



ARTICLE

Neural responses to cues paired with methamphetamine in healthy volunteers

Kathryne Van Hedger¹, Sarah K. Keedy¹, Leah M. Mayo², Markus Heilig² and Harriet de Wit¹

Drug cues, or conditioned responses to stimuli paired with drugs, are widely believed to promote drug use. The acquisition of these conditioned responses has been well characterized in laboratory animals: neutral stimuli paired with drugs elicit conditioned responses resembling the motivational and incentive properties of the drug itself. However, few studies have examined acquisition of conditioning, or the nature of the conditioned response, in humans. In this study, we used fMRI to examine neural responses to stimuli that had been paired with methamphetamine or placebo in healthy young adults. Participants first underwent four conditioning sessions in which visual-auditory stimuli were paired with either methamphetamine (20 mg, oral) or placebo. Then on a drug-free test day, the stimuli were presented during an fMRI scan to assess neural responses to the stimuli. We hypothesized that the stimuli would elicit drug-like brain activity, especially in regions related to reward. Instead, we found that the methamphetamine-paired stimuli, compared to placebo-paired stimuli, produced greater activation in regions related to visual and auditory processing, consistent with the drug's unconditioned effects on sensory processing. This is the first study to demonstrate conditioned neural responses to drug-paired stimuli after just two pairings of methamphetamine in healthy adults. The study also illustrates that conditioned responses may develop to unexpected components of the drug's effects.

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INTRODUCTION

Conditioned responses to stimuli paired with drugs, or drug cues, are known to promote drug use in animal models and human drug users [1–4]. In animal models, drug cues increase operant responding previously reinforced with cocaine, nicotine, amphetamine, or heroin [2, 5–7], and in humans, drug cues increase craving for cigarettes, alcohol, and other drugs [8, 9]. Indeed, drug cues retain their ability to elicit drug-seeking behavior long after cessation of drug use [10–12]. Decades of research indicates that, in laboratory animals, drug cues acquire motivational properties through Pavlovian conditioning [4]. In contrast, we know less about the acquisition of conditioned drug effects in humans. Studies investigating drug cues in humans typically utilize generic drug-related stim prior contiguity with the drug stimulus is mostly inferred, without explicit knowledge of how the responses were acquired [13, 14]. Recently, we developed a procedure to study the acquisition of conditioned drug responses in healthy young adults, showing that an initially neutral stimulus (e.g., an audiovisual cue) paired with a drug (e.g., amphetamine or alcohol) acquires motivational and incentive properties of the drug itself [15–17].

With the ability to control the process of acquisition of conditioned drug responses in humans, we can now investigate the nature of the conditioned responses and the neurobiological mechanisms underlying responses to cues. Conditioned responses to cues have been reported using different measures, such as subjective reports of 'liking' or 'preferring' the conditioned stimulus [18], behavioral indices of emotion (e.g., facial electromyography [15, 16]), attention to the stimulus (e.g., eye

movements [15, 16, 19]), striatal dopamine release using PET [20] and neural activity using functional magnetic resonance imaging (fMRI) measures [21]. The sensitivity of these indices varies across studies [22], and the outcome measures do not always co-vary [16]. Why certain components of a drug's effects become conditioned while others do not is unknown. The nature of the conditioned response may reflect procedural variations across studies, differences in sensitivity of measurement, or differences in brain or behavioral processes underlying conditioning.

Brain imaging studies in humans provide a novel way to study drug conditioning and the nature of conditioned responses. In this study we used fMRI to examine neural responses to a stimulus that had been paired de novo with a stimulant drug. Because conditioned responses typically resemble the unconditioned drug response [4, 23, 24], we would expect the conditioned neural response to a stimulant-paired cue to mirror the direct drug effect. Stimulant drugs increase activity in the mesolimbic dopamine system, commonly referred to as a reward circuit (e.g., refs. [25, 26]). Interestingly, stimulant drugs also affect brain activity in areas related to sensory processing, raising the possibility that sensory effects could also become conditioned [27, 28]. In this study we examined brain activation while viewing compound stimuli (visual images of a mountain or ocean scene accompanied by appropriate sounds of birds or waves) that had been previously paired two times each with oral methamphetamine or placebo. The scans occurred during a separate session several days after the last conditioning session, while subjects were drug-free. We examined BOLD responses to the stimuli using fMRI. We hypothesized that subjects would exhibit larger BOLD responses to the drug-paired

¹Department of Psychiatry and Behavioral Neuroscience, University of Chicago, Chicago, IL, USA and ²Center for Social and Affective Neuroscience, Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden
Correspondence: Harriet de Wit (hdew@uchicago.edu)

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cue, compared to the placebo-paired cue, in brain regions related to reward circuitry (e.g., ventral striatum) and sensory function.

