Cannabinoid Modulation of Frontolimbic Activation and Connectivity During Volitional Regulation of Negative Affect

Stephanie M Gorka¹, K Luan Phan^{1,2,3}, Maryssa Lyons⁴, Shoko Mori⁴, Mike Angstadt⁴ and Christine A Rabinak^{*,4,5}

¹Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, USA; ²Departments of Psychology and Anatomy & Cell Biology, Graduate Program in Neuroscience, University of Illinois at Chicago, Chicago, IL, USA; ³Mental Health Service Line, Jesse Brown Medical Center, Chicago, IL, USA; ⁴Department of Psychiatry, University of Michigan, Ann Arbor, MI, USA; ⁵Departments of Pharmacy Practice, Psychiatry & Behavioral Neurosciences, and Pharmaceutical Sciences, Translational Neuroscience Program, Wayne State University, Detroit, MI, USA

Behavioral and brain research indicates that administration of Δ^9 -tetrahydrocannabinol (THC) alters threat perception and enhances the suppression of conditioned fear responses via modulation of the frontolimbic circuit. No prior studies, however, have examined whether THC also affects volitional forms of emotion processing such as cognitive reappraisal. The aim of the current study was therefore to examine the effects of THC on frontolimbic activation and functional connectivity during cognitive reappraisal in a sample of healthy adults. The study was a randomized, double-blind, placebo-controlled, between-subject design and all participants ingested either an oral dose of synthetic THC ($n=41$) or placebo ($n=37$) before completion of an emotion regulation task during functional magnetic resonance imaging (fMRI). Functional connectivity was assessed using generalized psychophysiological interaction (gPPI) analyses. Results indicated that although there were no group differences in self-reported attenuation of negative affect during cognitive reappraisal, relative to placebo, THC increased amygdala activation and reduced amygdala and dorsolateral prefrontal cortex (dlPFC) functional coupling during cognitive reappraisal of emotionally negative pictures. This suggests that in addition to automatic emotional processes, THC affects frontolimbic functioning during cognitive reappraisal.

Neuropsychopharmacology (2016) **41**, 1888–1896; doi:10.1038/npp.2015.359; published online 6 January 2016

INTRODUCTION

The endogenous cannabinoid (CB) system is a robust neuromodulator of subjective affective states such as anxiety and aggression (Lutz, 2009; Martin *et al*, 2002). When ingested, Δ^9 -tetrahydrocannabinol (THC; the primary psychoactive ingredient in cannabis) binds to type 1 cannabinoid receptors (CB₁) in the brain and produces a variety of subjective effects including euphoria, dysphoria, increased and decreased anxiety, and sedation (Curran *et al*, 2002; McDonald *et al*, 2003). Notably, CB₁ receptors are also highly expressed in regions critical for emotion processing including the amygdala, hippocampus, and anterior cingulate cortex (ACC) (Eggan and Lewis, 2007; Mackie, 2005).

Through the use of functional magnetic resonance imaging (fMRI), THC has been shown to dampen limbic reactivity and enhance frontolimbic connectivity, thus leading to decreased anxiety and negative affect. For instance, THC attenuates amygdala activation (Phan *et al*, 2008), and

increases amygdala–rostral ACC/medial prefrontal cortex (mPFC) functional coupling (Gorka *et al*, 2015) during passive viewing of threatening faces. THC also dampens subgenual ACC (sgACC) reactivity to social and nonsocial negative images (Rabinak *et al*, 2012), and chronic, heavy cannabis users exhibit decreased amygdala and ACC activity during masked (or implicit) affective stimuli (*vs* controls) (Gruber *et al*, 2009).

However, THC can also increase limbic reactivity and either disrupt or have no impact on frontolimbic connectivity, as well as be anxiogenic and induce psychotic symptoms (D'Souza *et al*, 2004). Fusar-Poli *et al* (2009) reported that THC increased precuneus and primary motor cortex activation and reduced ACC and middle frontal gyrus activity during matching of negative emotional stimuli. In separate studies, the authors also demonstrated that THC increases amygdala activation (Bhattacharyya *et al*, 2010) and has no impact on amygdala–ACC functional connectivity (Fusar-Poli *et al*, 2010) during viewing of fearful faces. Overall, THC administration affects emotion processing via modulation of the frontolimbic circuit; however, data on the direction of these effects have been mixed.

The CB system is also involved in fear extinction and recall of extinction learning. Pharmacological blockade or genetic deletion of CB₁ receptors prevents consolidation of fear extinction (Chhatwal *et al*, 2005; Marsicano *et al*, 2002).

*Correspondence: Dr CA Rabinak, Department of Pharmacy Practice, Wayne State University, Eugene Applebaum College of Pharmacy & Health Sciences, 259 Mack Avenue, Suite 2190, Detroit, MI 48201, USA; Tel: +1 313 577 9875; Fax: +1 313 577 5369; E-mail: rabinak@wayne.edu

Received 11 September 2015; revised 23 November 2015; accepted 30 November 2015; accepted article preview online 9 December 2015

Meanwhile, acute administration of CB₁ agonists, including THC, facilitates fear extinction and its recall (Bitencourt *et al*, 2008; Chhatwal and Ressler, 2007; de Oliveira Alvares *et al*, 2008; Rabinak *et al*, 2013). Thus, THC may enhance suppression of conditioned fear responses, and we have shown that these effects are mediated by modulation of key nodes within the frontolimbic circuit (amygdala, hippocampus, and ventromedial PFC (vmPFC); Rabinak *et al*, 2014).

