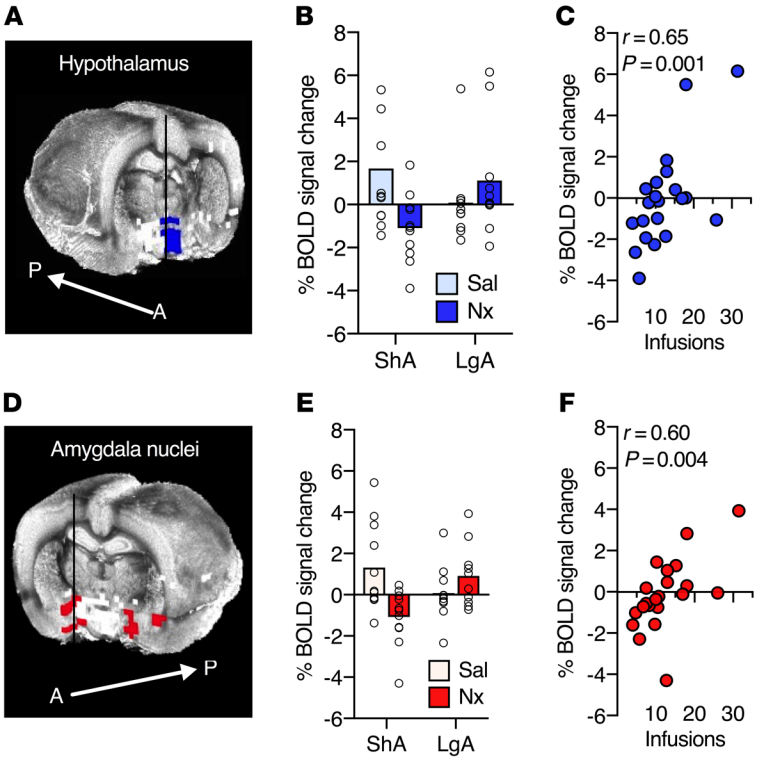

Figure 2. Withdrawal severity during conditioning is associated with changes in the hypothalamic and amygdala nuclei BOLD signal in response to the naloxone-paired cue. (**A**) Hypothalamic cluster extracted from the BOLD signal cue × heroin-access interaction shown in Figure 1C, with a 3D rendered whole-brain underlay. A, anterior; P, posterior. (**B**) Mean percentage of BOLD signal change from baseline in the hypothalamic cluster to the cue-only presentations. Dot plot displays individual data for each condition. Sal, saline. (**C**) Scatter plot of heroin intake during naloxone conditioning and BOLD signal response in the hypothalamic cluster to the naloxone-paired cue across both heroin-access groups (Pearson's correlation). (**D**) Amygdala nuclei extracted from the BOLD signal cue × heroin-access interaction shown in Figure 1C, with a 3D rendered whole-brain underlay. (**E**) Mean percentage of BOLD signal change from baseline in amygdala nuclei to the cue-only presentations. Dot plot displays individual data for each condition. (**F**) Scatter plot of heroin intake during naloxone conditioning and BOLD signal response in the amygdala nuclei to the naloxone-paired cue across both heroin-access groups (Pearson's correlation). n = 11 ShA rats; n = 10 LgA rats.

For fMRI data analysis, the averaged signal from white matter and the ventricles was removed by multiple regression analysis (34, 35). We conducted a whole brain 2 (heroin access, ShA versus LgA) × 2 (cue, saline- versus naloxone-paired odor) × 2 (cue presentation block, blocks 1 and 2 versus blocks 3 and 4) mixed-design ANCOVA of the percentage of BOLD signal change, using average respiration rate during the block as a covariate. No a priori regional hypotheses were tested. The 3dClustSim program in AFNI was used to estimate the probability of false-positive clusters, which was used to estimate the cluster size threshold for a given voxel-wise P value threshold to correct for multiple comparisons. A cluster size of 13 with a corrected $P < 0.01$ (uncorrected $P < 0.01$) was considered significant. The correlation between behavior (withdrawal severity as the number of heroin infusions during naloxone conditioning) and BOLD signal (in response to odor cues in each of the 19 ROIs that were extracted from the activation cluster, defined by a significant cue × heroin-access interaction) was evaluated using Pearson's test. P values were corrected for multiple comparisons using FDR ($q = 0.05$).

Study approval. All procedures were reviewed and approved by the NIDA Intramural Research Program's Institutional Animal Care and Use Committee in accordance with the National Research Council *Guide for the Care and Use of Laboratory Animals* (National Academies Press, 2011).

Author contributions

SAC, JCMV, and HL conducted the experiments. SAC, RJK, and EGLG analyzed the data. EAS and HL supervised the fMRI experiments. LFV and GFK supervised the behavioral experiments. SAC, GFK, and LFV conceptualized and designed the study and wrote the manuscript. All authors contributed to the manuscript writing.

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