Figure 1. Experimental timeline and regions of interest. A. Diagram of experimental timeline. Postnatal day 0 (P0) represents birth. Litters were culled to 6 hamsters on P7 and weaned to individual housing on P25. Socially Stressed or Control procedure exposure occurred from P28 to P42. Animals were sacrificed on P43. B. Depiction of regions punched for Tag-seq. Left to right, lateral hypothalamus, dorsomedial hypothalamus, and arcuate nucleus of the hypothalamus. Punches were taken bilaterally. Brain drawings here are from the Paxinos and Watson Rat Brain Atlas (Paxinos and Watson, 2007), though a hamster atlas (Morin and Wood, 2001) was used to guide tissue collection.

Figure 2. Boxplots of metabolic measures between Stress and Control subjects. A. Body weight at P42 in grams. B. Percent of weight at P28 gained by P42. C. Total mass of white adipose tissue pads collected in grams. D. Total food efficiency over stress period as grams of weight gained divided by grams of food eaten. Boxplots are depicted with median line and first and third quartile ranges in boxes, with 95% range as whiskers. Points represent the y-axis value of each subject. Significance results of Welch two-sample, two-tailed t-tests are reported for each measure.

Figure 3. Results of differential gene expression (DEGs) in A. lateral (LH), B. dorsomedial (DMH) and C. arcuate nucleus (ARC) subregions of the hypothalamus. Genes were determined to be differentially expressed if the log2 Fold Change was >= |0.2| and significant (eFDR) < 0.05. Top genes were identified as the greatest absolute value log2FC with an eFDR < 0.05 (dashed horizontal line). Dashed vertical lines at |0.75| and |1.5| are included to illustrate numbers of genes at different ranges of change. ↑↑ indicates increased relative expression, ↓↓ indicates decreased relative expression. Top genes were selected by filtering the highest fold change while having an eFDR < 0.05. Functional modules identified by Gene Ontology enrichment analysis (GO terms) are on the right.

Figure 4. Results of weighted gene coexpression network analysis (WGCNA) in A. lateral (LH), B. dorsomedial (DMH) and C. arcuate nucleus (ARC) subregions of the hypothalamus. All noted modules are significantly differentially expressed between groups, with boxplots of module eigengene value shown per region. Below are Gene Ontology (GO) terms associated with differentially expressed modules, and hub genes with highest module membership (MM).

Table 1. Summary of top highly differentially expressed genes across all regions analyzed. Top genes were selected by filtering the highest fold change while having an eFDR < 0.05. LH: lateral hypothalamus, DMH: dorsomedial hypothalamus, ARC: arcuate nucleus of the hypothalamus. log2FC: log2 fold change in expression between groups. Positive values indicate lower levels of transcript in Stressed subjects; negative values indicate higher levels in Stressed subjects. eFDR: enhanced false discovery rate, or permuted p-value.

Table 2. Differential gene expression overlap analysis. Direction indicates if gene transcripts increased or decreased in relative abundance in socially stressed hamsters and in which regions.

Table 3. Differential gene expression incongruence analysis. First organized by which regions conflicting genes were increased in expression in stressed subjects, then which regions they had decreased relative expression in.

Supplemental Figure 1. Principal Component Analysis by Region of Interest. Separation of normalized gene expression data of samples by region.

Supplemental Figure 2. Results of weighted gene coexpression network analysis (WGCNA) in A. lateral (LH), B. dorsomedial (DMH) and C. arcuate nucleus (ARC) subregions of the hypothalamus. Presented are each module and the number of genes in that module. Values in the table represent the Pearson correlation interaction term between Control and Stressed groups, with asterisks marking statistically significant differences: \* = p < 0.05, \*\* = p < 0.01. Positive values are higher expression of the module in the Stress group, negative values are lower expression in the Stress group.

Supplemental Figure 3. Spearman correlation between top differentially expressed genes and subjugation behaviors. Behaviors recorded during social stress were correlated with the top 10 most differentially expressed genes (the five genes with the highest positive and negative relative expression, determined by highest absolute log2FC and eFDR < 0.05). Only average attacks received by the intruder, tail-ups displayed by the intruder, and scent markings from the resident were correlated, as the other behaviors occurred with too little frequency for valid correlation. All comparisons were made with Spearman correlation (with r values displayed in table), as gene expression data was not normally distributed, and p values were Holm-corrected for multiple comparisons. No comparisons had a corrected p < 0.1.