Overall, THC affects passive and automatic forms of emotion processing such as threat perception and fear inhibition, and does so via frontolimbic modulation. However, no study has investigated the effects of THC on volitional emotional processes such as cognitive reappraisal that involves explicitly altering one's emotional state by reinterpretation of affective information. Cognitive reappraisal is highly effective at diminishing negative affect in response to aversive stimuli (Gross, 2002; Richards and Gross, 2000) and activates regions of the frontolimbic circuit including the medial, dorsolateral, and ventrolateral prefrontal cortices, and the ACC (Buhle *et al*, 2013; Phan *et al*, 2005; Ochsner *et al*, 2002). Engagement of these regions corresponds with reduced amygdala activity (Ochsner and Gross, 2005; Phan and Sripada, 2013) and the strength of amygdala–PFC functional coupling predicts the extent to which negative affect declines following reappraisal (Banks *et al*, 2007). Thus, successful downregulation of negative affect may rely upon effective inhibitory functioning of the frontolimbic circuit.

As CB₁ receptors are densely localized within the frontolimbic circuit (Eggan and Lewis, 2007) and THC affects automatic forms of emotion processing via frontolimbic neuromodulation (Gorka *et al*, 2015), THC may also acutely affect cognitive reappraisal processes that similarly rely on frontolimbic functioning. The aim of the current study was to examine the acute effects of THC on neural activation and functional connectivity during cognitive reappraisal. The study was a randomized, double-blind, placebo-controlled design in which healthy adult participants ingested either an oral dose of THC or placebo (PBO) before completing a cognitive reappraisal task during fMRI. Given prior conflicting findings on the effects of THC on neural and affective processing, we did not have specific hypotheses about how THC would affect the neural correlates of cognitive reappraisal; however, we speculated that THC would modulate frontolimbic activation and functional connectivity, particularly the amygdala and the medial and lateral prefrontal cortices (Bhattacharyya *et al*, 2010; Phan *et al*, 2008; Rabinak *et al*, 2014).

MATERIALS AND METHODS

Subjects

A total of 78 right-handed volunteers, recruited from the local community via print advertisements and flyers, participated in this study. To be eligible, volunteers had to be free of any current psychoactive medications, neurological or medical illness as confirmed by a physician evaluation, and any DSM-IV lifetime Axis I psychiatric disorders (including substance use disorders) as confirmed by the Structured Clinical Interview for DSM-IV (SCID-NP; First *et al*, 2002). Moreover, participants had a minimal history of

marijuana use (limited to <10 lifetime exposures) to limit prior THC exposure. No participant had used cannabis within the past 30 days, as verified by a negative urine toxicology screen at the time of study. No participant was a daily tobacco smoker. A total of 39 participants were randomly assigned to the THC group and the other 36 participants to the PBO group. Of note, data from 28 of the participants (THC = 14; PBO = 14) were collected at the University of Michigan (UM) as previously described on a different fMRI task (Rabinak *et al*, 2013) and data from the other 50 participants (THC = 27; PBO = 23) were collected at the University of Illinois at Chicago (UIC). Subject demographics for each drug group, by study site, is detailed in Table 1. For both sites, participants were required to be between the ages of 21 and 45 years in order to minimize developmental differences in brain maturation and cognitive function, and ensure that participants were old enough to provide consent to receive an illicit substance. All participants gave written informed consent after explanation of the experimental protocol, as approved by the UM and the UIC institutional review boards.

Experimental Protocol and Task

This study used a double-blind, placebo-controlled, between-subject design as previously described (Rabinak *et al*, 2013). Of note, the study protocol and experimental task were identical across both study locations. Approximately 120 min before scanning, participants ingested an opaque gelatin capsule (size 00) with dextrose filler that contained either synthetic THC (Marinol; 7.5 mg; Solvay Pharmaceuticals, Marietta, GA) or dextrose alone (PBO). The dose of 7.5 mg is considered a low dose and was chosen because it has previously been used in other pharmaco-fMRI studies of emotion processing (see, eg, Phan *et al*, 2008), and is the lowest effective dose that has been found to produce behavioral and subjective effects (Curran *et al*, 2002; Rabinak *et al*, 2012).

The Emotion Regulation Task (Banks *et al*, 2007; Phan *et al*, 2005) is a block-design variant of the reappraisal-based emotion regulation task developed in our laboratory based on paradigms previously validated by Ochsner and colleagues (Ochsner *et al*, 2002) and Davidson and colleagues (Urry *et al*, 2006). Stimuli consisted of 64 unpleasant and 32 neutral images from the International Affective Pictures System (IAPS; Lang *et al*, 2008). The task involved three conditions. In the 'Look' condition, participants simply looked at neutral images. In the 'Maintain' condition, participants were instructed to passively process (eg, experience naturally) unpleasant images. During the 'Reappraise' condition, participants were instructed to use the cognitive strategy of reappraisal to decrease negative affect evoked by unpleasant images.

Before scanning, participants were instructed to use two validated strategies of reappraisal (Ochsner *et al*, 2002; Phan *et al*, 2005): (1) conceptualizing the depicted scenario in a less negative way (eg, women crying outside of a church could be attending a wedding not a funeral); and (2) objectifying the content of the pictures (eg, a woman with facial bruises could be an actor in a movie). Participants were instructed not to look away from pictorial stimuli and understanding of the task was confirmed before scanning by

Table 1 Subject Demographics

	PBO (<i>n</i> = 37)			THC (<i>n</i> = 41)		
	UM (<i>n</i> = 14)	UIC (<i>n</i> = 23)	Total	UM (<i>n</i> = 14)	UIC (<i>n</i> = 27)	Total
Age	25.43 (5.05)	25.83 (5.53)	25.68 (5.29)	24.71 (3.75)	25.07 (3.99)	24.95 (3.87)
Gender (male/female)	9/5	9/14	18/19	4/10	12/15	16/25
Ethnicity						
Hispanic or Latino	0	2	2	1	10	11
Not Hispanic or Latino	14	21	35	13	17	30
Race						
American Indian/Alaskan Native	0	0	0	0	2	2
Asian	2	8	10	1	5	6
Black/African American	1	1	2	1	3	4
Native Hawaiian/Pacific Islander	0	1	1	0	0	0
White	11	12	23	10	9	19
More than 1 race	0	1	1	2	7	9
Unknown	0	0	0	0	1	1

Abbreviations: PBO, placebo; THC, Δ^9 -tetrahydrocannabinol; UIC, University of Illinois at Chicago; UM, University of Michigan. Age is mean (SD).

reviewing examples of reappraisal strategies generated by subjects with sample IAPS images not used in the ERT during scanning.

