The effect of a methadone-initiated memory reconsolidation updating procedure in opioid use disorder: A translational study

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Summary

Background Opioid use disorder (OUD) is a chronic relapsing psychiatric disorder. An unconditioned stimulus (US)-triggers a memory reconsolidation updating procedure (MRUP) that has been developed and demonstrated its effectiveness in decreasing relapse to cocaine and heroin in preclinical models. However, utilizations of abused drugs as the US to initiate MRUP can be problematic. We therefore designed a translational rat study and human study to evaluate the efficacy of a novel methadone-initiated MRUP.

Methods In the rodent study, male rats underwent heroin self-administration training for 10 consecutive days, and were randomly assigned to receive saline or methadone at 10 min, 1 h or 6 h before extinction training after 28-day withdrawal. The primary outcome was operant heroin seeking after reinstatement. In the human experimental study, male OUD patients were randomly assigned to get MRUP at 10 min or 6 h after methadone or methadone alone. The primary outcomes included experimental cue-induced heroin craving change, sustained abstinence and retention in the study at post intervention and the 5 monthly follow-up assessments. The secondary outcomes were changes in physiological responses including experimental cue-induced blood pressure and heart rate.

Findings Methadone exposure but not saline exposure at 10 min or 1 h before extinction decreased heroin-induced reinstatement of heroin seeking after 28-day of withdrawal in rats ($F_{(8,80)} = 8.26$, $p < 0.001$). In the human study, when the MRUP was performed 10 min, but not 6 h after methadone dosing, the MRUP promoted sustained abstinence from heroin throughout 5 monthly follow-up assessments compared to giving methadone alone without MRUP (Hazard Ratio [95%CI] of 0.43 [0.22, 0.83], $p = 0.01$). The MRUP at 10 min, but not at 6 h after dosing also decreased experimental cue-induced heroin craving and blood pressure increases during the 6-month study duration (group × months × cue types, $F_{(12, 63.3)} = 2.41$, $p = 0.01$).

Interpretation The approach of MRUP within about 1 to 6 h after a methadone dose potently improved several key outcomes of OUD patients during methadone maintenance treatment, and could be a potentially novel treatment to prevent opioid relapse.

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Research in context

Evidence before the study

We searched the PubMed using the terms of ((drug addiction) OR (drug memory)) AND ((unconditioned stimulus memory reconsolidation updating) OR (US memory retrieval extinction) OR (US memory reconsolidation updating)), with no language restrictions up to March 23, 2022. This search yielded 12 studies, with 4 studies from our group focusing on interventions of drug rewarding memories, 3 review articles, 4 on aversive memories, and 1 on a goal-tracking task in rodents. Among the 4 studies, we previously used US-initiated memory reconsolidation interventions to erase drug- or nicotine-associated memories. However, both 2 studies on drug memories were animal studies, in which the drug itself (heroin or cocaine) was used as US to trigger the memory reconsolidation updating, and the procedure could not translate to addicted patients. We found no studies using methadone-initiated MRUP to prevent opioid relapse in animal studies or human studies.

Added value of this study

In the study, we develop and validate the concept that a compound that shares similar pharmacological effects with heroin and that is used in clinical practice is able to initiate the process of reconsolidation of heroin memories. To our knowledge, this study is the first rat-to-human translational study to investigate the efficacy of methadone-initiated MRUP on heroin relapse. We show that the methadone-initiated MRUP significantly decreased heroin craving and increased sustained abstinence in opioid use disorder (OUD) patients, and promoted treatment retention during the study.

Implications of all the available evidence

The approach of MRUP within a specific time-frame after a methadone dose potently improved several key outcomes of OUD patients during methadone maintenance treatment (MMT) and could be a novel treatment used in conjunction with MMT to prevent opioid relapse in clinical practice.

Introduction

Opioid use disorder (OUD) is a severe global health problem and causes high rates of disability, transmission of infectious disease, criminality and early death.[1] Currently a primary treatment of OUD is methadone maintenance treatment (MMT).[2,3] MMT has shown efficacy for 5 decades in reducing opiate use-associated mortality and morbidity, HIV transmissions and criminal activities.[4] Nonetheless, retention in MMT is unsatisfactory and the risk of relapse is still high, ranging from 40% to 60% at month 6 after initiation.[5] Although a variety of psychosocial interventions are delivered in conjunction with MMT to improve its' outcomes, these psychosocial therapies have had limited efficacy.[6]

Drug relapse involves processes of learning and memory, in which repeated associations between drug rewarding effects (the unconditioned stimulus [US] of taking an opioid) and opioid associated cues (conditioned stimulus [CS]) gradually usurps normal neural circuits, and the persistence of the maladaptive memories causes drug craving and relapse even after prolonged abstinence.[7] Cue exposure therapy (CET), which is based on the memory extinction, has been assessed for reducing drug craving and relapse in conjunction with MMT, but the simple combination of CET with MMT did not improve retention rates and outcomes.[8]

