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Running Title: Neural Responses to Drug-Paired Cues

Neural responses to cues paired with methamphetamine in healthy volunteers

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ABSTRACT

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.

INTRODUCTION

Conditioned responses to stimuli paired with drugs, or drug cues, are known to promote drug use in animal models and human drug users (Berridge & Robinson, 2016; Perry et al, 2014; Stewart et al, 1984; Robinson & Berridge, 1993). In animal models, drug cues increase operant responding previously reinforced with cocaine, nicotine, amphetamine or heroin (Arroyo et al., 1998; Crombag et al, 2008; Palmatier et al, 2007; Perry et al, 2014), and in humans, drug cues increase craving for cigarettes, alcohol and other drugs (Ehrman et al, 1992; Monti et al, 1987). Indeed, drug cues retain their ability to elicit drug-seeking behavior long after cessation of drug use (Bedi et al, 2011; Meil and See, 1996; O'Brien et al, 1992). Decades of research indicates that, in laboratory animals, drug cues acquire motivational properties through Pavlovian conditioning (Stewart et al, 1984). 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 stimuli (e.g., images of cigarettes), and prior contiguity with the drug stimulus is mostly inferred, without explicit knowledge of how the responses were acquired (Robbins et al, 1999; Waters et al, 2009). 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 audio-visual cue) paired with a drug (e.g., amphetamine or alcohol) acquires motivational and incentive properties of the drug itself (Mayo et al, 2013; Mayo and de Wit, 2015; 2016).

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 (Childs and de Wit, 2016), behavioral indices of emotion (e.g., facial electromyography; Mayo and de Wit, 2015, 2016), attention to the stimulus (e.g., eye movements, Field and Cox, 2008; Mayo and de Wit, 2015, 2016), striatal dopamine release using PET (Boileau et al, 2007) and neural activity using fMRI measures (Kareken et al 2012). The sensitivity of these indices varies across studies (Cavallo et al, 2016a), and the outcome measures do not always co-vary (Mayo et al, 2016). 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 functional magnetic resonance imaging (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 (Martin-Soelch et al, 2007; Pavlov 1927; Stewart et al, 1984), 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., Breiter et al, 1997; Volkow et al 2001). Interestingly, stimulant drugs also affect brain activity in areas related to sensory processing, raising the possibility that sensory effects could also become conditioned (Jasinska et al, 2014; Yalachkov et al, 2012). 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 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 4 alcohol- or caffeine-containing drinks per day, body mass index outside the range 19-26 kg/m^2 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 counterbalanced. 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- and placebo-paired cues.

Session Procedures

The procedures used for this study are described in detail by Mayo and de Wit (2015). 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; Mayo and de Wit, 2015). We have previously reported conditioned effects with this dose (Cavallo et al, 2016a; Mayo and de Wit, 2015). 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; Johanson and Uhlenhuth, 1980; Morean et al, 2013), and the Profile of Mood States (POMS; McNair et al, 1971). Measurements were taken before and 15, 30, 70, 115, and 200 minutes 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 Mayo and de Wit (2015) 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 (Mayo and de Wit, 2015; Wardle et al., 2012). 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 a 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 11.2s 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 minutes. The cues were presented for the same amount of time for all participants.

Imaging was done on a Philips Achieva 3.0T scanner using a 32-channel headcoil and a gradient-echo echo-planar imaging sequence with acquisition parameters TR=2000; TE=30; 36 3.75mm thick axial slices aligned to the AC-PC line, no gap; 24x24cm FOV, (3.75mm^3 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 (Cox, 1996). 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 4mm 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-meaned and derivatives of motion parameters were estimated and included as covariates of no interest. Viewing/listening to the methamphetamine- 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 methamphetamine- and placebo-paired cues 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 Tziortzi et al. (2014).