Participants viewed two 20 s blocks of each condition interspersed with 20 s baseline blocks consisting of a white fixation cross on a black background to allow the blood oxygen level-dependent (BOLD) fMRI signal to return to baseline. During the baseline blocks, participants were asked to 'relax and clear your mind.' Each experimental block consisted of four images, presented for 5 s each in succession. Before each block, the instruction to 'Look,' 'Maintain,' or 'Reappraise' appeared in white text on a black screen for 5 s. Immediately following each task block, participants were asked to rate 'How negative do you feel?' on a 5-point scale (1 = not at all, 5 = extremely) via button response. The order of blocks was pseudorandomized over 4 separate runs of 5 m each.

Following the scanning session, participants viewed each of the 96 previously seen pictures and rated these images on Valence (1 = most unpleasant; 5 = neutral; 9 = most pleasant) and Arousal (1 = not at all arousing; 5 = somewhat arousing; 9 = extremely arousing).

Functional Imaging Acquisition

The fMRI scanning at UM Functional MRI Laboratory was performed on a 3T GE Signa System (General Electric Healthcare, Waukesha, WI) using a standard radiofrequency coil. Whole-brain functional images (ie, BOLD) were collected from 43 axial, 3-mm-thick slices using a T_2^* -sensitive gradient echo reverse spiral acquisition sequence (repetition time, 2000 ms; echo time, 30 ms; 64×64 matrix; 220 mm field of view; flip angle, 90), optimized to minimize susceptibility artifacts (signal loss) at the medial temporal lobe (including the amygdala) (Stenger *et al*, 2000).

The fMRI scanning at the Center for Magnetic Resonance Research at UIC was performed on a 3T GE MR 750 System (General Electric Healthcare) using an 8-channel phased-array radio frequency head coil. A standard T_2^* -sensitive gradient-echo echoplanar imaging (EPI) sequence was used (repetition time, 2000 ms; echo time, 22.2 ms; 64×64 matrix; 220 mm field of view; flip angle, 90; 44 axial slices).

Functional Imaging Analysis

Data from all participants met criteria for high quality and scan stability with minimum motion correction and were subsequently included in fMRI analyses (< 2 mm displacement in any one direction). The first four volumes were discarded to allow for T_1 equilibration effects. Statistical Parametric Mapping (SPM 8) software (Wellcome Trust Centre for Neuroimaging, London, www.fil.ion.ucl.ac.uk/spm) was used to perform conventional preprocessing steps. In brief, slice-time correction was performed to account for temporal differences between slice collection order, images were spatially realigned to the first image of the first run, functional images were normalized to a Montreal Neurological Institute (MNI) template using the EPI template, resampled to 2 mm^3 voxels, and smoothed with an 6 mm isotropic Gaussian kernel.

The general linear model was applied to the time series, convolved with the canonical hemodynamic response function and with a 128 s high-pass filter. Condition effects during the 20 s block of images were modeled with box-car regressors representing the occurrence of each block type, and effects were estimated at each voxel and for each subject. In addition, the six movement parameters obtained during realignment were included in the model as regressors to account for motion-related effects in BOLD. Of note, the preceding instruction screen and the following affect rating period were modeled separately and collapsed across

conditions. The individual SPMs were then analyzed at the second level in a random-effects statistical model using a two (group: PBO, THC) by three (condition: Look, Maintain, Reappraise) analysis of covariance (ANCOVA). Study site/scanner was included as a covariate.

To examine how THC would modulate frontolimbic activation and connectivity specifically during cognitive reappraisal, we conducted a partial brain search using a region of interest (ROI)-based analysis within the vmPFC, vlPFC, dmPFC, dorsolateral PFC (dlPFC), ACC, and amygdala; regions consistently implicated in cognitive reappraisal (Buhle *et al*, 2013). Prefrontal regions were defined using spherical masks centered on peaks independently defined based on a recent meta-analysis of 48 neuroimaging studies of reappraisal (Buhle *et al*, 2013) (see Table 2); in addition, a mask of vmPFC separately identified from another meta-analysis of cognitive reappraisal (Diekhof *et al*, 2011) was also included (see Table 2). Left and right amygdala ROIs were defined by anatomical landmarks using MARINA software (Walter *et al*, 2003) based on masks from the atlas of Tzourio-Mazoyer *et al* (2002).

To examine functional connectivity we used a generalized psychophysiological interaction analysis (gPPI). The deconvolved time series from a left and right anatomical amygdala

mask was extracted for each subject to create the physiological variable. Condition onset times for Look, Maintain, Reappraise, the preceding instruction screen, and the following affect rating period were separately convolved with the canonical hemodynamic response function for each condition, creating psychological regressors. Interaction terms (PPIs) were computed by multiplying extracted time series from the psychological regressors with the physiological variable. Activity within each amygdala seed was regressed on a voxel-wise basis against the interaction, with the physiological and psychological variables serving as regressors of interest. Individual contrast images for Reappraise vs Maintain were then entered into separate second-level *t*-tests. Like the focal activation model, study site/scanner was included as a covariate.