Recently, a memory reconsolidation-based updating procedure (MRUP) has been considered a promising method for prevention of drug relapse.[9] Reconsolidation is a concept that refers to a hypothetical time-limited process, which a CS or US reactivates, when the memory re-enters an unstable state and is available for pharmacological or behavioral interventions within a specific time interval (within 6 h after retrieval).[10,11] In animal to human translational studies, we and others showed that the strategy of CS-based MRUP, that is, repeated cue exposures during the reconsolidation window (10 min, but not 6 h) weakened the drug memory and permanently decreased drug craving.[12,13] We previously developed a novel US-based MRUP and demonstrated its superior effect over CS-based MRUP in

decreasing relapse to cocaine and heroin seeking in animal models.[14,15] In this procedure, drug reward memories are reactivated by an exposure to the drug itself (the US, e.g., cocaine or heroin) followed by repeated cue exposures (extinction training). However, both clinicians and regulatory agencies would be reluctant to expose addicted patients to their abused drug in clinical practice. Considering the similar pharmacologic effect between methadone and heroin, the purpose of the present study was to evaluate the efficacy of methadone-initiated MRUP in OUD. We had 3 hypotheses. 1) Repeated cue exposures within a reconsolidation time window-triggered by methadone could decrease heroin relapse in a rodent model. 2) Methadone-initiated MRUP is more efficacious than MMT alone in improving sustained abstinence, retention rate and cue-induced craving in OUD patients. 3) The methadone-initiated MRUP would have a time dependence such that the pairing of the methadone dose with the MRUP (or its rodent model equivalent) must be sufficiently close in the reconsolidation time window of less than 6 h.

Methods

Ethics

The protocol of rat study was approved by the Biomedical Ethics Committee for Animal Use and Protection of Peking University. The protocol of human study was approved by the institutional review boards of Peking University Biomedical Ethics Committee. All study participants provided written informed consent.

Study design and participants

We designed the present study to assess the efficacy of methadone-initiated MRUP in a rodent model of heroin relapse and in patients with heroin use disorders. We performed the rat study in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and reported according to the ARRIVE guidelines 2.0 checklist.

In a single blind (blinded outcomes assessor) experimental design, we randomly assigned participants to 1 of 3 groups. We recruited participants from 6 MMT clinics in Guangdong Province of China. The inclusion criteria were that participants had to be male outpatients aged between 18 and 55 years old who met the diagnostic criteria of heroin use disorder before starting MMT according to the criteria of *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV), with normal blood pressure and heart rate. They also were willing to provide urine samples for morphine testing, and had the capacity to give written informed consent. Participants were excluded if they had other drug or alcohol use disorders, serious somatic or psychiatric disorders in need of treatment that interfered with study participation, or cognitive impairment. We conducted the human study according to Good Clinical Practice and reported according to the CONSORT 2010 statement checklist.

Randomization and masking

We randomly assigned (1:1:1) participants to parallel groups receiving either MRUP starting 10 minutes (experimental group) or 6 hours (sham comparator) after methadone or methadone alone (control group). We used an urn randomization procedure to ensure that patients in 3 groups were similar with regard to education, methadone dose and addiction severity.[16] During the randomization period, an independent (blind) team member, who was only involved in the rat experiments, generated a table of randomization codes using Microsoft Excel software. Other team members who were responsible for the assessments, tests and entering data were blinded to the random allocation and interventions in both the human and rat studies. Unblinding did not occur after the database had been locked by the statistician.

Procedures

The timeline of the rat experiments is provided in Figure 1A. We used male Sprague-Dawley rats weighing 260−280 g for all the experiments (n = 49). The training, extinction and test procedures were similar to our previous studies.[12,14,15] For the US retrieval manipulation, the dose of methadone was based on previous studies investigating the safety of acute methadone exposure.[17] During the drug-priming reinstatement tests, we injected heroin (0.10 mg/kg or 0.25 mg/kg, i.p.) or saline (0.50 ml, i.p.) 5 min before the start of the sessions based on our previous studies.[12,15] This information is described in the Supplementary Data.

The timeline of the human study is provided in Figure 2A. Eligible candidates were scheduled for a screening interview during which they read the study protocol and signed the informed consent form. There were 3 phases (baseline phase, intervention phase, follow-up assessment phase) in the human study. After ensuring stable MMT without illicit drug use for at least 2 weeks, we collected baseline demographic and clinical data including urine morphine and conducted baseline tests of craving for heroin induced by CS. During the 28-day intervention phase, patients randomized to MRUP occurring either 10 min or 6 h after methadone dosing received the intervention 3 times per week for a total of 12 sessions. When randomized to MRUP, patients received methadone administration (US for memory retrieval) either 10-minute or 6-hour before the 30-min exposure to drug-related stimuli (memory extinction). Patients who were randomized to