METHODS AND MATERIALS

Participants

Healthy men and women ($N=59$) aged 18–35 years were recruited via flyers and online advertisements. Respondents were excluded if they had a current psychiatric disorder (DSM 5; APA 2013), past year substance dependence, current use of prescription medication (excluding hormonal birth control), abnormal electrocardiogram, night shift work, left-handedness, less than high school education, lack of fluency in English, consuming more than four alcohol-containing or caffeine-containing drinks per day, body mass index outside the range 19–26 kg/m², and contraindications for fMRI scanning (e.g., claustrophobia, copper IUD). Of the 59 participants who enrolled, four did not complete the fMRI scan, two were excluded for falling asleep during the fMRI scan, and two were excluded for excessive movement during the fMRI scan, leaving a final study sample of 51 participants. This study was approved by the University of Chicago Biological Sciences Division Institutional Review Board.

Study Design

This study used a within-subjects design with four conditioning sessions followed by an imaging session. During the conditioning sessions participants received either methamphetamine (20 mg oral) or placebo, each paired with distinctive audiovisual cues consisting of a mountain scene with bird sounds or an ocean scene with wave sounds. Cues and drug order were counter-balanced. Preferences for the two cues were assessed before and after the conditioning, using measures of behavioral preference, subjective liking, and attentional bias. During the final fMRI session (no drug administration), we examined patterns of brain activation to the methamphetamine-paired and placebo-paired cues.

Session Procedures

The procedures used for this study are described in detail in ref. [15]. Our modification included adding an fMRI scan to the post conditioning session, and we did not collect data on emotional reactivity to the cues. Otherwise the procedures were identical.

Drug

Methamphetamine (Desoxyn, Lundbeck) was crushed and mixed with 10 ml of combined Ora-Plus and Ora-Sweet syrups (Paddock Laboratories, Minneapolis, MN [15]). We have previously reported conditioned effects with this dose [15, 22]. Placebo consisted of 10 ml of equal parts Ora-Plus and Ora-Sweet. Syrups were administered in clear 1 oz plastic cups.

Drug Effects Measures

Subjective Measures. Subjective effects were assessed using the Drug Effects Questionnaire (DEQ [29, 30]), and the Profile of Mood States (POMS [31]). Measurements were taken before and 15, 30, 70, 115, and 200 min after drug administration.

Cardiovascular Measures. Heart rate (HR) and blood pressure (BP) were monitored (Omron, Lake Forest, IL) during the conditioning sessions at the same intervals as the subjective measures.

Conditioning Measures

Participants completed the same behavioral preference, subjective liking and attentional bias tasks described by [15] with minor modifications detailed below.

Behavioral Preference Task. For the behavioral preference task participants were given a choice between images containing the methamphetamine- and placebo-paired cues and selected the

one they preferred. During the post-conditioning session participants completed this task twice (i.e., 30 trials) while in the scanner. However for consistency, behavioral data from only the first 15 trials were used to compare to pre-test.

Attentional Bias Task. Attentional bias was measured using a modified dot-probe task with images of the two cues [15, 32]. In addition to the previous outcome measures of orienting and sustained attention we also measured reaction time (RT) to the probe.

fMRI Task. fMRI data were obtained while subjects completed a modified version of forced choice preference task, and neural activation was recorded while subjects experienced the methamphetamine-paired and placebo-paired cues. The task consisted of 30 trials. Each trial was comprised of 11.2 s of each cue plus task image from the conditioning sessions, with the matched soundtrack. Subjects were instructed to passively view and listen. Trials alternated between ocean and mountain backgrounds. Then both images were shown side by side, and participants indicated which image they preferred using a button box in their right hand. The task varied slightly in length because it was self-paced, but it lasted about 18 min. The cues were presented for the same amount of time for all participants.

