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SUPPLEMENTARY INFORMATION

Power analysis

Power analysis was conducted using G*Power¹. Type I and type II error rates smaller than 0.05 can be achieved by N=23 given a large effect size of change between two sessions (Cohen's $d=0.8$). We slightly over-sampled in anticipation of possible exclusion of subjects due to, for example, concurrent use of other substances or excessive head motion during fMRI scanning.

Stimuli

Drug cues were of two sub-categories: heroin and prescription opioids. Participants who used heroin exclusively or as their drug of choice (N=14) were shown heroin-related images as drug cues; participants who used prescription opioids exclusively or as their drug of choice (N=10) were shown images of common prescription opioid pills (Vicodin, Percocet, Oxycontin, etc) and preparation for their use. All drug-related images were selected from our laboratory archive and were validated in previous studies^{2,3}. The neutral stimuli were from our collection of non-drug images (e.g., building facades, people engaged in everyday activities, etc.) that have been previously used in studies of cue-reactivity in substance use disorders^{4,5}. For male and female participants, sexual stimuli were selected from the erotic pictures in the International Affective Picture System (IAPS), and from our own stimulus archive, that fell into the top quartile of pleasantness based on the male and female IAPS normative ratings, respectively. Aversive stimuli were selected from IAPS pictures that fell into the bottom quartile of pleasantness based on the overall normative ratings.

The hedonic value of each stimulus was determined by an unrelated group of OUD patients on a 9-point scale (1=the least pleasant; 9=the most pleasant). The mean (\pm SD) pleasantness ratings were 3.75 (\pm 0.38) and 3.77 (\pm 1.24) for the heroin and prescription opioid stimuli; 7.33 (\pm 0.90) and 7.72 (\pm 1.60) for the sexual stimuli for male participants and the sexual stimuli for female participants; 1.07 (\pm 0.17) for the aversive stimuli; and 3.84 (\pm 1.21) for the neutral stimuli.

Study medication

To ensure completeness of opioid detoxification, XR-NTX was preceded by a challenge with 0.6 mg of naloxone hydrochloride IV. Participants were offered up to three monthly intramuscular injections of XR-NTX (380 mg of naltrexone-HCl gradually released from dissolvable polymer microspheres over a period of one month, manufactured by Alkermes Inc, Cambridge, MA, under the brand name Vivitrol®). As part of the consent procedure, participants were briefed about the expected loss of pharmacological

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effects of opioids resulting from the XR-NTX treatment, and the dangers of attempting to overcome the opiate receptor blockade with higher than usual opioid doses^{6,7}. Medication was provided in the context of ongoing psychosocial support (two weekly sessions of professional drug counseling and anti-relapse strategies by trained clinical psychologists) and twice-weekly urine drug screen (UDS) monitoring. Plasma concentrations of naltrexone and 6- β -naltrexol (an active metabolite of naltrexone) were measured on the day of the on-treatment session, using established liquid chromatography and tandem mass spectrometry technique^{2,8}. Upon study completion, continuation of care was discussed with the participants and they were given referrals to treatment providers in the community.

Additional behavioral assessments

Additional baseline assessments included the Edinburgh Handedness Scale⁹, DSM-IV-TR¹⁰, Addiction Severity Index 5h Edition (ASI)¹¹, Mini-International Neuropsychiatric Interview¹², Wechsler Abbreviated Scale of Intelligence (WASI)¹³, Hamilton Anxiety Rating Scale (HAM-A)¹⁴, the 24-item version of the Hamilton Depression Rating Scale (HAM-D)^{15,16}, and the Smoking History Questionnaire comprised of the Fagerstrom Test for Nicotine Dependence¹⁷ with 25 added items¹⁸.

