

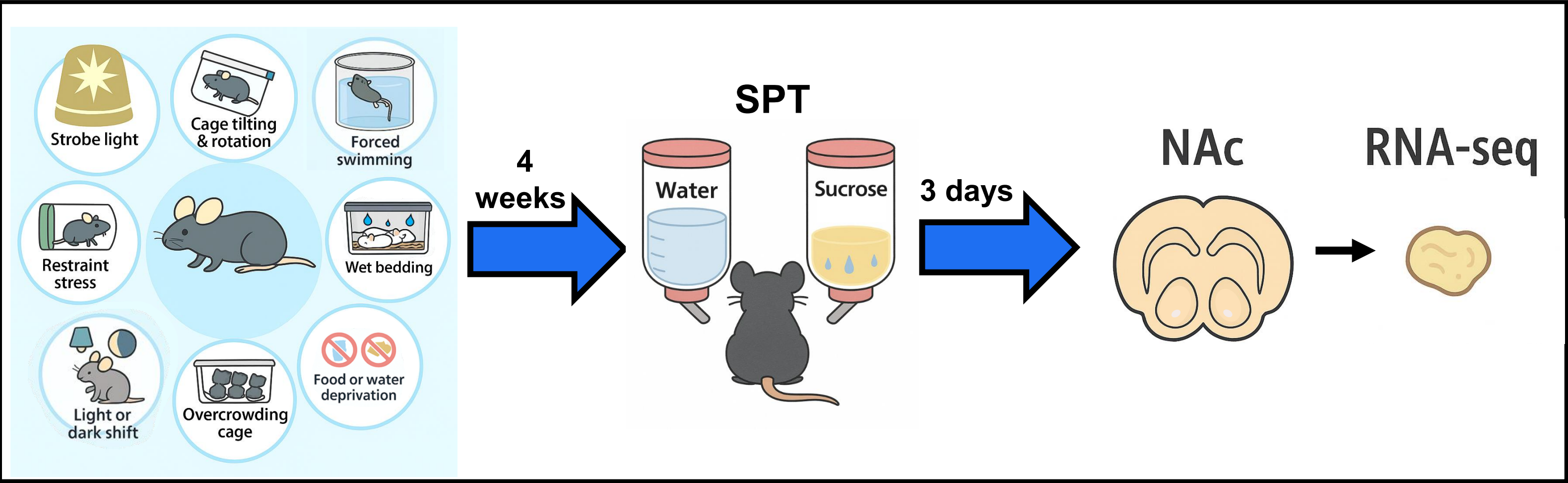
Chronic Stress-Induced Alterations in Feeding Behavior and Emotional Phenotypes via *Mc4r* Downregulation in the Nucleus Accumbens