Imaging was done on a Philips Achieva 3.0 T scanner using a 32-channel headcoil and a gradient-echo echo-planar imaging sequence with acquisition parameters TR=2000; TE=30; 36 3.75 mm thick axial slices aligned to the AC-PC line, no gap; 24 × 24 cm FOV, (3.75mm³ voxels); SENSE factor=2, Flip angle=90°. Four images were acquired and discarded just prior to task start. A high resolution T1-weighted image (MPRAGE sequence) was also acquired to assess for incidental findings, and for alignment and spatial standardization of the functional data. Subject head motion was minimized with foam packing around the head. Stimuli were viewed via projection onto a mirror system mounted on the headcoil. Sounds were delivered via earbud headphones and played at ~80 dB.

Subject-Level Functional Image Processing

Neuroimaging data analysis was conducted with AFNI [33]. Preprocessing steps included alignment of the time series to the last image (as it was closest in time to acquisition of the structural image), spatial registration of the aligned time series data to the anatomical scan, anatomical scan warping to Talairach space and warp applied to functional data, functional data spatially smoothed with a 4 mm fwhm Gaussian kernel, and intensity normalization. With this preprocessed data, neural activation was then estimated for each subject to the methamphetamine- and placebo-paired cues using a linear regression analysis as implemented in AFNI's 3dDeconvolve program. De-means and derivatives of motion parameters were estimated and included as covariates of no interest. Viewing/listening to the methamphetamine-paired and placebo-paired cues were modeled as block functions. No other aspect of the imaging task (e.g., the choice-making) was of interest for analysis. Mean activation for each cue were extracted from bilateral ventral striatum for each subject for primary analysis of impact of drug-associated conditioning of reward systems. The mask for ventral striatum was that of the "7 subdivisions" striatum probabilistic atlas of ref. [34].

Statistical Analysis

Drug Effects. We used repeated-measures analysis of variance (RM-ANOVA) to examine subjective and cardiovascular effects during the two methamphetamine sessions and the two PBO sessions. Time (before and 15, 30, 70, 115, and 200 min after drug administration) and drug (methamphetamine and placebo) were treated as within-subjects factors. To explore relationships

between subjective drug effects (e.g., drug liking) and our conditioning measures, we calculated peak change scores from baseline averaging across the two methamphetamine sessions and across the two placebo sessions to create single scores for methamphetamine and placebo.

Behavioral Effects of Conditioning. Our behavioral index of conditioning was the difference in responses to the audiovisual cues at pre-test and post-test on the forced choice task, subjective liking task, and attentional bias task. For each task we conducted RM-ANOVA with time and drug as within-subjects factors.

Imaging. Analyses were conducted as within subject comparisons, directly contrasting the methamphetamine-paired and placebo-paired cues, using age, sex, and methamphetamine-paired cue (whether ocean or mountain was the methamphetamine-paired cue) as covariates. Parameter estimates for extracted ventral striatum activation were compared between methamphetamine-paired and placebo-paired cues using paired *t*-tests. Secondary whole-brain analyses were conducted using AFNI's 3dmvm, implementing an ANCOVA with sex, age, methamphetamine-paired cue as covariates and comparing methamphetamine- versus placebo-paired cue activation on a voxelwise basis. Significant clusters of voxels were identified if they met a $p > .001$ familywise error correction threshold, where at least 20 contiguous voxels each met $p > .001$. This threshold was determined using 3dClustSim, which ran 10,000 Monte Carlo simulations of randomly generated data of the same resolution and in-brain inclusion mask (34,990 3.5 mm³ voxels) as the preprocessed data, and utilizing the average spatial autocorrelation function of the subjects (obtained via 3dFWHMx). These procedures avoid concerns around high false-positive rates in fMRI [35]. Finally, Pearson correlations were conducted between behavioral data and activation in bilateral ventral striatum and any other regions identified by the whole-brain analysis showing differences for methamphetamine- versus placebo-paired cues.

RESULTS

Participants

Table 1 summarizes the demographic characteristics and drug use patterns of the 51 participants who completed the study.

