Whole Transcriptome Digital Spatial Profiling of Pain-Induced DRGs

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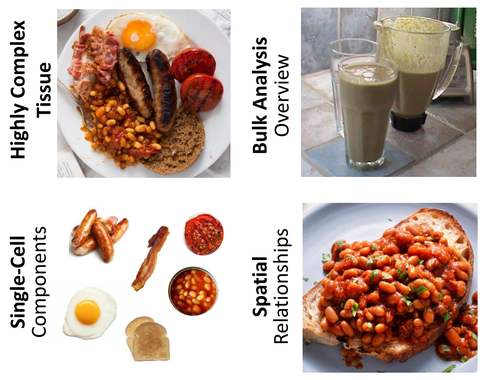
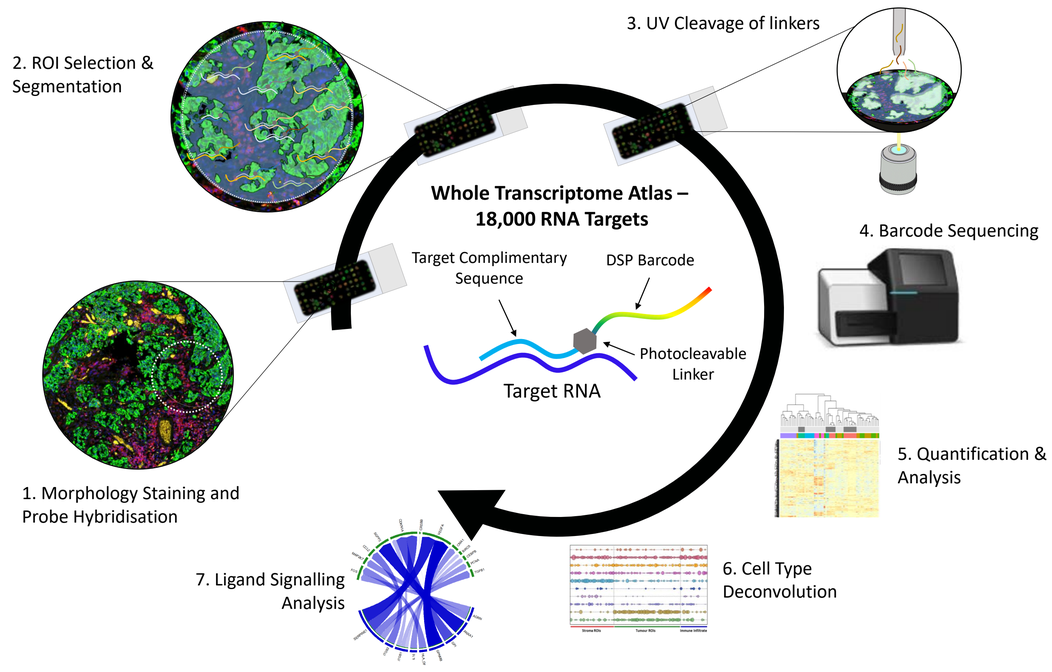
Whole Transcriptome Digital Spatial Profiling

Spatial Analysis - Tissues are not homogenous but are made of multiple discrete compartments. Bulk sequencing methods take a homogenized snapshot, resulting in a huge loss of complexity. Single-cell sequencing methods allow for the cataloguing of individual components within a tissue. However, it is only with spatial techniques that the relationships between these components can be analysed. GeoMx Digital Spatial Profiling (DSP) enables quantitative, non-destructive, whole transcriptome profiling in a single FFPE tissue section.



