*)	Similarities between monkeys and humans regarding progressive auditory damage, which increases in severity with aging[15].	High cost and lower availability; negative psychosocial effect[16]. Use of anesthesia.
Mouse	Wide availability of tests to assess hearing damage.	There is a relationship between age and resistance of the tympanic membrane, which may lead to alteration of the middle ear response[27].
Sheep	Its characteristics allow visualization through the histological analysis of cellular aspects, ear architecture, intracavitary spaces and anatomy[11]; docile behavior; can be maintained in farms, increasing animal comfort and reducing susceptibility to disease infection; wide availability; access paths in surgery are preserved[16]. Similarity of size between sheep and human structures.	There might be a lower availability of the animal.
Rat	Presence of tectorial membrane, Reissner's membrane, organ of Corti and cochlea[1,17].	It is not so easy to manipulate; frequently have otitis media; fragile junction of the tympanic bulla[1,17].
Guinea Pig	Full bullas, incus and malleus fused, three and a half cochlear turns, Hensen's cells, tectorial Membrane, Reissner's membrane and organ of Corti; manipulation for surgical experiments that involve oval window, tympanic membrane and stapes; microsection given their temporal bones' robustness and size[17]. Easy manipulation, docile, temporal bone anatomy similar to humans[9].	Few studies on the vestibulocochlear nerve are described in the literature[9].
Paca	Favorable anatomo-physiological characteristics[12].	There might be a lower availability of the animal.
Quail	Small size, large egg production and early sexual maturity[24].	Difficulty of access to inner ear structures.
Locust (*Locusta migratoria)*	Easy manipulation, due to its size and anatomical characteristics.	Few studies using this model; few techniques when compared to other animal models.
Turtle (*Caretta caretta*)	Advantageous auditory evoked potentials as it can be conducted in a few hours and with untrained animals[38].	There might be a lower availability of the animal.
Pig	Temporal bone is in the same position as in humans, and tympanic membrane, middle ear and ossicular chain also have similarities in structural dimensions; easy vision and manipulation of the incudostapedial joint[8].	Temporal line, spina suprameatum, external ear canal and mastoid cells were not identified in this animal; difficulty accessing the middle ear[8].

With respect to the difference between the number of turns in rats and guinea pigs, the authors of the study analyzed suggest that, in research using drugs that influence the cochlea, it would be better to use guinea pigs, since it is the model with a larger number of turns[17]. This study does not justify such an assertion, but there may be a relationship between the greater number of turns and a larger basilar membrane size, which would generate a larger spectrum of frequencies and more hair cells.

Despite the absence of an auditory organ and some results that are not applicable in mammalian animal models, zebrafish are widely cited in auditory evaluation analyses, mainly in the assessment of hair cells in the lateral line of this animal model, demonstrating that anatomical characteristics, reproducibility and habitat are relevant for their choice in different assessment methods.

As an option to the animal models already established in the literature and commonly used, the occurrence of alternative models was verified. Some animals have anatomical similarities, others have been chosen for practicality, size, reproducibility and even for the possibility of transgenic reproduction.

The *Rede de Métodos Alternativos ao Uso de Animais*[2] (RENAMA) highlights the application of the 3R principles: reduction: the use of the smallest possible number of animals to obtain the necessary information to the experiment; refinement: the pain, suffering or stress of the animal used in the experiment should be minimized; and replacement: when the required level of information is acquired without the use of live vertebrate animals.

In accordance with the normative resolutions of *Conselho Nacional de Controle de Experimentação Animal*[3] (CONCEA), it is essential to aim for the possibility of alternatives to the use of animals. If they do not exist, the best techniques proposed should be considered, in order to refine the study and reduce the number of animals used.

With regard to anatomy, isolated attributes may not be decisive for the determination of the animal model used; the advantages and disadvantages of the animals' auditory system characteristics should be evaluated for the appropriate choice.

In order to achieve the objectives proposed in the research, the ideal is that there is a balance between the auditory evaluation method, its feasibility in relation to access to equipment, the presence of a trained professional to evaluate the animal model, and a considerable number of advantages in anatomical and structural terms and greater possibility of generalization for the human auditory system.

It was found, in the literature, a great availability of alternatives for conducting auditory evaluations, such as DPOAE, auditory evoked potentials, scanning electron microscopy (SEM) and cytocochleograms.

In relation to the methods of hearing evaluation, DPOAE and BAEP were the most used, showing themselves to be important research tools. These tests, being objective and non-invasive methods, allow the characterization of hearing damage more reliably than behavioral techniques, especially in research with animal models. However, they are limited to the possible use of sedation or anesthesia, which may interfere with wave latency.

Histologically, cellular morphology characterization may facilitate the analysis of the pathophysiology of hearing loss. However, the choice of the animal model interferes with the type of evaluation to be performed. The counting of hair cells in alternative animal models, such as fish, has been performed by methods such as time-lapse imaging, a technique less widespread than optical or electron microscopy, common in mammalian and avian studies.

Variations in studies may be justified by the research objective, as well as by the animal model chosen and the access to equipment. Therefore, it is essential to know the characteristics of the auditory system of the chosen model, its advantages, disadvantages and limitations in experimental practice. Considering all these aspects, the determination of the number of animals should be as small as possible, respecting, in particular, the standards proposed by RENAMA.

CONCLUSION

The choice of the experimental animal to evaluate the auditory system depends on anatomical, physiological, economic, spatial, psychosocial factors and the evaluation's objective. Rodents are still the most commonly used animal models, and the most frequently cited hearing evaluations are distortion product otoacoustic emissions and brainstem auditory evoked potential.

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