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Tetrahydrocannabivarin (THCv) reduces Default Mode Network and increases Executive Control Network Resting State Functional Connectivity in Healthy Volunteers.

Running title:
Effects of THCv on Resting State Functional Connectivity.

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Abstract:

Background: The cannabinoid CB1 Neutral Antagonist Tetrahydrocannabivarin (THCv) has been suggested as a possible treatment for obesity but without the depressogenic side-effects of inverse antagonists such as Rimonabant. However, how THCv might affect the resting-state functional connectivity of the human brain is as yet unknown.

Method: We examined in 20 healthy volunteers the effects of a single 10mg oral dose of THCv and placebo in a randomized, within-subject, double-blind design. Using resting-state functional magnetic resonance imaging and seed based connectivity analysis we selected amygdala, insula, OFC and dmPFC regions of interest. Mood and subjective experience were also measured before and after drug administration using self-report scales.

Results: Our results revealed, as expected, no significant differences in subjective experience with a single dose of THCv. However we found reduced resting-state functional connectivity between the amygdala seed region and the DMN and increased resting-state functional connectivity between the amygdala seed region and the dACC and between the dmPFC seed region and the IFG/MFG. We also found a positive correlation under placebo for amygdala-precuneus connectivity with BMI this correlation was not apparent under THCv.

Conclusion: Our findings are the first to show that treatment with the CB1 neutral antagonist THCv decreases resting state functional connectivity in the Default Mode network and increases connectivity in the Cognitive Control network and Dorsal Visual Stream network. This effect profile suggests possible therapeutic activity of THCv for obesity where functional connectivity has been found to be altered in these regions.

Key words: fMRI, resting-state, cannabinoids, obesity, reward, default mode
Introduction

Despite severe health and economic consequences of excess body weight (Andreyeva et al., 2004), the neurobiological mechanism of disordered eating in humans remains unclear. The endocannabinoid system in the human brain which involves cannabinoid type 1 (CB1) receptors has been implicated in reward processing in animals and humans (Solinas et al., 2007) and in regulating feeding behavior by modulating brain reward signals related to appetite and consumption of food (Solinas et al., 2008). For example, when tetrahydrocannabinol (THC) which is a psychoactive constituent of the cannabis plant and a partial agonist at the CB1 receptor was administered in rats it led to an increase in the hedonic response to sucrose and a decrease in aversive reactions to bitter solutions (Jarrett et al., 2005). Moreover, Solinas and Goldberg reported that administration of THC, during food consumption, increased the motivation to respond for the food in rats.

Rimonabant, an antagonist and possible inverse agonist (Pertwee, 2005) at the CB1 receptor, was licensed in Europe for the treatment of obesity in 2006 but was withdrawn from clinical use in 2008 due to depression like side effects. Rimonabant was found to promote weight loss by decreasing food intake (Scheen et al., 2006) however, it also presented with depression- and anxiety- related side effects. In an attempt to try and understand how these treatments might be having their effects we did a study in 2010 that examined the neural response to reward and aversion in humans after a 7 day treatment with rimonabant. We found that rimonabant reduced neural responses to pleasant chocolate taste in reward areas of the brain such as the ventral striatum and orbitofrontal cortex whilst increasing brain responses to aversive sights and tastes in regions such as the lateral orbitofrontal cortex (Horder et al., 2010). We concluded that although reduced food intake might be due to reduced neural response to reward this may also have been a mechanism by which depression side-effects where induced, given that reduced neural responses to reward have been found in depressed patients (Keedwell et al., 2005; Wacker et al., 2009) and in
those at risk of depression (McCabe et al., 2009; McCabe et al., 2012) and thought to be a possible biomarker for depression (Hasler and Northoff, 2011).

THCv, a neutral antagonist that acts on CB1 receptors has been suggested as a potentially safer alternative with fewer side effects (Le Foll et al., 2009; Pacher and Kunos, 2013). Animal studies have shown that like rimonabant, THCv reduces weight gain and food consumption but unlike rimonabant does not increase activity in basolateral amygdala and ventral tegmental area- brain regions involved in emotion regulation (Meye et al., 2013).

Moreover, our recent fMRI study, the first to investigate THCv effects on the reward and aversion in the healthy human brain, found that relative to placebo THCv increased activation to pleasant chocolate stimuli in the anterior cingulate cortex, caudate, putamen, and midbrain (Tudge et al., 2014) opposite to the profile of those “at risk” of depression (McCabe et al., 2009; McCabe et al., 2012). Thus our results supported the idea that THCv does not impair reward function, and this may be related to a potentially safer side-effect profile.