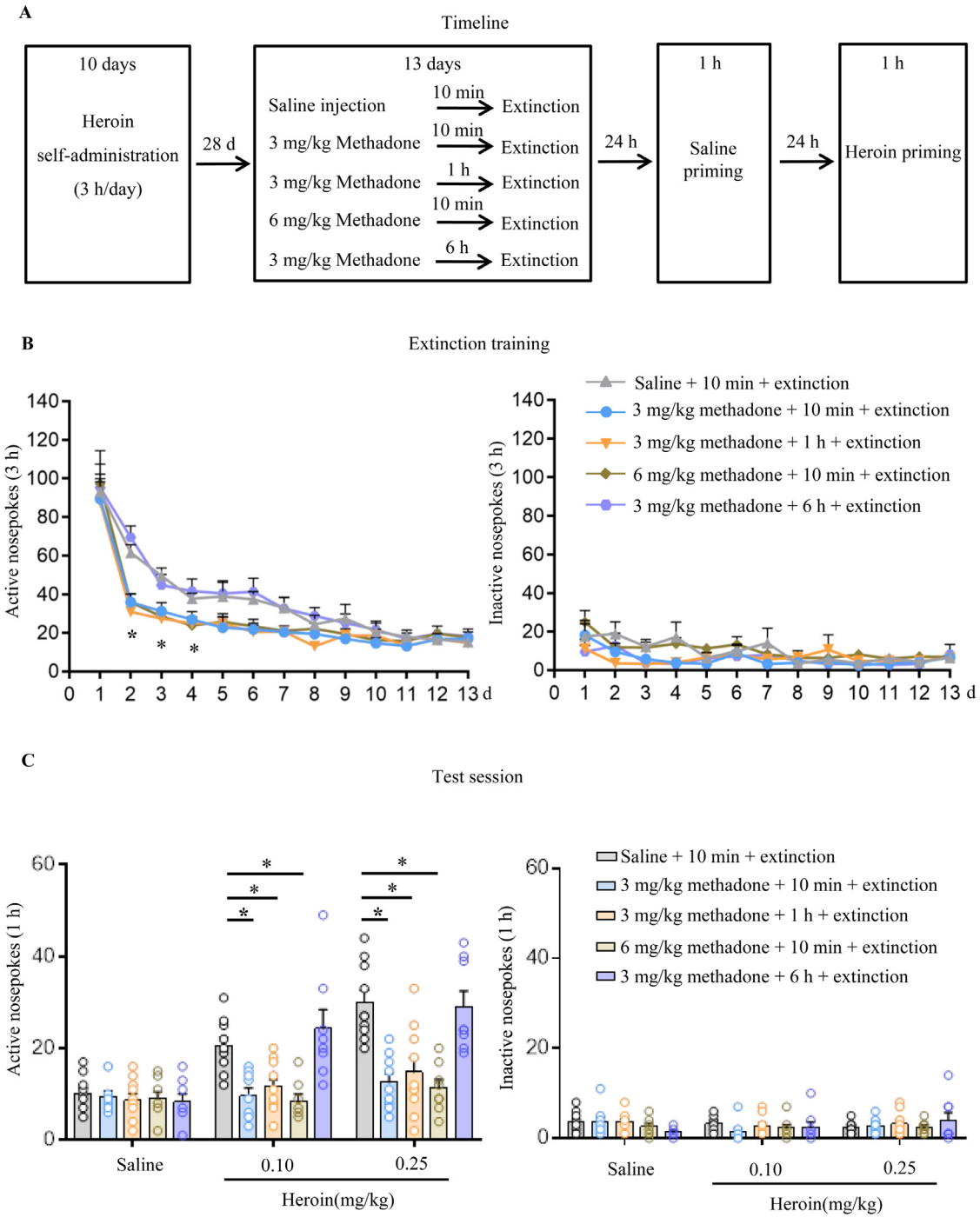

Figure 1. ***In rats, methadone-initiated memory reconsolidation updating procedure (MRUP) facilitated extinction responding and decreased heroin-priming-induced reinstatement of heroin seeking after prolonged withdrawal.*** (**A**) Experimental timeline of the procedure. Rats first underwent heroin self-administration training for 10 consecutive days. After that they were randomly assigned to intraperitoneally receive saline or 3 mg/kg methadone 10 min, 1 h or 6 h before extinction training. Another group received 6 mg/kg methadone 10 min before extinction training to assess the dose effect of methadone. After a 28-day withdrawal period, all groups underwent reinstatement testing during 3 consecutive days: saline priming test, 0.10 mg/kg heroin priming test, and 0.25 mg/kg heroin priming test. (**B**) Nose-poke responses during the extinction session on the active operandum (left) and inactive operandum (right). (**C**) Nose-poke responses during the test session on the active operandum (left) and inactive operandum (right). *$p < 0.05$, compared with 'Saline + 10 min + extinction' group (mixed ANOVA). Error bars represent SEM. Saline + 10 min + extinction, n=10; 3 mg/kg methadone + 10 min + extinction, n=9; 3 mg/kg methadone + 1 h + extinction, n=10; 6 mg/kg methadone + 10 min + extinction, n=8; 3 mg/kg methadone + 6 h + extinction, n=8.