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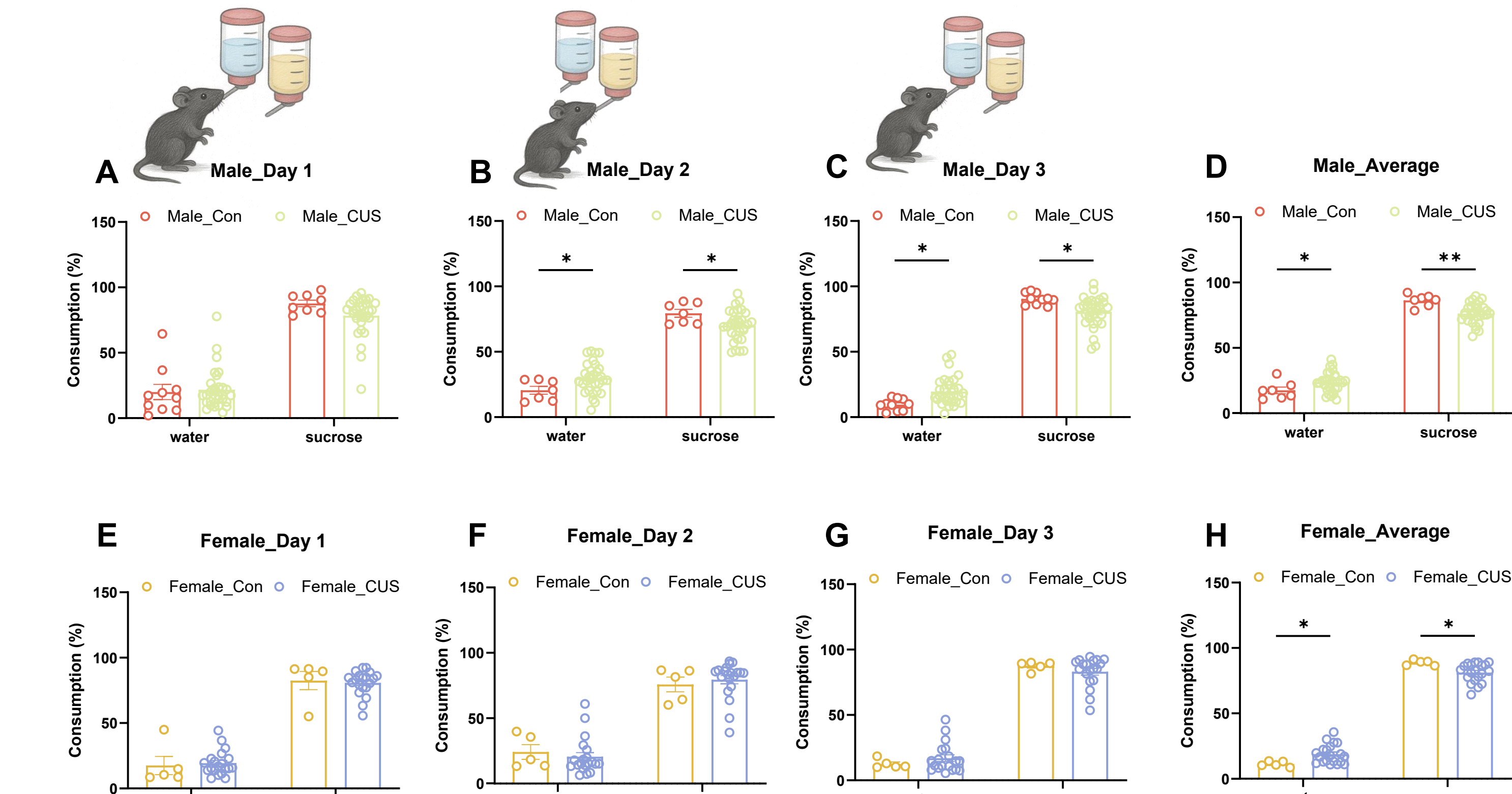
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Materials and Methods

