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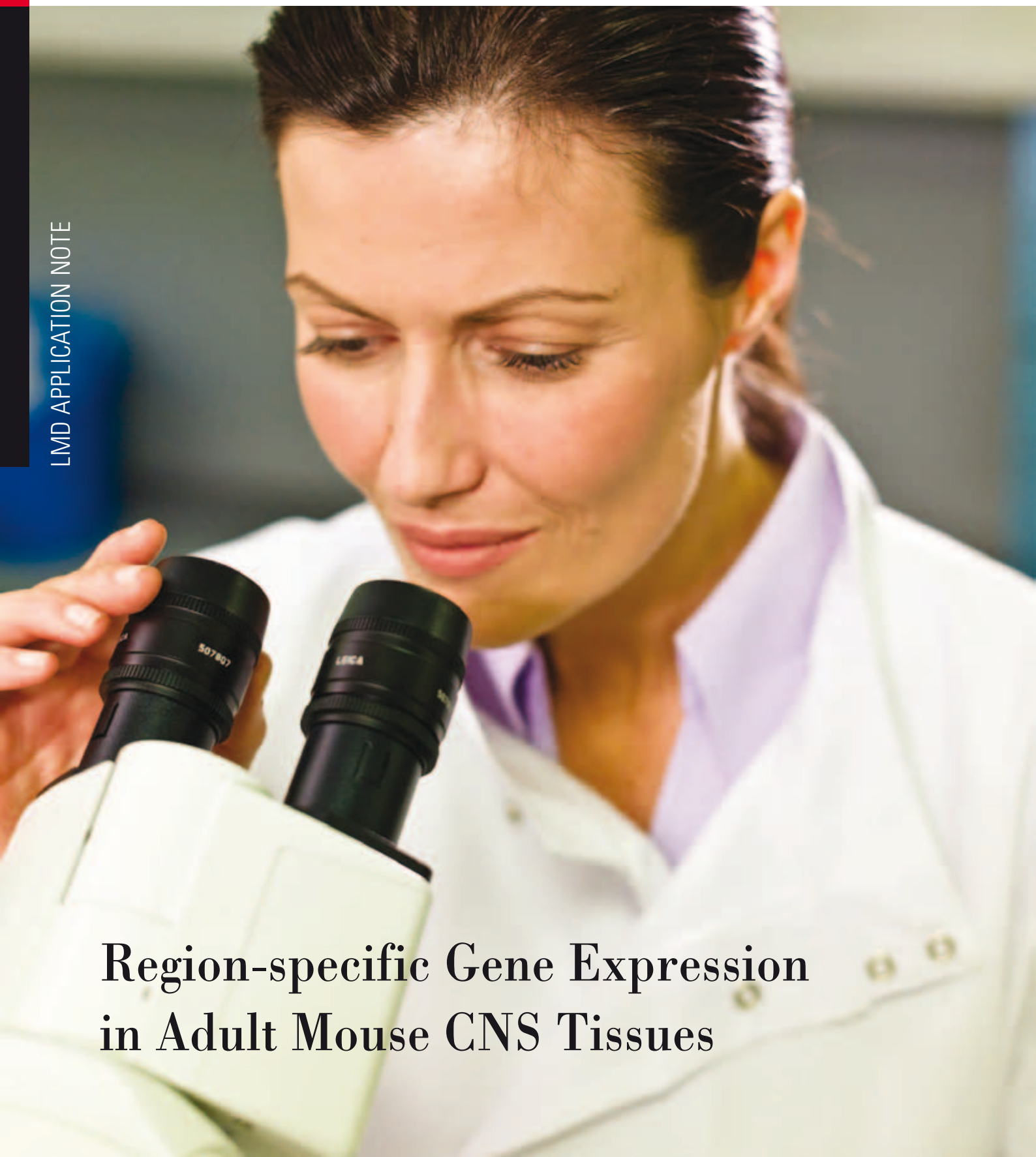

Region-specific Gene Expression in Adult Mouse CNS Tissues

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Abstract

Different areas of the Central Nervous System (CNS) display specific and selective gene expression profiles. Here, we used the Leica Laser Microdissection system LMD6500 to study region-specific mRNA expression in the adult mouse retina and hippocampus. Quantitative real-time reverse transcription PCR (qRT-PCR) and microarray analyses were performed to verify the specificity of the microdissection procedure and the purity of the extracted RNAs.