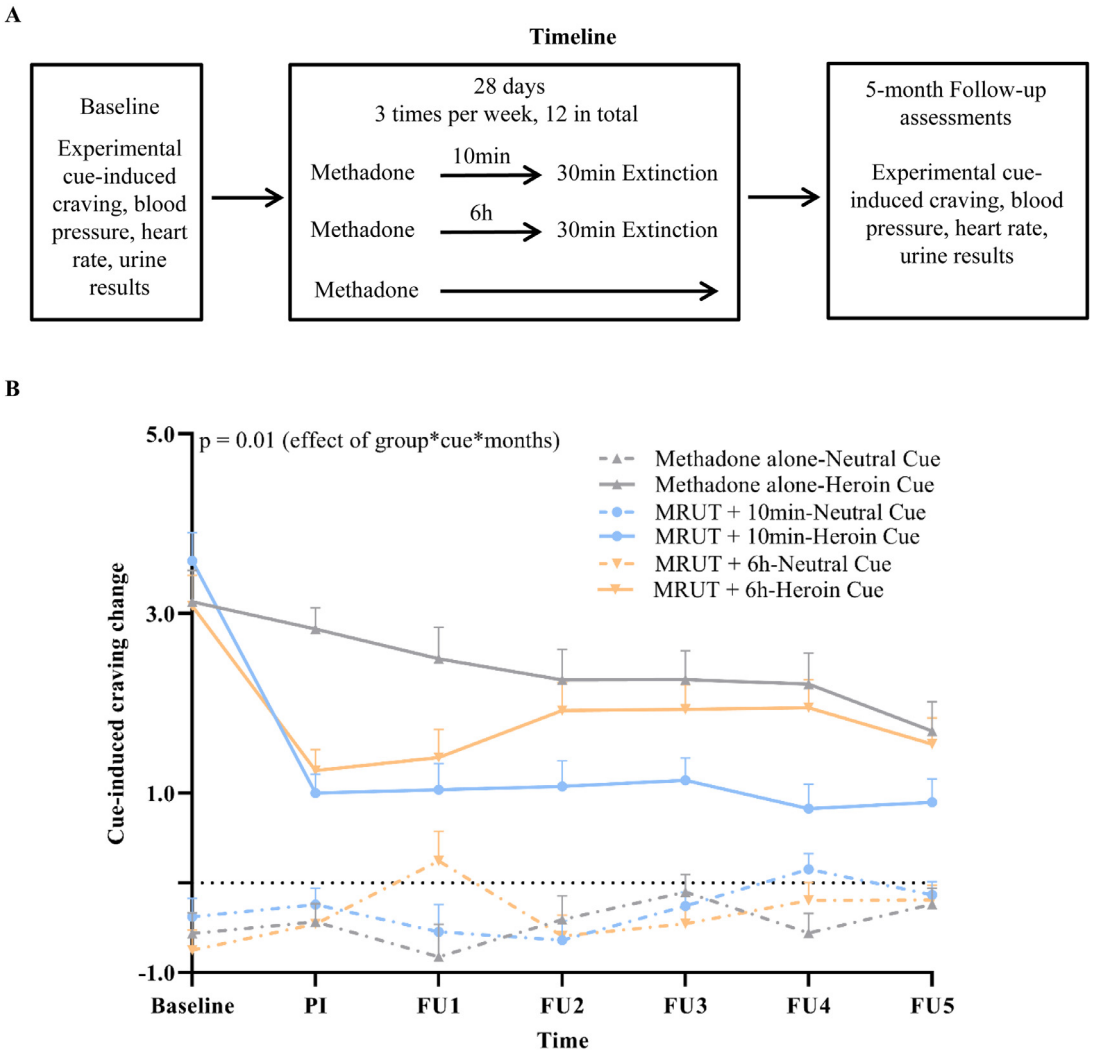

Figure 2. ***In humans, methadone-initiated memory reconsolidation updating procedure (MRUP) caused a long-lasting attenuation of cue-induced heroin craving***. (**A**) Timeline of the experimental procedure in the human study. Neutral- and heroin-cue-induced drug craving in abstinent heroin addicts was measured with VAS, sympathetic activation (heart rate and blood pressure), and urine results were obtained on day 1 before intervention (baseline). Twenty-four hours later, participants were randomly divided into 3 groups, and given MRUP 3 times a week (12 in total) or methadone alone for 28 days. During the extinction sessions, the participants were given 2 consecutive sessions of repeated exposures to 3 different heroin-related cues. Cue-induced heroin craving, sympathetic activation, and urine results were assessed again at the post-intervention and at 5 monthly follow-ups by using a procedure identical to that used at baseline. (**B**) Experimental cue-induced heroin craving changes in groups before intervention (baseline), after intervention, at the 5 monthly follow-ups, the interaction effects of group × cue types × months, cue types × months, group × cue types, and the main effect of cue types and months: all $p < 0.001$; the interaction effect of group × months and the main effect of group: $p < 0.05$. Error bars represent SEM. Methadone alone: methadone alone without memory reconsolidation updating procedure, MRUP + 10 min: methadone-initiated memory reconsolidation updating procedure with 10-min delay, MRUP + 6 h: methadone-initiated memory reconsolidation updating procedure with 6-h delay. PI: post intervention, FU1: 1st month follow-up, FU2: 2nd month follow-up, FU3: 3rd month follow-up, FU4: 4th month follow-up, FU5: 5th month follow-up.

methadone alone, received methadone administration as usual without MRUP. We did the post intervention tests of craving for the experimental heroin CS and urine morphine on the next day after the 28-day intervention for MRUP groups or after a 28-day interval for the methadone alone group. During the 5 monthly follow-up assessments phase, we conducted the tests of craving for the experimental heroin CS and urine morphine identically to the baseline phase, and recorded the retention of participants in the study. The detailed information is described in the Supplementary Data.

Outcomes

The primary outcomes were operant heroin seeking after reinstatement in rats, and experimental cue-induced heroin craving change, sustained abstinence and retention in the study after the intervention and at 5 monthly follow-up assessments. Heroin craving was assessed using a visual analog scale with 0 ("extremely low") and 10 ("extremely high") at the left and right ends. Sustained abstinence required that patients had biochemical confirmation of heroin abstinence by a urine morphine test at each follow-up session. Patients who were lost to the follow-up assessments or did not provide biochemical validation were classified as not being abstinent in the primary analysis. Retention in the study was defined as patients who took part in each of the 5 follow-up interviews.

The secondary outcomes were changes in physiological responses including experimental cue-induced blood pressure change and heart rate change monitored using a portable electrocardiogram monitor. Exploratory outcomes included the number of the 12 intervention sessions completed and the dropout rate from the 5-month follow-up assessments.

Statistical analysis

We determined the sample size based on previous studies, in which the power to detect significant effects was typically a Cohen d' of 1 or above.[12,14,15] In the present study, we used a total of 49 rats to detect a minimum effect size of 0·60 for heroin-priming-induced reinstatement of heroin seeking after prolonged withdrawal, while providing 90% power at $\alpha \leq 0{\cdot}05$. We excluded 4 drug-experienced rats due to illness (n=3) or failure to acquire self-administration (n=1). We expressed the data as mean (± SEM) and analyzed using mixed-measures analysis of variance (ANOVA) in the rat study. Data distribution was assumed to be normal, but this was not formally tested. Significant main effects and interactions in the ANOVA were followed by Bonferroni *post hoc* testing. We performed these analyses using SPSS 22·0 software.