We also found that THCv increased activation to the aversive stimulus in amygdala, insula and orbitofrontal cortex (Tudge et al., 2014). We suggested this might be a mechanism by which food intake is reduced by increasing the salience of food, and perhaps then decreasing time to satiety. This is plausible in light of a study by Tallett et al. (2008), which found that the behavioral satiety sequence (time to stop feeding) was accelerated under a rimonabant and naloxone combination in rats (Tallett et al., 2008). However how THCv might affect activity in these regions at rest in healthy individuals is as yet unknown.

Therefore in this study we aimed to investigate the effects of a single oral 10mg dose of THCv on resting-state functional connectivity between regions of interest; amygdala, insula and the orbitofrontal cortex identified from our previous task findings (Tudge et al., 2014). Further these regions have also been identified as altered in connectivity in studies of resting state in obese individuals (Lips et al., 2014; Coveleskie et al., 2015; Zhang et al., 2015). We also selected the dorsal medial PFC region as we are interested in THCv effects on prefrontal regions, especially those known to be involved in resting state functional connectivity in depression (Sheline et al., 2010). We hypothesized that relative to placebo,
THCv would increase region of interest functional connectivity, in line with our effects found during a task (Tudge et al., 2014).
Methods

Participants

Nineteen participants (9 female), aged 20-36 were included in a within-subjects, double-blind, placebo-controlled, crossover design to receive a single dose of oral treatment with THCv (10mg/day) or placebo. Participants completed the resting state scan once with the drug (10mg THCv approximately 1 hour before scan to allow for peak blood plasma levels to occur) and then again 1 week later with the placebo, or vice versa. Both participant and experimenter were blind to the treatment condition. Ethical approval was provided by Oxford Research Ethics Committee and written informed consent was obtained from all participants before screening and after a complete description of the study was given. Participants were recruited from the university volunteer register and via internet adverts. Volunteers were assessed with the Structured Clinical Interview for DSM-IV Axis I Disorders Schedule (SCID-I) (First et al., 1997) to exclude a current or previous history of major depression or any other Axis 1 disorder. Participants also had no history of drug or alcohol misuse and did not smoke more than 5 cigarettes a day. Participants were right handed, had normal or corrected to normal vision and were not on medications apart from the contraceptive pill. Participants had no neurological disorders or contraindications for MRI examination.

Baseline ratings of mood and anhedonia were collected using the Beck Depression Inventory (BDI) (Beck et al., 1961), the Fawcett-Clarke Pleasure Scale (FCPS) (Fawcett et al., 1983), the Snaith-Hamilton Pleasure Scale (SHAPS) (Snaith et al., 1995) and the Temporal Experience of Pleasure Scale (TEPS) (Gard et al., 2007). Body mass index (BMI) and an Eating Attitudes questionnaire were used to rule out eating disorders (EAT) (Garner et al., 1982). Participants were scanned twice, once with THCv or placebo and then 1 week later with the other. To assess the effects of the treatment the following questionnaires were taken before and after the treatment: Visual Analogue Scales (VAS) of happiness, sadness, anger, disgust alertness and anxiety, and the Befindlichkeit Scale of mood and energy (BFS) (von Zerssen et al., 1974).
Experimental Design

MRI derived measures of brain function, based on blood-oxygenation-level-dependent (BOLD) contrast were used to compare brain responses at rest across the THCv and the placebo groups at approximately 1 hour after treatment. The resting-state data were acquired before any other scans including the structural scan. Subjects were instructed while lying in the dimmed light of the scanner to keep their eyes open, looking at a black screen.

fMRI Data Acquisition

Images were acquired with a 3.0-T VARIAN/SIEMENS whole-body scanner at the Oxford Centre for Functional Magnetic Resonance Imaging (FMRIB), where T2*-weighted echo planar imaging (EPI) slices were acquired every 2.41 seconds (TR=2.41). Imaging parameters were selected to minimize susceptibility and distortion artifacts in the orbitofrontal cortex (Wilson et al., 2002). Coronal slices with in-plane resolution of 3.0 x 3.0 mm and between plane spacing of 3 mm were obtained. The matrix size was 64 x 64 and the field of view was 192 x 192 mm. Acquisition was carried out during the resting scan, yielding 128 volumes in total. A whole brain T2*-weighted EPI volume of the above dimensions, and an anatomical T1 volume with coronal plane slice thickness 3 mm and in-plane resolution of 1.0 x 1.0 mm were also acquired to improve the registration process.