MRI data acquisition

Blood-oxygen-level dependent (BOLD) fMRI was performed, using a whole-brain, single-shot gradient-echo echo-planar sequence with the following parameters: repetition time (TR)/echo time (TE)=2000/30 ms, field of view (FOV)=220×220 mm², matrix=64×64, slice thickness/gap=4.5/0 mm, 32 slices, effective voxel resolution of 3.4×3.4×4.5 mm³, flip angle (FA)=90°. After BOLD fMRI, MPRAGE T1-weighted images were acquired with the following parameters: TR/TE=1510/3.71 ms, FOV=256×192 mm², matrix=256×192, slice thickness/gap=1/0 mm, 160 slices, effective voxel resolution of 1×1×1 mm³, FA=9°. An oblique acquisition, oriented along the anterior commissure–posterior commissure line allowed coverage of the entire brain with the exception of the lower cerebellum.

MRI data analyses

Functional MRI images were adjusted for slice timing, realigned to the first scan to correct for head motion, spatially smoothed by a Gaussian filter with full-width/half-maximum parameter (FWHM) set to 8 mm, and normalized into stereotactic Montreal Neurological Institute (MNI) space with 2-mm cubic voxels. Individual-level statistical analyses were performed voxel-wise by modeling drug, sexual, aversive, and neutral stimuli using a canonical hemodynamic response function as well as its derivatives with respect to time and dispersion. Effects of drug, sexual and aversive stimuli were contrasted with the neutral stimuli.

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The NAcc was anatomically defined according to the Harvard-Oxford Atlas (<http://www.fmrib.ox.ac.uk/fsl>) (see Figure S1 left). The mOFC was defined as a spherical region with radius of 5 mm centered at $x/y/z=3/41/-12$, a point derived from a meta-analysis that showed peak activation to hedonic experience at this coordinate¹⁹ (see Figure S1 right). Contrast values for drug, sexual and aversive stimuli during the pre-treatment and on-treatment sessions were extracted from the NAcc and mOFC ROIs using MarsBaR 0.42 (<http://marsbar.sourceforge.net>). After the ROI analyses, a whole-brain ANOVA was performed using GLM Flex (http://mrtools.mgh.harvard.edu/index.php/GLM_Flex). Significant activation was identified at a corrected threshold of $p < 0.05$ (using a combined threshold of voxel-level $p < 0.005$ and cluster extent > 137 voxels, determined by a 2000-iteration Monte-Carlo simulation).

Procedure and analyses of the post-treatment session

As in the pre-treatment and on-treatment sessions, COWS, self-reported craving, and self-reported withdrawal were assessed before the fMRI cue-reactivity task (pre-fMRI) and immediately after the task (post-fMRI).

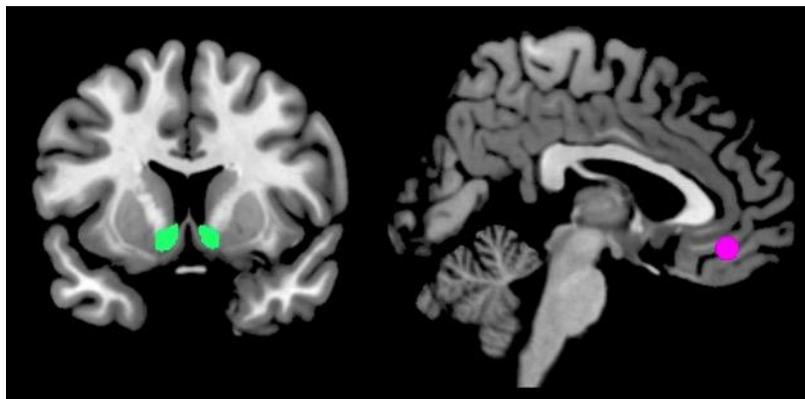


Figure S1. A priori regions of interest. Green: bilateral nucleus accumbens ($y=10$). Violet: medial orbitofrontal cortex ($x=3$) (in MNI space).

We examined the effect of pre-treatment vs. post-treatment sessions and the effect of on-treatment vs. post-treatment sessions on the COWS scores, self-reported craving scores, self-reported withdrawal scores, and the NAcc and mOFC responses to opioid-related cues. Due to the limited sample size for the post-treatment session (see “Participant attrition and missing data” below), we conducted the

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comparisons using nonparametric, permutation tests^{20,21}. Before the permutation tests, the pre-fMRI and post-fMRI scores were averaged for each of the COWS, self-report craving, and self-report withdrawal measures. For the pre-treatment vs. post-treatment comparison, each permutation randomly reassigned the session labels (i.e. pre-treatment or post-treatment) to the scores. Using 10,000 permutations, a null distribution of pre-treatment vs. post-treatment difference was generated for each variable. The two-tailed p value was calculated as the percentage of the absolute value of the observed between-session difference score being larger than that of the randomly generated difference scores. The same procedure was used for the on-treatment vs. post-treatment comparison. We also compared the patients who completed the post-treatment session to those who did not, using the same permutation test procedure as described above.