We calculated to enroll 82 participants to detect a minimum effect size of 0·60 for the cue-elicited craving during the post-intervention test session, while providing 90% power at $\alpha \leq 0.05$ and with a 20% dropout rate during the follow-ups in human study.[12] We reported the continuous variables with non-normal distributions (such as age, weight, education, months of heroin use, abstinent months, depressive symptom, anxiety symptom, working memory and addiction severity) as median (interquartile range) and analyzed the data by Kruskal-Wallis H test. The MMT dose had a normal distribution, and we analyzed these data by ANOVAs. The categorical variables were used as proportions (%) and analyzed using Chi-square tests (χ^2 test) at baseline. Finally, a total of 83 patients were enrolled in the present study, and data on all of them were analyzed to compare demographic and clinical differences at baseline.

Seven patients were terminated from the study because they completed less than 80% of the 12-time interventions. Per protocol analysis was conducted to analyze outcomes (data from 76 patients) such as experimental cue-induced heroin craving and physiological changes, sustained abstinence and retention in the study after the intervention and at 5 monthly follow-up assessments. We calculated sustained abstinence from the first follow-up after the 1-month intervention or from baseline until the patient left, relapsed to illicit opioid abuse or until the end of 5-month follow-up using Kaplan Meier survival analyses (results given as median and 95% confidence interval [95%CI]). The Log Rank test was used to detect the difference of sustained abstinence between 3 groups, and multivariable Cox regression model (Cox model) was further controlled other covariates and presented as hazard ratios (HR). We also did the collinearity test for independent variables in the Cox model. We estimated the retention rate using the generalized linear mixed model (GLMM) with fixed effects of groups and timepoints (months). We analyzed the exploratory outcomes by χ^2 test. We performed these analyses using SPSS 22.0 software.

Missing data occurred in less than 20% of the cue-induced craving and physiological changes, and in the baseline addiction severity index (ASI). For missing cue-induced craving and physiological parameters, we employed the SAS mixed procedure, which uses a maximum-likelihood approach for incomplete repeated-measures data.[12,18] In this model, we used the between-subject factor of groups, and the within-subject factors of months (baseline, post-intervention and 5 months follow-ups) and cue types (heroin-related cue and neural cue). We did the above analyses using SAS software. Missing data on the ASI were imputed. We compared the ASI results from imputed data to the results without the original missing data and found that these statistical imputations did not bias the results. Values of p less than or equal to 0.05, 2-tailed, were considered statistically significant.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Methadone-initiated MRUP decreases heroin relapse in rats

We first investigated the effect of the methadone-initiated memory retrieval-extinction procedure (a rodent

model of MRUP) on heroin-priming-induced reinstatement of heroin seeking after a 28-day withdrawal period (Figure 1A). Statistical analysis included the between-subject factor of groups and within-subject factor of priming doses (0, 0.10, 0.25 mg/kg), and it showed that the methadone exposure facilitated extinction responding, with a significant group × extinction session interaction during extinction training (Figure 1B, $F_{(48,480)} = 1.48$, $p < 0.05$). The reinstatement test showed a significant group difference (Figure 1C, $F_{(4,40)} = 9.71$, $p < 0.001$) and priming dose difference (Figure 1C, $F_{(2,80)} = 49.36$, $p < 0.001$), as well as group × priming dose interaction (Figure 1C, $F_{(8,80)} = 8.26$, $p < 0.001$). The *post hoc* analysis showed that heroin-priming-induced relapse occurred in the saline group and the 6-h methadone group but not in the 10-min methadone group and 1-h methadone group.

These results suggest that exposure to methadone at 10–60 min, but not at 6 h, before extinction decreased the heroin-priming-induced reinstatement of heroin seeking even after prolonged withdrawal.

Enrollment and baseline characteristics of OUD patients

A total of 167 male patients with heroin use disorders were recruited and screened with 68 ineligible, and 16 who declined to enroll in the experimental study. We then randomized 83 participants (30 to MRUP with a 10-min methadone dosing delay, 26 to MRUP with a 6-h delay, and 27 to methadone alone) at baseline, and included 76 for the 5 monthly follow-up assessments with 7 dropouts from the 1-month intervention: 1 from the 10-min delay, 2 from the 6-h delay, and 4 from the methadone alone group (Supplementary Figure 1).

The median age of participants was 44.00 (11.00) years and all were males. The median years of education was 9.00 (3.00) years. Most participants (42.17%) were married or living married, 31 (37.35%) were single or never married, and 17 (20.48%) were divorced or separated. Clinically the median number of years using heroin was 19.50, and the median months of heroin abstinence was 23.00. The baseline methadone dose was 61.63 ± 28.20 mg. Most (57.80%) participants had visible depressive symptoms, and 25 (30.12%) showed significant anxiety symptoms. The study groups showed no significant differences in baseline demographics, clinical characteristics (Table 1) or addiction severity (Supplementary Table 1).

Methadone-initiated MRUP causes a long-lasting attenuation of experimental cue-induced heroin craving and physiological changes

The MRUP groups received 12 consecutive interventions of methadone plus extinction trainings within a 28-day period. The control group only received methadone as usual (Figure 2A). A total of 96.67% of patients completed all the sessions of MRUP with the 10-min delay, while 84.62% of patients completed all the sessions of MRUP with the 6-h delay. The completion rates for the 2 intervention groups showed no significant difference ($\chi^2_{(1,56)} = 2.49$, $p = 0.12$). During the 5 months after interventions, the number of patients dropping out was: 5 (17.24%) for MRUP with a 10-min delay, 5 (20.83%) for MRUP with a 6-h delay, and 8 (34.78%) for methadone alone ($\chi^2_{(2,76)} = 2.34$, $p = 0.31$) (Supplementary Table 3).