Drug Effects During Conditioning Sessions

Subjective Measures. Subjective responses to methamphetamine did not differ across the first and second drug sessions, thus responses for the two methamphetamine sessions and two placebo sessions were averaged. As expected, methamphetamine increased ratings of feel drug, like drug, feel high, and want more compared to placebo (Table 2). Methamphetamine also increased feelings of friendliness, anxiety, elation, and vigor, and decreased feelings of fatigue and confusion (POMS). These results are in line with previous studies from this laboratory [15, 17, 36, 37].

Cardiovascular Measures. The cardiovascular effects of methamphetamine versus placebo did not differ across the two administrations, so the two methamphetamine and two placebo sessions were averaged. Methamphetamine significantly increased both blood pressure (reported here as mean arterial pressure) and heart rate compared to placebo (Table 2). Again, these results are similar to those previously reported [15].

Behavioral Effects on Post-Conditioning Test Session Forced Choice Task. Preference for the methamphetamine-paired versus placebo-paired cues did not change from before to after

Table 1. Demographic information and nonmedical drug use (N = 51). Mean values for current drug use are reported from only current users, and number of current users is indicated in parentheses

	Percent (N) or Mean (SEM)
<i>Gender</i>	
Male/Female	25/26
Age (years)	23.5 (0.6)
Education (years)	14.7 (0.3)
BMI	22.9 (0.2)
<i>Race</i>	
Caucasian	51.0% (26)
African-American	21.6% (11)
Asian	7.8% (4)
Other	15.7% (8)
Unknown	3.8% (2)
<i>Current drug use</i>	
Caffeinated drinks per day (n = 33)	1.3 (0.1)
Cigarettes per week (n = 9)	10.3 (2.9)
Alcoholic drinks per week (n = 46)	7.7 (0.6)
<i>Lifetime drug use (ever used)</i>	
Marijuana	82.4% (42)
Opiates	25.5% (13)
Stimulants	45.1% (23)
Hallucinogens	49.0% (25)
MDMA	33.3% (17)
Sedatives	29.4% (15)

Table 2. Subjective and cardiovascular effects of methamphetamine (MA) and placebo (PBO; N = 51)

<i>Subjective Effects</i>	PBO		MA		<i>T-value</i>
	Mean	SEM	Mean	SEM	
<i>DEQ</i>					
Feel	19.86	3.09	50.39	3.30	9.81***
Like	23.77	2.94	68.85	3.33	12.55***
Dislike	32.47	4.23	34.90	3.31	0.61
High	11.21	2.22	42.05	3.79	9.50***
More	20.38	2.90	65.12	3.99	11.31***
<i>POMS</i>					
Friendliness	-2.06	0.50	2.21	0.70	5.63***
Anxiety	-0.27	0.33	1.68	0.66	2.71**
Elation	-1.24	0.30	2.78	0.59	6.43***
Anger	0.33	0.36	0.28	0.51	-0.10
Fatigue	1.04	0.49	-1.74	0.59	-3.97***
Depression	-0.36	0.48	-0.03	0.39	0.50
Confusion	0.36	0.28	-0.52	0.34	-2.16*
Vigor	-2.10	0.58	4.89	0.84	7.31***
<i>Cardiovascular effects</i>					
Blood pressure	-4.04	1.21	10.95	1.38	8.74***
Heart rate	-5.32	1.58	7.43	2.43	6.07***

DEQ drug effects questionnaire, POMS profile of mood states Note: DEQ and POMS values are averaged peak change scores over the two PBO and two MA sessions. Blood pressure is represented as mean arterial pressure * $p < .05$, ** $p < .01$, *** $p < .001$

