It is never as good the second time around: Brain areas involved in salience processing habituate during repeated drug cue exposure in treatment engaged abstinent methamphetamine and opioid users

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A B S T R A C T

The brain response to drug-related cues is an important marker in addiction-medicine. However, the temporal dynamics of this response in repeated exposure to cues are not well known. In an fMRI drug cue-reactivity task, the presence of rapid habituation or sensitization was investigated by modeling time and its interaction with condition (drug-neutral) using an initial discovery-sample. Replication of this temporal response was tested in two other clinical populations all abstinent during their early recovery (treatment). Sixty-five male participants (35.8 ± 8.4 years-old) with methamphetamine use disorder (MUD) were recruited as the discovery-sample from an abstinence-based residential treatment program. A linear mixed effects model was used to identify areas with a time-by-condition interaction in the discovery-sample. Replication of these effects was tested in two other samples (29 female with MUD from a different residential program and 22 male with opioid use disorder from the same residential program as the discovery sample). The second replication sample was re-tested within two weeks. In the discovery-sample, clusters within the VMPFC, amygdala and ventral striatum showed both a main effect of condition and a condition-by-time interaction, indicating a habituating response to drug-related but not neutral cues. The estimates for the main effects and interactions were generally consistent between the discovery and replication-samples across all clusters. The re-test data showed a consistent lack of drug × neutral and habituation response within all selected clusters in the second cue-exposure session. The VMPFC, amygdala and ventral striatum show habituation in response to drug-related cues which is consistent among different clinical populations. This habituated response in the first session of cue-exposure and lack of reactivity in the second session of exposure may be important for informing the development of cue-desensitization interventions.

1. Introduction

Drug craving has been considered a motivational state that activates drug seeking behavior, potentiating relapse (Ekhtiari et al., 2016; Wise, 1988), and is one of the diagnostic criteria for substance use disorders in the DSM 5 (American Psychiatric Association, 2013). Incentive sensitization theory describes drug craving as the attribution of salience to the drug-related cues that can activate the hypersensitized learning associated with rewarding properties of drugs (Robinson and Berridge, 1993). Drug craving and subsequent relapse can be triggered by exposure to the conditioned drug related cues whether they are pictures, mental images, smells, words, or actual drugs and their paraphernalia (Ekhtiari et al., 2010). In this context, drug cue exposure has been developed as an ecologically valid experimental to examine the mechanistic and predictive value of subjective, behavioral and biological responses to drug cues (Drummond, 2000; Carter and Tiffany, 1999).

FMRI drug cue reactivity (FDCR) explores the neural response to drug cues presented to subjects inside the scanner (Ekhtiari et al., 2016). As of 2019, there are over 300 original published studies with FDCR. These studies reported that FDCR in certain brain areas is associated with severity of the drug addiction (Sjoerd et al., 2014; Smolka et al., 2006), defines prognosis of long-term recovery (Kosten et al., 2006; Janes et al., 2010), predicts the response to specific interventions (Courtney et al., 2016; Mann et al., 2014) and can be used as a proxy measure for effectiveness of different interventions in clinical trials (Lukas et al., 2013; Sadraee et al., 2019).

In FMRI drug cue reactivity studies, conventionally, the average response to drug cues in contrast to control conditions (neutral cues, fixation cross etc.) is reported/utilized as the main contrast/signal of interest (Hartwell et al., 2011; Van Hedger et al., 2018; Schacht et al., 2013). However, drug cue reactivity triggers a motivational/affective response that has a temporal behavior (Ekhtiari et al., 2016). With contempo-
rary methodological conventions for fMRI cue reactivity tasks, there are multiple exposures to drug cues as blocks or events within each experiment. This provides a great opportunity to include time in the analysis to explore the temporal behavior of the response to drug cues. We expect different regions to have habituating or potentiating responses during repeated trials of cue exposure. The dynamic response to affective cues have been reported previously in different brain areas including the amygdala, ventral striatum and prefrontal cortices (emotional faces (Wright et al., 2001) and aversive images (Phan et al., 2003)). It has been reported frequently in fMRI studies that the amygdala habituates to emotionally salient stimuli (emotional faces) (Breiter et al., 1996; Fischer et al., 2003; Sladky et al., 2012) and its habituation is a more reliable index than its mean signal (Plichta et al., 2014). The ventral striatum also habituates with multiple exposures to rewarding stimuli (monetary reward) (Moses-Kolko et al., 2011). The first fMRI drug cue reactivity study that explored the temporal response in the amygdala, ventral striatum, ventromedial prefrontal cortex (VMPFC) and anterior cingulate cortex implemented prolonged exposure to drug cues (10 minute videos) with fMRI and subjective measurements collected during and after the exposure (Murphy et al., 2018). This study found a positive correlation between the temporal response in the amygdala and subjective report of craving. Previous investigations had not considered the possibility of habituation/sensitization to the cues and therefore did not examine the effects of repeated presentation of drug cues on brain activation, however, we expect areas that are involved in reward and saliency processing to present a time by condition (drug>neutral) interaction indicating a temporally dynamic response to the repeated presentation of drug cues.

This investigation examined three major questions. First, what is the time course of brain activation to repeated exposure of drug cues? Second, is there individual variability of the time course of brain activation that can be related to clinical characteristics, i.e., duration of dependence or abstinence? Third, what is the between-session reliability of the time course of brain activation in repeated drug cue exposure? To provide methodological resources to answer these questions, we have recently developed and validated a large pictorial database of drug and controlled neutral cues with 320 images and a series of fMRI drug cue reactivity tasks for opioid and methamphetamine users (Ekhrtiari et al., 2020). Having 8 blocks of cues makes it possible to explore the temporal dynamic response to the drug cues by adding time as a factor in the analysis models. This experimental resource also provides an opportunity to test and compare drug cue reactivity in both opioid and methamphetamine users between or within different sample populations multiple times with distinct but equivalent drug cue sets. To answer questions one and two, we have recruited a discovery sample of methamphetamine users and conducted both whole-brain and ROI-based analyses to identify whether there are brain regions exhibiting time by condition (drug>neutral) interactions. Using clusters identified in this discovery sample, we examined whether the time by condition interaction effects replicated in two other replication samples, one with methamphetamine use disorder, but in a different clinical setting with longer duration of abstinence, and the other with opioid use disorder within the same clinical setting and same duration of abstinence as the discovery sample. To answer the third question, the second group was re-tested with a distinct but equivalent set of cues in the fMRI task within two weeks (Fig. 1).