Participant attrition and missing data

Two participants were missing the HAM-A and HAM-D scores.

Participant characteristics

Participants reported average lifetime opioid use of 8.54 years (SD=8.01, range=1–34) and used opioids on 9.88 days (SD=9.46, range=0–30) out of 30 days before the enrollment. Average time between last opioid use and enrollment was 14.08 days (SD=10.74, range=1–30; a record of more than 30 days between last opioid use and enrollment was coded as “30 days”). Participants reported abstinence from opioids for 22.83 days (SD=13.38, range=5–49) before the first XR-NTX injection.

The average ASI Drug Composite Score was 0.26 (SD=0.10, range=0.09–0.45). The scores on the HAM-A and HAM-D were 5.09 (SD=5.08, range=0–20) and 5.35 (SD=4.27, range=0–17), respectively, indicating no to moderate anxiety and depression. Participants’ average IQ as assessed by WASI was 101.79 (SD=11.10, range=81–126). Twenty-three participants were daily tobacco smokers who smoked 10.65 cigarettes per day (SD=8.43, range=1–40) and had 5.85 pack-years of tobacco exposure (SD=6.87, range=0.2–32). At the time of the on-treatment session, plasma concentrations of naltrexone and 6-β-naltrexol were 3.06 ng/ml (SD=1.54, range=1.24–7.93) and 6.81 ng/ml (SD=2.89, range=2.69–15.34), respectively.

Results from the post-treatment session

Despite the small number of participants in the post-treatment imaging session (N=11 for behavioral assessments, N=9 for the fMRI task), there have been interpretable changes in participants’ opioid craving, withdrawal, and MCL cue-reactivity in the pre-treatment vs. post-treatment sessions and the on-treatment vs. post-treatment sessions. Physical opioid withdrawal symptomatology (assessed by

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COWS) on the day of the post-treatment session was significantly lower than on either the pre-treatment or the on-treatment sessions ($p=0.009$ & 0.04 ; see Table S1 and Figure S2A). Self-reported craving at the post-treatment session was significantly lower than the pre-treatment session ($p=0.0002$), and was comparable to the on-treatment session ($p=0.35$) (see Table S1 and Figure S2B). Self-reported opioid withdrawal in the post-treatment session was significantly lower than the pre-treatment session ($p=0.02$), and was comparable to the on-treatment session ($p=0.24$) (see Table S1 and Figure S2C). The NAcc response to drug cues at the post-treatment session (mean \pm SD=1.95 \pm 2.54) was comparable to the pre-treatment session ($p=0.17$) and significantly greater than the on-treatment session ($p=0.01$) (see Figure S2D). The mOFC showed a similar trend as the NAcc, but the difference between the post-treatment session (mean \pm SD=1.52 \pm 7.63) and the first two sessions did not reach statistical significance ($p=0.76$ & 0.15) (see Figure S2E).

Table S1. COWS and self-reported craving and withdrawal scores at the post-treatment session

	Pre-fMRI (mean \pm SD)	Post-fMRI (mean \pm SD)
COWS	0.82 \pm 1.47	0.64 \pm 1.29
Self-report craving	0.55 \pm 0.82	1.27 \pm 1.90
Self-report withdrawal	0.00 \pm 0.00	0.18 \pm 0.40

Pre-/post-fMRI: before/after the fMRI task; COWS: the Clinical Opiate Withdrawal Scale