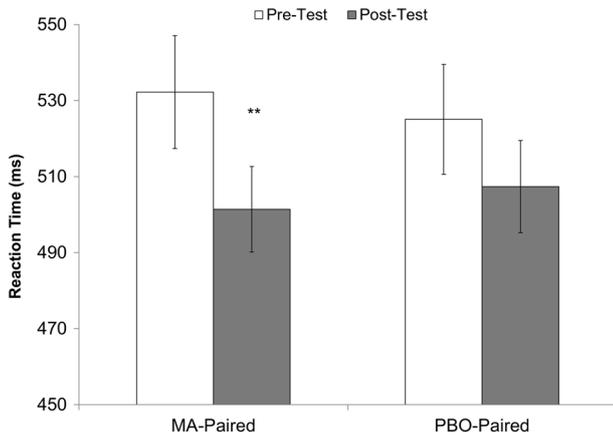


Fig. 1 Reaction times on a modified dot-probe task with the methamphetamine-paired and placebo-paired cues (attentional bias) before and after conditioning. Participants responded significantly faster to the probe when it appeared in the same location as the methamphetamine-paired cue following conditioning. ** $p = .01$

conditioning (mean preference before 4.37 ± 0.33 SEM and after 4.47 ± 0.33 SEM).

Subjective Liking Task. Liking ratings of both methamphetamine-paired and placebo-paired cues declined from before to after conditioning (time, $F(1, 50) = 30.8, p < .001$). Before conditioning mean liking of to-be-methamphetamine-paired cues was 78.97 ± 2.47 SEM, and to-be-placebo-paired cues was 82.89 ± 2.04 and after conditioning liking of methamphetamine cues was 71.67 ± 2.93 SEM, and placebo cues was 70.71 ± 3.03 . There was no main effect for drug or interaction with drug.

Attentional Bias Task: After conditioning, RT's were significantly faster, $F(1,50) = 4.61, p < .05$ for both stimuli. However, participants were faster to respond to the probe when it appeared in the same location as the methamphetamine-paired cue, compared to the PBO-paired cue (time by drug interaction, $F(1,50) = 4.16, p < .01$; mean pre RT 501.40 ± 11.25 SEM and post RT 532.21 ± 14.87 SEM; Fig. 1). The RT's for the to-be-conditioned stimuli did not differ at pre-test and RT's did not change from pre- to post-conditioning for the placebo image. Data for orienting attention and sustained attention measures were lost for 6 participants either because of equipment malfunction (5) or participant constraints (1). All remaining subjects had at least 50% of trials with valid gazes. On the measure of orienting attention there was a marginally significant interaction, $F(1,44) = 3.77, p = .06$. Subjects directed slightly more initial gazes at the methamphetamine-paired cue at post-test (18.27 ± 0.49 SEM) compared to pre-test (16.62 ± 0.57 SEM; Fig. 2). On the measure of sustained attention (dwell time) participants spent marginally more time looking at both cues at post-test compared to pre-test (time, $F(1,44) = 3.77, p = .06$), but methamphetamine-paired and placebo-paired cues did not differ on this measure. There was no main effect for drug and no significant interaction

Neuroimaging results

All subjects met motion threshold correction criteria of <3 mm displacement. The extracted activation during the methamphetamine- versus placebo-paired cues did not differ in the ventral striatum, but for the whole-brain analysis, activation for methamphetamine-paired cues relative to placebo-paired cues increased significantly in bilateral visual and auditory cortex, and insula (Table 3, Fig. 2). No clusters showed significantly higher activation for placebo-paired relative to methamphetamine-paired cues. Pearson correlations were conducted between behavioral

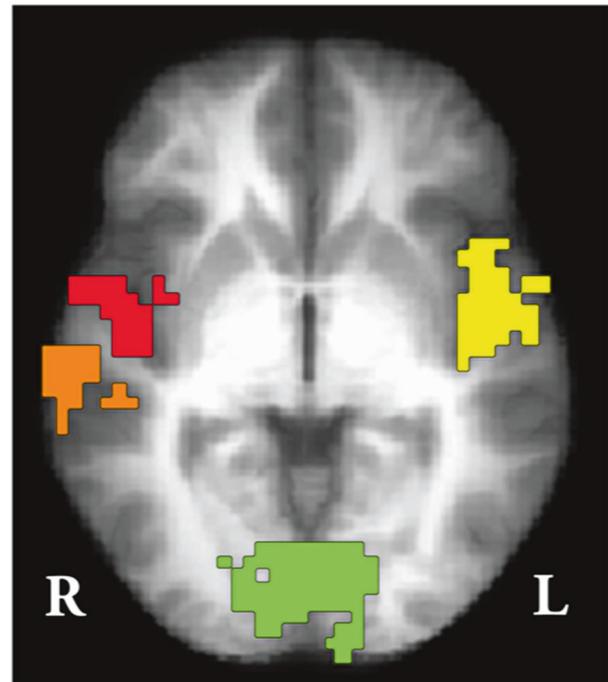


Fig. 2 Images in neurological convention (left is right). Methamphetamine-paired images evoked significantly greater activation than placebo-paired images in clusters in bilateral primary/secondary visual cortex (green), left auditory cortex and insula (yellow), and right auditory cortex (orange, red) and insula (red)