Statistical Analysis

Drug Effects: We used repeated-measures ANOVA (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 minutes after drug administration) and drug (methamphetamine, 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- 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 10000 Monte Carlo simulations of randomly generated data of the same resolution and in-brain inclusion mask (34,990 3.5mm^3 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 (Eklund et al, 2016). Lastly, 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 (Mayo et al, 2013; Mayo and de Wit, 2015; Söderpalm et al, 2003; Wachtel et al, 2002).

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 (Mayo and de Wit, 2015).

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 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; Figure 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; Figure 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- 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 <3mm 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, Figure 2). No clusters showed significantly higher activation for placebo-paired relative to methamphetamine-paired cues. Pearson correlations were conducted between behavioral 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 (Mayo and de Wit, 2015). 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. Based on prior knowledge that stimulant drugs activate dopaminergic reward circuits (Breiter et al, 1997), 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 (Uftring et al, 2001) and drug cues (Jasinska et al, 2014). Uftring et al (2001) 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 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 (Eikelboom and Stewart, 1982). 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 (Cavallo et al, 2016a), positive emotional responses to the cue (measured by facial electromyography), behavioral preference for the cue or attentional bias toward the cue (Mayo et al, 2013; Mayo and de Wit, 2015; Cavallo et al, 2016a). 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 (Heal et al, 2013), 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 (Moeller et al, 2014; Nestler et

al 2009; Tomasi et al, 2011). Interestingly, in another example of unanticipated conditioned drug responses in humans, Rheker et al (2017) 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 simulant 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 (Uftring et al, 2001).

The drug-paired cue also increased activation in the insula, suggesting that additional neural coding of salience may have contributed to this effect (Koob and Volkow, 2016). The posterior insula is known to receive sensory input from auditory and

visual cortex (Ghaziri et al, 2017), 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 (Field and Cox, 2008), 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 (Mayo and de Wit, 2015). 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 (Cavallo et al, 2016b). 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 euphorigenic effects. Additionally, 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 (Jasinska et al 2014). Indeed, Jasinska et al. 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 (Uftring et al, 2001) 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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REFERENCES

Arroyo M, Markou A, Robbins TW, Everitt BJ (1998). Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. *Psychopharmacology* **140:** 331-344.

Bedi G, Preston KL, Epstein DH, Heishman SJ, Marrone GFm Shaham Y et al (2011). Incubation of cue-induced cigarette craving during abstinence in human smokers. *Biol Psychiatry* **69:** 708-711.

Berridge KC, Robinson TE (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *Am Psychol* **71:** 670-679.

Boileau I, Dagher A, Leyton M, Welfeld K, Booji L, Diksic M, et al (2007). Conditioned dopamine release in humans: a positron emission tomography [^{11}C]raclopride study with amphetamine. *J Neurosci* **27*:*** 3998-4003.

Breiter HC, Gollub RL, Weisskoff RM, Kennedy DN, Makris N, Berke JD, et al (1997). Acute effects of cocaine on human brain activity and emotion. *Neuron* **19:** 591-611.

Cavallo JS, Mayo LM, de Wit H (2016a). Acquisition of conditioning between methamphetamine and cues in healthy humans. *PLOS ONE* **11:** e0161541.

Cavallo JS, Ruiz NA, de Wit H (2016b). Extinction of conditioned responses to methamphetamine-associated stimuli in healthy humans *Psychopharmacology* **233:** 2489-2502.

Childs E, de Wit H (2016). Alcohol-induced place conditioning in moderate social drinkers. *Addiction* **111:** 2157-2165.

Cox RW (1996). AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* **29:** 162-173.

Crombag HS, Bossert JM, Koya E, Shaham Y (2008). Context-induced relapse to drug seeking: a review. *Philos Trans R Soc B Biol Sci* **363:** 3233-3243.

Ehrman RN, Robbins SJ, Childress AR, O'Brien CP (1992). Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharmacology (Berl)* **107:** 523-529.

Eikelboom R, Stewart J (1982). Conditioning of drug-induced physiological responses. *Psychol Rev* **89:** 507-528.

