Table 1. Mean percentage change in BOLD signal from baseline in response to saline and naloxone-paired cue-only presentation for ShA and LgA rats in ROIs

	ShA		LgA	
ROI	Saline cue	Naloxone cue	Saline cue	Naloxone cue
Amygdala nuclei	1.3	−1.1	0.1	0.9
Anterior hypothalamus	−0.4	−1.5	0.7	2.0
Auditory cortex	0.1	−1.0	−0.5	0.3
Dorsomedial thalamus	0.5	−0.6	−0.1	0.4
Dorsal striatum	−0.1	−1.1	−1.1	0.6
Ectorhinal cortex	0.5	−1.1	−1.0	0.2
Habenula	−0.6	−0.6	1.0	0.1
Hippocampus	−0.3	−0.7	−0.4	0.0
Insula	0.1	−1.0	−0.5	0.1
Lateral hypothalamus	1.3	−1.1	−0.3	0.4
Lateral geniculate	0.3	−0.7	−1.4	0.2
PAG and PVG	−0.2	−1.4	−0.5	0.5
Peduncle	−0.4	−3.5	−1.9	0.5
Precommisural nucleus	0.6	−0.4	−0.1	0.5
Pretectal nucleus	−0.1	−1.1	−0.8	0.6
PVN/VM hypothalamus	1.7	−1.1	0.1	1.1
Reticular formation	0.2	−1.4	−1.0	0.2
S1/S2	0.1	−0.6	−0.1	0.6
Ventrolateral thalamus	0.5	−1.2	−0.8	0.3

We anatomically segregated the cluster defined by the statistically significant heroin-access (ShA versus LgA) × cue (saline paired versus naloxone paired) interaction (shown in Figure 1C) into 19 ROIs using a standard rat atlas (14). S1/S2, primary and secondary somatosensory cortex.

As part of a habit-learning circuit, the thalamus and dorsal striatum are implicated in reward and incentive salience (2, 25). Additionally, the naloxone-paired cue activated the precommissural nucleus (PRC), periaqueductal gray (PAG)/periventricular gray (PVG), and the pretectal nucleus in LgA rats, whereas these same regions were deactivated in ShA rats. The saline-paired cue deactivated these regions in LgA rats. The PRC and PAG/PVG are anatomically connected to the extended amygdala, VM hypothalamus, and pretectal nucleus and play a key role in negative emotional learning (5, 26).

Finally, both odor cues activated the anterior hypothalamus and a region consistent with the habenula in LgA rats, whereas opposite effects were observed in ShA rats. The habenula can inhibit the mesolimbic dopamine system and modulate emotional and motivational states (27). Together with the hypothalamus and amygdala, the habenula serves to maintain hedonic homeostasis (2, 19, 26). Although the anatomical resolution of fMRI and the small size of the rat habenula limited our ability to confirm its activation, potential engagement of the habenula during negative conditioned responding warrants further investigation.

We found that conditioned heroin withdrawal motivated heroin intake and engaged brain regions that are associated with negative emotional learning, particularly extrahypothalamic and hypothalamic stress/arousal circuitries. These circuits are consistent with and extend fMRI findings in individuals with OUD on drug cue reactivity tasks (22, 23). Thus, we argue that conditioned cues can maintain compulsive drug use by removing aversive states (conditioned negative reinforcement) as well as by producing positive incentive states (conditioned positive reinforcement) (2, 22, 28) and that both forms of learning contribute to allostatic changes in emotional processes that perpetuate opioid addiction.

Exposure to conditioned withdrawal stimuli may drive craving and provoke relapse in individuals with OUD by inducing a powerful aversive stress state that is relieved by opioid use (7, 29). The stimuli that trigger conditioned withdrawal are likely the same as those that convey learned tolerance (30). Thus, individuals with OUD are especially susceptible to overdose death when they use opioids in unfamiliar contexts or with unfamiliar cues (e.g., different administration procedure or opioid) and their bodies fail to engage learned compensatory mechanisms (30) or when they encounter conditioned withdrawal stimuli and their drug tolerance is reduced, such as following detoxification in a treatment facility or release from incarceration (31). Critically, the 3 United States Food and Drug Administration-approved medications for OUD, which target opioid receptors (8), may not fully alleviate cue-conditioned withdrawal.

Therefore, we propose that understanding and targeting the brain circuits that underlie conditioned withdrawal and downstream emotional circuitries (e.g., brain stress circuits) provides an innovative conceptual framework for novel treatment and the prevention of opioid overdose deaths. Examining fMRI, and in parallel, psychological responses to drug-related cues provide a potentially powerful approach to understanding individual differences leading to and maintaining compulsive opioid use. Indeed, dysfunction in brain circuits of negative emotional learning is a potential biomarker for tracking progression and remission of OUD.

Methods

Further information can be found in Supplemental Methods and Supplemental Figures 1–5.

Subjects. Adult male Long-Evans rats (Charles River Laboratories) were group housed (2 to 3/cage) on a 12-hour light/12-hour dark cycle at the NIDA animal facilities in the Biomedical Research Center. At approximately 6 weeks of age (250–275 g), rats were implanted with chronic indwelling i.v. catheters in the right jugular vein under isoflurane anesthesia, as previously described (9). Rats underwent MRI scanning between 3 and 3.5 months of age (32, 33). Standard rat chow and water were available ad libitum in home cages and throughout the self-administration experiments, but not during the other experimental procedures. The plurality of experimental procedures was performed in the dark cycle, with few extending into the light cycle.

Drugs. Heroin hydrochloride was obtained from NIDA and dissolved in 0.9% sterile saline for i.v. infusions (60 μg/kg/0.1 ml). Naloxone hydrochloride was obtained from Hospira and dissolved in 0.9% sterile saline for a s.c. injection in a volume of 1 ml/kg and 120 μg/kg.

Statistics. Heroin self-administration data were analyzed using 2-way ANOVA, with heroin access (ShA versus LgA) as a between-subjects factor and session or cue (saline versus naloxone paired) as the within-subjects factor. Statistical significance was set at $\alpha = 0.05$. Post hoc comparisons were conducted when appropriate, and P values were corrected for multiple comparisons using Bonferroni's method. The statistical analyses for behavioral experiments were performed using GraphPad Prism 7 software. One LgA rat was excluded from the MRI study for failed catheter patency.