Within our *a priori* ROIs we identified significant activations and connectivity patterns that survived small-volume correction ($P < 0.05$, family-wise error-corrected (FWE)) that balances the risk of type I and II errors in the context of strong *a priori* regionally based hypotheses (Lieberman and Cunningham, 2009) and is comparable to thresholds used in prior fMRI studies of cognitive regulation of emotion (Buhle *et al*, 2013). For completeness and to generate hypotheses in future studies, we report all significant activations at a whole-brain voxel-wise threshold of $P < 0.05$, FWE.

To clarify the direction of effects, we extracted parameter estimates/ β -weights representing BOLD response activation/connectivity strength (parameter estimates, in arbitrary units (a.u.) in terms of mean $\pm$ SD) averaged across all voxels within a 10 mm (5 mm for amygdala) sphere surrounding the peak activation/connectivity clusters of each participant. Follow-up tests were performed using paired or independent sample *t*-tests, as appropriate. We calculated Hedges' *g*, an index of effect size corrected for different sample sizes, on drug effects based on the following: Hedges' $g = \frac{\text{mean}_{\text{PBO}} - \text{mean}_{\text{THC}}}{\text{SD}^*_{\text{pooled}}}$, where $\text{SD}^*_{\text{pooled}} = \sqrt{\frac{(n_{\text{PBO}} - 1)\text{SD}^2_{\text{PBO}} + (n_{\text{THC}} - 1)\text{SD}^2_{\text{THC}}}{n_{\text{PBO}} + n_{\text{THC}} - 2}}$.

Subjective Ratings Analysis

Subjective ratings were assessed using a two (group: PBO, THC) $\times$ three (condition: Look, Maintain, Reappraise) $\times$ two (study site: UM, UIC) ANOVA. Follow-up tests were performed using paired or independent sample *t*-tests, as appropriate.

RESULTS

Subjective Ratings

Subjective ratings were missing from one THC participant and valence and arousal ratings were missing from five participants (three THC) because of computer malfunction. There was a main effect of instruction ($F_{(2, 146)} = 122.40$, $P < 0.001$) for the 'online' subjective ratings; there was no main effect of group ($F_{(1, 73)} = 0.40$, $P = 0.53$), no main effect of scanner site ($F_{(1, 73)} = 0.53$, $P = 0.47$), no group $\times$ scanner site interaction ($F_{(2, 73)} = 1.15$, $P = 0.29$), no group $\times$ instruction interaction ($F_{(2, 146)} = 2.24$, $P = 0.11$), no scanner site $\times$ instruction interaction ($F_{(2, 146)} = 0.03$, $P = 0.97$), and no group $\times$ scanner site $\times$ instruction interaction ($F_{(2, 146)} = 0.14$,

Table 2 Coordinates Used in ROI Analysis from Buhle *et al* (2013)

Brain region	MNI coordinates		
	x	y	z
Dorsomedial prefrontal cortex	9	30	39
	0	15	63
	0	6	63
	0	-9	63
	0	18	42
	-9	12	69
Anterior cingulate cortex	-3	24	30
Dorsolateral prefrontal cortex	51	15	48
	51	6	48
	42	21	45
	42	30	39
	-33	3	54
	-36	22	-2
	-42	18	9
Ventrolateral prefrontal cortex	-51	12	21
	-51	21	9
	60	24	3
	48	24	9
	48	16	6
Ventromedial prefrontal cortex ^a	-42	45	-6
	6	40	-22
	0	38	-18

Abbreviation: MNI, Montreal Neurological Institute.

ROI analyses were conducted by creating a 10 mm radius sphere around the peak coordinate and identifying significant activations that survived small-volume correction ($P < 0.05$, corrected).

^avmPFC coordinate from Diekhof *et al* (2011).

$P=0.87$). For the valence ratings, there was a main effect of image type ($F_{(1, 69)}=213.89$, $P<0.001$); there was no main effect of group ($F_{(1, 69)}=1.43$, $P=0.24$), no main effect of scanner site ($F_{(1, 69)}=0.23$, $P=0.63$), no group $\times$ scanner site interaction ($F_{(1, 69)}=1.19$, $P=0.28$), no group $\times$ image type interaction ($F_{(1, 69)}=0.33$, $P=0.57$), no scanner site $\times$ image type interaction ($F_{(1, 69)}=0.91$, $P=0.34$), and no group $\times$ scanner site $\times$ instruction interaction ($F_{(1, 69)}=1.45$, $P=0.23$). For the arousal ratings, there was also a main effect of image type ($F_{(1, 69)}=122.01$, $P<0.001$); there was no main effect of group ($F_{(1, 69)}=0.24$, $P=0.62$), no main effect of scanner site ($F_{(1, 69)}=0.34$, $P=0.56$), no group $\times$ scanner site interaction ($F_{(1, 69)}=3.63$, $P=0.06$), no group $\times$ image type interaction ($F_{(1, 69)}=0.33$, $P=0.57$), no scanner site $\times$

image type interaction ($F_{(1, 69)}=0.91$, $P=0.34$), and no group $\times$ scanner site $\times$ instruction interaction ($F_{(1, 69)}=0.22$, $P=0.64$).

Participants reported feeling less negative affect following Reappraise compared with Maintain condition (Maintain > Reappraise: PBO, $t_{(36)}=4.09$, $P<0.001$; THC, $t_{(39)}=5.55$, $P<0.001$; Table 3), and the magnitude of reappraisal-related reductions in negative affect did not differ between groups ($t_{(75)}=0.38$, $P=0.71$; Table 3). Both groups reported greater negative affect following Maintain compared with Look blocks (Maintain > Look: PBO, $t_{(36)}=10.41$, $P<0.001$; THC, $t_{(37)}=8.73$, $P<0.001$; Table 3). Unpleasant images were rated as less pleasant and more arousing than neutral images (Valence: PBO, $t_{(34)}=-15.94$, $P<0.001$; THC, $t_{(37)}=-8.54$, $P<0.001$; Arousal: PBO, $t_{(34)}=7.33$, $P<0.001$; THC, $t_{(37)}=8.80$, $P<0.001$; Table 3)

Table 3 Subjective Ratings of Negative Affect and Postscanning Valence and Arousal Ratings

	PBO		THC	
	Mean	SD	Mean	SD
'Online' (during scanning)				
Negative affect rating				
Look	1.10	0.15	1.27	0.42
Maintain	2.64	0.92	2.52	0.82
Reappraise	2.17	0.79	2.00	0.61
Maintain-Reappraise	0.47	0.69	0.52	0.60
After scanning				
Valence rating				
Neutral	5.88	0.78	5.49	1.18
Unpleasant	3.06	0.97	2.96	1.13
Arousal rating				
Neutral	2.24	1.19	1.71	1.16
Unpleasant	4.75	1.94	4.70	2.07

Abbreviations: PBO, placebo; THC, Δ^9 -tetrahydrocannabinol.