2. Methods

In this study, the dynamic response to drug cues was explored in a discovery sample using an fMRI drug cue reactivity (FDCR) task and then the replication of the results was tested in two independent samples. The second replication sample was re-tested within two weeks with a distinct but equivalent version of the FDCR task to test the replication of the results within a sample population (Fig. 1).

2.1. Participants

2.1.1. Discovery sample

The discovery sample (n = 65) consisted of men (mean age: 35.86, standard deviation (SD): 8.47) with methamphetamine use disorder who were admitted to a residential abstinence-based treatment center (128&12 center) in Tulsa, Oklahoma and were abstinent in the residential program or its aftercare transitional living programs. Inclusion criteria were (1) English speaking, (2) diagnosed with methamphetamine use disorder (last 12 months), (3) current abstinence from methamphetamine for at least one week and (4) willingness and ability to interact in the informed consent process. Exclusion criteria were (1) unwillingness or inability to complete any of the major aspects of the study protocol, including magnetic resonance imaging (e.g., due to claustrophobia), drug cue rating, or behavioral assessment, (2) abstinence from methamphetamine for more than 6 months based on self-report, (3) schizophrenia or bipolar disorder based on the MINI interview, (4) active suicidal ideation with intent or plan determined by self-report or assessment by PI or study staff during the initial screening or any other phase of the study, (5) positive drug test for amphetamines, opioids, cannabis, alcohol, phencyclidine, or cocaine confirmed by breath analyzer and urine tests.

2.1.2. First replication sample

The first replication sample (n = 29) was recruited from men with methamphetamine use disorder from the Women in Recovery (WIR) program at Family and Children’s Services located in Tulsa, Oklahoma. WIR is an intensive outpatient “alternative to punishment” program for eligible women facing long prison sentences for non-violent drug-related offenses. The inclusion/exclusion criteria for recruitment were the same as the discovery sample, however the recruited sample is 1 year less educated (11.10 (SD:1.92) years compared to 12.12 (SD:1.81) years of education in the discovery sample). They had also been abstinent for a longer period time (112.73 (SD:49.21) days compared to 61.28 (SD:36.29) days of abstinence in the discovery sample) (Table 1). In the MINI assessments, the first replication sample reported opioid use disorder and alcohol use disorder in a significantly lower level compared to the discovery sample (Table 1).

2.1.3. Second replication sample

The second replication sample (n = 22) was recruited from men with opioid use disorder who were admitted to the same clinical setting as the discovery sample (128&12 residential abstinence-based center in Tulsa, Oklahoma) with the same inclusion/exclusion criteria except for being diagnosed with opioid use disorder (last 12 months) and had no significant difference in age, education and duration of abstinence when compared to the discovery sample (Table 1). The second replication sample was recruited after the termination of recruitment for the discovery sample without any time overlap in the recruitment process.

2.2. Procedure

All three recruited samples in this study were tested with the fMRI drug cue reactivity task introduced below at Laureate Institute for Brain Research (LIBR), Tulsa, OK. Data collection in these three groups was conducted as the baseline and pre-intervention assessments in three randomized clinical trials at LIBR between January 2018 and December 2019 (discovery sample from ClinicalTrials.gov Identifier: NCT03382379, replication sample 1 from NCT03922646 and replication sample 2 from NCT03907644). All participants signed written IRB approved consent forms and were assessed comprehensively including psychiatric assessments with the DSM-V Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998).
2.3. fMRI task

All three recruited samples in this study were tested with the same fMRI drug cue reactivity task structure (Ekhtiari et al., 2020). Pictorial cues within the FDCR task were selected from opioid or meth pictures based on the group of participants. In the fMRI task, participants were presented with blocks of cues that are either drug-related or neutral. Each block contains six images presented for five seconds each, with 200 ms of blank screen between cues so that an entire block lasts 31 s. After every block, participants were asked to rate their current urge to use drug on a one to four scale, with one being “No Urge” and four being “Strong Urge” using a 4 keys response box. The time between blocks varied between 8 and 12 s, and blocks alternated between drug and neutral, starting with neutral. A total of 8 blocks were presented, four of each condition. Total scan time for this task was approximately 6.5 min (Fig. 2).

Participants are trained how to use the response box during the task both outside scanner and inside scanner before the task. Participants are instructed to “remember, some images will be related to drug use and may induce “drug craving” inside you. This is a normal response in some people and we ask you focus on the image and try not to suppress or avoid your feelings. Let your brain respond to them normally.” (Fig. S1).

There is no significant difference between blocks within each subset in terms of craving, valence, arousal and physical features (value, hue and contrast) that was assessed in a validation study before (Ekhtiari et al., 2020). Among the equivalent image sets, there is no significant difference between image sub-sets for craving, valence, arousal and physical features (value, hue and contrast). There is also no significant difference in physical features (value, hue and contrast) between drug and neutral subsets within each set. The stimuli and codes for the fMRI tasks are available in this link (https://github.com/rukplicki/LIBR_MOCD) and in the validation publication of the dataset (Ekhtiari et al., 2020).