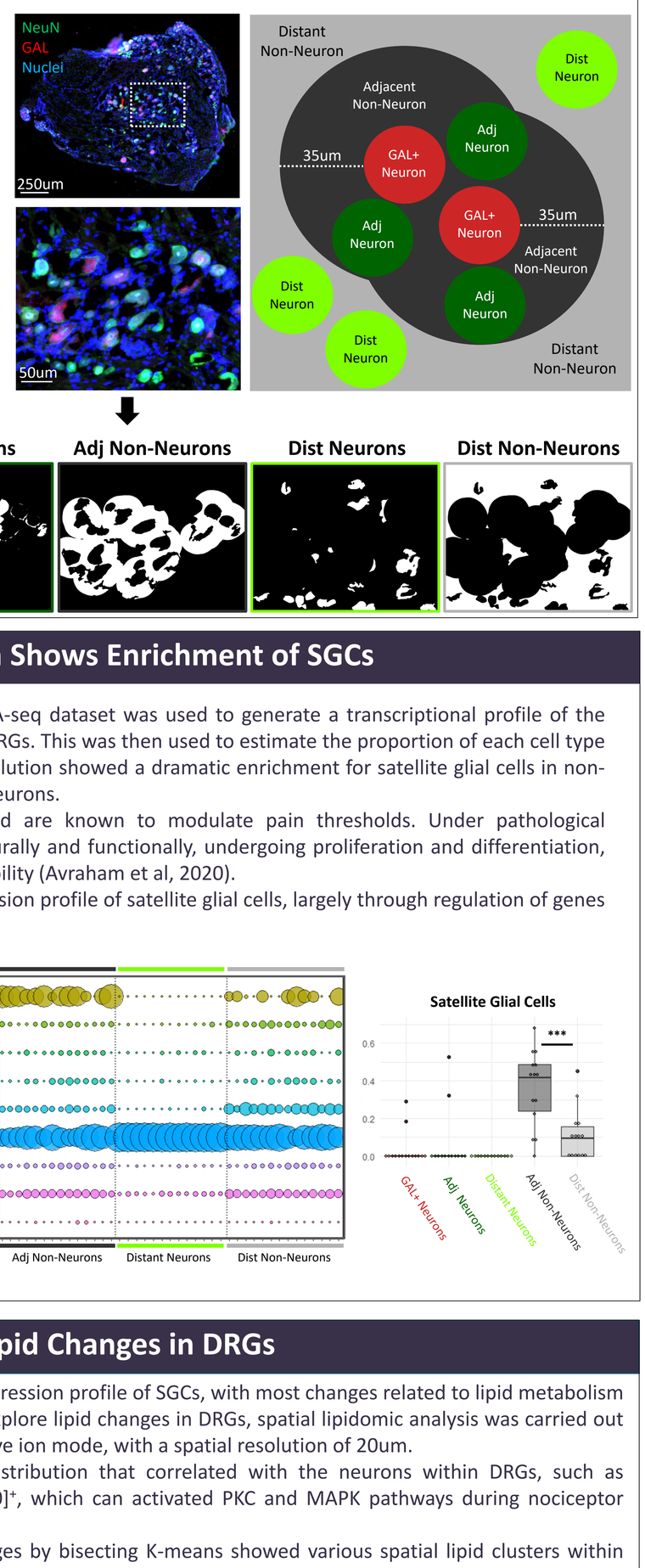


Selecting Regions in Relation to GAL+ve Neurons

Neuropathic pain is pain that originates from pathology of the nervous system, such as through nerve trauma or autoimmune diseases and is thought to affect ~10% of the population.

The Galanin ligand is induced in some neurons in dorsal root ganglia (DRGs) under pain conditions, and plays an inhibitory role in pain processing, with high doses of GAL shown to reduce pain.

To investigate the relationship between these GAL+ve neurons and the surrounding tissue a custom ImageJ script was developed at MDC in order to select regions of interest (ROIs) adjacent to and distant from GAL positive neurons in a pain induced rat model. Regions were further split into neural and non-neural regions based on NeuN staining.



Cell Type Deconvolution Shows Enrichment of SGCs

A publicly available single-cell RNA-seq dataset was used to generate a transcriptional profile of the different cell types present in rat DRGs. This was then used to estimate the proportion of each cell type within each ROI. Cell type deconvolution showed a dramatic enrichment for satellite glial cells in non-neuron ROIs adjacent to GAL+ve Neurons.