For cue-induced heroin craving, a mixed procedure with the between-subject factor of groups, and the within-subject factors of months and cue types showed a significant triple interaction effect between group × months × cue types (Figure 2B, $F_{(12,\ 63.3)} = 2.41$, $p = 0.01$). As for heroin cue-induced craving changes, after the 1-month intervention, the craving change scores significantly dropped in the MRUP groups (1.00 ± 0.23 for 10-min delay group, and 1.25 ± 0.25 for 6-h delay group, respectively, both $p < 0.001$) compared with the control group (2.83 ± 0.26, $p = 0.43$). During the whole experiment, the craving change of participants in MRUP with a 10-min delay kept stable up to the final assessment. However, the craving change for participants in MRUP with a 6-h delay started to be lost after 1 month, and no significant differences were found between the craving changes of participants in MRUP with a 6-h delay and the control group at the 2nd month (1.83 ± 0.27 and 2.24 ± 0.30, $p = 0.46$) or the 5th month of follow-ups.

The cue-induced systolic blood pressure changes showed a significant interaction of group × cue types ($F_{(2,\ 68.6)} = 7.76$, $p < 0.001$). We observed a similar change trend in the heroin cue-induced systolic blood pressure changes in the 3 groups (Supplementary Table 4) with the main effects of cue types and group being significant ($F_{(1,\ 68.9)} = 101.88$, $p < 0.001$, and $F_{(2,\ 66.9)} = 4.79$, $p = 0.01$). The main effects on diastolic blood pressure and heart rate increases of cue types ($F_{(1,\ 61.3)} = 36.67$, and $F_{(1,\ 73.2)} = 34.52$, both $p < 0.001$) and months ($F_{(6,\ 64.9)} = 2.58$, $p = 0.03$, and $F_{(6,\ 64)} = 4.53$, $p < 0.001$) were both significant (Supplementary Table 4, Supplementary Figure 2).

These results showed that relapse back to craving occurred in the MRUP with a 6-h delay group after a relatively brief period. However, the intervention of MRUP with a 10-min delay could induce long-lasting reductions in heroin cravings and physiological changes for at least 5 months after the intervention.

Methadone-initiated MRUP promotes sustained abstinence and treatment retention

The results showed that the median months of sustained abstinence during the 28-day intervention and 5 monthly post-intervention assessments were significantly different

Variables	Participants (*n* = 83)	Methadone alone group (*n* = 27)	MRUP + 10 min group (*n* = 30)	MRUP + 6 h group (*n* = 26)	*p*
Age, median (interquartile range), y	44 (11)	42 (12)	45 (12)	47 (10.25)	0.36
Weight, median (interquartile range), kg	61 (11)	60 (14)	65 (11)	60 (13.75)	0.32
Education, median (interquartile range), y	9 (3)	9 (3)	9 (3)	9 (0.75)	0.66
Marital status, n (%)					0.51
Married, living as married	35 (42.17%)	14 (51.85%)	13 (43.33%)	8 (30.77%)	
Single, never married	31 (37.35%)	8 (29.63%)	10 (33.33%)	13 (50.00%)	
Divorced, separated	17 (20.48%)	5 (18.52%)	7 (23.33%)	5 (19.23%)	
Having offspring, n (%)	38 (45.78%)	16 (59.26%)	13 (43.33%)	9 (34.62%)	0.19
History of heroin use, median (interquartile range), months	234 (103.50)	224 (79)	253 (83.50)	248.50 (129.25)	0.26
Abstinent months, median (interquartile range)	22 (57)	26 (52)	20·50 (52.50)	21 (73.25)	0.92
Methadone dose, Mean ± SD, mg	61.63 ± 28.20	60.03 ± 26.98	68.30 ± 28.88	55.58 ± 28.12	0.23
BDI score, median (interquartile range)	9 (12)	6 (11)	11.50 (10.25)	10 (12)	0.08
≥ 8, n (%)	48 (57.80%)	11 (40.74%)	20 (66.67%)	17 (65.38%)	0.09
HAMA score, median (interquartile range)	10 (12)	5 (12)	10 (8)	10 (12.75)	0.13
≥ 14, n (%)	25 (30.12%)	7 (25.93%)	8 (26.67%)	10 (38.46%)	0.53
DST score, median (interquartile range)	11 (3)	11 (3)	11 (3)	12 (3)	0.31

***Table 1*: Demographic and clinical characteristics of heroin users.**
Notes: abbreviations, methadone alone: methadone alone without memory reconsolidation updating procedure; MRUP + 10 min: methadone-initiated memory reconsolidation updating procedure with 10-min delay; MRUP + 6 h: methadone-initiated memory reconsolidation updating procedure with 6-h delay; BDI: Beck Depression Inventory; HAMA: Hamilton Anxiety Scale; DST: Digit Span Test.

among the 3 interventions ($p < 0.001$): 6 months for the 10-min delay MRUT [95% CI 5.54, 6.46], 5 months for the 6-h delay MRUT [95% CI 4.57, 5.43] and 4 months for the methadone alone [95% CI 3.51, 4.50] (Figure 3A). We further confirmed the efficacy of MRUT on sustained abstinence using a Cox model adjusted for marital status, employment, usage years of heroin, ever injecting heroin, heroin dose, MMT admission age, methadone dose, and addiction severity using the methadone alone group as reference. We found that the 10-min delay MRUP could be a protective factor for sustained abstinence with a hazard ratio of 0.43 [0.22, 0.83] ($p = 0.01$), while the 6-hour delay MRUP showed no significant effect on sustained abstinence (0.57 [0.29, 1.11], $p = 0.10$) (Figure 3B). The multicollinearity test in the Cox model showed no multicollinearity with all correlation coefficients less than 0.70 (Supplementary Table 2).