findings and extracted activation for the differences between methamphetamine- versus placebo-paired cues in ventral striatum, primary/secondary visual cortex, auditory cortex, and insula. No significant associations were found (F -values <0.2 and $>-0.2, p$ -values $>.05$ corrected).

DISCUSSION

In the present study, we used fMRI to detect conditioned responses to sensory cues paired with methamphetamine or placebo in healthy young adults [15]. After just two drug pairings, we observed greater brain activation to the methamphetamine-paired, compared to the placebo-paired stimuli, consistent with a conditioning effect. The scanning session was conducted several days after the last conditioning session, while subjects were in a drug-free state. Surprisingly, the conditioned brain activation corresponded to regions involved in visual and auditory processing, rather than reward regions.

To our knowledge, this is the first study investigating conditioning of neural responses to initially neutral drug-paired cues in healthy humans. On the basis of prior knowledge that stimulant drugs activate dopaminergic reward circuits [25], we hypothesized that the methamphetamine-paired cue would elicit activity in reward-related regions. However, the methamphetamine-paired cue did not activate regions related to reward. Instead, it activated regions involved in sensory processing. Interestingly, this is consistent with other reports from imaging studies of amphetamine responses [38] and drug cues [27, 38] used fMRI to show that amphetamine enhances brain responses to sensory stimuli. Consistent with these reports, we observed robust activation in visual and auditory regions (i.e., bilateral primary/secondary visual cortex, auditory cortex, and insula) upon presentation of the methamphetamine-paired stimulus, relative to the placebo-paired stimulus. The behavioral indicators of conditioning were less clear: the stimulus-drug

Table 3. Brain areas in which drug-paired stimuli elicited greater activation than placebo-paired stimuli

	Cluster size (number of 3.5 mm ³ voxels)	Center of Mass x	Center of Mass y	Center of Mass z	Peak T-value
Bilateral cuneus and lingual gyri (BA 17, 18)	533	1.0	-80	2	8.7
Left superior temporal gyrus and Insula	112	-46	-4	-1	6.3
Right superior temporal gyrus	109	61	-31	6	5.9
Right superior temporal gyrus and Insula	90	-50	-3	-3	6.6

pairings did not change behavioral preference for the cues, although there was a small increase in attentional bias toward the methamphetamine-paired stimulus. We conclude from these findings that the conditioned neural responses to the cues were related more to the arousing and attentional effects of methamphetamine than to its rewarding effects.

The Pavlovian conditioning procedure allows drug-like responses to become conditioned to neutral stimuli, but it does not provide the experimenter with control over which drug effects become conditioned [39]. Stimulant drugs, like other drugs, produce a range of effects in the brain and on behavior, any of which might become conditioned. Indeed, using this procedure we have previously reported conditioning with various measures, including liking of the drug-paired cue [22], positive emotional responses to the cue (measured by facial electromyography), behavioral preference for the cue or attentional bias toward the cue [15, 17, 22]. However, the responses that exhibited conditioning has varied across studies. In the present study one hypothesis was that the conditioned stimulus would activate the same dopamine-rich brain areas activated by methamphetamine [40], and that it would produce conditioned, reward-like responses. Instead, we observed conditioned neural activation that may be more closely related to the effects of methamphetamine on arousal, vigilance and reactivity to environmental stimuli [41–43]. Interestingly, in another example of unanticipated conditioned drug responses in humans [44], studied conditioned responses to a taste that had been repeatedly paired with the antidepressant amitriptyline or placebo. The authors detected no conditioned antidepressant-like mood effects (as measured by standardized mood rating scales), but instead found that the amitriptyline-paired taste elicited more antidepressant-like side effects such as dry mouth, dizziness and fatigue. Thus, although the functional aspects of Pavlovian conditioning can be established in a controlled study, the responses that become conditioned are not under experimental control. This has implications for understanding the role of conditioned cues in motivated, or addictive behaviors: It is not always clear which responses will be conditioned, or which come to influence future motivated behavior.

