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Supplementary Materials for

Transcriptional signatures of heroin intake and relapse throughout the brain reward circuitry in male mice

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The PDF file includes:

Figs. S1 to S5
Legends for supplementary files S1 to S9

Other Supplementary Material for this manuscript includes the following:

Supplementary files S1 to S9

Supplementary Materials

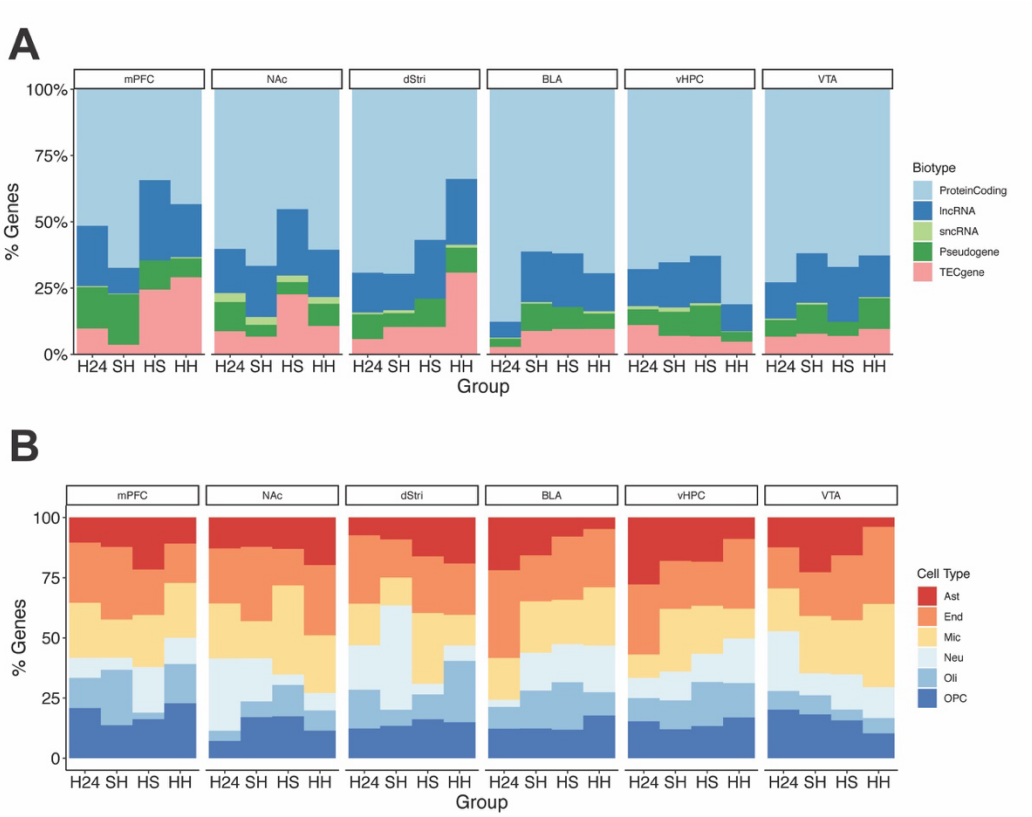

Fig S1. Heroin self-administration causes region-specific transcriptional regulation across gene biotypes and cell types throughout the brain's reward circuitry. (**A**) Proportion of transcripts belonging to particular biotypes (protein coding, long noncoding RNA (lncRNA), short noncoding RNA (sncRNA), pseudogene, or to be experimentally confirmed (TEC)) presented across experimental conditions faceted by brain region. (**B**) Proportion of DEGs enriched for cell-type-specific markers of astrocytes (Ast), endothelial cells (End), microglia (Mic), neurons (Neu), oligodendrocytes (Oli), or oligodendrocyte precursor cells (OPC) presented across experimental conditions faceted by brain region.