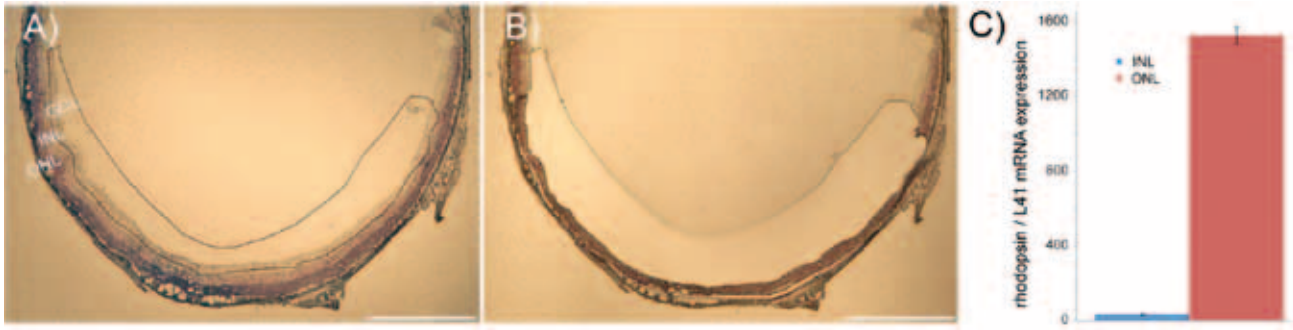

Fig.1: LCM of adult mouse retinal layers. A) uncut section, B) microdissection of ONL, . C) rhodopsin qRT-PCR from microdissected INL and ONL. Abbreviations: GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer; Scale bars (A, B): 400 µm

AUTHORS

Andrea Messina[1], Mark Dunleavy[2,3], Paola Sgadò[2], Valentina Adami[1,2], Yuri Bozzi[2,4], Simona Casarosa[1,4]

[1]Laboratory of Developmental Neurobiology, Centre for Integrative Biology (CIBIO), University of Trento, Italy

[2]Laboratory of Molecular Neuropathology, Centre for Integrative Biology (CIBIO), University of Trento, Italy

[3]Department of Physiology, Royal College of Surgeons (RCSI), Dublin, Ireland

[4]Neuroscience Institute, National Research Council (CNR), Pisa, Italy

1. Laser capture microdissection of adult mouse retinal layers.

Sections from C57BL/6 adult mouse eyes were cut (15 µm) with a Leica CM1850UV cryostat and placed on Leica PEN-membrane slides. After hematoxylin staining, LCM was performed with a Leica LMD6500 microdissector using a 5x objective in brightfield with TL-BF contrast method. The laser parameters used for the dissection were: power 60, aperture 7, speed 7, specimen balance 46 and offset 25. Retinal layers were microdissected using the pencil function to border each single layer (Fig.1A-B). Microdissected layers derived from three mice were pooled, and total RNA was extracted using Macherey-Nagel reagents and protocols. To test the precision of microdissecting single layers, we performed qRT-PCR for rhodopsin, a protein involved in phototransduction specifically expressed of photoreceptors, whose nuclei are located in the outer nuclear layer (ONL) (Fig. 1C).

2. Laser capture microdissection of adult mouse hippocampal subfields.

Sections from C57BL/6 adult mouse dorsal hippocampi were cut (25 µm) with a Leica CM1850UV cryostat and mounted onto Leica PEN-membrane slides. CA1 and CA3 subregions were dissected using the "Draw and Cut" function of the Leica LMD6500 microdissector (Figure 2A-D). The 10x objective with TL-BF contrast was used. The laser parameters used for the dissection were: power 60, aperture 12, speed 10, specimen balance 45 and offset 40. To improve the quality and accuracy of cutting, the concave side of the area to be dissected was cut first to prevent the remaining tissue from falling out of the focal plane. The total volume of tissue dissected from each subregion was CA1: 3.1 ± 0.3 mm3, CA3: 3.8 ± 0.4 mm3 (approximately corresponding to 75 sections per brain). Total RNA was extracted from each pool of CA1/CA3 tissue using Qiagen reagents and protocols and used for hybridization of mouse gene expression arrays (Agilent 4X44K). Microarray data analyses by the Agilent GeneSpring GX software and DAVID functional annotational tools showed a significant regulation of genes involved in synaptic function (Fig. 2E-F).

