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Transcriptional Profiling of Somatostatin Interneurons in the Spinal Dorsal Horn

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The spinal dorsal horn (SDH) is comprised of distinct neuronal populations that process different somatosensory modalities. Somatostatin (SST)-expressing interneurons in the SDH have been implicated specifically in mediating mechanical pain. Identifying the transcriptomic profile of SST neurons could elucidate the unique genetic features of this population and enable selective analgesic targeting. To that end, we combined the Isolation of Nuclei Tagged in Specific Cell Types (INTACT) method and Fluorescence Activated Nuclei Sorting (FANS) to capture tagged SST nuclei in the SDH of adult male mice. Using RNA-sequencing (RNA-seq), we uncovered more than 13,000 genes. Differential gene expression analysis revealed more than 900 genes with at least 2-fold enrichment. In addition to many known dorsal horn genes, we identified and validated several novel transcripts from pharmacologically tractable functional classes: Carbonic Anhydrase 12 (*Car12*), Phosphodiesterase 11A (*Pde11a*), and Protease-Activated Receptor 3 (*F2rl2*). *In situ* hybridization of these novel genes showed differential expression patterns in the SDH, demonstrating the presence of transcriptionally distinct subpopulations within the SST population. Overall, our findings provide new insights into the gene repertoire of SST dorsal horn neurons and reveal several novel targets for pharmacological modulation of this pain-mediating population and treatment of pathological pain.

Mechanical pain is one of the chief symptoms in many pathological pain conditions[1]. Accordingly, understanding the spinal circuits underlying this component of pain perception has been a central aim of preclinical pain research[2,3]. Recent studies employing genetic tools to manipulate specific spinal circuits have greatly expanded our understanding of this question[4–7]. In this way, it was discovered that a population of Somatostatin-expressing (SST) excitatory interneurons in the superficial dorsal horn is required for mechanical pain, as demonstrated by the complete absence of mechanical pain when SST neurons were ablated[6]. It appears that other sensory modalities such as thermosensation and innocuous touch were left undisturbed, indicating that SST interneurons play a specific and restricted role. With the functional role of SST neurons now well-established, it would be advantageous to comprehensively characterize the repertoire of genes expressed by SST neurons, thus providing a basis for their unique properties and highlighting potential targets for selective pharmacological manipulation.

RNA-sequencing (RNA-seq) is a powerful tool to uncover the transcriptome of cells and tissues. To date, gene expression studies of the dorsal horn have used RNA isolated from bulk spinal tissue, which represents a mixture of genes expressed by multiple neuronal and non-neuronal cell types[8,9]. To examine the transcriptional profile of a single neuronal population, isolating RNA solely from those cells is necessary[10].

To access the transcriptome of specific cell types, various methods have been developed. Dissociation of intact neurons from neural tissue coupled with fluorescence activated cell sorting (FACS) has been used to profile neurons and glia in the CNS, but this method requires relatively harsh protease treatments at warm temperatures, which induces artifactual gene expression signatures due to processing[11–13].

To obviate the need to dissociate neural cells, a cell type-specific method called Isolation of Tagged Nuclei from Specific Cell Types (INTACT) was developed[14,15]. In this method, nuclei conditionally express a fusion

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