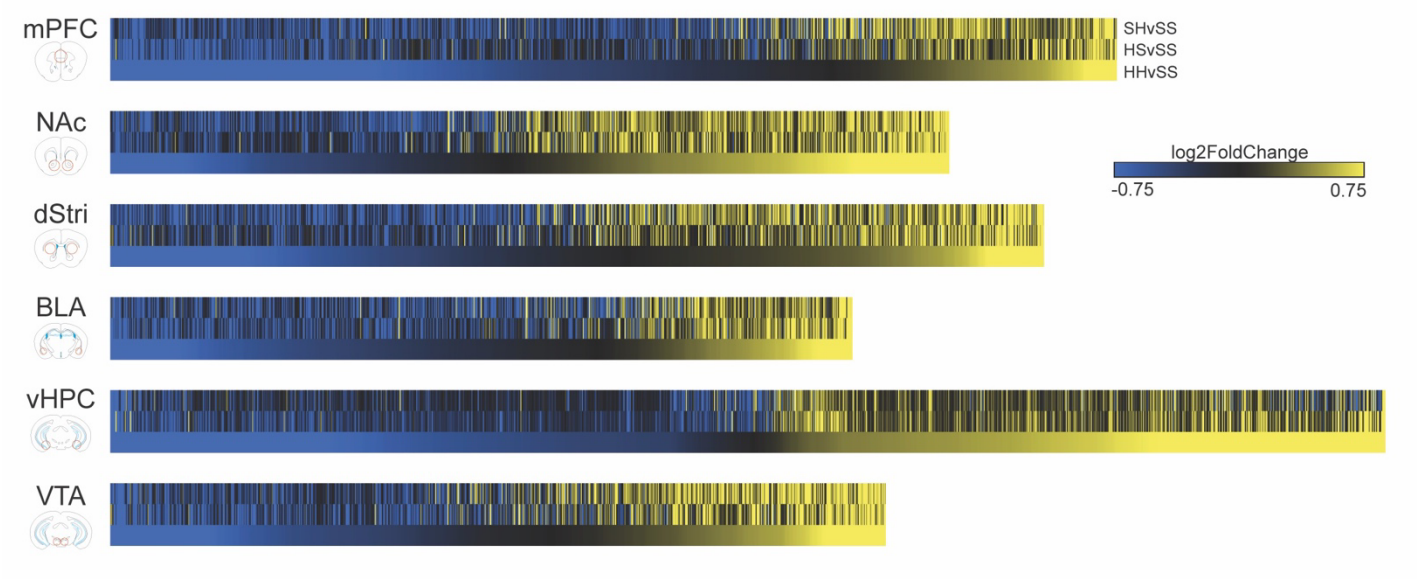

Fig S2. Differential gene expression induced by heroin IVSA after prolonged abstinence. Union heatmaps seeded to log2FoldChange of the HH condition (heroin IVSA followed by 30 d withdrawal and an acute heroin challenge) showing broadly similar transcriptional regulation for up- or downregulated genes across the SH condition (acute heroin exposure: saline IVSA, 30 d withdrawal and an acute heroin challenge) and the HS condition (heroin withdrawal: heroin SA followed by 30 d withdrawal and an acute saline challenge) across all brain regions.