2.4. fMRI data

MR images were acquired using two GE MRI 750 3T scanners at LIBR. The FDCR task took 6 min 32 s of scan time with the following parameters: TR/TE = 2000/27 ms, FOV/slice=240×2.9 mm, 128×128 matrix producing 1.875×1.875×2.9 mm voxels, 39 axial slices, and 196 repetitions. High-resolution structural images were acquired through an axial T1-weighted magnetization-prepared rapid acquisition with gradient-echo (MPRAGE) sequence, using the following parameters: TR/TE=5/2.012 ms, FOV/slice=240×192/0.9 mm, 256×256 matrix producing 0.938×0.928×0.9 mm voxels, and 186 axial slices.

2.5. Data analysis

2.5.1. fMRI data preprocessing

First level processing was done in AFNI and included removal of the first three pre-steady state images, despiking, slice timing correction, realignment, transformation to MRI space, and 4 mm of Gaussian FWHM smoothing. Then, regression was carried out including nuisance regressors for the first three polynomial terms and the six motion parameters. TRs with excessive motion (defined as the Euclidean norm of derivative of the six motion parameters being greater than 0.3) were censored during regression.

2.5.2. Block-wise temporal dynamic fMRI analysis

In order to evaluate the temporal dynamics of the BOLD response, we included separate regressors for each block of images, so that each participant had eight beta coefficients of interest computed (four neutral and four drug). At the group level, data were fit using a linear mixed
effect in R. The model was “beta ~ condition * time + motion” with a random effect for subject. Condition was either drug or neutral, with neutral in the intercept, and time was treated as a continuous variable with integer values of 1 through 4 (for the first four through blocks of each image type) which were mean centered for analysis. The mean Euclidean norm across the entire imaging run was used as a summary of head motion for each participant and was included because of the potential for residual effects of head motion after standard correction procedures. We also repeated all analyses without including the motion term and results (not shown) were nearly identical. The voxel-wise whole-brain analysis was used to identify clusters with a significant time by condition interaction ($p < 0.001$). Clusters in a priori regions of interest (ROIs; amygdala and ventral striatum (VStr), defined as Brainnetome ROIs 211 + 213 for left and 212+214 for right amygdala and ROIs 219, 220, 223, 224 for the VStr including the nucleus accumbens and ventral caudate) (Fan et al., 2016) were identified using a threshold of $p < 0.05$.

Individual-level dynamic responses (habituation slopes) were estimated for each ROI exhibiting a condition by time interaction as well as self-reported craving. Slopes were computed by fitting separate linear models for each subject with beta ~ condition * time + motion for BOLD responses and craving ~ condition * time for self-reported craving. From each linear model, we took the beta for time in each condition (drug or neutral) to be the subject’s temporal response to that condition. The condition by time interaction was taken to be the subject’s selective habituation to drug related images, with negative values indicate a decreasing response to drug related images when compared to the change in response to neutral images.

2.5.3. Replication testing
The temporal response to four drug and neutral blocks within the selected masks (significant clusters) discovered from the time by condition (drug/neutral) interaction in the discovery sample was explored in two replication samples. We fit four independent LME (linear mixed effect) models in three sample populations (one assessed twice) for the average beta estimates within each mask. These models were identical to the model applied at the voxel level. For model comparison, the estimates for the effect of condition and the condition by time interaction between each replication sample and the discovery sample were compared using Z-tests.

As an alternative approach, we also extracted the preprocessed time-series (i.e. including all steps except for task modeling) from each selected ROI. Then we fit a single LME model with an ARMA(1,1) covariance structure per dataset which was specified as: BOLD ~ drug * time + neutral * time + motion with a random effect for subject. The drug and neutral regressors were generated using AFNI to convolve a 31 s BLOCK function with the appropriate stimulus timing. In this case, we estimated both overall and linearly time-varying responses to each image category. The main effect of drug then became a post-hoc test of comparing the difference between drug and neutral coefficients to zero. Similarly, the drug by time interaction reported in the primary analysis became a post-hoc test comparing the difference between the drug by time and neutral by time interactions to zero. This approach has the advantage of including much more data per regressor (i.e. instead of 31 s of BOLD data per beta, now we include 124 s per subject, multiplied by the number of subjects). Results become a bit more difficult to interpret, however, as it is no longer possible to examine the mean response by block. We refer to this alternative approach as the combined model.

3. Results

3.1. Discovery sample results

3.1.1. Main effect of condition
Using a whole brain analysis in the discovery sample, the main effect of cue type (condition: drug-neutral) is significant in areas reported in previous cue reactivity studies including the prefrontal cortex, amygdala, striatum, insula and secondary visual processing areas. The main effect of condition is depicted in Fig. 3 panel a.

3.1.2. Main effect of time
Several clusters in areas like the dorsolateral prefrontal (DLPFC), anterior insula cortex (AIC) and dorsal anterior cingulate cortex (dACC) show a negative main effect of time but no condition by time interaction, indicating attenuated responses to both categories of cues throughout the task. The main effect of time is depicted in Fig. 3 panel b.

3.1.3. Condition ‘time interaction
Clusters within the ventromedial prefrontal cortex (VMPFC), bilateral superior temporal gyrus (STG) (Fig. 4), right amygdala and bilateral ventral striatum (Fig. 5) show a significant condition by time interaction with an attenuating response for drug related cues (habituation). No area shows a significant condition by time interaction with an escalating response to drug related cues (sensitization). Masks from these six clusters were generated to test the response within the replication samples.

3.1.4. Individual habituation slopes
The correlation matrix between the clinical characteristics, i.e., duration of methamphetamine use, monthly cost of methamphetamine use, and duration of abstinence and craving self-reports before and after scanning (visual analogue scale 0-100) with individual habituation slopes within the six cluster masks and also subjective reports of craving inside the scanner after each block shows no significant correlation
a. Main effect of condition (drug>neutral)

b. Main effect of time

Fig. 3. Whole brain response to the fMRI drug cue reactivity task in the discovery sample (n = 65). Panel a. main effect of condition (craving>neutral) and b. main effect of time.