Eklund A, Nichols TE, Knutsson H (2016). Cluster failure: why fMRI inferences for spatial extent have inflated false-positive rates. *Proc Natl Acad Sci* **113:** 7900-7905.

Everitt BJ, Robbins TW (2016). Drug addiction: updating actions to habits to compulsions ten years on. *Annu Rev Psychol* **67:** 23-50.

Field M, Cox W (2008). Attentional bias in addictive behaviors: a review of its development, causes, and consequences. *Drug Alcohol Depend* **97:** 1-20.

Ghaziri J, Tucholka A, Girard G, Houde JC, Boucher O, Gilbert G, et al (2017). The corticocortical structural connectivity of the human insula. *Cereb Cortex* **27:** 1216–1228.

Heal DJ, Smith SL, Gosden J, Nutt DJ (2013). Amphetamine, past and present – a pharmacological and clinical perspective. *J Psychopharmacol* **27:** 479-496.

Jasinska AJ, Stein EA, Kaiser J, Naumer MJ, Yalachkov Y (2014) Factors modulating neural reactivity to drug cues in addiction: a survey of human neuroimaging studies. *Neurosci Biobehav Rev* **38:** 1-16.

Johanson CE, Uhlenhuth EH (1980). Drug preference and mood in humans: diazepam. *Psychopharmacology (Berl)* **71:** 269-273.

Kareken DA, Grahame N, Dzemidzic M, Walker MJ, Lehigh CA, O'Connor SJ (2012). fMRI of the brain's response to stimuli experimentally paired with alcohol intoxication. *Psychopharmacology* **220:** 787-797.

Koob GF, Volkow ND (2016) Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry* **3:** 760-73

Martin-Soelch C, Linthicum J, Ernst M (2007). Appetitive conditioning: neural bases and implications for psychopathology. *Neurosci Biobehav Rev* **31:** 426-440.

Mayo LM, de Wit H (2015). Acquisition of responses to a methamphetamine-associated cue in healthy humans: self-report, behavioral, and psychophysiological measures. *Neuropsychopharmacology* **40:** 1734-1741.

Mayo LM, de Wit H (2016). Acquisition of conditioned responses to a novel alcohol-paired cue in social drinkers. *J Stud Alcohol Drugs* **77:** 317-326.

Mayo LM, Fraser D, Childs E, Momenan R, Hommer DW, de Wit H, et al (2013). Conditioned preference to a methamphetamine-associated contextual cue in humans. *Neuropsychopharmacology* **38:** 921-929.

McNair D, Lorr M, Droppleman L (1971). *Profile of Mood States.* Educational and Industrial Testing Service: San Diego, CA.

Meil WM, See RE (1996). Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. *Behav Pharmacol* ***7*****:** 754–763.

Moeller SJ, Honorio J, Tomasi D, Parvaz MA, Woicik PA, Volkow ND, et al (2014). Methylphenidate enhances executive function and optimizes prefrontal function in both health and cocaine addiction. *Cereb Cortex* **24:** 643-653.

Monti PM, Binkoff JA, Abrams DB, Zwick WR, Nirenberg TD, Liepman MR (1987) Reactivity of alcoholics and nonalcoholics to drinking cues*. J AbnormPsychol* **96:** 122–126.

Morean ME, de Wit H, King AC, Sofuoglu M, Rueger SY, O'Malley SS (2013) The drug effects questionnaire: psychometric support across three drug types. *Psychopharmacology* **227:**177-92.

Nestler EJ, Hyman SE, Malenka RC (2009). Chapter 13: Higher cognitive function and behavioral control. In *Molecular Neuropharmacology: A foundation for clinical neuroscience* (2nd ed.) McGraw-Hill Medical: New York, NY. pp 313-334

O'Brien, CP, Childress AR, McLellan AT, Ehrman R (1992). Classical conditioning in drug-dependent humans. *Ann N Y Acad Sci* **654:** 400-415.