Functional MRI Results

The ANOVA revealed a group $\times$ instruction interaction for the left amygdala ($-28, 4, -18$; volume = 688 mm³; $Z=3.00$, $P=0.05$, corrected; Figure 1a). There were no other significant group $\times$ condition interactions identified in any of the other *a priori* ROIs (vmPFC, vlPFC, dmPFC, dlPFC, ACC, right amygdala) or from the whole-brain analysis. There was also no effect of scanner site.

Follow-up inspection of ROI-extracted BOLD signal (β weights) revealed that within the THC group, left amygdala activation increased during Maintain compared with Look ($t_{(39)}=4.56$, $P<0.001$), but the increase to Reappraise did not reach significance ($t_{(39)}=-1.86$, $P=0.07$). Within the PBO group, there was no difference in left amygdala activation between Maintain and Look ($t_{(35)}=0.65$, $P=0.52$) or Maintain and Reappraise ($t_{(35)}=0.13$, $P=0.90$).

Between-group comparisons revealed that THC increased left amygdala activity during Reappraise compared with PBO (mean $\beta \pm$ SD: THC, 0.48 ± 0.46 vs PBO, 0.24 ± 0.40 ; $t_{(74)}=2.35$, $P=0.02$; Hedges' $g=0.55$; Figure 1b). There was no significant difference in left amygdala activation between the drug groups during Maintain (mean $\beta \pm$ SD: THC,

Figure 1 Significant group $\times$ instruction interaction in left amygdala regional activation. (a) Voxel-wise statistical F-maps overlaid on a canonical brain rendering (MINI sagittal) showing significant group by instruction interaction in the left amygdala. Threshold for displaying the image is set at $P=0.05$ and masked; color bars represent statistical F scores. (b) Mean BOLD response (β weights, arbitrary units (a.u.)) from the left amygdala ($-28, 4, -18$) from each condition showing increased activation during Reappraise in the THC group as compared with the PBO group. PBO (green bars) and THC (red bars). Error bars indicate SEM. Significant differences are marked with brackets and asterisk. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

0.36 ± 0.31 vs PBO, 0.25 ± 0.31 ; $t_{(74)} = 1.64$, $P = 0.11$) or Look (mean $\beta \pm$ SD: THC, 0.09 ± 0.33 vs PBO, 0.21 ± 0.30 ; $t_{(74)} = -1.56$, $P = 0.12$).

The gPPI functional connectivity analyses revealed a significant group $\times$ condition interaction between the left amygdala and dlPFC ((36, 22, 54); volume = 3312 mm³; $Z = 3.95$, $P = 0.05$, corrected; Figure 2a). Similarly, there was a significant group $\times$ condition interaction between the right amygdala and dlPFC ((48, 0, 48); volume = 1024 mm³; $Z = 3.14$, $P = 0.05$, corrected; Figure 2c). There were no other significant group $\times$ condition interactions between the left or right amygdala seed regions and any of the other *a priori* ROIs (vmPFC, vlPFC, dmPFC, ACC) or from the whole-brain analysis. There was also no effect of scanner site.

The *post hoc* comparisons of extracted gPPI parameter estimates from the dlPFC revealed no significant difference in left amygdala–dlPFC or right amygdala–dlPFC functional coupling between Maintain and Look or Maintain and Reappraise within either drug group (P s > 0.10).

Compared with the PBO group, THC decreased left amygdala–dlPFC and right amygdala–dlPFC functional coupling during Reappraise (left amygdala–dlPFC: mean $\pm$ SD: THC, 0.02 ± 0.38 vs PBO, 0.30 ± 0.47 ; $t_{(74)} = -2.64$, $P = 0.01$; Hedges' $g = 0.65$; Figure 2b; right amygdala–dlPFC: mean $\pm$ SD: THC, 0.10 ± 0.24 vs PBO, 0.32 ± 0.27 ; $t_{(74)} = -3.70$, $P < 0.001$; Hedges' $g = 0.86$; Figure 2d). In addition, THC decreased right amygdala–dlPFC functional coupling during Maintain (mean $\pm$ SD: THC, 0.02 ± 0.29 vs PBO, 0.36 ± 0.34 ; $t_{(74)} = -2.27$, $P = 0.03$; Hedges' $g = 1.08$; Figure 2d), but THC had no significant effect on left amygdala–dlPFC functional coupling during Maintain between drug groups (mean $\pm$ SD: THC, 0.14 ± 0.39 vs PBO, 0.12 ± 0.35 ; $t_{(74)} = 0.25$, $P = 0.80$). Compared with PBO, THC increased left amygdala–dlPFC functional coupling during Look (mean $\pm$ SD: THC, 0.21 ± 0.42 vs PBO, 0.01 ± 0.42 ; $t_{(74)} = 2.10$, $P = 0.04$; Hedges' $g = 0.48$; Figure 2b), but THC had no significant effect on right amygdala–dlPFC functional