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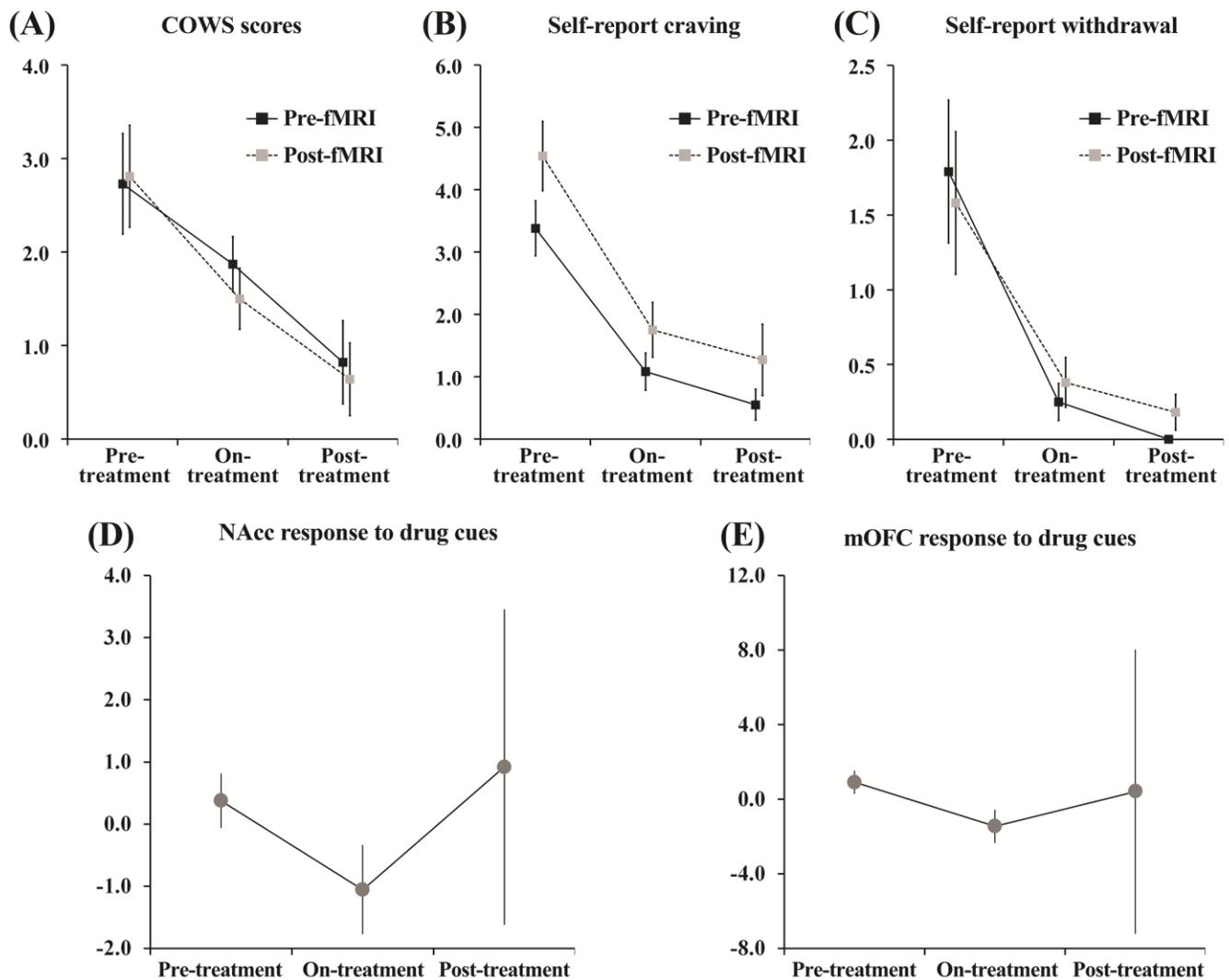


Figure S2. Exploratory comparisons between the pre-treatment, on-treatment, and post-treatment sessions. (A) COWS scores (objective assessment of withdrawal symptoms). (B) Self-reported opioid craving. (C) Self-reported opioid withdrawal. (D) NAcc response to drug cues. (E) mOFC response to drug cues. Pre-/post-fMRI: before/after the fMRI task; COWS: the Clinical Opiate Withdrawal Scale; NAcc: nucleus accumbens; mOFC: medial orbitofrontal cortex. (Error bar: standard error of mean)