As for retention rate, the main effects of group and months were both significant ($F_{(2, 523)} = 7.30$, $p = 0.001$, $F_{(6, 523)} = 3.49$, $p = 0.002$, respectively), but the interaction effect of group × months was not significant ($F_{(12, 511)} = 0.20$, $p = 0.99$). Retention rates of MRUP with a 10-min delay and MRUP with a 6-h delay were higher than that in the methadone alone group ($t = 3.50$, $p < 0.001$, $t = 2.81$, $p < 0.05$, respectively). The retention rate showed significant differences among the 3 interventions at the first month follow-up ($t = -2.75$, $p = 0.006$), but at the longer follow-ups, the retention rate became worse ($F_{(6, 523)} = 3.49$, $p = 0.002$) (Figure 3C, Supplementary Table 3).

These results showed that the intervention of MRUP with a 10-min delay offered greater protection from relapse behavior and better adherence to the study than no MRUP or a 6-h delay in providing MRUP.

Discussion

We investigated the efficacy of methadone-initiated MRUP in a translational rat and human study. Using a rat relapse model, methadone-initiated MRUP accelerated the extinguishing of heroin craving and produced lasting inhibition of heroin relapse after prolonged withdrawal. We also observed the methadone-initiated MRUP promoted sustained abstinence, treatment retention, and persistently decreased subsequent heroin craving and blood pressure induced by the preexisting heroin-related cues during the whole 5-monthly follow-up of assessments in outpatients on MMT.

The CS-based MRUP was developed based on memory reconsolidation theory, and it was found to persistently inhibit return of fear and drug memories within a specific time-window (within 6 hours post retrieval, a 'reconsolidation window') in rats and humans.[12,13,19,20] We developed a US-based MRUP, during which memory extinction is preceded by an acute exposure to the addicted drug, and we showed that it is superior to a CS-based memory updating intervention.[14,15] However, a critical concern in the translation of the US-based MRUP is the ethical and legal aspects of exposing patients to the abused drug. Methadone is a full

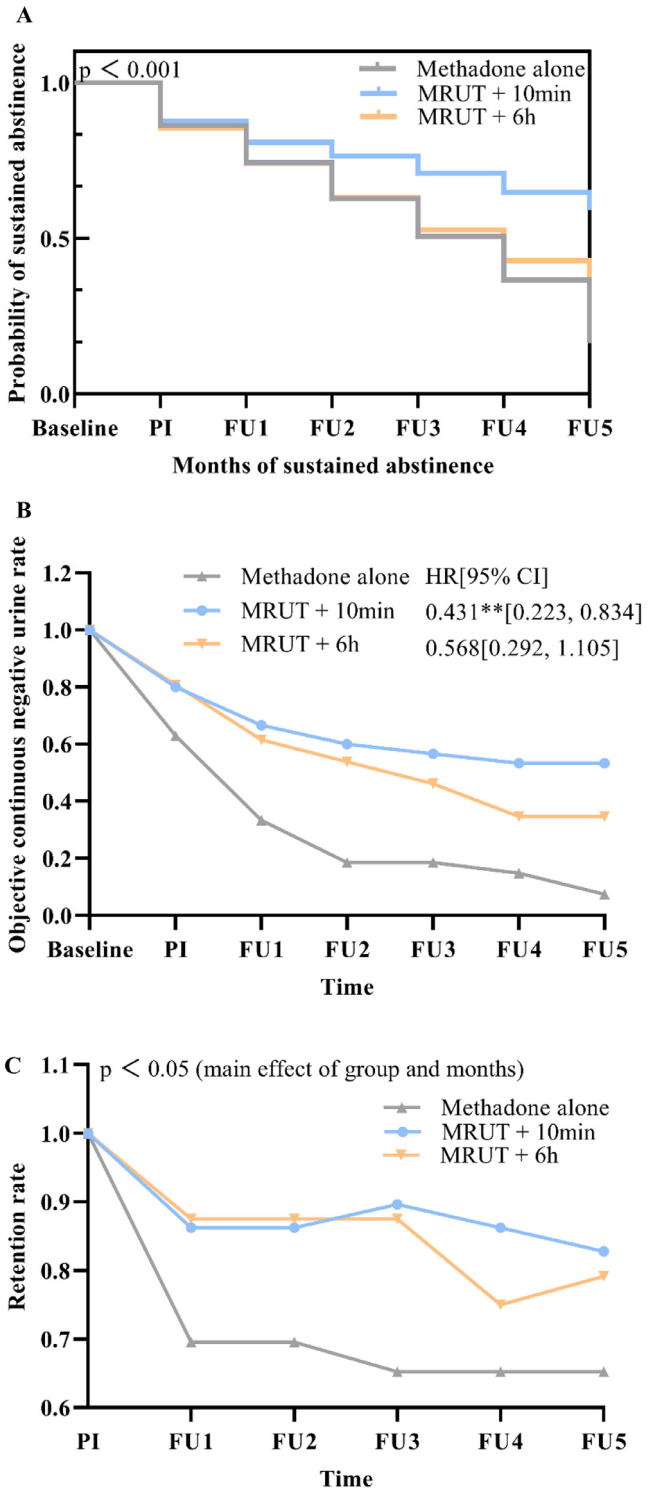

Figure 3. ***Methadone-initiated memory reconsolidation updating procedure (MRUP) increased sustained abstinence and the retention rate in heroin addicts***. (**A**) Kaplan-Meier survival curve of sustained abstinence in participants among different groups ($p < 0.001$). (**B**) Objective continuous negative urine rate at different months in different groups combined with hazard ratio [HR, 95% CI] from Cox results (adjusted for marital status, employment, usage year of heroin, ever injecting heroin, heroin dose, methadone maintenance treatment admission age, methadone dose, addiction severity, $p < 0.05$, methadone alone group as reference). (**C**)