Analysis methods

Pre-processing

Imaging data were pre-processed and analysed using FSL tools (www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004). fMRI data pre-processing was carried out using FEAT (FMRI Expert Analysis Tool, Version 6.0, a part of FSL software), and included
the following steps: non-brain removal (Smith, 2002), motion correction using MCFLIRT (Jenkinson and Smith, 2001), spatial smoothing using a Gaussian kernel of full-width at half maximum (FWHM) of 5mm, grand mean intensity normalization of the entire 4D dataset by a single multiplicative factor and high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with sigma=64.0s). fMRI volumes were registered to the individual’s structural scan and the MNI-152 standard space image (Montreal Neurological Institute, Montreal, QC, Canada) using FMRIB’s Linear Image Registration Tool (FLIRT) (Jenkinson et al., 2002).

**Time series extraction and higher level analysis**

To study resting-state functional connectivity, a seed-based correlation approach was used with the amygdala, OFC and insula as selected seeds using the Harvard-Oxford subcortical structural atlas (Kennedy et al., 1998). To maximize the exact coverage, the masks of these seed regions were threshold by 20% to include voxels having at least 20% of being in these particular regions. We also created seeds for the dmPFC (18 34 29; -24 35 28) (8 mm radius) coordinates from (Sheline et al., 2010) as they showed this region to have increased connectivity within the default mode and affective network in depressed patients. The dmPFC seeds were created with Wake Forest University Pickatlas tool in SPM8 see (McCabe et al., 2010).

The mean time course within the left and right seeds of each ROI (except for the OFC, only comprising one medial seed) was calculated and used as a regressor in a general linear model for each of the networks. In addition, white matter and cerebrospinal fluid were segmented using FSL’s FAST and the 6 motion parameters (3 translations and 3 rotations), and the global signal obtained from the pre-processing steps were all used as nuisance regressors. The resulting segmented white matter and cerebrospinal fluid images were then thresholded to ensure 80% tissue type probability. For each individual, the general linear model was analyzed by using the FMRI Expert Analysis Tool [version 5.4, part of
FMRIB’s Software Library (Smith et al., 2004)]. The resulting parameter estimate maps were then analyzed using higher level 1 sample t-tests for group averages and paired samples t-tests for treatment effects. Whole-brain $z$-statistical images were thresholded with an initial cluster-forming threshold of $z > 2.3$ and a corrected cluster significance threshold of $P < 0.008$ (i.e. $P < 0.05$ Bonferroni corrected for the 6 regions of interest: R/L amygdala, R/L insula, OFC and dmPFC (Worsley, 2001)). The % BOLD signal change in the graphs is the PE/COPE values converted to mean % BOLD signal change viaFeatquery (FSL) (www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004) for the regions that had significant correlations with the seeds (Table 2).

**BMI correlations:**

To examine the relationship with BMI we took the % BOLD signal change from voxels identified as significantly different between drug and placebo (Table 2) usingFeatquery in each individual subject and then correlated this with the BMI scores for each individual.

**Results**

**Demographic details and mood ratings**

Demographic data analysis (Table S1) revealed participants had low depression scores, as well as normal EAT scores. One-way ANOVAs revealed no significant effects ($p>.05$) of gender on any of the demographic measures. Repeated-measures ANOVAs were employed to examine the effect of drug (placebo/THCv) and time (pre-scan/post-scan) on scores of mood, energy and affect, as measured by the BFS and VAS. Results revealed there was no main effect of drug on mood, energy, or affect ($p>.05$) (Table S2). In order to assess any potential confounding effects of gender or order on mood, energy, and affect scores, gender and order were included in the analyses as independent variables. No main effects of gender or order, and no gender x drug or order x drug interactions were revealed, suggesting
that the order of drug condition and the gender of the participant did not have an effect on mood, energy and affect ratings.

Functional connectivity: placebo

The functional connectivity for the seed regions is described in Supplementary Table S3 for the placebo group alone (baseline). Overall, the patterns of connectivity associated with each of the seed regions are consistent with resting-state and functional connectivity experiments in healthy controls and obese patients or depressed patients (Anand et al., 2005; Greicius et al., 2007; Leh et al., 2007; Robinson et al., 2009; Sheline et al., 2010; Cauda et al., 2011; Kullmann et al., 2012).