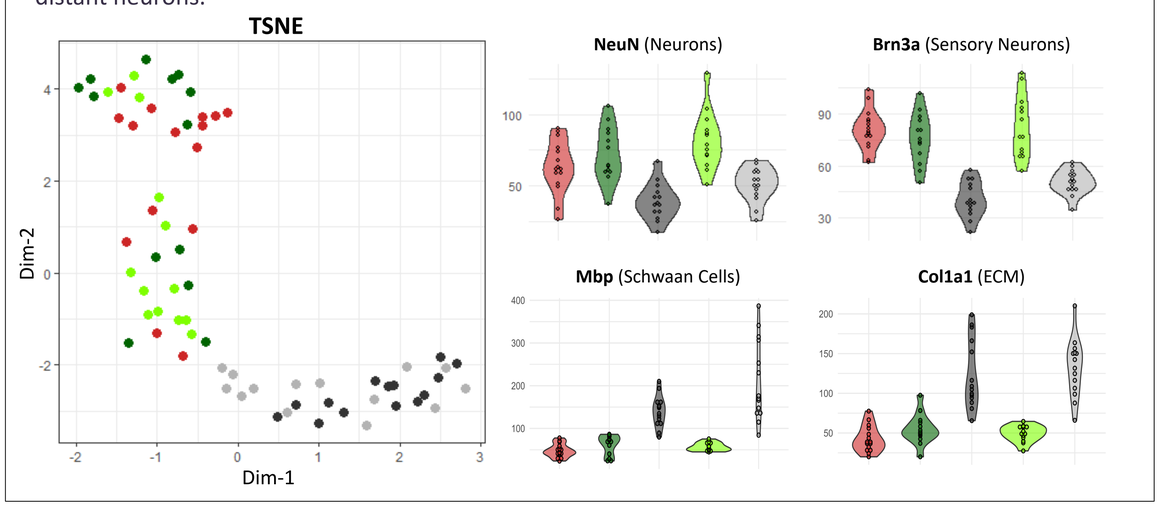
SGCs envelop neuronal soma and are known to modulate pain thresholds. Under pathological conditions they are altered structurally and functionally, undergoing proliferation and differentiation, which may impact neuronal excitability (Avraham et al, 2020).

Nerve injury alters the gene expression profile of satellite glial cells, largely through regulation of genes involved in lipid metabolism.

ROIs from Different Regions are Transcriptionally Distinct

Neuron ROIs were enriched for neuronal markers such as NeuN and Brn3a, while non-neural ROIs were enriched for markers of Schwaan cells and ECM genes.

Dimensional reduction of normalized counts showed that neurons and non-neuron ROIs were transcriptionally distinct from one another. Additionally, GAL adjacent neurons tended to cluster with GAL+ve neurons rather than GAL distant neurons.