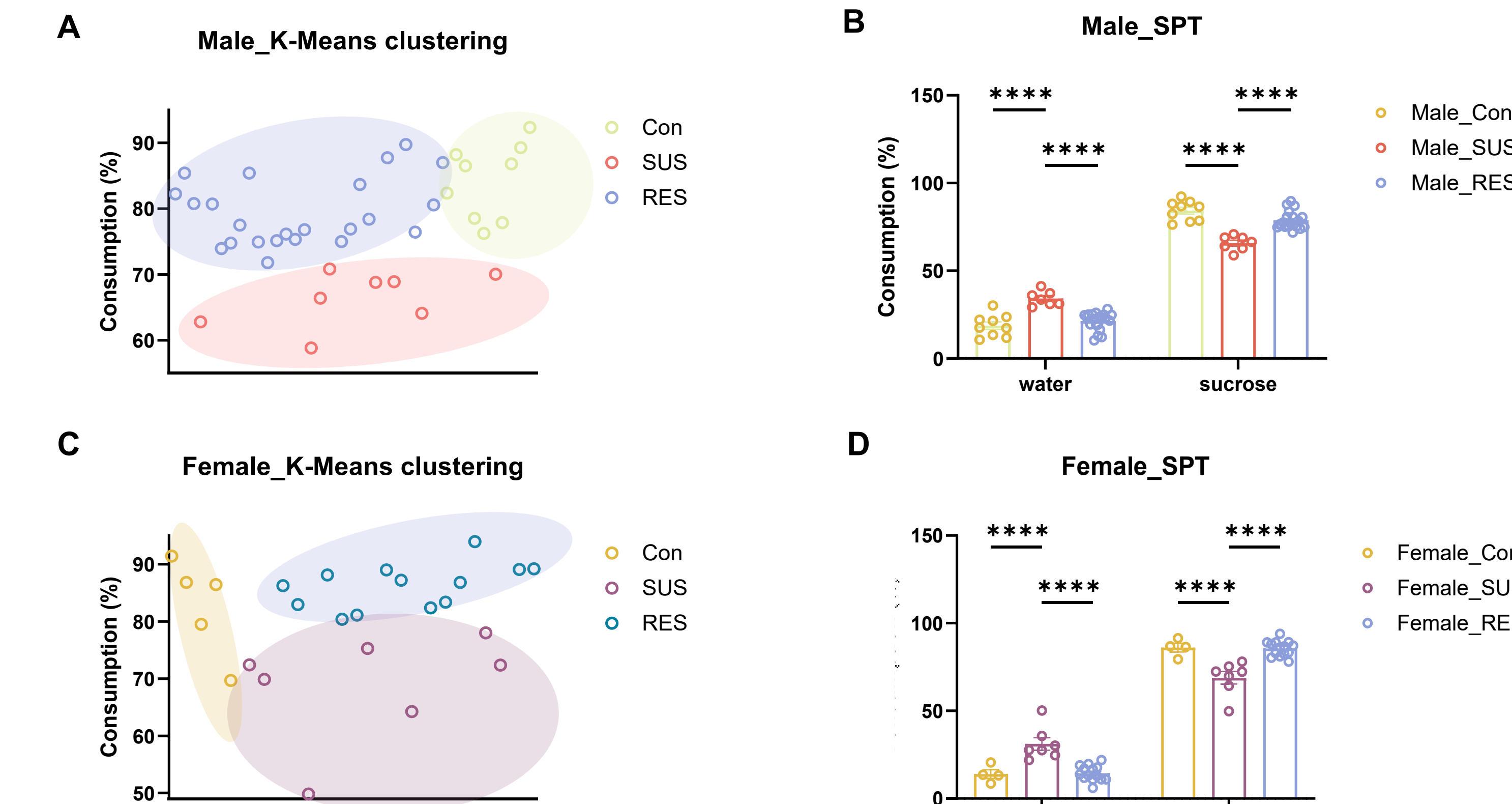


1. Chronic unpredictable stress reduces sucrose preference in stress-susceptible mice



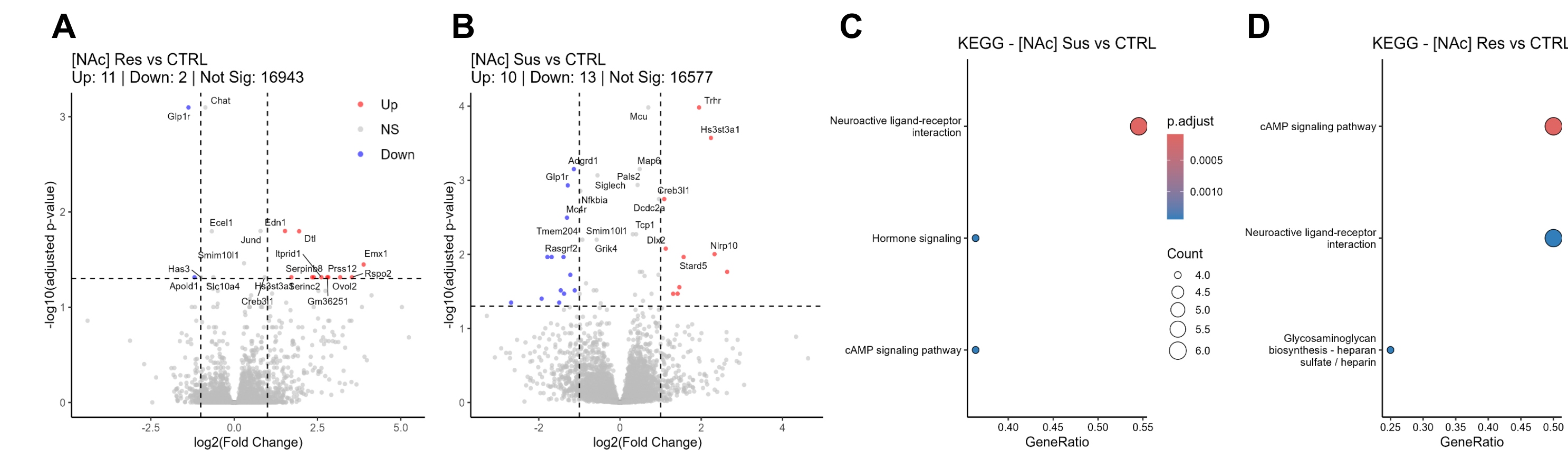
(A–D) Results of a 3-day sucrose preference test (SPT) in male mice following chronic unpredictable stress (CUS). On day 1, CUS-exposed groups showed only a trend toward reduced sucrose intake (A), whereas on days 2 (B) and 3 (C) a marked decline in sucrose consumption was observed, indicating that overall sucrose preference is progressively and robustly diminished after CUS exposure (D). (E–G) In female mice exposed to CUS, sucrose intake during the 3-day SPT showed only a trend toward reduction on days 1–3 (E, F, G) without a marked daily decline. However, when averaged across the 3 days (H), CUS-exposed females displayed a significant decrease in sucrose intake compared to controls, indicating that although the effect was less pronounced than in males, stress also reduced sucrose preference in females.