Functional connectivity of the A-priori seeds:

Left amygdala seed

Compared to the placebo group there was reduced functional connectivity between the left amygdala seed and the left precuneus and the left posterior cingulate cortex (PCC), key areas constituting the Default Mode Network (DMN) in the THC\textsuperscript{v} group (Fig. 1). There was also decreased connectivity with the lateral occipital cortex but this did not meet the bonferroni correction.

There was also increased functional connectivity between the left amygdala seed and the dorsal anterior cingulate gyrus/premotor area, which overlaps with the executive control network, in the THC\textsuperscript{v} group (Table I, Fig. 2).

Functional connectivity for the exploratory seeds:

Right dmPFC seed

Compared to the placebo group there was an increased functional connectivity between right dmPFC seed and the right IFG/MFG, which overlaps with the right dorsal visual stream network in the THC\textsuperscript{v} group (Table I, Fig. 3).
Correlation with BMI

We found a positive correlation between the % BOLD signal change extracted from the precuneus (the area that correlated with the amygdala seed region) and BMI ($r = 0.649$, $p=0.003$) in the placebo group that was not present under THCv ($r = 0.38$, $p=0.1$) (Fig4) with the difference scores between Placebo and THC plotted in Fig 4b. Further when the outlier that was higher than 2 SD from the mean (% BOLD change 1.5) was removed from the placebo group the correlation with the BMI was still significant at $r = 0.591$, $p=0.01$. We did not find a significant correlation between the BMI and brain connectivity for any of the other seed ROIs.
**Discussion:**

Our study reveals that administration of a single THCv oral dose modulates resting-state functional connectivity in a double-blind placebo controlled design, in healthy volunteers despite no significant effects on mood. Specifically we found that resting-state functional connectivity was reduced between the left amygdala and parts of the DMN (left precuneus and the PCC) for the THCv group when compared with the placebo group.

Previous studies in obese individuals have revealed increased activity in DMN regions, specifically in the precuneus and PCC, when compared with healthy subjects (Tregellas et al., 2011). Further these brain areas have been highlighted in studies investigating obese versus lean individuals visual responses to food cues and tastes (DelParigi et al., 2005; Cornier, 2009) suggesting an involvement in feeding behaviour. Moreover, posterior cingulate cortex has been found to play a crucial role in switching spatial attention to targets of salient and motivational value i.e. directing attention to food stimuli in subjects experiencing hunger (Mohanty et al., 2008). Additionally, correlational analysis of the key nodes within the DMN revealed that the posterior cingulate cortex is involved in retrieving and integrating information from other subsystems implicated in self-referential thoughts and episodic memory (Hassabis et al., 2007; Buckner et al., 2008). Thus, increased activation of DMN in obese individuals has been suggested as a reflection of greater attention to internal states and memories influenced by past experience with food (Tregellas et al., 2011). **Further our reduced DMN connectivity under THCv might also be related to the reported safer side effect profile of THCv (less depression) as depressed patients have been found to have greater DMN connectivity (Sheline et al., 2010). Therefore it would be interesting to see if our result of decreased connectivity with the DMN under THCv could be replicated in obese patients and how this might relate to food intake and depression symptoms.**

We also found increased connectivity between the left amygdala and the dorsal anterior cingulate cortex extending into the supplementary motor cortex, part of the executive control network, as well as an increased connectivity between the dmPFC and the IFG/MFG, part of the dorsal visual stream network. The executive control network is consistently
recruited by cognitively demanding tasks of attention, working memory, and response
selection (Seeley et al., 2007). Specifically, the dorsal regions of PFC have been implicated in
response activation and inhibition as investigated by GO/NOGO tasks (Garavan et al., 2002).
Additionally, a study investigating neural activations during a food specific GO/NOGO task
in obese adolescents found a reduced activation in medial PFC when the participants were
required to inhibit proponent responses to food suggesting hypofunctioning of inhibitory
control in obese individuals (Batterink et al., 2010). Thus our results of increased connectivity
between the amygdala seed region and regions such as the anterior cingulate in the executive
control network under THCv might help us understand how, at a mechanistic level, THCv
could allow greater control over food intake.

