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Laser Microdissection and Pressure Catapulting is superior to conventional manual dissection for isolating cellular compartments of the cochlea

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Background/aims: Isolating a pure cell pool by conventional methods from a specific cellular compartment of the cochlea for gene expression analysis and other molecular biological studies has proven to be inefficient due to contamination by unwanted other cell types. In this study we set out to demonstrate that Laser Microdissection and Pressure Catapulting (LMPC) is much more reliable than conventional manual cochlea dissection for this purpose.

Methods: Spiral ganglions (SG) were isolated from postnatal rat cochleae by manual dissection and LMPC. Also, modioli from rats were manually dissected. Total RNA was isolated from all three cell pools. The presence of type II iodothyronine deiodinase (D2), claudin 11 (Cld-11), neurofilament light chain (NF-L) and glyceraldehide-3-phosphate dehydrogenase (GAPDH) transcripts were determined in all sample pools by means of RT-PCR and agarose gel electrophoresis.

Results: The similar levels of GAPDH indicated the same amount and quality of RNA in each sample. The transcript of NF-L, a neural tissue marker was detected in all three samples, indicating that they contained RNA from SG cells. Transcripts of the Cld-11 and D2 genes (which are never expressed in the SG cells) were not present in the laser microdissected SG cell pool, but could be detected in both manually dissected pools. This indicates that LMPC is capable of providing a pure SG cell pool as opposed to conventional manual dissection.

Conclusions: We conclude that Laser Microdissection and Pressure Catapulting is superior to conventional manual dissection for isolating cellular compartments of the cochlea. LMPC opens up a vast new frontier for tissue preparation which will have dramatic impact on molecular biological studies of the inner ear.