Fig. 4. Temporal behavior of clusters with time by condition interaction in whole brain analysis in the discovery sample. VMPFC: ventromedial prefrontal cortex, RSTG: right superior temporal gyrus, LSTG: left superior temporal gyrus (p < 0.001).
between the habituation slopes and clinical characteristics or craving self-reports outside the scanner that can survive \( p < 0.05 \) threshold corrected for false discovery rate (FDR) (Fig. S2). There is also no correlation between activations in the six clusters in the first block, first two blocks and all blocks and the clinical characteristics surviving corrected threshold (Fig. S2).

3.2. Region of interest results

Independent LME models were tested in the discovery sample population and two replication samples (one assessed twice) for the average beta estimates within the six cluster masks generated from the discovery sample.

3.2.1. VMPCF

In the discovery sample, the LME model within the VMPFC mask shows highly a significant estimate for the condition by time interaction (\( t = -4.97, p\text{-value} < 0.001 \)). There is also a significant effect of condition (\( t = 4.55, p\text{-value} < 0.001 \)) and marginally significant effect of time (\( t = 1.98, p\text{-value} = 0.04 \)). The condition by time interaction is significant in both replication sample 1 (\( t = -2.54, p\text{-value} = 0.01 \)) and the first assessment of replication sample 2 (\( t = -3.24, p\text{-value} = 0.01 \)), but not in the retest in replication sample 2 (\( t = -0.35, p\text{-value} = 0.72 \)) (Table 2 and Fig. 6). The effect of motion is not significant in any of the 4 models. In the model comparison by contrasting the estimates for the condition by time interaction between each replication sample and the discovery sample using Z-tests, there is no significant difference (\( p\text{-values} > 0.16 \)) between the discovery sample and replication samples 1 and 2 in both masks; however the difference between the discovery sample and replication sample 2-retest is significant (\( Z = -2.44, p\text{-value} = 0.01 \) and left: \( Z = -2.22, p\text{-value} = 0.02 \)).

3.2.2. STG

There is no significant effect of condition in the STG in the discovery or replication samples 1 and 2. However, in the replication sample 2 re-test, there is a significant negative (neutral-drug) effect of condition in both masks (right: \( t = -2.35, p\text{-value} = 0.028 \) and left: \( t = -2.18, p\text{-value} = 0.03 \)) (Fig. S3). The LME model within both right and left STG masks shows a highly significant estimate for the condition by time interaction (right: \( t = -5.53, p\text{-value} < 0.001 \) and left: \( t = -5.33, p\text{-value} < 0.001 \)). The condition by time interaction is significant in both masks in the replication sample 2 (right: \( t = -2.71, p\text{-value} = 0.007 \) and left: \( t = -3.27, p\text{-value} = 0.001 \)) but not in the re-test in the same sample. In replication sample 1 the condition by time interaction is marginally significant in both masks (right: \( t = -1.90, p\text{-value} = 0.059 \) and left: \( t = -1.88, p\text{-value} = 0.06 \)) (Table 2 and Fig. S3). There is no effect of motion in the STG masks. In the model comparison by contrasting the estimates for the condition by time interaction between each replication sample and the discovery sample using Z-tests, there is no significant difference (\( p\text{-values} > 0.16 \)) between the discovery sample and replication samples 1 and 2 in both masks; however the difference between the discovery sample and replication sample 2-retest is significant (\( Z = -2.44, p\text{-value} = 0.01 \) and left: \( Z = -2.22, p\text{-value} = 0.02 \)).

3.2.3. Amygdala

The discovery sample LME model within the amygdala mask shows a highly significant estimate for the condition by time interaction (\( t = -3.43, p\text{-value} = 0.001 \)). There is also a significant effect of condition (\( t = 8.45, p\text{-value} < 0.001 \)). In three other replication samples, the effect of condition by time interaction is significant in replication
Table 2
Replication of Temporal Dynamic Response to Drug-related Cues Compared to Neutral Cues. Columns represent beta coefficients, standard errors (SEs) and p-values from four independent LME (linear mixed effect) models in three sample populations (one assessed twice) for the average beta estimates within the six masks obtained from the initial discovery phase. VMPFC: ventromedial prefrontal cortex, RSTG: right superior temporal gyrus, L VStr: left ventral striatum.