2. Stress-susceptible and -resilient phenotypes were distinguished using K-means clustering analysis



(A, C) K-means clustering of sucrose preference test (SPT) data in male and female mice. Using sucrose consumption (%) as the input variable, data were partitioned into three clusters (k = 3) with Euclidean distance and k-means++ initialization, which corresponded to control (Con), stress-susceptible (SUS), and stress-resilient (RES) groups. (B, D) SPT results of male and female mice classified by K-means clustering. Animals assigned to the Con, SUS, and RES clusters based on sucrose consumption patterns showed that the SUS cluster exhibited significantly reduced sucrose intake compared to Con and RES clusters.

3. Transcriptomic analysis revealed downregulation of *Glp1r* and *Mc4r* in the NAc associated with stress susceptibility

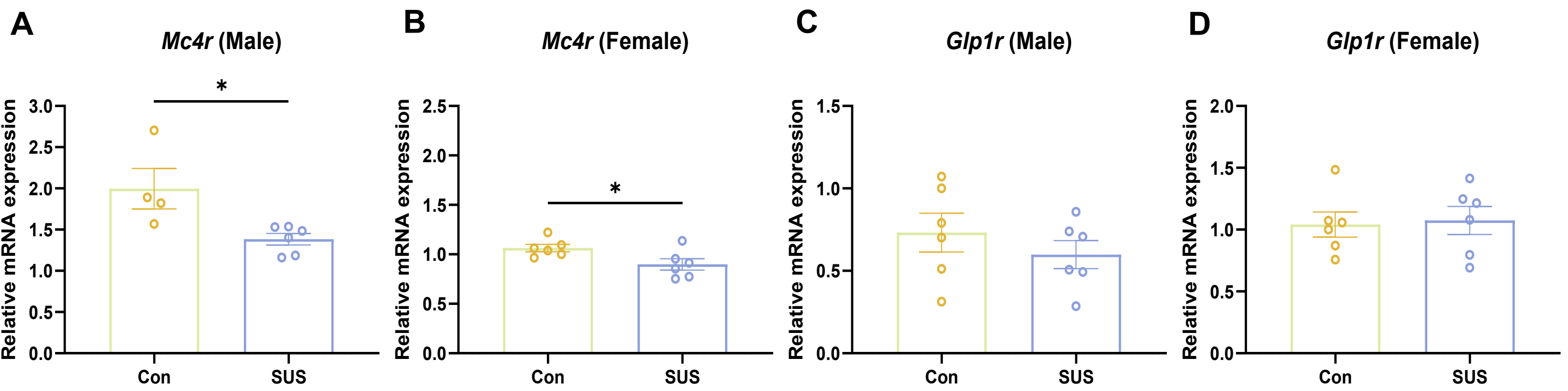


(A) Volcano plot showing differentially expressed genes (DEGs) in the nucleus accumbens (NAc) of stress-resilient (RES) versus control (CTRL) mice. A total of 11 genes were upregulated and 2 genes were downregulated, with notable downregulation of *Glp1r*. (B) Volcano plot for stress-susceptible (SUS) versus CTRL mice, identifying 10 upregulated and 13 downregulated genes, including significant downregulation of *Glp1r* and *Mc4r*. (C) KEGG pathway enrichment analysis for DEGs in SUS versus CTRL, highlighting significant enrichment in *Neuroactive ligand-receptor interaction*, *Hormone signaling*, and *cAMP signaling pathway*. (D) KEGG pathway enrichment in RES versus CTRL, showing enrichment in cAMP signaling pathway, Neuroactive ligand-receptor interaction, and Glycosaminoglycan biosynthesis - heparan sulfate / heparin. DEG threshold: $|\log_2FC| > 1.2$, adjusted $p < 0.05$. Data visualized using DESeq2 and clusterProfiler in R.