Figure 2 Significant group $\times$ instruction interaction in amygdala functional connectivity with the dorsolateral prefrontal cortex. (a) Voxel-wise statistical F-maps overlaid on a canonical brain rendering (MNI sagittal) showing significant group by instruction interaction between the left amygdala 'seed' region and the dorsolateral prefrontal cortex (dlPFC). Threshold for displaying the image is set at $P = 0.05$ and masked; color bars represent statistical F scores. (b) Mean functional connectivity (gPPI parameter estimates; arbitrary units (a.u.)) from the dlPFC (36, 22, 54) from each condition showing decreased functional coupling between the left amygdala 'seed' and dlPFC during Reappraise, increased functional coupling between these two regions during Look in the THC group compared with the PBO group, and little change in connectivity during Maintain in either group. (c) Voxel-wise statistical F-maps overlaid on a canonical brain rendering (MNI sagittal) showing significant group by instruction interaction between the right amygdala 'seed' region and the dlPFC. Threshold for displaying the image is set at $P = 0.05$ and masked; color bars represent statistical F scores. (d) Mean functional connectivity from the dlPFC (48, 0, 48) from each condition showing decreased functional coupling between the right amygdala 'seed' and dlPFC during Reappraise and Maintain in the THC group compared with the PBO group, and little change in connectivity during Look in either group. PBO (green bars) and THC (red bars). Error bars indicate SEM. Significant differences are marked with brackets and asterisk. A full color version of this figure is available at the *Neuropsychopharmacology* journal online.

coupling during Look between drug groups (mean $\pm$ SD: THC, 0.27 ± 0.33 vs PBO, 0.27 ± 0.40 ; $t_{(74)} = -0.03$, $P = 0.98$).

DISCUSSION

The aim of the current study was to examine whether acute THC administration modulates frontolimbic activation and functional connectivity during a volitional emotion regulation task. All participants, regardless of drug condition, self-reported successful downregulation of negative affect using cognitive reappraisal. However, the THC group exhibited greater left amygdala activation, and less amygdala–dlPFC functional coupling during cognitive reappraisal (vs PBO group). In addition, within the THC group, left amygdala activation was higher during both Reappraise and Maintain relative to Look. Compared with PBO, THC also decreased *right* amygdala–dlPFC functional coupling during Maintain, but increased *left* amygdala–dlPFC functional coupling during Look. Broadly, these results suggest that THC modulates amygdala, but not PFC, activation and amygdala–dlPFC functional connectivity during cognitive reappraisal and negative emotion processing in general.

In the current study THC increased amygdala activation during cognitive reappraisal, whereas others have shown that THC decreases amygdala activation during fear inhibition (Rabinak *et al*, 2013), and can increase or decrease amygdala activation during threat perception (Bhattacharyya *et al*, 2010; Phan *et al*, 2008). This suggests that the effects of THC on amygdala activation may differ depending on whether the emotional process is volitional or automatic (cognitive reappraisal vs fear inhibition). Besides being more effortful and goal-directed than fear inhibition, cognitive reappraisal also occurs later in the temporal unfolding of an emotional response over time. More specifically, fear inhibition (and threat perception) occurs quickly, at the initial appraisal stage of an emotional response, whereas cognitive reappraisal occurs later, once affective responses have already been generated (Gross, 1998, 2001, 2013). Cognitive reappraisal is therefore a relatively slower process that involves active manipulation of cognitive, physiological, and motivational responses themselves. Considering the current findings, the ability of THC to dampen amygdala reactivity may be specific to fast-acting, automatic regulation strategies such as inhibiting conditioned fear responses during fear extinction and recall (Rabinak *et al*, 2014). When the strategy unfolds more slowly over time and requires active cognitive modulation of emotional experiences, THC may actually have the opposite effect and enhance amygdala reactivity.

In addition to focal activation changes THC decreased amygdala–dlPFC functional coupling during cognitive reappraisal. The dlPFC is a key node of the frontolimbic circuit and is critically involved in the generation and maintenance of alternative interpretations of stimulus content (Ochsner *et al*, 2012). Moreover, during cognitive reappraisal, dlPFC and amygdala activation covary in samples of healthy adults (Banks *et al*, 2007; Erk *et al*, 2010), and it has been suggested that during volitional regulation efforts, the dlPFC exerts inhibitory influences on the amygdala to facilitate the attenuation of negative affect (Banks *et al*, 2007). In the current study, the THC group exhibited greater amygdala reactivity during cognitive reappraisal (vs PBO) and it is

plausible that these effects were mediated by changes in amygdala–dlPFC functional connectivity. More specifically, because THC diminished amygdala–dlPFC functional coupling, amygdala response to negative stimuli may have been unregulated, creating sustained hyperactivity. Others have also found that THC increases amygdala reactivity in response to negative stimuli, and it is possible that THC-induced amygdala–dlPFC decoupling leads to sustained amygdala reactivity in several affective contexts (Bhattacharyya *et al*, 2012a). Importantly, in the current study, despite this neural pattern of results, the THC group reported less negative affect following Reappraise relative to Maintain, suggesting that their regulation efforts were indeed successful. Future studies should investigate the factors and processes that may contribute to this dissociation between self-reported affect and neural patterns.

Related to the points above, within the THC group, amygdala activation was greater during both Maintain and Reappraise relative to Look and implies that to some extent, THC dampened amygdala activation to neutral images relative to negative images. Interestingly, THC also increased (left) amygdala–dlPFC functional connectivity during Look, and decreased (right) amygdala–dlPFC functioning connectivity during Maintain. This pattern of results broadly implies that in response to neutral images, THC increases amygdala–dlPFC functional coupling that may attenuate amygdala reactivity. However, in response to negative images, and during attempts to actively reinterpret negative stimuli, THC decreases amygdala–dlPFC functional coupling that may enhance amygdala reactivity. The mechanisms underlying the direction of these effects are unclear. However, one possibility is that the THC group had difficulties engaging the dlPFC in response to negative stimuli, and then overcompensated or misemployed dlPFC in response to neutral stimuli. Alternatively, given that acute THC administration is associated with impairments in affective stimuli perception (Ballard *et al*, 2012), and can increase the salience of nonsalient stimuli (Bhattacharyya *et al*, 2012b), the THC group may have had difficulties accurately decoding visual and affective information and subsequently employing the appropriate regulatory response, even to emotionally neutral stimuli. These alternative hypotheses require further investigation.