| Discovery Sample | VMPFC | RSTG | LSTG | R Amygdala | R VStr | L VStr |
|------------------|-------|------|------|------------|--------|-------|
|                  | Beta  | SE   | P value | Beta  | SE   | P value | Beta  | SE   | P value | Beta  | SE   | P value |
| Intercept        | 0.057 | 0.071| 0.425 | 0.006 | 0.051| 0.906 | -0.008 | 0.05 | 0.868 | 0.223 | 0.069| 0.002 |
| Motion           | -0.992| 0.582| 0.093 | -0.53  | 0.42 | 0.211 | -0.801 | 0.407| 0.054 | -0.568 | 0.576| 0.327 |
| Condition (drug>neutral) & 0.192 | 0.042 | <0.001 | -0.037 | 0.031 | 0.228 | -0.044 | 0.032 | 0.169 | 0.292 | 0.035 | <0.001 |
| Time             | 0.051 | 0.026 | 0.048 | 0.095 | 0.02 | <0.001 | 0.1 | 0.02 | <0.001 | -0.005 | 0.022 | 0.818 |
| Condition:Time   | -0.182 | 0.037 | <0.001 | -0.153 | 0.028 | <0.001 | -0.152 | 0.029 | <0.001 | -0.105 | 0.031 | 0.001 |
| Replication Sample 1 | Intercept | -0.049 | 0.088 | 0.579 | -0.139 | 0.095 | 0.156 | -0.17 | 0.104 | 0.113 | 0.166 | 0.089 | 0.07 |
| Motion           | -0.025 | 0.751 | 0.974 | 1.175 | 0.832 | 0.169 | 1.862 | 0.899 | 0.048 | 0.022 | 0.765 | 0.978 |
| Condition (drug>neutral) & 0.187 | 0.06 | 0.004 | -0.172 | 0.052 | 0.003 | -0.182 | 0.064 | 0.005 | 0.246 | 0.055 | <0.001 |
| Time             | 0.084 | 0.038 | 0.027 | 0.055 | 0.03 | 0.069 | 0.079 | 0.04 | 0.052 | 0.048 | 0.029 | 0.099 |
| Condition:Time   | -0.136 | 0.053 | 0.012 | -0.081 | 0.043 | 0.099 | -0.108 | 0.057 | 0.06 | -0.073 | 0.041 | 0.074 |
| Replication Sample 2 | Intercept | 0.069 | 0.108 | 0.525 | 0.069 | 0.078 | 0.389 | 0.007 | 0.096 | 0.941 | 0.278 | 0.092 | 0.006 |
| Motion           | -1.405 | 0.828 | 0.105 | -0.696 | 0.612 | 0.268 | -0.286 | 0.747 | 0.706 | -1.17 | 0.726 | 0.123 |
| Condition (drug>neutral) & 0.145 | 0.076 | 0.058 | -0.06 | 0.048 | 0.218 | -0.113 | 0.06 | 0.063 | 0.145 | 0.049 | 0.004 |
| Time             | 0.066 | 0.048 | 0.174 | 0.091 | 0.031 | 0.003 | 0.131 | 0.038 | 0.001 | 0.018 | 0.031 | 0.564 |
| Condition:Time   | -0.22 | 0.068 | 0.001 | -0.117 | 0.043 | 0.007 | -0.176 | 0.054 | 0.001 | -0.126 | 0.044 | 0.004 |
| Replication Sample 2 Retest | Intercept | 0.078 | 0.127 | 0.542 | -0.031 | 0.135 | 0.823 | -0.036 | 0.148 | 0.81 | 0.249 | 0.117 | 0.043 |
| Motion           | -0.274 | 1.225 | 0.825 | 0.689 | 1.322 | 0.608 | 0.315 | 1.47 | 0.832 | -0.102 | 1.121 | 0.928 |
| Condition (drug>neutral) & 0.019 | 0.072 | 0.789 | -0.152 | 0.064 | 0.028 | -0.124 | 0.057 | 0.03 | 0.076 | 0.071 | 0.295 |
| Time             | 0.006 | 0.046 | 0.895 | -0.008 | 0.034 | 0.825 | 0.015 | 0.036 | 0.682 | -0.045 | 0.033 | 0.176 |
| Condition:Time   | -0.023 | 0.065 | 0.721 | -0.017 | 0.048 | 0.72 | -0.022 | 0.051 | 0.66 | -0.023 | 0.047 | 0.618 |
3.5. Alternative combined model

For all 6 ROIs, 4 datasets and three terms (condition, time, and their interaction), the combined model produced results that are qualitatively similar to the primary analysis (see supplemental Figs. S4 and S5). Coefficients in the combined model tend to have been estimated more precisely (with smaller confidence intervals) and have smaller magnitude (supplemental Fig. S5).

3.6. Subjective self-report of craving

There is a significantly higher level of momentary craving (urge) self-report after seeing drug related blocks compared to neutral blocks in all four groups (p-value < 0.001 in all groups) (Fig. 8). The difference in response to the drug related blocks compared to the neutral blocks were higher in replication sample 2 and its retest (opioid cues) compared to two other groups (methamphetamine cues) (p-value < 0.01 in all two groups comparisons). There is a small time by condition interaction in the discovery sample (Estimate = -0.1, p-value = 0.014) that was not replicated in other groups. The individual habituation slope for the time by condition interaction in the discovery sample was not correlated with individual habituation slopes in the 6 cluster masks (Fig. S2). There is no significant difference in response to the drug related blocks compared to the neutral blocks test in test-retest in the replication sample 2 (Z = -0.13, p-value = 0.89) with a reasonable test-retest reliability (ICC = 0.69, 95%CI 0.40–0.86).

4. Discussion

This investigation examining the time course of brain activation in response to drug cue exposure in a group of individuals with methamphetamine and opioid use disorder while they are abstinent during their early recovery (treatment) yielded three main results. First, we found the VMPCF, right amygdala and bilateral ventral striatum respond to drug cues with higher activation compared to neutral cues and then show a significant habituation in this response in the repeated cue presentation. We found that none of the brain areas shows an increased response to drug cues over time (sensitization). Second, we found the habituation response in these areas replicated consistently in independent samples of opioid and methamphetamine users. Third, we found that the areas exhibiting the habituation response in baseline sessions are not responsive to drug cues in a second session of cue exposure few days later.

Habituation in response to repeated presentation of conditioned cues is a well-known phenomenon in behavioral neuroscience in both animal and human models (Quirk and Mueller, 2008; Phelps et al., 2004). The role of the VMPCF, amygdala and ventral striatum, as the central hubs for dynamic processing the affective/reinforcing value of the environmental cues (Phan et al., 2003; Sescousse et al., 2013; Phan et al., 2002), is reported frequently in the habituation response to the salient
cues (Wright et al., 2001; Breiter et al., 1996; Fischer et al., 2003; Sladky et al., 2012; Strauss et al., 2005; Ishai et al., 2004). Novelty detection, rapid processing of the saliency prediction errors and dynamic evaluation of the environment instead of a sustained processing can be foundation of this rapidly habituating response. Consistent with our findings, the right amygdala is shown to respond dynamically to salient cues, while the left amygdala is specialized for sustained evaluations of salient cues (Wright et al., 2001; Fischer et al., 2003).