3. Conclusions

Our results confirm that different CNS regions are characterized by specific gene expression profile. LCM, using the Leica system, is an efficient, contact- and contamination-free approach to further investigate CNS differential gene expression, hopefully providing greater insight into a number of CNS pathologies.

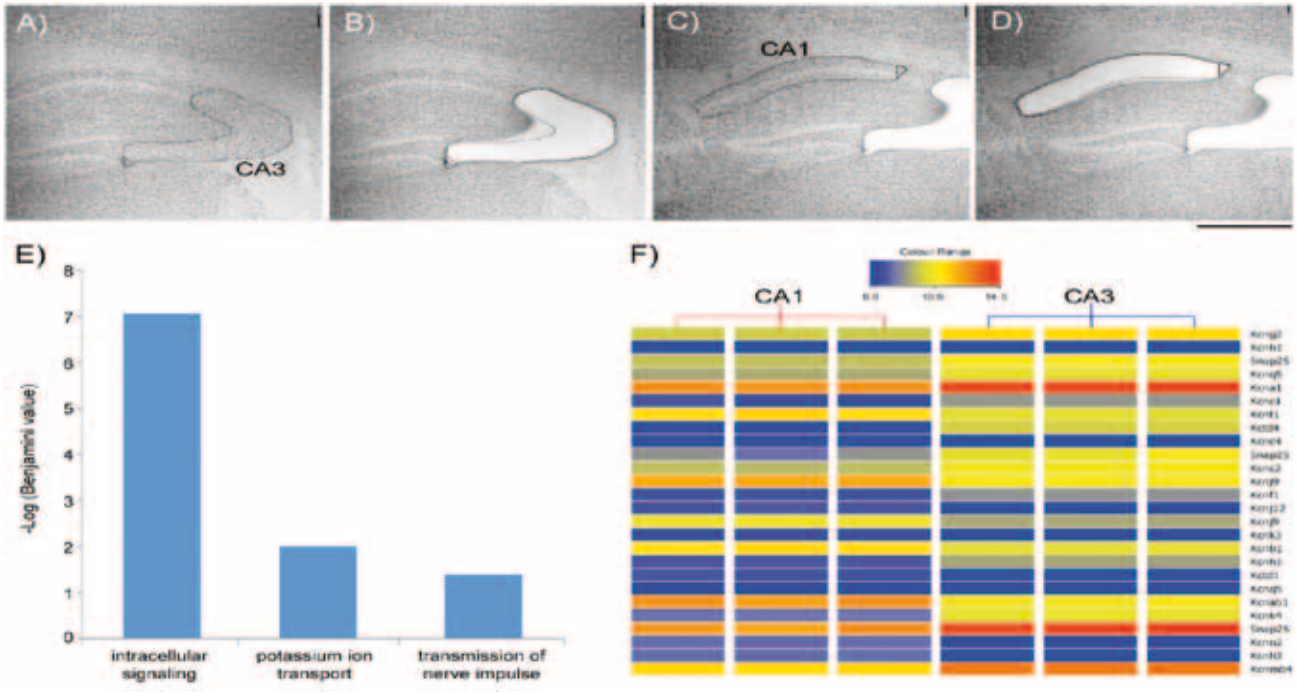

Fig.2: LCM of adult mouse hippocampal subfields. A-D) Section of dorsal hippocampus showing the outline and post-cut of the CA3 and CA1 subregions (scale bar: 275 µm). E-F) Microarray data: bar graph displaying the major biological processes associated with regulated genes (E) and expression heatmap of genes associated with potassium ion transport (F).

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