Palmatier MI, Liu X, Matteson GL, Donny EC, Caggiula AR, Sved AF (2007). Conditioned reinforcement in rats established with self-administered nicotine and enhanced by noncontingent nicotine. *Psychopharmacology* **195:** 235-243.

Pavlov IP (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex*. Dover Publications: New York, NY.

Perry CJ, Zbukvic I, Kim JH, Lawrence AJ (2014). Role of cues and contexts on drug-seeking behaviour. *Br J Pharmacol* **171:** 4636-4672.

Rheker J, Winkler A, Doering BK, Rief W (2017). Learning to experience side effects after antidepressant intake – results from a randomized, controlled, double-blind study. *Psychopharmacology* **234:** 329-338.

Robbins SJ, Ehrman RN, Childress AR, O'Brien CP (1999). Comparing levels of cocaine cue reactivity in male and female outpatients. *Drug Alcohol Depend* **53:** 223-230.

Robinson TE, Berridge KC (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Res Rev* **18:** 247-291.

Söderpalm, A, Nikolayev L, de Wit H (2003). Effects of stress on responses to methamphetamine in humans. *Psychopharmacology (Berl)* **27:** 1270-1277.

Stewart J, de Wit H, Eikelboom R (1984). Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. *Psychol Rev* **91:** 251-268.

Tomasi D, Volkow N, Wang G, Wang R, Telang F, Caparelli E, et al (2011). Methylphenidate enhances brain activation and deactivation responses to visual attention and working memory tasks in healthy controls. *NeuroImage* **54:** 3101-3110.

Tziortzi AC, Haber, SN, Searle, GE, Tsoumpas, C, Long, CJ, Shotbolt, P, et al (2014). Connectivity-based functional analysis of dopamine release in the striatum using diffusion-weighted MRI and positron emission tomography. *Cereb Cortex* **24:** 1165-1177.

Uftring SJ, Wachtel SR, Chu D, McCandless C, Levin DN, de Wit H (2001). An fMRI study of the effect of amphetamine on brain activity. *Neuropsychopharmacology* **25:** 925-935.

Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L, et al (2001). Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci* **21:** RC121.

Wachtel SR, Ortengren A, de Wit H (2002). The effects of acute haloperidol or risperidone on subjective responses to methamphetamine in healthy volunteers. *Drug Alcohol Depend* **68:** 23-33.

Wardle MC, Garner MJ, Munafó MR, de Wit H (2012). Amphetamine as a social drug: effects of d-amphetamine on social processing and behavior. *Psychopharmacology (Berl)* **223:** 199-210.

Waters AJ, Carter BL, Robinson JD, Wetter DW, Lam CY, Kerst W, et al (2009). Attentional bias is associated with incentive-related physiological and subjective measures. *Exp Clin Psychopharm* **17:** 247-257.

Yalachkov Y, Kaiser J, Naumer MJ (2012). Functional neuroimaging studies in addiction: multisensory drug stimuli and neural cue reactivity. *Neurosci Biobehav Rev* **36:** 825–835.

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)
Gender	
Male/Female	25/26
Age (years)	23.5 (0.6)
Education (years)	14.7 (0.3)
BMI	22.9 (0.2)
Race	
Caucasian	51.0% (26)
African-American	21.6% (11)
Asian	7.8% (4)
Other	15.7% (8)
Unknown	3.8% (2)
Current Drug Use	
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)
Lifetime Drug Use (ever used)	
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)

	PBO		MA		
Subjective Effects	Mean	SEM	Mean	SEM	T-value
DEQ					
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***
POMS					
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***
Cardiovascular Effects					
Blood Pressure	-4.04	1.21	10.95	1.38	8.74***
Heart Rate	-5.32	1.58	7.43	2.43	6.07***

Abbreviations: 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. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