The role of the bilateral superior temporal gyrus (STG) in drug cue reactivity was reported in previous studies (Kang et al., 2012; McClernon et al., 2008; Yalachkov et al., 2012). In our study, the STG did not show any positive or negative main effect of condition (drug>neutral), however, it consistently showed a significant time by condition interaction with a response to drug-related images that begins around zero and becomes negative, while the response to neutral images begins negative and quickly moves toward 0. The role of the STG in visuotemporal attentional processing of environmental stimuli might be one explanation for this finding (Li et al., 2012). It is reported that lesions in the STG are associated with more prolonged deployment of visuotemporal attention (Shapiro et al., 2002). Drug cues might engage the STG as a rapidly responding area to attentionally salient stimuli, however, this attentional saliency will drop rapidly for drug cues and
overall there is not a higher signal for drug cues compared to the neutral cues in the STG.

Differential habituation in response to the drug cues (and not neutral cues) in our experimental setting while cue presentation is not associated with rewards could be a representation of Pavlovian extinction based on the incentive sensitization theory. Extinction in response to repeated presentation of conditioned cues is a foundation for cue exposure therapy in psychiatric disorders like phobias, PTSD and substance use disorder. Habituation in amygdala response to emotional cues is reported as an outcome of cue exposure therapy in people with phobia (Goossens et al., 2007) and VMPPC activity during early extinction could predict the outcome of the cue exposure therapy in people with phobia (Lange et al., 2020). Furthermore, stimulating the VMPPC with repetitive transcranial magnetic stimulation (rTMS) in a placebo-controlled trial improved treatment outcome of cue exposure therapy. Habituation in response to affective stimuli in the VMPPC, amygdala and ventral striatum is also being targeted in the fMRI neurofeedback studies for reduction of affective response among people with depression (Young et al., 2014), PTSD (Nicholson et al., 2017; Zotev et al., 2018) and healthy participants (Zotev et al., 2011, 2013, Paret et al., 2016; Haller et al., 2013). However, these extinction-based interventions have not been as successful in addiction medicine as cue exposure therapies in areas like phobias or PTSD (Marissen et al., 2007; Martin et al., 2010; Mellentin et al., 2017). One of the explanations for this lack of clinical translation for Pavlovian extinction-based interventions is the involvement of other cognitive and learning processes such as instrumental extinction and hierarchical instrumental expectancies (Hogarth et al., 2014; Thewissen et al., 2006). A study on the brain response to a cue exposure therapy, reported higher reduction in response to alcohol cues only in few clusters across the brain including ventral striatum after nine sessions of cue exposure in people with alcohol use disorder (Vollstädt-Klein et al., 2011). In the same direction, we found in this study that from many areas of activation due to the exposure to the drug cues, only the VMPPC, amygdala and ventral striatum showed significant habituation in response to repeated exposure to drug cues in the temporal window of our fMRI task. It can be expected that conventional cue exposure interventions will cause habituation and learning extinction across few neuro/cognitive processes from many that are involved in drug craving (Ekhtiari et al., 2016; Byrne et al., 2019).

The results of this study should be cautiously interpreted as extinction in the context of incentive sensitization theory as participants in all three sample populations were recruited from active abstinence-based treatment programs. As it could be expected in this setting, repeated exposure to the drug cues were not associated with any consequent drug use or expectation to drug use while participants have been learning skills to overcome drug craving in their treatment programs. However, with change in the clinical context or drug use expectation we can expect different outcomes. We have not found any strong relationship between duration of abstinence and habituation slopes that would survive the corrected threshold, however, the role of different treatment interventions in changing the habituation slope should be explored more in the future studies.

Habituation of response to drug cues during fMRI tasks and then the lack of response in the habituated areas in the second session of imaging 1–2 weeks after the first imaging session would have implications for any study using within subject measurements of drug cue reactivity as a “treatment monitoring biomarker”. Lack of reliability within task or in test-retest will undermine the ability of a measure to detect the desired outcome (Elliott et al., 2020). Investigators should be cautious in selecting areas we have reported in this study with habituation in their response to drug cues (i.e., ventral striatum, right amygdala and VMPPC) as pre-registered regions of interest (ROIs) for outcome measures in clinical trials. However, there might be potentials in interindividual variations in this habituated response as “predictive biomarker” as it has been reported recently (Regier et al., 2021). In an article that is published during the review process of this work, Regier et al., reported that habituation in response to drug related stimuli in amygdala, hippocampus, fusiform gyrus, mid brain and posterior cingulate gyrus predicted treatment outcome in cocaine users in 8 weeks as people with higher habituation showed better response to the treatment (Regier et al., 2021). Further exploration of this interindividual variation within the goal-track vs. sign tracker spectrum of the incentive salience theory of addiction and its implications in treatment development and selection will be worth further investigations (Colaizzi et al., 2020; Pitchers et al., 2017).

We have not found any consistent habituation or sensitization in the subjective craving responses after being exposed to the drug blocks compared to the neutral blocks. We have also not found any significant correlation between craving self-reports and brain activations or habituation slopes in the six cluster masks. Phan et al., 2003 have also reported lack of a time by condition interaction in the subjective reports to the emotionally salient stimuli while they have found habitual responses in the medial aspects of PFC, amygdala and hippocampus (Phan et al., 2003). One possibility could be that self-report is a subjective response of the individual to the drug cue and does not reflect various dynamic changes that are actually occurring in the brain. There is a large body of

Fig. 8. Subjective Report of Craving after Drug-related and Neutral Blocks. Middle panels show the subjective report of momentary craving (urge) after to four drug and neutral blocks in four levels, 1: no urge, 2: slight urge, 3: moderate urge and 4: strong urge; error bars represent ± one standard error. The right panels show coefficients from four independent LME (linear mixed effect) models in three sample populations (one assessed twice) for the subjective reports. Whiskers depict 95% confidence intervals.
evidence that the brain might be processing drug cues to generate motivated behavior in a way that is not accessible to verbal report in the substance using individual (Parvaz et al., 2016). This gap between subjective self-report of craving and brain response to the drug cues, due to the multi-dimensional nature and implicit aspects of craving, opens the door for specific applications for the brain-based biomarkers of craving to provide additional diagnostic, predictive and treatment monitoring values to both research and clinical practice (Paulus et al., 2005).

