Morphology and electrophysiology of the vestibular organ in the guinea pig
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The vestibular organ of the guinea pig: anatomy and function.

Anatomy

The inner ear of the guinea pig is located in the temporal bone and comprises both the acoustic end organ (the cochlea), which protrudes in the bulla and the vestibular end organ (the vestibule with the utricle and saccule and three semicircular canals) which is embedded in the bone (figure 1).

Figure 1 The guinea pig vestibular inner ear structures.  
ES: endolymphatic sac,  
ED: endolymphatic duct,  
CC: crus communis,  
PSC: posterior semicircular canal,  
PA: posterior ampulla,  
CD: cochlear duct,  
SSC: superior semicircular canal,  
LSC: lateral semicircular canal,  
SA: superior ampulla,  
LA: lateral ampulla,  
UM: utricular macula,  
U: utricle,  
S: saccule;  
SM: saccular macula

There is one cochlear sensory area, the organ of Corti, and there are five vestibular sensory areas, the saccular and utricular macula and three cristae ampullaris in the semicircular canals. All sensory organs lie within the membranous labyrinth, which is filled with endolymph. Endolymph is an extracellular fluid, but its composition resembles an intracellular fluid with high potassium and low sodium concentration. Surrounding the membranous labyrinth to the bone (the bony labyrinth) is the perilymphatic space, filled with perilymph, which has a high sodium and low potassium concentration. The cochlear and vestibular parts of the membraneous labyrinth are connected by the ductus reuniens. This makes the cochlea and vestibular labyrinth part of the same anatomical and functional entity.
Otolith organs

The two otolith organs, utricle and saccule, are located in the vestibule. The endolymphatic compartment of the saccule is connected to the scala media of the cochlea through the ductus reuniens. The saccule and utricle are connected by the utriculo–saccular duct that leads to the endolymphatic duct and the intradural endolymphatic sac. In a normal position of the head, the utricular macula is positioned more or less in a horizontal plane, while the saccular macula is positioned vertically, perpendicular to the utricular macula (figure 2).

The sensory areas are located in the macula of the utricle and saccule. The macula of the utricle is triangular (figure 3), the macula of the saccule hook–shaped. They are covered by the otolithic membrane.

The otolithic membrane consists of different layers (figure 4 & 5). On top are the otoconia, which in the guinea pig and other mammals are calcium carbonate crystals. These crystals have a high density. The otoconia are embedded in a gelatin layer and beneath this layer is the subcupular meshwork. The hair bundles of the sensory hair cells are connected to this meshwork, which is flexible and makes flexion and deflection of hair cell bundles possible.
Figure 4. LM cross-section of the otolithic membrane. On top are the otoliths (O) embedded in the gel layer (GL). The hair cell bundles (HCB) of the sensory cells are embedded in the subcupular meshwork (SM). Bar = 10 µm.

Figure 5. SEM view of the otolithic membrane (for abbreviations see figure 4).

Figure 6. SEM overview of the ampulla. In vivo the cupula (C) reaches the roof of the ampulla, but shrinks due to fixation artefacts. The long hair bundles in the crista ampullaris (CA) are visible. The transitional epithelium (TE) surrounds the crista.

Figure 7. LM picture of the crista ampullaris. Note the higher density and length of hair cell bundles (HCB) in the peripheral part. Next to the crista are the regions of transitional epithelium (TE) and dark cells (DC). MF: Myelinated nerve fibres, C: capillaries, bar = 20 µm.
Semicircular canals

The three semicircular canals are positioned approximately at perpendicular planes to one another. Near the vestibule is a widening of the canals, the ampulla (figure 6). In the ampulla is a saddle shaped area where the sensory hair cells are located, the crista ampullaris.

Compared to the otolith organs, the sensory hair cell bundles of the crista ampullaris are much longer than those in the otolith organs. The density of hair cells is greater in the periphery than in the central part of the crista and the kinocilia are longer in the periphery (figure 7). The sensory hair cells are covered by the cupula, which is a gelatinous mass with a large number of openings which are filled by the sensory hair cell bundles. The cupula extends from the crista ampullaris to the roof of the ampulla.

Sensory hair cells

The sensory epithelium of the macula consists of sensory hair cells surrounded by supporting cells. There are two types of sensory hair cells, who differ in shape and innervation (figure 8).