μ-opioid agonist and is a standard opioid-substitution treatment, which has been shown to reduce relapse in patients who remain in treatment.[2] Therefore, in the present study, we reactivated heroin-related memories using methadone exposure followed by extinction to induce the memory updating process. We demonstrated that in both rats and humans the intervention was more efficacious if the delay between methadone administration and repeated cue exposure was 10 min or 1 h but not 6 h. These results are consistent with the time-window of memory reconsolidation[21] and support our previous findings on US-based memory reconsolidation interventions.[14,22,23]

We used methadone as the US to trigger the reconsolidation of heroin memory, because as a full μ-opioid agonist, it has a similar pharmacologic effect to heroin, and is a standard opioid-substitution treatment. However, another contributing biological mechanism for this effect of methadone specifically on memory reconsolidation may be related to its d-methadone component of this racemic methadone mixture. d-methadone (dextro-methadone) is a noncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonist that binds to the dizocilpine (MK-801)-binding site of the receptor with an affinity comparable with that of well-established NMDAR antagonists.[24] This time action of d-methadone and the 6-h time boundary for obtaining the sustained positive therapeutic effect of its combination with MRUT suggests another interesting mechanism for the retrieval extinction that may depend on the NMDAR antagonism from d-methadone. Considering that NMDAR antagonists have certainly proven productive in depression and post-traumatic stress disorder,[25] it is interesting to develop more rapid and sustained extinction processes by combining other NMDAR antagonists with MRUP.

Besides substitution treatment for OUDs, psychotherapy using CET is applied to reduce cue-induced drug craving and relapse in the clinic.[26,27] Unfortunately, CET alone might increase dropout and relapse rates among abstinent patients with OUD,[28] and the combination of CET and MMT did not improve retention rates and outcomes.[8,29] Our methadone-initiated MRUP with a 10-minute delay after dosing significantly prevented the relapse to heroin, and the patient adherence with MRUP in our study was good. Even when extinction was applied 6 hours after methadone exposure, heroin cue-induced craving still decreased during the first month of follow-up, however, it was not sustained at the second month of follow-ups. This may result from the temporal inhibitory effect of extinction, which are consistent with a temporal effect of CET.[28] These results support the superior efficacy of MRUP using cue-exposure interventions.

The present study had several limitations. First, we focused only on male rats and humans. In China, male heroin patients are relatively common. Demographics and drug-use characteristics of patients in the current study were consistent with a previous study,[30] indicating that this cohort may be considered representative of the wider population of interest within the context of this research. However, because females may respond differently compared with males to drug-related cues and to drug use, MRUP with methadone should also be tested in females.[31,32] Second, we did not use a double-blinded design, because the blinding of patients would have been unachievable. However, the staff responsible for the outcome measurement and analysis were blind to the experiment design. Third, all patients included in the present study had reached stable methadone doses in MMT, and it is unknown whether the treatment will be efficacious for patients during the induction phase of MMT. Fourth, we used monthly morphine-containing urines as the indicator of relapse to heroin, and further research is required with more frequent urine monitoring or perhaps hair and related tissues as more longitudinal indicators of opioid use including fentanyl use.

This rat-to-human translational study demonstrated the efficacy of methadone-initiated MRUP. The methadone-initiated MRUP significantly decreased heroin craving and increased sustained abstinence of OUD patients, and promoted treatment retention during the study. MRUP could be a novel treatment used in conjunction with MMT to prevent opioid relapse.

Contributors

Lin Lu, Ping Wu and Yan-Xue Xue designed the whole experiments. Kai Yuan and Lu Cao conducted the preclinical study, Jing-Li Yue, Shi-Qiu Meng, Qing Fang, Xiao-Jie Guo and Ye-Kun Sun performed the clinical study. Jing-Li Yue, Kai Yuan, Ping Wu, Yao-Ping Bao, Na Zeng and Jie Sun analyzed the data. Jing-Li Yue and Kai Yuan wrote the manuscript draft. Yan-Xue Xue, Ping Wu, Yao-Ping Bao, Le Shi, Ying Han, Tang-Sheng Lu, Wei Yan, Jie Shi and Thomas Kosten revised the manuscript. Lin Lu and Thomas Kosten made the final confirmation of the manuscript.

Data sharing statement

Due to the nature of this research, to protect the participants' privacy and confidentiality, raw data are not

Retention rate at different months in different groups, main effect of months: $p = 0.002$; main effect of group: $p = 0.001$. Methadone alone: methadone alone without memory reconsolidation updating procedure, MRUP + 10 min: methadone-initiated memory reconsolidation updating procedure with 10-min delay, MRUP + 6 h: methadone-initiated memory reconsolidation updating procedure with 6-h delay. PI: post intervention, FU1: 1st month follow-up, FU2: 2nd month follow-up, FU3: 3rd month follow-up, FU4: 4th month follow-up, FU5: 5th month follow-up.

shared publicly. Relevant data supporting the findings and conclusions of this study are available within the article and/or supplementary materials. Upon a justifiable request, the share of de-identified data should be approved by the board of an investigational ethics committee.

Declaration of interests

We declare no competing interests.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.104283.

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