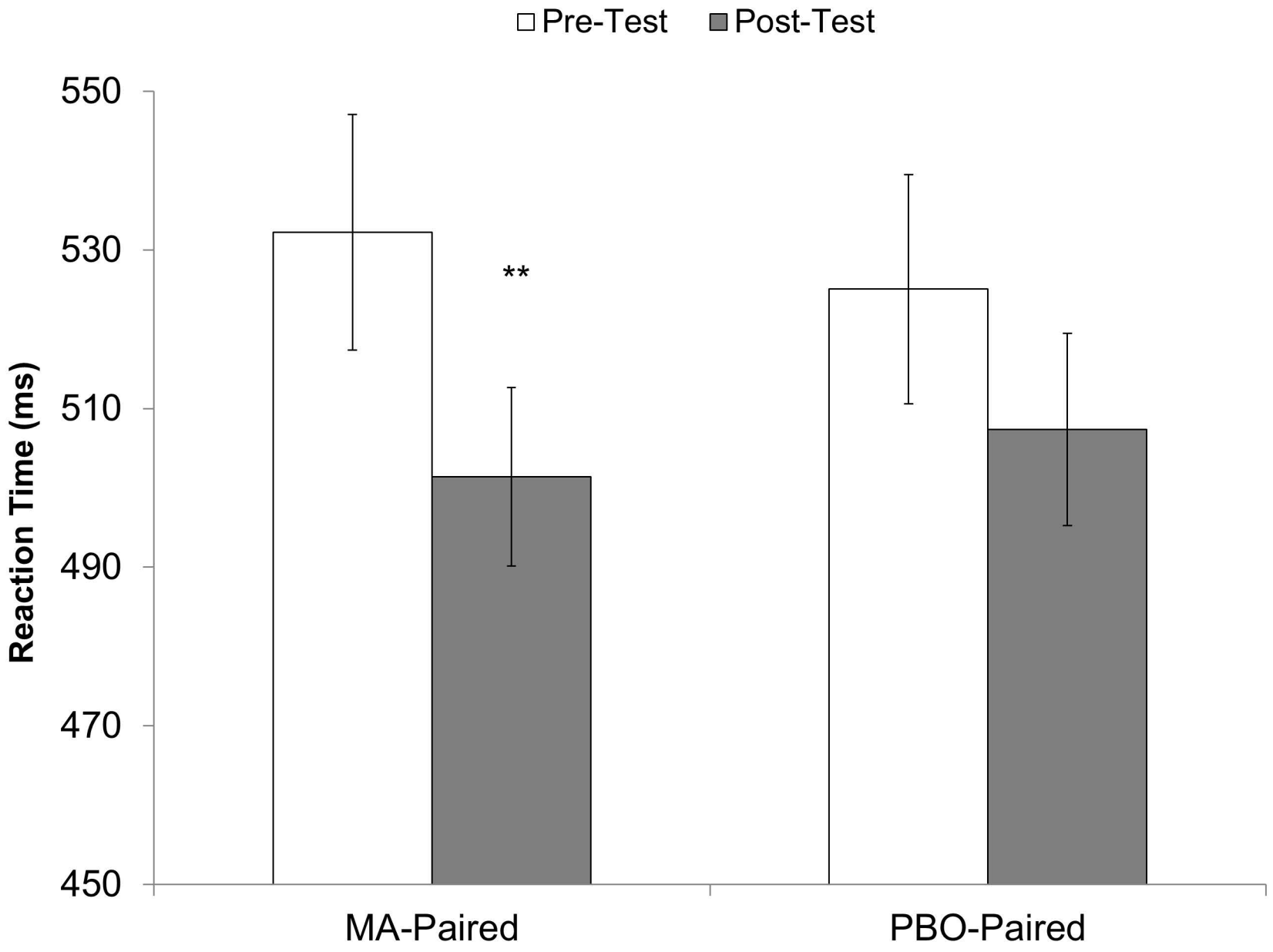
Table 3. Brain areas in which drug-paired stimuli elicited greater activation than placebo-paired stimuli