The type I sensory hair cell is flask shaped and completely surrounded by the terminal end (calyx) of an afferent nerve. The type II sensory hair cell is cylindrical in shape and both afferent and efferent nerve endings synapse with the lower part of the cell. The upper surface of both types contains 20–100 stereocilia and one kinocilium (figure 9). The stereocilia are considered as specialized microvilli containing actin filaments extending into the cuticular plate. They are arranged in a step-like fashion according to length, the longest adjacent to the kinocilium. The kinocilium is longer than the longest stereo-
cilium (figure 10) and is attached to respectively the subcupular meshwork of the otolithic membrane in the otolith organs and the cupula in the cristae ampullares. The stereocilia in a hair bundle are linked by tip–links and side–links (figure 11). Movement of cupula or otolithic membrane results therefore in a simultaneous deflection of the hair cell bundle.

**Supporting cells, transitional epithelium and dark cells**

The sensory hair cells are surrounded by supporting cells, which run from the epithelial surface down to the basal lamina. The supporting cells have no stereocilia or kinocilium and are attached to each other and the sensory hair cells with tight junctions.

The transitional cells surround the sensory epithelium (figure 6). They are similar to the supporting cells, but differ in size and location of the nucleus. The transitional cell is smaller and the nucleus more central than in supporting cells.

The dark cells are only present in the utricle and crista (figure 7). They lie peripheral from the transitional cells, are flat and have the shape of a polygon. In the utricle degenerating otoconia are sometimes found on the

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*Figure 9* SEM picture of hair cell bundles (HCB). The sensory hair cells are surrounded by supporting cells (SC). Note the position of the long kinocilia which are all approximately located on the same side of the hair cell bundle (polarization).

*Figure 10* Cross–section of the utricular macula without otolithic membrane. The dotted lines outline the type I (I) and type II (II) sensory cells.

*Figure 11* EM picture of the tip–links in the hair bundle (arrows). The links and hair bundles are covered by the glycoprotein.
surface. The cytoplasm stains darkly and is filled with large vacuoles and small mitochondria. The basal portion of the cell shows extensive interdigitation of the cytoplasm.

**Glycocalyx**

The glycocalyx is a surface coat, which appears on the surface of most epithelial cells (figure 11). It also covers the sensory epithelial cells of the vestibular system. The glycocalyx consists of a fuzzy layer comprised of glycoconjugates such as mucopolysaccharides, glycoprotein and glycolipid material. In the sensory hair cell bundle, it is thought to have a function in the mechanical coupling of the stereocilia in the transduction process and in the structural integrity of the sensory hair cell bundle.

**Inner ear fluids**

The perilymph surrounds the membranous labyrinth (figure 12). Its contents are high in sodium and low in potassium. Perilymph consists partly of filtration of cerebrospinal fluid and partly of filtration from blood vessels in the ear. The cochlear aqueduct is a narrow channel from the base of the scala tympani directly to the cerebrospinal fluid.
The membraneous labyrinth is filled with endolymph. It is high in potassium and low in sodium content. Endolymph is produced in the stria vascularis in the cochlea. The endolymphatic sac is generally thought to have a function in the resorption process. The current necessary for sensory transduction is mainly carried by potassium ions. The maintenance of potassium concentration in the vestibular organ is attributed to secretion of potassium by the vestibular dark cells, similar to functioning of the stria vascularis in the cochlea. However, how exactly the inner ear fluids are regulated is not known yet and the subject of extensive research.

Function

The sensory hair cells with their hair cell bundle in the macula of the utricle and saccule and in the crista ampullaris of the semicircular canals are built to detect acceleration. The hair cell bundles play a key role in this function. The (long) kinocilium in the hair cell bundle determines the polarization of the cell. In a resting state with the hair cell bundle in a neutral position, the discharge rate of the individual afferent vestibular nerves is stable (figure 13). A displacement of the hair cell bundle towards the kinocilium results in an increase in the firing rate of the afferent nerve fibres that are in contact with the sensory hair cell. Displacement away from the kinocilium decreases the firing rate. A single sensory hair cell can detect acceleration in the direction of the polarization. Consequently, when more cells are present with a different polarization, different directions of motions can be detected.

The otolith organs are specifically built to detect linear acceleration and to detect head orientation. The maculae are surrounded by endo-lymph. The surface of the otholithic membrane is covered by the otoliths with a high mass. When a linear acceleration occurs, the position of the stereocilia of the macular sensory hair cells changes immediately. Since the ooliths have a higher mass and are surrounded by endolymph, the movement of the oolith membrane will be delayed.