One of the strengths of this study is the adoption of a discovery-replication model in testing hypotheses with brain imaging data. Serious concerns are mounting regarding the replicability of fMRI results (Poldrack et al., 2017). Using conventional exploratory methods in the whole brain or many ROIs can be associated with an increased risk of false positives that were not well controlled in many fMRI studies before (Eklund et al., 2016). In this study, we have used a discovery sample to find the areas that have significant effects in our analysis model, then used masks based on that analysis as a priori ROIs to test for replication in two independent samples. This kind of discovery-replication analysis pipeline will be helpful in reducing the risk for false positive results in the field. Replication of the same findings in other clinical populations and other labs across the world will be the next step for confirmation of our findings. To facilitate this confirmatory phase, we have shared our fMRI drug cue reactivity task (Ekhtiari et al., 2020) and our activation masks to help other labs to test the replication of the results in other research environments/groups (https://github.com/rkuplicki/LIBR_FDCR_Dynamic).

There are several limitations in this study. First, neither our study participants in the three sample populations nor our drug cues in the fMRI task have a clear label for being purely opioid or meth related. As it is obvious in Table 1 and also with anyone who has a clinical experience in addiction medicine, opioid and meth addiction have significant overlaps in both drug use rituals and also people who are affected in many parts of the world including the US. Therefore, attributing the results of this study for being meth or opioid specific seems complex. However, as shown in table one, there are significant variations in terms of opioid and meth use profile among sample populations. Replication of response in this diverse set of clinical samples provides support for having a general response in both meth and opioid users instead of a drug specific behavior. Another limitation of this study is the limited temporal window of the experimental paradigm for 6.5 min and 4 blocks of cue exposure. This temporal window limits our sampling capacity to cover just rapid habituation response. Increase in the temporal window might bring potentials to detect areas with a more subtle habituation response. Another limitation to be considered is that participants were receiving treatment in between test and retest time points. This treatment could have contributed to the habituation observed to the repeated cue exposures (test-retest). In addition, it is important to keep in mind that we used the term “repeated exposure” to refer to the repeated exposure to different drug cues; participants were not repeatedly exposed to the same drug cues. This type of repeated exposure to similar but distinct cues may more closely resemble the drug cues that occur in real life.

This investigation is a one of the first studies on the temporal dynamics of responsiveness to drug cues. There are still many questions to be answered in future studies. One of the main remaining question is whether areas with or without habituation in response to drug cues represent different cognitive constructs composing the craving phenomenon and how to modulate these cognitive processes with different cognitive or neuromodulatory interventions to stop the habituation process in the habituating areas or promote habituation in non-habituating areas. In the same context, we know that the VMPFC, amygdala and VS are highly connected areas (Paret et al., 2016), however the distinct role of these areas in the habituation response is still unclear. The clinical implication of these findings is another remaining question. Potentials for changing this dynamic response and their clinical significance and clinical value of this habituation as a potential biomarker or intervention development tool should be explored in the future.

In this preliminary study, we have explored the temporally dynamic response to the repeated presentation of drug cues among people with methamphetamine and/or opioid use disorders. We found that the VMPFC, right amygdala and ventral striatum show a rapid habituation in response to drug cues, but not in the neutral cues. This habituation response was replicated in two other clinical populations. These areas will remain habituated to drug cues in a second session of cue exposure a few days later. This habituation in response could be a foundation to explore potentials for cue exposure interventions to modulate cue induced carving and reduce risks for potential relapse.

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Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request, however, the study analysis codes and cluster masks are available in https://github.com/rkuplicki/LIBR_FDCR_Dynamic.

Declaration of Competing Interest
Authors reported no conflict of interest.

Credit authorship contribution statement
Hamed Ekhtiari: Conceptualization, Methodology, Funding acquisition, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. Rayus Kuplicki: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. Robin L. Aupperle: Conceptualization, Methodology, Funding acquisition, Data curation, Writing – review & editing. Martin P. Paulus: Conceptualization, Methodology, Funding acquisition, Writing – original draft, Writing – review & editing, Supervision.

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References
Ekhtiari, H., Nasserli, P., Yavari, F., Mokri, A., Monterono, J., 2016. Neuroscience of drug craving for addiction medicine: From circuits to therapies. Prog. Brain Res. 223, 115–141.
Wise, R.A., 1988. The neurobiology of craving: implications for the understanding and treatment of addiction. J. Abnorm. Psychol. 97 (2), 118.
American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Pub.
Robinson, T.E., Berridge, K.C., 1993. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res. Rev. 18 (3), 247–291.
Ekhtiari, H., Alam-Mehrdjerdi, Z., Nouri, M., George, S., Mokri, A., 2010. Designing and evaluation of reliability and validity of visual cue-induced craving assessment task for methamphetamine smokers. Basic Clin. Neurosci. 1 (4), 34–37.
Drummond, D.C., 2000. What does cue-reactivity have to offer clinical research? Addiction 95 (8/2), 129-144.

Carter, B.L., Tiffany, S.T., 1999. Meta-analysis of cue-reactivity in addiction research. Addict. Behav. 24 (12), 1279-1287.

Ekhtiari, H., Faghih, A., Ogibahan, M.A., Paulus, M.P., 2016. Functional neuroimaging for addiction medicine: from mechanisms to practical considerations. Prog. Brain Res. 224, 129-153 Vol.

Sjoerd, Z., van den Brink, W., Beekman, A.T., Penninx, B.W., Veltman, D.J., 2014. Cue reactivity is associated with duration and severity of alcohol dependence: an fMRI study. PLoS One 9 (1), e84560. doi:10.1371/journal.pone.0084560.

