

Aβ-mechanoreceptor neurons undergo early molecular changes following induction of cortical spreading depolarization

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INTRODUCTION: Regenerative pain medicine is a nascent subfield within orthopedic research aiming to use the body's own capacity for repair [1]. Of interest, is the trigeminal ganglia (TG), a group of sensory nerves that innervate the skull and play a role in bone healing. Previous work has demonstrated that these nerves overlap with those from the trigeminovascular system which innervate the meninges and surrounding blood vessels [2,3]. They have long been implicated in the propagation of pain, leading to headaches like migraine due to nociceptor innervation and their secretion of neuropeptides responsible for sensation of pain [1,2]. Whether these same nerves contribute to both regeneration and injury is unknown. To begin addressing this question, we sought to investigate models of pain sensation in the skull. One model is cortical spreading depolarization (CSD), a method that induces migraine in animals and is characterized by a wave-like depolarization across the cortex with hyperpolarization [4]. Peptidergic neuronal subtypes have been the focus of cellular contributors to migraine as they secrete the pain-linked neuropeptide calcitonin gene-related peptide (CGRP). However, the role of Aβ-mechanoreceptor neurons in migraines has received relatively less attention, even though they also innervate the meninges [6]. Here, we re-analyzed a recently published snRNAseq dataset of the TG following CSD induction [5] to define the transcriptional response of neural subtypes to migraine-inducing stimulation.

METHODS: Previously published filtered matrices were collected from GSE197289. Processing was performed similarly as to the original publication. Briefly, using Seurat [7], nuclei with > 400 features AND < 15,000 UMIs AND < 5% of mitochondrially-encoded transcripts were kept for analysis, data was normalized by (LOG) and scaled to 10,000 transcripts while regressing %MT RNA and total RNA counts (UMI). Harmony [8] was used to integrate the different timepoints and samples while accounting for batch effects. Cell cluster annotations were assigned based on the classification used in the original publication. Pseudobulk was performed by collapsing all single cell UMIs into one cell for all cell types in the dataset. EdgeR [9] was then used to calculate differentially expressed genes by comparing each time point post CSD induction to the 0hr timepoint control. Significance threshold was set to ≥ 1 and ≤ -1 logFC for significantly Up- and Downregulated genes respectively. GSEA enrichment analysis was done using PathFindR [10], using gene lists obtained from edgeR. Pathway datasets were GO Biological Processes and Reactome Pathways within PathFindR.

RESULTS: In order to explore transcriptional differences in neuronal subtypes after CSD induction we conducted pseudobulk differential gene expression (DGE) analysis using edgeR across all clusters in the dataset (Fig. 1a). Out of the 15 cell clusters within the dataset, only 3 clusters showed substantial gene changes post CSD induction at early (1.5hrs) and late (6hrs) time points. This included two cell clusters of Aβ-mechanoreceptors (NF1 and NF2) and the Satellite Glial cells. At the early timepoint the NF clusters showed small upregulated transcriptional changes, although NF1 exhibited increased transcription relative to NF2 (Fig. 2a). At the late time point there was a noticeable increase in transcriptional activity across both cell clusters. In the NF1 population, upregulated transcripts included genes associated with interferon stimulation (*Rsad* and *Ifit1*), inhibitor of BMP signaling (*Nog*) and a neurofilament protein (*Ina*). In the NF2 population top genes included a metal-chelator (*Mt3*), a thyroid response gene (*Thrsp*), and *Calcb* an isoform of CGRP (Fig. 2b). We next performed gene set enrichment analysis using PathFindR to understand biological processes either up- or downregulated after CSD induction. At the early-stage processes involving transcription were enriched (*Chromatin Remodeling*^{NF1/2}, *RNA splicing*^{NF1/2}, *viral entry into the host cell*^{NF1} and *double-strand break repair*^{NF2}) (Fig. 3a). Gene interaction networks generated by PathFindR highlight genes implicated in these processes are downregulated (*data not shown*). We next used the Reactome database to identify signaling pathways enriched in these cells. We identified enrichment of the *RhoA*^{NF1}, *Cdc42*^{NF1/2}, and *Rac1 GTPase Cycle*^{NF1/2} (Fig. 3b). Gene interaction networks highlight genes in the *Cdc42* and *Rac1 GTPase cycle* are downregulated in both populations, whereas for the *RhoA GTPase cycle*, 1/3 of its genes were upregulated (*data not shown*), suggesting that the *RhoA GTPase Cycle* might be transitioning into an activated state.

DISCUSSION: Using a previously published snRNAseq dataset, we identified three cell clusters (NF1, NF2, Satellite Glial) that exhibited transcriptional changes following CSD induction. The NF1 population exhibited increased DEGs relative to the NF2 population although both appeared to be low, which can be explained due to early time point (1.5 hrs) collection. It is worth noting however, both cell populations exhibited downregulated biological processes essential for transcription, which may suggest cellular stress. Exploring enrichment of signaling pathways at the early timepoint suggested both populations had downregulation of *Cdc42* and *Rac1 GTPase cycling*, pathways that are essential for neural stem/progenitor cells' dendritic development, growth and spine maturation [12]. *RhoA*, on the other hand is considered to act antagonistically on these processes, and instead causes axonal and dendritic retraction [11]. 1/3 of *RhoA* pathway genes were upregulated, suggesting it may have been transitioning into a state of activation. If true, this would suggest axonal and dendritic remodeling/retraction, leading to loss of innervation along portions of the meninges, in turn also affecting the vasculature, given their bi-directional communication during development [13]. Taken together, here we show for the first time preliminary transcriptional changes of Aβ-mechanoreceptors in an animal model of headache pain, suggesting their implication in the pathogenesis of migraine pain. Confirmation by *in situ*, staining, or qRT-PCR is necessary to confirm pre-liminary data. Specifically, confirming both axonal/dendritic remodeling via *RhoA* and subsequent loss of meningeal innervation. Not only will this provide new directions for further study, but confirming the involvement of *RhoA* may lead to it being a novel target to treat migraine pain.

SIGNIFICANCE/CLINICAL RELEVANCE: Development of treatment and/or prevention methods for migraine pain has been difficult due to the complexity and lack of complete understanding of the condition. Here we shed light on a novel subset of nerves which appear to activate early on in an animal model of migraine, further study of this population may provide insight into development of new candidate targets for headache pain.

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IMAGES AND TABLES:

Fig1. UMAP of TG dataset split by timepoint

Fig2. Volcano plots of Up- and Downregulated genes within early (A) and late (B) timepoints

Fig3. Enrichment plots of Biological Processes (A) and Reactome signaling pathways (B) in the early time point