Figure 13 Discharge rates of vestibular nerve fibres are dependant on the flexion or deflection of the hair cell bundle. The polarization is directed towards the kinocilium (to the right in the drawing)
The relative position of the otolithic membrane to the macula shifts and since all kinocilia are embedded in the subcupular meshwork of the otolith membrane, the sensory hair cell bundles deflect, some towards and some away from the kinocilium and thereby change the nerve fibres discharge rates (figure 14). When the acceleration ends, the elastic subcupular meshwork and gel layer bring the otolithic membrane back in its resting position. To detect different directions of acceleration, the polarization of the kinocilia on both maculae is complex (figure 15). In the utricular macula, the kinocilia are positioned towards the midline (striola) of the macula, while in the saccule they are positioned away from the striola. Moreover, since both maculae are curved areas and the position of macula of the utricle and saccule is approximately perpendicular, any head tilt or linear acceleration direction will result in a stimulation or inhibition of some hair cells and therefore makes the vestibular system sensitive to linear acceleration and gravity detection in all directions.

The semicircular canals are specifically adapted to respond to angular acceleration. While the structure of the sensory hair cells is the same, the hair cell bundle is characterized by the long stereocilia and kinocilium which is embedded in the cupula. In contrast with the otolith organs, all sensory hair cells in a crista are polarized in the same direction over the complete surface. In the lateral crista they are oriented towards the utricle, in the other cristae away from the utricle. When the head tilts or turns the position of the semicircular canals changes immediately (figure 14). The movement of the endolymph is delayed and the cupula with the sensory hair cell bundles deflects. A single crista ampullaris can give information about one plane of angular acceleration. Since the planes of the semicircular canals are almost perpendicular, angular accelerations in all planes can be detected by the vestibular system.
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For animal experimental purposes, the use of routine clinical tests of the human vestibular function is limited. The two most widely spread clinical test methods, caloric and rotating chair test, give an impression of the function of the vestibular system as a whole. Both test methods measure the responses to a vestibular stimulus by means of a nystagmogram, which is an indirect method, relying not only on the peripheral vestibular organs but also on the vestibular nerve, brain–stem nuclei system and an intact oculomotor system. Furthermore, only the responses of the horizontal semicircular canal are measured. Also, in the rotating chair test the resulting response from both vestibular organs is measured.

In the ideal animal experimental design, individual parts of the vestibular organ can be stimulated and measured quantitatively, longitudinally and can be reproduced. One of the main problems of vestibular function testing is the presentation of a measurable physiological vestibular stimulus. As mentioned before, the vestibular system is sensitive to linear and angular acceleration. Since the vestibular system is located inside the skull, administering such a stimulus accurately and reproducible requires an elaborate mechanical test set–up. A rigid coupling of the stimulator to the skull is necessary to ensure that given stimuli actually reach the vestibular organ. Both angular and linear accelerations are possible. Angular acceleration excites the semicircular canals, which is a slower system than the otolith organs that are stimulated by linear acceleration. Pulse–like linear acceleration, however, is similar to vibration which also elicits a response in the cochlea, which has to be separated from the vestibular response.

In the last two decades, the development of short latency vestibular evoked potentials has extended the possibilities to investigate the vestibular system. The principle is simple. High intensity, repeatable angular or linear acceleration impulses with a short rise time are applied to the head of the subject. The rapid acceleration elicits a synchronous response from a high number of neurons, which can be measured by implanted or skin electrodes. Since the responses remain very small and responses to a single stimulus disappear in noise, a high number of identical stimuli are given and the responses averaged, which separates the response from the noise.

Elidan, Plotnik, Honrubia et al. have used angular and linear acceleration to elicit VsEPs measured with skin electrodes in several species. The origins to linear and angular acceleration were found to be mainly from the otolith organs, respectively semicircular canals. However, there is always stimulation

**Short latency vestibular evoked potentials**

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and a response of all vestibular organs, although small. After destruction of the vestibular organ, the responses disappear, which confirms the vestibular origin. Böhmer et al have measured the responses to linear acceleration by inserting an electrode in the facial nerve canal. This made reliable longitudinal measurements possible with a higher strength and signal/noise ratio.

To differentiate the responses from the cochlea and the vestibular organ, the most reliable method is to destruct the cochlea by drilling away the modiolus and the cochlear nerve. Unfortunately, this crude method has a large negative influence on the function of the vestibular organ as well. The most common method of differentiating the vestibular from the cochlear response is acoustic masking. Loud white noise is used to (over)stimulate the cochlear hair cells so that an added vestibular stimulus can no longer elicit a response from the cochlea. Unfortunately, the noise can influence the vestibular response as well.

Vestibular evoked responses have already been used to investigate several functional and pathological processes in the vestibular system. This includes fetal development, otoconia deficient mice and the effect of aminoglycosides, diuretics, head orientation and gravity. The application of short latency evoked responses in vestibular research promises to be a valuable tool for examining vestibular function in animal experiments.
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