Smolka, M.N., Bühler, M., Klein, S., et al., 2006. Severity of nicotine dependence modulates cue-induced brain reactivity in regions involved in motor preparation and imagery. Psychopharmacology 184 (3-4), 577-588 (Berl.).

Kosten, T.R., Scanley, B.E., Tucker, K.A., et al., 2006. Cue-induced brain activity changes and relapse in cocaine-dependent patients. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 31 (3), 644-650.

Janes, A.C., Fizzarella, D.A., Richard, S., et al., 2010. Brain reactivity to smoking cues prior to smoking cessation predicts ability to maintain tobacco abstinence. Biol. Psychiatry 67 (8), 722-729.

Courtney, K.E., Schacht, J.P., Hutchison, K., Roche, D.J., Ray, L.A., 2016. Neural substrates of cue reactivity: association with treatment outcomes and relapse. Addict. Biol. 21 (1), 3-22.

Mann, K., Vollstädt-Klein, S., Reinhardt, I., et al., 2014. Predicting naloxone response in alcohol-dependent patients: the contribution of functional magnetic resonance imaging. Alcohol. Clin. Exp. Res. 38 (11), 2754-2762.

Lukas, S.E., Lown, S.B., Lindsay, K.P., et al., 2013. Extended-release naltrexone (XR-NTX) attenuates brain responses to alcohol cues in alcohol-dependent volunteers: a bold fMRI study. Neuropsychopharmacol. 38 (7), 176-185.

Saddzeee, A., Paulus, M., Ekhtiari, H., 2019. fMRI as an outcome measure in clinical trials: a systematic review in clinicaltrials.gov. medRxiv, 201902972.

Hartwell, K.J., Johnson, K.A., Li, X., et al., 2011. Neural correlates of craving and resisting craving for tobacco dependent smokers. Addict. Biol. 16 (4), 654-666.

Van Hedger, K., Keedy, S.K., Mayo, L.M., Heilig, M., de Witt, H., 2011. Responses to cues paired with methamphetamine in healthy volunteers. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 43 (4), 1752-1737.

Schacht, J.P., Anton, R.F., Myrick, H., 2013. Functional neuroimaging studies of alcohol cue reactivity: a quantitative meta-analysis and systematic review. Addict. Biol. 18 (1), 121-133.

Wright, C.L., Fischer, H., Whalen, P.J., McIverney, S.C., Shin, L.M., Rauch, S.L., 2001. Different prefrontal cortex and amygdala habitation to repeatedly presented emotional stimuli. Neuroreport 12 (2), 379-383.

Phan, K.L., Liberson, I., Welsh, R.C., Britton, J.C., Taylor, S.F., 2003. Habituation of rostral anterior cingulate cortex to repeatedly emotionally salient pictures. Neuropsychopharmacol 28 (7), 1344-1350.

Breiter, H.C., Enot, N.L., Whalen, P.J., et al., 1996. Response and habituation of the human amygdala during visual processing of facial expression. Neuron 17 (5), 875-887.

Fischer, H., Wright, C.L., Whalen, P.J., McIverney, S.C., Shin, L.M., Rauch, SL, 2003. Brain habituation during repeated exposure to fearful and neutral faces: a functional MRI study. Brain Res. 59 (5), 387-392.

Sladky, R., Höflich, A., Atanov, J., et al., 2012. Increased neural habituation in the amygdala and prefrontal cortex in social anxiety disorder revealed by fMRI. PLoS One 7 (11), e45005. doi:10.1371/journal.pone.0045005.

Plichta, M.M., Grimm, O., Morgen, K., et al., 2014. Amygdala habituation: a reliable fMRI phenotype. Neuroimage 103, 383-390.

Mones, C., Kolb, E.L., Frern, J., Becker, S., et al., 2011. Rapid habituation of ventral striatal response to reward in perceptual biasation. Biol Psychiatry 70 (4), 395-399.

Murphy, A., Lubman, D.I., McKeon, S., et al., 2018. Time-dependent neural changes associated with craving in opioid dependent: an fMRI study. Addict. Biol. 23 (5), 1168-1178.

Ekhtiari, H., Kuplicki, R., Pruthi, A., Paulus, M., 2020. Methamphetamine and opioid cue database (MCDM): development and validation. Drug Alcohol Depend., 107941.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., et al., 1998. The Mini-international neurological psychiatric interview (MINI): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (20), 22-33.

Fan, L., Li, H., Zhuo, J., et al., 2016. The human brainstem atlas: a new brain atlas based on connectomic architecture. Cereb. Cortex 26 (8), 3598-3526.

Quirk, G.J., Mueller, D., 2008. Neural mechanisms of extinction learning and retrieval. Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol. 33 (1), 56-72.

Phipps, E.A., Delgado, M.R., Nearig, K.L., LeDoux, J.E., 2004. Extinction learning in humans: role of the amygdala and vmPFC. Neuron 43 (6), 897-905.

Sescousse, G., Caldö, X., Segura, B., Dreher, J.C., 2013. Processing of primary and secondary rewards: a quantitative meta-analysis and review of human functional neuroimaging studies. Neurosci. Biobehav. Rev. 37 (4), 681-696.

Phan, K.L., Wagner, T., Taylor, S.F., Liberson, I., 2002. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage 16 (2), 331-348.

Strawn, M., Makris, N., Abrar, I., et al., 2005. fMRI of sensitization to angry faces. Neuroimage 26 (2), 389-413.

Ishai, A., Pesonen, A., Bilek, P.C., Ungerleider, L.G., 2004. Suppression of suppression is mediated by modulation. Proc. Natl. Acad. Sci. 101 (26), 9827-9832.

Kolb, E.B., Chang, D.S., John, G.H., et al., 2012. Individual differences in smoking-related cue reactivity in smokers: an eye-tracking and fMRI study. Prog. Neuropsychopharmacol. Biol. Psychiatry 38 (2), 285-293.