The pre-treatment vs. post-treatment and on-treatment vs. post-treatment comparisons suggest that the decline in opioid craving and withdrawal was less likely to be a direct pharmacodynamic effect of

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XR-NTX than the indirect effect of reduced expectancy and pharmacologically enforced abstinence²². The rebound of the NAcc (and to a lesser degree the mOFC) response at the post-treatment session suggests that three months of XR-NTX are not sufficient to produce a durable reduction in MCL cue-reactivity after the treatment ends. Nevertheless, the number of participants with post-treatment assessments was so small that the findings from this self-selected group should be considered strictly exploratory.

A comparison between the patients who completed the post-treatment session and those who did not showed that the two groups did not differ significantly in terms of demographic characteristics, behavioral results, and brain response (see Table S2).

Table S2. A comparison of the patients who completed the post-treatment session vs. those who did not

Variable	mean±SD	p value
Sample size	11 vs. 13	
Gender	6 female vs. 3 female	p=0.11 [#]
Age	31.18±9.80 vs. 29.38±7.49	p=0.60
Years of education	14.45±1.51 vs. 13.38±2.96	p=0.28
Years of lifetime opioid use	9.82±7.18 vs. 7.46±9.00	p=0.50
ASI Drug Composite Score	0.22±0.08 vs. 0.30±0.11	p=0.08
HAM-A	5.00±5.61 vs. 5.15±4.91	p=0.94
HAM-D	5.22±5.14 vs. 5.46±3.78	p=0.88
IQ (measured by WASI)	99.18±9.60 vs. 104.00±12.16	p=0.29
Plasma naltrexone concentration (ng/ml)	3.27±2.02 vs. 2.89±1.04	p=0.58
Plasma 6-β-naltrexol concentration (ng/ml)	7.33±3.59 vs. 6.38±2.19	p=0.45
COWS		
(pre-treatment, pre-fMRI)	2.20±1.81 vs. 3.17±2.82	p=0.34
(pre-treatment, post-fMRI)	2.00±1.80 vs. 3.42±2.75	p=0.18
(on-treatment, pre-fMRI)	1.64±1.29 vs. 2.08±1.38	p=0.35
(on-treatment, post-fMRI)	1.10±0.99 vs. 1.83±1.75	p=0.20
Self-reported craving		
(pre-treatment, pre-fMRI)	3.45±2.42 vs. 3.31±2.02	p=0.85

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(pre-treatment, post-fMRI)	4.18±2.56 vs. 4.85±2.94	p=0.55
(on-treatment, pre-fMRI)	1.27±1.62 vs. 0.92±1.38	p=0.51
(on-treatment, post-fMRI)	1.73±1.79 vs. 1.77±2.49	p=0.93
Self-reported withdrawal		
(pre-treatment, pre-fMRI)	1.27±1.68 vs. 2.23±2.77	p=0.31
(pre-treatment, post-fMRI)	1.18±1.66 vs. 1.92±2.81	p=0.45
(on-treatment, pre-fMRI)	0.18±0.60 vs. 0.31±0.63	p=0.52
(on-treatment, post-fMRI)	0.36±0.92 vs. 0.38±0.77	p=0.83

Pre-/post-fMRI: before/after the fMRI task; ASI: the Addiction Severity Index; HAM-A/HAM-D: the Hamilton Anxiety/Depression Rating Scale; WASI: the Wechsler Abbreviated Scale of Intelligence; COWS: the Clinical Opiate Withdrawal Scale. #p value from χ^2 test.

Discussion – negative contrast values for the NAcc and mOFC responses to cues

As shown in Figure 1A&B, the NAcc and mOFC responses to the cues were mostly negative (with the exception of responses to drug cues in the pre-treatment session). Given the relative nature of BOLD signal, it might have been caused by the relatively elevated MCL response to neutral stimuli, which served as a baseline in all contrasts (i.e. drug vs. neutral, sexual vs. neutral, and aversive vs. neutral)²³. Our paradigm did not allow us to examine the absolute baseline brain activity, which would ideally be tested using arterial spin labeling fMRI. We hope that future research will shed light on this interesting issue.

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