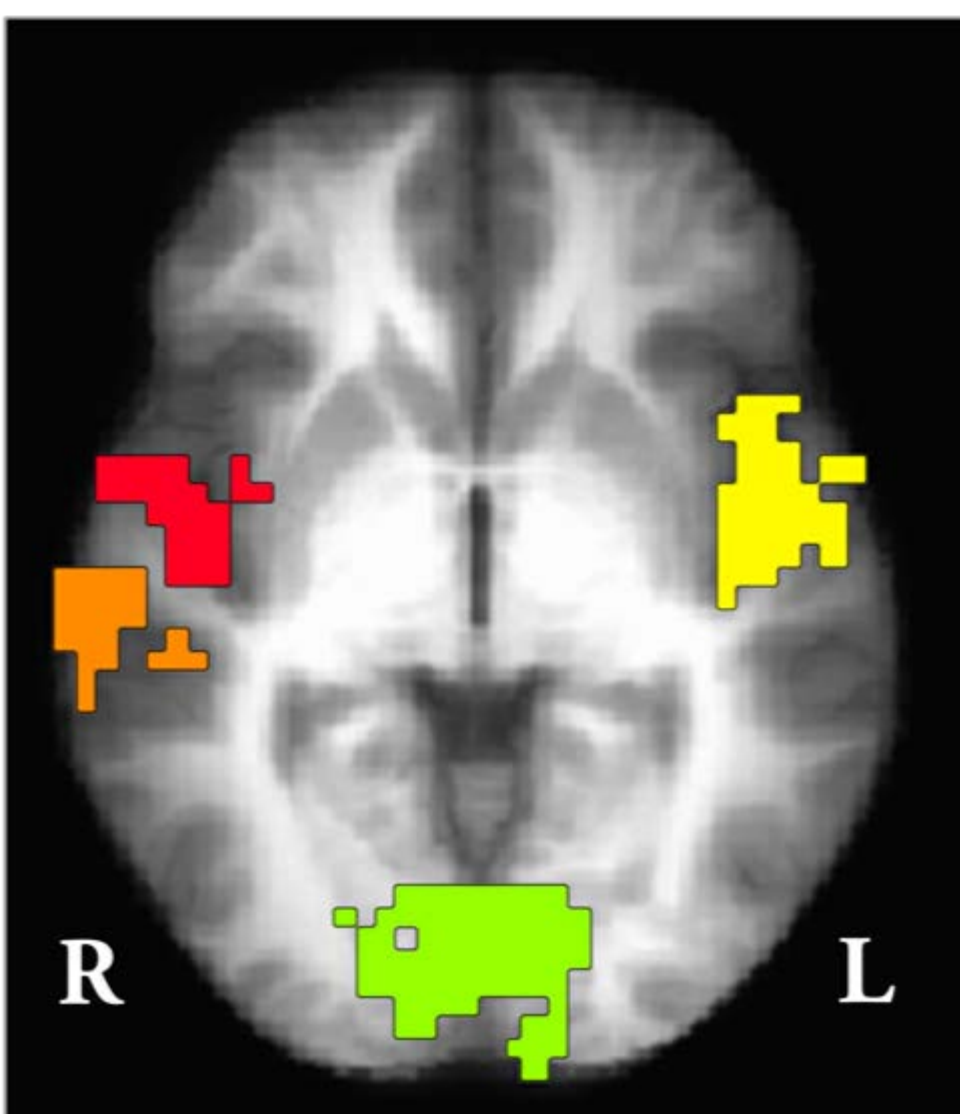
	Cluster size (number of $3.5mm^3$ voxels)	Center of Mass x	Center of Mass y	Center of Mass z	Peak t value
Bilateral cuneus and lingual gyri (BA 17, 18)	533	1.0	-80	2	8.7
Left superior temporal gyrus and Insula	112	-46	-4	-1	6.3
Right superior temporal gyrus	109	61	-31	6	5.9
Right superior temporal gyrus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

FIGURE CAPTIONS

Figure 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 = 0.01.

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


Pre-Test
Post-Test
550
530
510
490
470
450
Reaction Time (ms)
**
MA-Paired
PBO-Paired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