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Title: Analysis of the transcriptional and translational profile of Agrp neurons

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The Agouti-Related Protein neurons (herein Agrp neurons), located in the arcuate nucleus of the hypothalamus, are key regulators of the feeding behavior. Despite abundant evidence of their importance for homeostatic control of energy balance, there is a lack of evidence, at both the transcriptional and the translational levels, of genes activated or suppressed in a scenario of negative energy balance, i.e. food deprivation (FD). Therefore, the aim of this project is to provide a broad view of the transcriptional and translational dynamics of Agrp neurons upon FD. The Agrp neuron transcriptome was obtained from two public datasets: GSE93374 and GSE87544. Raw sequencing data was processed independently for both datasets with the software pipeline: 1) SRA Toolkit (v.2.9.2); 2) FastQC (v.0.11.7); 3) UMI-tools extract (v.0.5.5); 4) STAR (v.2.5.4); 5) Piccard (v.2.18.17); 6) featureCounts (v.1.6.3); 7) UMI-tools count (v.0.5.5). Cells with less than 800 expressed genes were filtered. To identify Agrp neurons, the datasets were merged using the scran (v.1.10.2) MNNcorrect function and clustered using buildSNNgraph function. Next, cells with expression of *Agrp* and *Npy* higher than 0.05 were considered Agrp neurons. Additionally, cells within GSE93374 with raw counts > 0 for both *Agrp* and *Npy* were also considered Agrp neurons. Using *ad libitum* samples as reference, an enrichment fold metric (EF = FD / Fed) was calculated and genes with EF < 0.5 or EF ≥1.105 were considered altered by FD at the transcriptional level. The Agrp neuron translatome was obtained using Agrp-RiboTag mice submitted to *ad libitum* (n=6) or 16-hour food deprivation (n=7). Following immunoprecipitation, sequencing was performed, and raw data was processed with the following pipeline: 1) FastQC (v.0.11.7); 2) STAR (v.2.5.4); 3) HTSeq (v.0.11.1); 4) DESeq2 (v.3.8). Genes with false discovery rate < 0.05 and fold change (LFC) < -0.5 or LFC > 0.5 were considered altered by FD at the translational level. String (v.11) and IPA (v.01-14) were used to analyze pathways and biological processes in both levels. In the transcriptome, 1190 Agrp cells were identified and 1005 genes were considered altered by FD. We observed processes associated to synaptic plasticity and pathways associated to synaptogenesis and glutamate signaling. There is evidence in literature showing that a negative energy balance can induce spine formation in Agrp neurons, and glutamate receptors are important for such plasticity. In the translatome, 529 genes were considered diferentially expressed upon FD. We observed processes like circadian regulation of gene expression and pathways like leptin signaling in obesity and endoplasmic reticulum (ER) stress. Literature shows the importance of circadian clock for the Agrp and feeding behavior regulation and also that ER stress (e.g by FD) can lead to resistance to leptin and increased levels of Agrp and Npy peptides. The transcriptome and translatome of Agrp neurons shared 59 genes, they were associated with ER stress and leptin signaling. In brief, our results showed a clear distinction between transcriptome and translatome levels. The first could respond to FD by forming new spines and facilitating neuron activation while the second could lead to direct changes (protein level), in pathways like the circadian clock and the regulation of neuron sensibility to leptin.