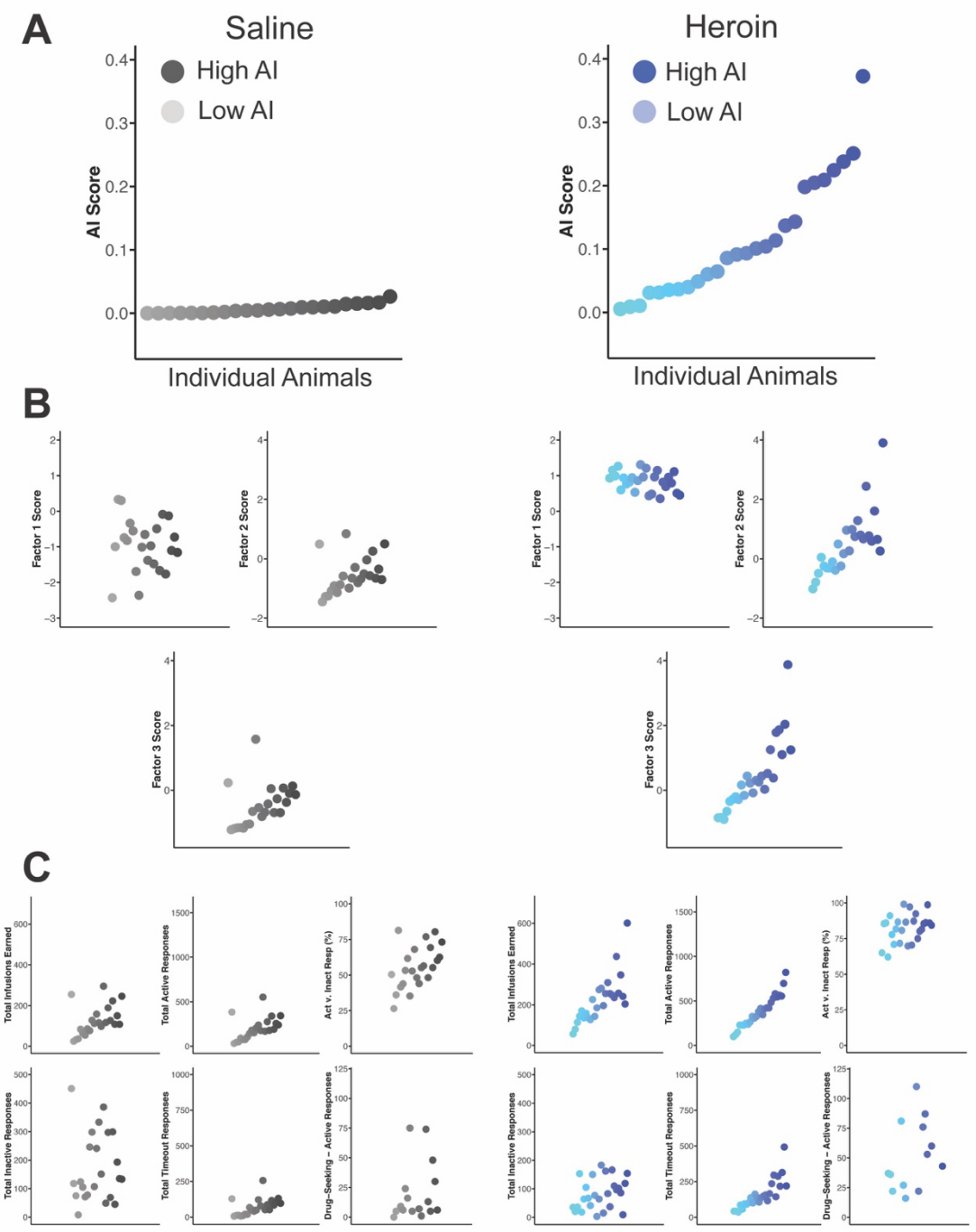

Fig S3. The addiction index (AI) effectively separates animals with and without a history of heroin intake across behavioral domains and latent variables identified through Factor Analysis. (**A**) Plots show the AI score attributed to individual animals represented as gray (saline IVSA; left) or blue (heroin IVSA; right) dots, staggered for visibility. Saturation of symbol color represents relative AI score in each group, and is carried through the following figure panels. (**B**) Plots show individual animals colored by AI score (saturation, as in panel A) in either saline (gray; left) or heroin (blue; right) mapped onto values for individual Factors identified, showing that higher AI (more saturation of symbol) is associated with higher Factor. Note that Factor scores are generally higher for heroin animals compared to saline animals. (**C**) Plots show individual animals colored by AI score (saturation, as in panel A) in either saline (gray; left) or heroin (blue; right) mapped onto individual behavioral measures used for Factor Analysis. Note

that with the exception of Total Inactive Responses heroin animals show higher scores for all behavioral measures, and animals with higher AI tend to score higher on each behavioral variable.