Abstract

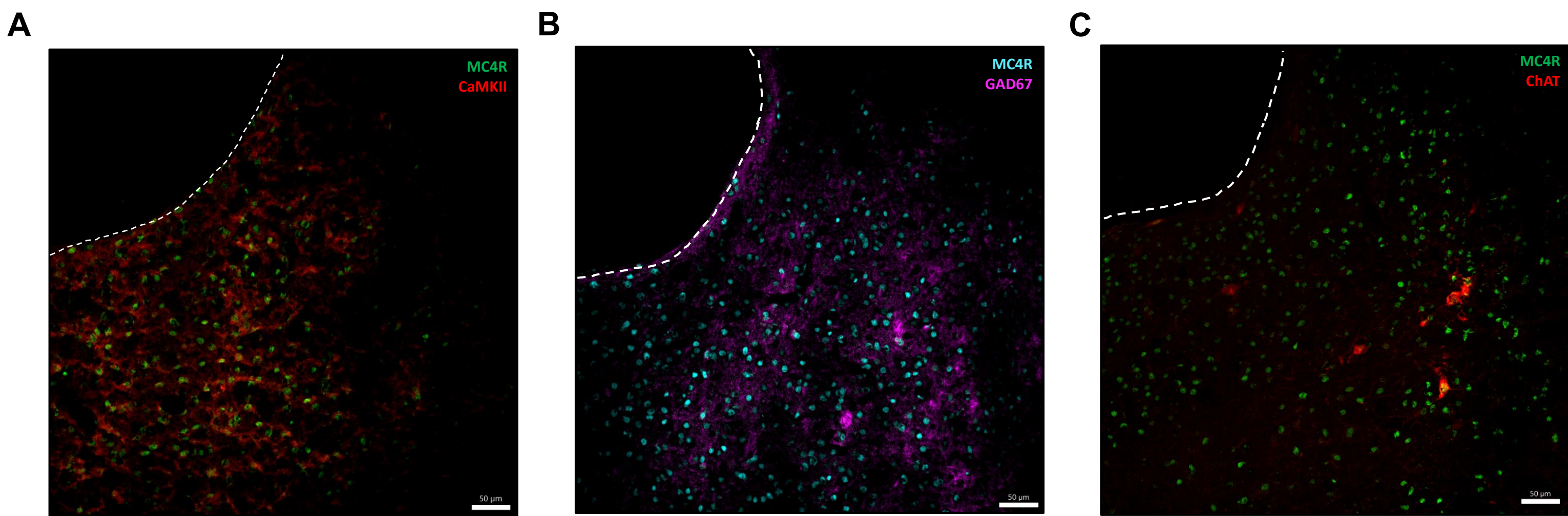
Stressful events are well-established risk factors for psychological disorders, particularly depression. Nevertheless, a substantial subset of individuals demonstrate resilience, maintaining stable psychological functioning despite repeated exposure to adverse stressors. While stress-induced symptoms such as anhedonia, dysregulated feeding behaviors, and behavioral despair are frequently observed, the precise synaptic and molecular mechanisms underlying these diverse phenotypes remain largely unknown. In this study, we examined the effects of chronic unpredictable stress (CUS) on behavior and gene expression in an inbred mouse model. Mice exposed to CUS exhibited significant behavioral alterations, including reduced sucrose preference (anhedonia), abnormal feeding patterns, and increased behavioral despair. Transcriptomic analysis using RNA sequencing revealed stress-specific gene expression changes in the nucleus accumbens (NAc), a key brain region involved in motivation and emotional regulation. Notably, we observed significant downregulation of glucagon-like peptide-1 receptor (Glp1R) and melanocortin 4 receptor (MC4R) genes in the NAc of stressed mice. Both genes play essential roles in appetite regulation, energy balance, and mood modulation. These findings suggest that stress-induced suppression of Glp1R and MC4R signaling within the NAc may contribute to disrupted feeding behavior and emotional dysregulation. Our study highlights a potential molecular pathway linking chronic stress to neurobehavioral outcomes and identifies promising targets for intervention in stress-related disorders.