Compared to the placebo-paired cue, the methamphetamine-paired cue enhanced activity in both the visual and auditory cortices. These regions correspond directly to the visual and auditory aspects of the stimuli used, and are typically involved in passive sensory processing. As expected with a stimulant drug, methamphetamine increased ratings of vigor, and increased heart rate and blood pressure during the conditioning sessions. Thus, our finding of increased activity upon presentation of the drug-paired cue reflects the combined conditioning effect of the sensory stimulation of the cue and the stimulating effect of the drug [38].

The drug-paired cue also increased the activation in the insula, suggesting that additional neural coding of salience may have contributed to this effect [45]. The posterior insula is known to receive sensory input from auditory and visual cortex [46], and may integrate this input with other sensory and motivational inputs, thus influencing the afferent reciprocal connections of insula back to motivational/limbic structures, which could reflect an early stage of stimulus salience learning. Alternatively, it is also possible that the apparent involvement of the insula is an artifact

of its proximity to auditory cortex, where the signal was strong and the insula appeared to be involved because of spatial blurring.

The other conditioned effect detected in the present study was an increase in attentional bias toward the drug-paired stimuli. Attentional bias toward drug-related cues is a strong indicator of drug use in established drug users [19], and the present findings suggest that this type of attentional bias may develop early in the process of conditioning. Participants in this study exhibited faster RTs to the methamphetamine-paired cue after conditioning, and marginal increases in orienting attention to the methamphetamine-paired cue. However, we did not replicate previous findings of increases in sustained attention to the methamphetamine-paired cue and decreases in sustained attention to the placebo-paired cue [15]. This difference may be a result of the fact that post-conditioning measures were collected after the fMRI scan where the cues were presented without drug pairings. One previous study using these cues found that conditioned responses extinguish quickly when the cues are presented without drug [47]. Exploratory post hoc analysis indicated that attentional bias toward the methamphetamine-paired cue was not correlated with the magnitude of brain activation, suggesting that the conditioned increase in vigilance to the drug-paired cue was independent of the cue-induced brain activation.

This study had several limitations, including a modest sample size. It is possible that with a larger sample we might have detected conditioning on other behavioral measures, and conditioned activation in reward-related regions of the brain, consistent with the drug's euphorogenic effects. In addition, compared to a natural setting where drug users experience many pairings, our participants experienced very few pairings between the cues and the drug itself. Thus, some conditioned effects may only develop after more pairings. Further, we used a low, oral dose of methamphetamine in this study. Drug users typically use much higher doses, and administer the drugs by routes that involve faster onset. These conditions also are likely to enhance conditioning. Finally, we collected fMRI data only after conditioning, thus we do not have fMRI data on the direct effects of methamphetamine on responses to the cues. Future studies should assess neural responses to the cues at all three phases: before drug exposure, in the presence of drug, and after conditioning.

We draw several conclusions from this study. First, it is possible to establish a Pavlovian conditioned neural response to cues paired with low, oral doses of methamphetamine in healthy adults. This finding underscores the notion that classical conditioning develops extremely rapidly, supporting its key role in the development of addiction. Second, the conditioned neural responses may extend beyond the expected, dopamine-rich reward areas, to brain areas involved in sensory processing of a drug-paired cue [27]. Indeed, Jasinska et al. [27], suggested that drugs of abuse may facilitate sensory processing of cues, thus partially explaining the increases in sensory and perceptual cortices often reported in cue imaging studies. Interestingly we found in a previous fMRI study [38] that administration of amphetamine enhanced activity in the visual cortex elicited by a visual stimulus, and enhanced activity in the motor cortex that

was elicited by a motor task. The present study appears to have conditioned this drug-enhanced activation. It remains to be determined what role conditioned responses play in future drug-seeking. By studying this early stage of acquisition of conditioning, however, we may be poised to intervene earlier with prevention or treatment strategies.

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ADDITIONAL INFORMATION

Conflict of interest The authors declare that they have no conflict of interest.

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