Finding increased dmPFC connectivity with the IFG/MFG part of the dorsal visual
stream resting state network under THCv is interesting given that a recent study suggests that
just thinking about eating food (shown in images) increases activation in visual and prefrontal
cortical regions in females with anorexia nervosa (Brooks et al., 2012). The authors
concluded that such activations might underlie biases toward controlling food intake
commonly observed in individuals with anorexia nervosa (Brooks et al., 2012). Thus it is
possible that increased connectivity between dmPFC and visual networks under THCv might
further enable THCv as a treatment to control food intake. Although there are as yet no other
resting state studies on THCv in humans, there has been a study showing that the CB1
receptor agonist δ9-tetrahydrocannabinol, THC, increases functional connectivity between
the dmPFC and the right dorsal visual stream network (Klumpers et al., 2012) therefore
directly opposite to our findings but consistent as THCv is a neutral antagonist. Klumpers et
al discussed their findings with THC in relation to the known involvement of the dmPFC in
decision making and cognitive control with previous behavioural studies also showing how
THC can interfere with these processes.

Examining the relationship between BMI and brain results we found a significant
positive correlation between amygdala-precuneus connectivity under placebo but not under
THCv. This seems to indicate that as BMI increases, functional connectivity between these
regions increases, and that THCv removes this effect especially at the top end of the BMI (see Fig 4). However as our group were not obese individuals a future study relating a greater range in BMI and resting state functional connectivity with pharmacological treatment effects would be very interesting.

Thus, in conclusion our results show that THCv can modulate resting state functional connectivity in key networks such as the default mode network and the cognitive control network and this might be relevant to the development of THCv as an anti-obesity medication. Future studies are needed to examine the relationship between resting state functional connectivity after repeated treatment with THCv in overweight individuals and how this relates to control over food intake.
Acknowledgments

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References:


Legends:

Fig 1. Coronal, sagittal and axial slices showing amygdala seed region, functional connectivity maps for the difference between placebo and THCv groups, overlaid on the DMN. The right graph displays the % BOLD signal change extracted from the region of significant treatment effect precuneus: \([-8 -72 24 p = 0.0009]\) for the placebo group and THCv group.

Fig 2. Coronal, sagittal and axial slices showing amygdala seed region, functional connectivity maps for the difference between placebo and THCv groups, overlaid on the Executive Control Network. The right graph displays the % BOLD signal change extracted from the region of significant treatment effect: dACC \([6 4 52 p = 0.00001]\) for the placebo group and THCv group.

Fig 3. Coronal, sagittal and axial slices showing RdmPFC seed region, functional connectivity maps for the difference between placebo and THCv groups, overlaid on the Right Dorsal Visual Stream Network. The right graph displays the % BOLD signal change extracted from the region of significant treatment effect: IFG/MFG \([52 28 22 p = 0.003]\) for the placebo group and THCv group.

Fig 4. Graph of the % BOLD signal change extracted from the region of significant treatment effect: precuneus \([-8 -72 24 p = 0.0009]\) and correlated with BMI score for placebo and THCv.
Table 1: Resting state functional connectivity for brain seed regions and the effects of THCv.

<table>
<thead>
<tr>
<th>Brain region</th>
<th>MNI coordinates</th>
<th>z-score</th>
<th>Number of voxels</th>
<th>Significance (p-value)</th>
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<tbody>
<tr>
<td></td>
<td>X</td>
<td>Y</td>
<td>Z</td>
<td></td>
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<tr>
<td><strong>Placebo &gt; THCv</strong></td>
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<tr>
<td>L Amygdala seed</td>
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<tr>
<td>Precuneus</td>
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<td>-72</td>
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<tr>
<td>PCC</td>
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<td>-58</td>
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<td>2.74</td>
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<tr>
<td>Lateral Occipital Cortex</td>
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<td>-70</td>
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<td>4</td>
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<td><strong>THCv &gt; Placebo</strong></td>
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<tr>
<td>L Amygdala seed</td>
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<tr>
<td>dACC/Supplementary motor cortex</td>
<td>6</td>
<td>4</td>
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<tr>
<td>R dmPFC seed</td>
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<tr>
<td>IFG/MFG</td>
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<td>28</td>
<td>22</td>
<td>3.47</td>
</tr>
</tbody>
</table>

MNI, Montreal Neurological Institute; Gender was added as a covariate of no interest. All z values were cluster corrected for multiple comparisons (z>2.3, p<0.008) except*.
Figure 1
Figure 2
Figure 3
Figure 4

(a) Placebo $r = 0.642, p = 0.003$
(b) THCv $r = 0.38, p = 0.1$

Placebo minus THCv Difference score
$r = 0.244, p = 0.314$