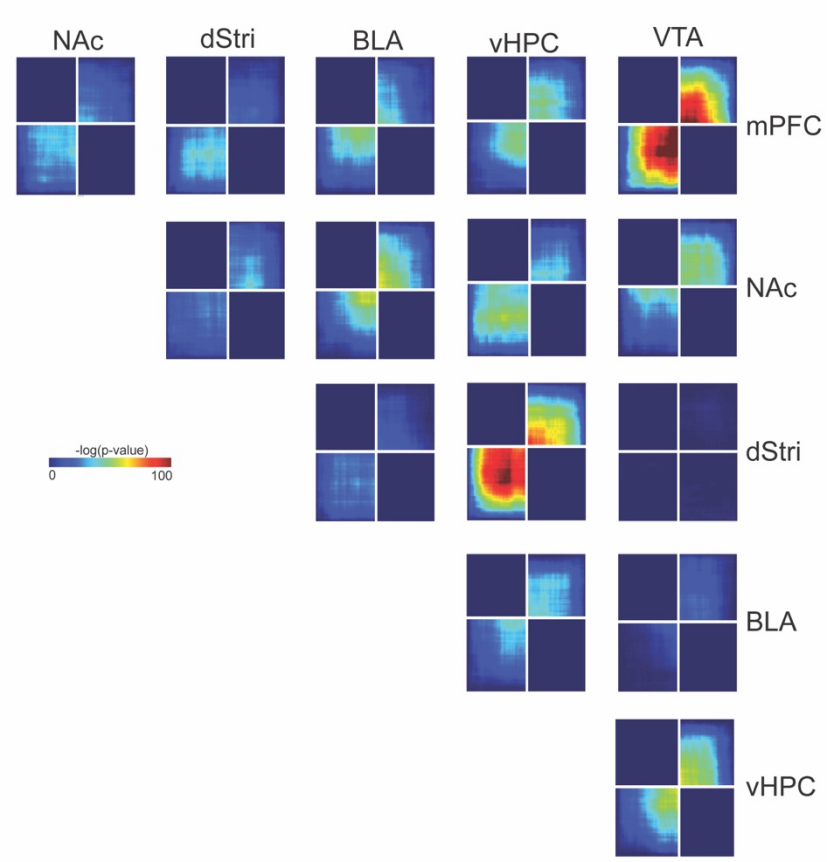

Fig S4. Genes identified to be associated with the Addiction Index are largely independent across brain regions. Rank-rank hypergeometric overlap plots comparing all brain regions to one another reveal generally independent AI-associated gene expression patterns, but identify a strong concordance between the VTA and mPFC, and the vHPC and dStri (lower-left quadrant: genes upregulated in both brain regions, upper-right quadrant: genes downregulated in both brain regions).

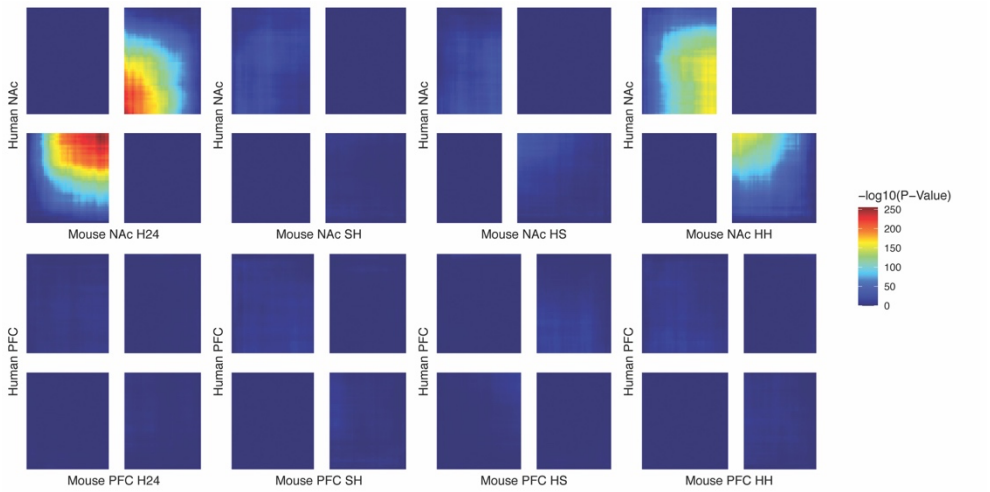

Fig S5. Threshold-free transcriptomic overlap across human and mouse datasets. Rank-rank hypergeometric overlap plots comparing transcriptomic overlap between human OUD and experimental conditions in mice (H24, heroin 24 h; SH, saline-heroin; HS, heroin-saline; HH, heroin-heroin) reveal a strong concordant transcriptomic response between human OUD and H24, but a discordant relationship for HH. No concordance is apparent for PFC (human dorsolateral PFC, mouse mPFC). Lower-left quadrant: genes upregulated in both human and mouse, upper-right quadrant: genes downregulated in both human and mouse, upper left quadrant, genes upregulated in mouse but downregulated in human; lower-right quadrant, genes downregulated in mouse but upregulated in human.

Supplementary file S1. Differential gene expression overlap lists for GO:BP analysis, related to Fig. 2.

Supplementary file S2. GO:BP analysis results for all groups and brain regions, related to Fig. 3.

Supplementary file S3. Ingenuity Pathway Analysis: Upstream Regulator Analysis results for all groups and brain regions, related to Fig. 3 and Fig. 6.

Supplementary file S4. Alluvial pattern analysis groupings, related to Fig. 4.

Supplementary file S5. GO:BP analysis results and gene membership for hierarchical clustering of heatmaps, related to Fig. 5.

Supplementary file S6. Behavioral data used to generate Addiction Index, related to Fig. 7.

Supplementary file S7. Addiction Index association gene lists, and summarized GO:BP analysis of positively- or negatively-associated Addiction Index genes, related to Fig. 7.

Supplementary file S8. Genes overlapping across mouse and human RNAseq datasets, related to Fig. 8.

Supplementary file S9. LDSC GWAS input sources, related to Fig. 8.