4. *Mc4r* expression was reduced in the NAc of stress-susceptible mice



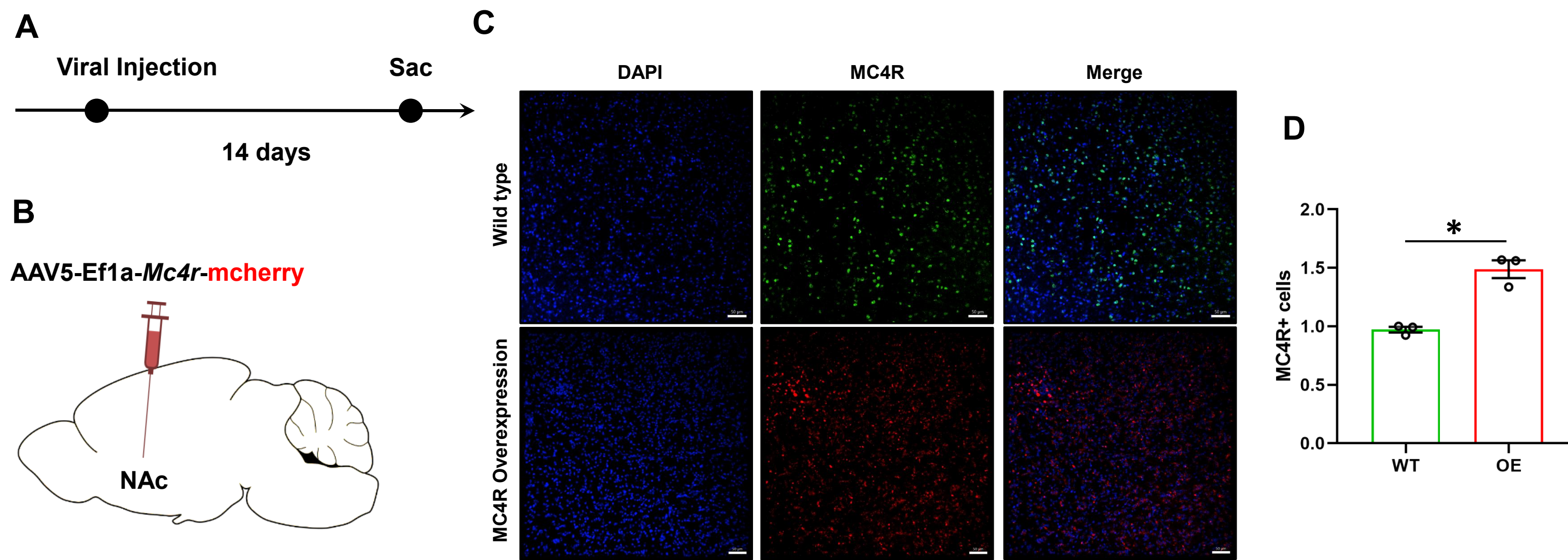
(A–D) Quantitative PCR (qPCR) analysis of *Mc4r* and *Glp1r* expression in the nucleus accumbens (NAc) of male and female mice. (A, B) Expression of *Mc4r* was significantly reduced in SUS mice compared to Con in both males (A) and females (B). (C, D) In contrast, *Glp1r* expression did not differ significantly between Con and SUS groups in either males (C) or females (D). Data are presented as mean $\pm$ SEM with individual values overlaid, and statistical significance was determined by unpaired two-tailed t-tests. * $p < 0.05$.

5. MC4R was broadly expressed across neuronal subtypes in the WT NAc



(A–C) Representative immunofluorescence images from wild-type mouse NAc showing MC4R with neuronal subtype markers. (A) MC4R (green) and CaMKII (red; excitatory neurons). (B) MC4R (cyan) and GAD67 (magenta; inhibitory neurons). (C) MC4R (green) and ChAT (red; cholinergic neurons). The dashed line outlines the NAc boundary. Scale bar, 50 µm.

6. Efficient AAV-mediated *Mc4r* overexpression in the NAc for behavior validation



Conclusion

In the CUS model, sequencing of the nucleus accumbens revealed distinct transcriptional signatures of resilience and susceptibility. *Glp1r* was downregulated in both phenotypes, whereas *Mc4r* reduction was specific to susceptible mice. KEGG analysis implicated neuroactive ligand-receptor interactions, hormone signaling, and cAMP signaling in stress adaptation. qPCR confirmed this selective downregulation, and immunofluorescence showed that MC4R is expressed in CaMKII⁺, GAD67⁺, and ChAT⁺ neurons in the WT NAc. To further explore its functional role, an AAV5-Mc4r overexpression model was established, and IHC verified successful Mc4r expression in the NAc, providing a basis for future behavioral studies on melanocortin signaling in stress adaptation.

Acknowledgement

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