

# Application of quantitative time-resolved confocal imaging for investigating proteins involved in potential modulation of stereocilia length and stiffness

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Previous data indicate that the length and stiffness of outer hair cell stereocilia are actively regulated (1). To find the mechanisms that underlie this stiffness modulation, we studied the membrane-cytoskeleton linker protein radixin, which is present in high concentration in stereocilia (2, 3). The quinocarmycin analog DX-52-1 binds strongly and specifically to radixin and has previously shown considerable selectivity and faster kinetics for radixin over the other ERM proteins (4). Temporal bones were excised from young normal hearing guinea pigs, and a small opening was made at the cochlea's base and at the apex (5). Special double-barreled electrodes were used for cochlear microphonic recording, electrical stimulation, staining of the bundle membrane with the membrane dye Di-3-ANEPPDHQ, and delivery of radixin inhibitor DX-52-1 (1.0mM). Quantitative confocal imaging was performed on a Zeiss LSM 780. In order to visualize the sound-evoked motion of stereocilia, sequences of confocal images were acquired during sound stimulation (6). Pixels acquired during the same phase of the stimulus were extracted using a Fourier series technique, and arranged into movies showing the sound-evoked motion of the bundle (5, 6). Image acquisition triggered acoustic and electric stimulus. The extracellular potentials are tuned to a particular sound stimulus frequency near 220Hz to get maximum response. The acquired image sequences were low-pass filtered and motion quantified through optical flow analysis using Matlab.

When the DX-52-1 radixin blocker was introduced, the amplitude of the sound - evoked electrical potentials decreased substantially, and a pronounced change in the sound - evoked bundle deflections was observed (7). Motion at both the base and the tip of the bundle decreased, and there was a paradoxical increase in electrically evoked motion suggesting the function of radixin protein is important role for the bundle movements (8). Radixin influences several aspects of hair cell function, including sound-evoked stereocilia deflections and electromotility (9). Inhibition of radixin affect the bundle movement which might lead to reduced hearing (10).

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