Methodology

Xenopus laevis tadpoles (stage 52) or heads were fixed with 4 % paraformaldehyde and embedded in paraffin. Transversal 20-µm-thick sections were cut on a microtome. Slices were transferred to a water bath and immediately mounted onto slides. Slides were left overnight at 37 °C. Afterwards sections were incubated in Xylol for paraffin removal before performing the Nissl staining. Therefore sections were rehydrated with decreasing alcohol concentration and stained with thionin solution. Thionin binds to nucleic acids and therefore stains cell nuclei (DNA) and rough endoplasmic reticulum (RNA). Sections were again dehydrated with increasing alcohol concentration, incubated in Xylol and finally coverslipped. Images were taken with an Olympus BX63 microscope. For overview images a 4x objective and for the detail view images a 20x objective was used.

Captions

<u>Scheme</u>

Schematic drawing of a Xenopus inner ear, redrawn after Bever et al., 2003. In black the sensory epithelia of the different sensory end organs are highlighted. In magenta the respective planes corresponding to the different Nissl pictures are indicated. aa, anterior ampulla; ac, anterior canal; ap, amphibian papilla; bp, basilar papilla; ha, horizontal ampulla; l, lagena; pa, posterior ampulla; pc, posterior canal; s, saccule; u, utricle.

<u>Tadpole</u>

Nissl stained transversal sections showing the right side of a *Xenopus laevis* stage 52 tadpole with focus on the inner ear. Dorsal is up, midline is to the left.

- (1) corresponds to plane b in the scheme, showing the utricle. The insert shows the magnification of the macula, the sensory epithelium of the utricle. The overlying crystals are otoconia, components of the otolithic membrane.
- (2) corresponds to plane c in the scheme, showing the saccule. The insert shows the magnification of the macula of the saccule.
- (3) corresponds to plane d in the scheme, showing the lagenar recess. The insert shows the magnification of the macula of the lagena.
- (4) corresponds to plane e in the scheme, showing the amphibian papilla recess. The insert shows the magnification of the sensory epithelium and the overlying tectorial membrane.
- (5) corresponds to plane f in the scheme, showing the basilar papilla recess. The insert shows the magnification of the sensory epithelium and the overlying tectorial membrane.

<u>Adult</u>

Nissl stained transversal sections showing the left side of a *Xenopus laevis* head with focus on the inner ear. Dorsal is up, midline is to the right.

- (1) corresponds to plane a in the scheme, showing the ampullae of the anterior and horizontal canal. The insert shows the magnification of the anterior ampulla with its crista ampullaris.
- (2) corresponds to plane b in the scheme, showing the utricle. The insert shows the magnification of the macula.

- (3) corresponds to plane c in the scheme, showing the saccule. The insert shows the magnification of the macula of the saccule.
- (4) corresponds to plane d in the scheme, showing the lagenar recess. The insert shows the magnification of the macula of the lagena.
- (5) corresponds to plane e in the scheme, showing the amphibian papilla recess. The insert shows the magnification of the sensory epithelium and the overlying tectorial membrane.
- (6) corresponds to plane f in the scheme, showing the basilar papilla recess. The insert shows the magnification of the sensory epithelium.

Sectioning, staining and imaging was done by me in the lab of Prof. Dr. Hans Gerd Nothwang (Neurogenetics group, University of Oldenburg)