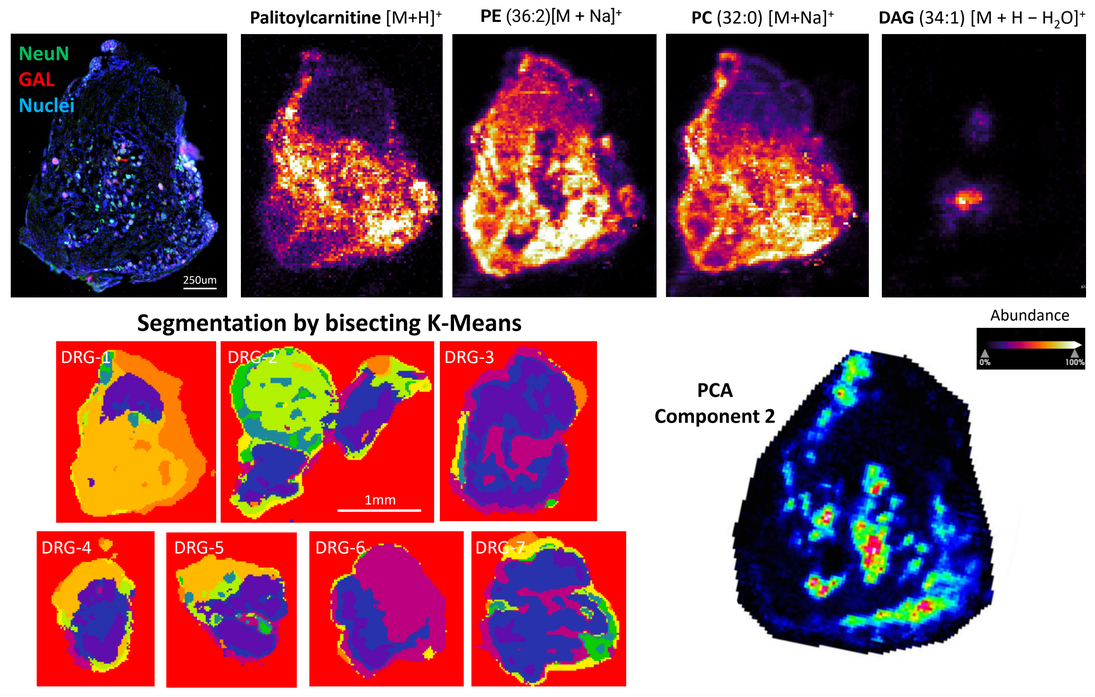


Mass Spec Imaging of Lipid Changes in DRGs

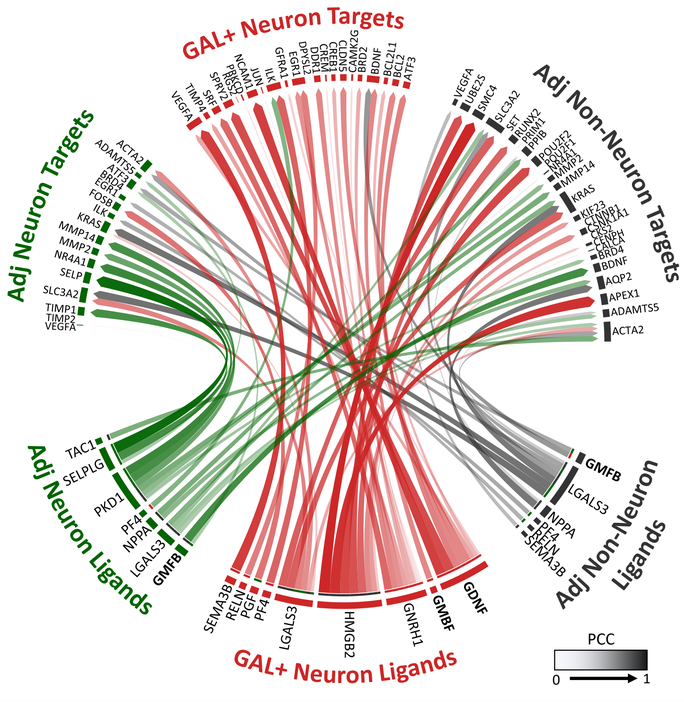
Nerve cell injury alters the gene expression profile of SGCs, with most changes related to lipid metabolism (Avraham et al, 2020). To further explore lipid changes in DRGs, spatial lipidomic analysis was carried out by DESI mass spec imaging in positive ion mode, with a spatial resolution of 20um.

Various lipids showed a spatial distribution that correlated with the neurons within DRGs, such as diacylglycerol DAG(34:1) $[M+H-H20]^+$, which can activated PKC and MAPK pathways during nociceptor sensitization (Loo et al, 2015).

Segmentation of the total ion images by bisecting K-means showed various spatial lipid clusters within DRGs, some of which correlated with GAL+ve neurons. Taken together, these results implicate satellite glial cells and lipid signalling events in response to pain induction around GAL positive neurons.



Ligand Signalling Analysis Reveals GAL+ve Neuron Specific Events



Ligand signalling analysis between GAL+ve neuron adjacent regions revealed a range of ligands with high activity in these regions.

Intensity of the lines between ligand and target gene indicates the Pearson correlation of the two genes in the indicated regions.

Many of these ligands have previously been linked to neurons and pain processing, such as Galectin-3 (LGALS3). Inhibition of Galectin-3 has been linked to attenuation of neuropathic pain after nerve injury.

Both Glial Maturation Factor B (GMFB) and Glial Cell Derived Neurotrophic Factor (GDNF) showed high activity in GAL+ve adjacent regions, further linking changes in glial cells to Galanin induction following pain.

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