This study had numerous strengths, including having a double-blind, placebo-controlled design in a relatively large sample for a pharmacofMRI study, and the use of a well-validated cognitive reappraisal task consistently shown to engage a specific frontal circuit. There are also several limitations worth noting. First, although participants received extensive instruction, and were tested on their cognitive reappraisal abilities before the task, the extent to which they engaged in this strategy or individual differences in strategies employed during Reappraise is unclear. Second, participants rated their self-reported negative affect at the end of each task block, instead of after each individual image, and this may have led to biased reports because of the relative retrospective nature of the assessments. Third, data were collected at two different sites, using two different scanners, and although site was included as a covariate in all analyses, it is possible this influenced the pattern of results. Fourth, gPPI analyses are correlational and therefore directionality between the amygdala and dlPFC cannot be

inferred. Future work is therefore needed to replicate and extend this line of research.

In sum, the present results suggest that relative to PBO, THC increases amygdala activation and reduces amygdala–dlPFC functional coupling during cognitive reappraisal. These results are in contrast with data suggesting that THC reduces amygdala reactivity, and enhances amygdala–PFC functional connectivity, during fear inhibition (Rabinak *et al*, 2014), but are consistent with some studies (but not all) on the neural effects of THC during threat perception (Bhattacharyya *et al*, 2012a). Together, this literature suggests that THC may facilitate automatic forms of emotion processing but disrupt, at least on a neural level, volitional attempts to regulate negative affect. Although additional work is needed to elucidate under what conditions, and for whom, THC potentiates vs attenuates frontolimbic and emotional functioning, this pattern of results could indicate that during acute intoxication, recreational cannabis users experience deficits in effortful attempts to dampen negative affect.

FUNDING AND DISCLOSURE

This material is based on work supported by a grant from the National Center for Research Resources (UL1RR024986 and UL1RR029879) and from the National Institute of Mental Health (1R21MH093917) awarded to KLP. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center for Research Resources or the National Institutes of Health. The authors declare no conflict of interest.

ACKNOWLEDGMENTS

We thank Donald McNair for his assistance with data collection at the University of Illinois at Chicago.

REFERENCES

- Ballard ME, Bedi G, de Wit H (2012). Effects of delta-9-tetrahydrocannabinol on evaluation of emotional images. *J Psychopharmacol* **26**: 1289–1298.
- Banks SJ, Eddy KT, Angstadt M, Nathan PJ, Phan KL (2007). Amygdala frontal connectivity during emotion regulation. *Soc Cogn Affect Neurosci* **2**: 303–312.
- Bhattacharyya S, Atakan Z, Martin-Santos R, Crippa JA, McGuire PK (2012a). Neural mechanisms for the cannabinoid modulation of cognition and affect in man: a critical review of neuroimaging studies. *Curr Pharm Des* **18**: 5045–5054.
- Bhattacharyya S, Crippa JA, Allen P, Martin-Santos R, Borgwardt S, Fusar-Poli P *et al* (2012b). Induction of psychosis by delta-9-tetrahydrocannabinol reflects modulation of prefrontal and striatal function during attentional salience processing. *Arch Gen Psychiatry* **69**: 27–36.
- Bhattacharyya S, Morrison PD, Fusar-Poli P, Martin-Santos R, Borgwardt S, Winton-Brown T *et al* (2010). Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. *Neuropsychopharmacology* **35**: 764–774.
- Bitencourt RM, Pamplona FA, Takahashi RN (2008). Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. *Eur Neuropsychopharmacol* **18**: 849–859.
- Buhle JT, Silvers JA, Wager TD, Lopez R, Onyemekwu C, Kober H *et al* (2013). Cognitive reappraisal of emotion: A meta-analysis of human neuroimaging studies. *Cereb Cortex* **24**: 2981–2990.
- Chhatwal JP, Davis M, Maguschak KA, Ressler KJ (2005). Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* **30**: 516–524.
- Chhatwal JP, Ressler KJ (2007). Modulation of fear and anxiety by the endogenous cannabinoid system. *CNS Spectr* **12**: 211–220.
- Curran VH, Brignell C, Fletcher S, Middleton P, Henry J (2002). Cognitive and subjective dose-response effects of acute oral Δ 9-tetrahydrocannabinol (THC) in infrequent cannabis users. *Psychopharmacology* **164**: 61–70.
- de Oliveira Alvares L, Pasqualini Genro B, Diehl F, Molina VA, Quilfeldt JA (2008). Opposite action of hippocampal CB1 receptors in memory reconsolidation and extinction. *Neuroscience* **154**: 1648–1655.
- D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT *et al* (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* **29**: 1558–1572.
- Diekhof EK, Geier K, Falkai P, Gruber O (2011). Fear is only as deep as the mind allows: a coordinate-based meta-analysis of neuroimaging studies on the regulation of negative affect. *Neuroimage* **58**: 275–285.
- Eggan SM, Lewis DA (2007). Immunocytochemical distribution of the cannabinoid CB1 receptor in the primate neocortex: a regional and laminar analysis. *Cereb Cortex* **17**: 175–191.
- Erk S, Mikschl A, Stier S, Ciaramidaro A, Gapp V, Weber B, Walter H (2010). Acute and sustained effects of cognitive emotion regulation in major depression. *J Neurosci* **30**: 15726–15734.
- First MB, Spitzler RL, Gibbon M, Williams JB (2002). *Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-Patient Edition (SCID-I/NP)*. Biometrics Research Department, New York State Psychiatric Institute: New York.
- Fusar-Poli P, Allen P, Bhattacharyya S, Crippa JA, Mechelli A, Borgwardt S *et al* (2010). Modulation of effective connectivity during emotional processing by Δ 9-tetrahydrocannabinol and cannabidiol. *Int J Neuropsychopharmacol* **13**: 421–432.
- Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, Martin-Santos R *et al* (2009). Distinct effects of Δ 9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Arch Gen Psychiatry* **66**: 95–105.
- Gorka SM, Fitzgerald DA, de Wit H, Phan KL (2015). Cannabinoid modulation of amygdala subregion functional connectivity to social signals of threat. *Int J Neuropsychopharmacol* **18**: 1–6.
- Gross JJ (1998). Antecedent- and response-focused emotion regulation: divergent consequences for experience, expression, and physiology. *J Pers Soc Psychol* **74**: 224–237.
- Gross JJ (2001). Emotion regulation in adulthood: timing is everything. *Curr Dir Psychol Sci* **10**: 214–219.
- Gross JJ (2002). Emotion regulation: affective, cognitive, and social consequences. *Psychophysiology* **39**: 281–291.
- Gross JJ (ed). *Handbook of Emotion Regulation*. Guilford Publications, 2013.
- Gruber SA, Rogowska J, Yurgelun-Todd DA (2009). Altered affective response in marijuana smokers: an fMRI study. *Drug Alcohol Depend* **105**: 139–153.
- Lang PJ, Bradley MM, Cuthbert BN (2008). *International Affective Picture System (IAPS): Affective Ratings of Pictures and Instruction Manual, Technical Report A-8*. University of Florida: Gainesville, FL.
- Lieberman MD, Cunningham WA (2009). Type I and type II error concerns in fMRI research: re-balancing the scale. *Soc Cogn Affect Neurosci* **4**: 423–428.
- Lutz B (2009). Endocannabinoid signals in the control of emotion. *Curr Opin Pharmacol* **9**: 46–52.

- Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. In: Pertwee RG (ed). *Cannabinoids*. Springer: Berlin Heidelberg, 2005, pp 229–325.
- Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG et al (2002). The endogenous cannabinoid system controls extinction of aversive memories. *Nature* **418**: 530–534.
- Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2002). Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology* **159**: 379–387.
- McDonald J, Schleifer L, Richards JB, de Wit H (2003). Effects of THC on behavioral measures of impulsivity in humans. *Neuropsychopharmacology* **28**: 1356–1365.
- Ochsner KN, Bunge SA, Gross JJ, Gabrieli JDE (2002). Rethinking feelings: an fMRI study of the cognitive regulation of emotion. *J Cogn Neurosci* **14**: 1215–1229.
- Ochsner KN, Gross JJ (2005). The cognitive control of emotion. *Trends Cogn Sci* **9**: 242–249.
- Ochsner KN, Silvers JA, Buhle JT (2012). Functional imaging studies of emotion regulation: a synthetic review and evolving model of the cognitive control of emotion. *Ann NY Acad Sci* **1251**: E1–E24.
- Phan KL, Angstadt M, Golden J, Onyewuenyi I, Popovska A, de Wit H (2008). Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J Neurosci* **28**: 2313–2319.
- Phan KL, Fitzgerald DA, Nathan PJ, Moore GJ, Uhde TW, Tancer ME (2005). Neural substrates for voluntary suppression of negative affect: a functional magnetic resonance imaging study. *Biol Psychiatry* **57**: 210–219.
- Phan KL, Sripada CS. Emotion regulation. In: Armony J, Vuilleumier P (eds). *The Cambridge Handbook of Human Affective Neuroscience*. Cambridge University Press: Cambridge, UK, 2013, pp 375–400.
- Rabinak CA, Angstadt M, Lyons M, Mori S, Milad MR, Liberzon I et al (2014). Cannabinoid modulation of prefrontal-limbic activation during fear extinction learning and recall in humans. *Neurobiol Learn Mem* **113**: 125–134.
- Rabinak CA, Angstadt M, Sripada CS, Abelson JL, Liberzon I, Milad MR et al (2013). Cannabinoid facilitation of fear extinction memory recall in humans. *Neuropharmacology* **64**: 396–402.
- Rabinak CA, Sripada CS, Angstadt M, de Wit H, Phan KL (2012). Cannabinoid modulation of subgenual anterior cingulate cortex activation during experience of negative affect. *J Neural Transm* **119**: 701–707.
- Richards JM, Gross JJ (2000). Emotion regulation and memory: the cognitive costs of keeping one's cool. *J Pers Soc Psychol* **79**: 410–424.
- Stenger VA, Boada FE, Noll DC (2000). Three-dimensional tailored RF pulses for the reduction of susceptibility artifacts in T* 2-weighted functional MRI. *Magn Reson Med* **44**: 525–531.
- Tzourio-Mazoyer N, Landeau B, Papathanassiou D, Crivello F, Etard O, Delcroix N et al (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* **15**: 273–289.
- United Nations Office on Drugs and Crime (2012). *World Drug Report 2012*. United Nations: New York, USA.
- Urry HL, van Reekum CM, Johnstone T, Kalin NH, Thurow ME, Schaefer HS et al (2006). Amygdala and ventromedial prefrontal cortex are inversely coupled during regulation of negative affect and predict the diurnal pattern of cortisol secretion among older adults. *J Neurosci* **26**: 4415–4425.
- Walter B, Blecker C, Kirsch P, Sammer G, Schienle A, Stark R et al (2003). MARINA: an easy tool for the creation for MASKs for Region of INterest Analyses. Paper presented at the 9th International Conference on Functional Mapping of the Human Brain, New York, NY.