

Delta-9-tetrahydrocannabinol reduces the performance in sensory delayed discrimination tasks. A pharmacological-fMRI study in healthy volunteers

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ABSTRACT

Background: Cannabis proved to be effective in pain relief, but one major side effect is its influence on memory in humans. Therefore, the role of memory on central processing of nociceptive information was investigated in healthy volunteers.

Methods: In a placebo-controlled cross-over study including 22 healthy subjects, the effect of 20 mg oral Δ^9 -tetrahydrocannabinol (THC) on memory involving nociceptive sensations was studied, using a delayed stimulus discrimination task (DSDT). To control for nociceptive specificity, a similar DSDT-based study was performed in a subgroup of thirteen subjects, using visual stimuli.

Results: For each nociceptive stimulus pair, the second stimulus was associated with stronger and more extended brain activations than the first stimulus. These differences disappeared after THC administration. The THC effects were mainly located in two clusters comprising the insula and inferior frontal cortex in the right hemisphere, and the caudate nucleus and putamen bilaterally. These cerebral effects were accompanied in the DSDT by a significant reduction of correct ratings from 41.61% to 37.05% after THC administration (rm-ANOVA interaction “drug” by “measurement”: $F(1,21) = 4.685$, $p = 0.042$). Rating performance was also reduced for the visual DSDT (69.87% to 54.35%; rm-ANOVA interaction of “drug” by “measurement”: $F(1,12) = 13.478$, $p = 0.003$) and reflected in a reduction of stimulus-related brain deactivations in the bilateral angular gyrus.

Conclusions: Results suggest that part of the effect of THC on pain may be related to memory effects. THC reduced the performance in DSDT of nociceptive and visual stimuli, which was accompanied by significant effects on brain activations. However, a pain specificity of these effects cannot be deduced from the data presented.

Introduction

Exogenous cannabinoid-based medications modulating neuronal signaling via activation of cannabinoid CB₁ and CB₂ receptors (Rodriguez de Fonseca et al., 2005) have been approved for several medical indications, including the treatment of pain (Di Marzo, 2006; Koppel et al., 2014; Lotan et al., 2014; Pacher et al., 2006). However, while preclinical evidence consistently supported an analgesic action of cannabinoids (Woodhams et al., 2017), findings from human experimental settings showed various cannabinoid effects, comprising a reduction of the affective but not sensory dimension of pain, moderate antinociceptive effects, and occasional hyperalgesic effects (Lötsch et al., 2017). Moreover, while controlled studies failed to show a robust

analgesic effect, in clinical settings it appeared that cannabis was in most cases associated with analgesia in open-label studies or retrospective reports, which may be compatible with an unspecific effect (Lötsch et al., 2017). Nevertheless, an expert committee found the available evidence sufficient to conclude that cannabinoids are an effective treatment in chronic pain, especially with a neuropathic component (Abrams, 2018).

The results of pharmacological functional magnetic resonance imaging (pharm-fMRI) studies suggest that a reproducible cannabis action in humans consists in a modulation of the central processing of the nociceptive input. Two independent studies showed that Δ^9 -tetrahydrocannabinol (THC) reduced the connectivity between brain areas frequently shown to be involved in the perception and processing of

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pain (Lee et al., 2013; Walter et al., 2016). However, subjective pain intensity is influenced by various factors such as aversive hedonic properties (Wiech et al., 2010), arousal (Ring et al., 2013), expectations (Keltner et al., 2006), attention bias (Tracey et al., 2002) or reward (Navratilova and Porreca, 2014). A descending modulatory network, including the periaqueductal gray (PAG), the frontal lobe, the anterior cingulate cortex (ACC), the insula and the amygdala (Tracey and Mantyh, 2007), is involved in these modulations. Attention possibly influences this system as activity in the PAG has been shown to be increased during distraction, as compared to paying full attention to painful stimuli (Tracey et al., 2002). This is supported by the finding of anatomical connections between cortical and brainstem regions (Hadjipavlou et al., 2006).

In line with various specific and unspecific modulators of pain, further consistent findings regarding central actions of cannabinoids were negative effects on memory, which became manifest either as a common side effect in humans (Ranganathan and DöSouza, 2006), reduction of the encoding of pictorial memories in humans (Bossong et al., 2012), or as an effect that reduced aversive memories in mice (Marsicano et al., 2002). Among established experimental paradigms to study effects on memory are delayed stimulus discrimination tasks (DSDT) (Rainville et al., 2004; Sahgal and Iversen, 1978; Terry et al., 1996), which have been widely used (321 findings in a PubMed search performed on October 25, 2017) and can be implemented with various sensory stimuli. DSDT involve the presentation of pairs of stimuli separated by an interstimulus interval (ISI) with the subsequent rating by the subject which stimulus was more intense. DSDT has proven its ability to study the encoding of stimulus intensity, the storage of this information in memory and the comparison with a second sensation (Rainville et al., 2004). An investigation on five regular marijuana users, applying a delayed matching task similar to DSDT which requires to match one of four differently shaded squares to a formerly presented square, showed impairment of delay-dependent but not delay-independent discrimination (Lane et al., 2005). To our knowledge, this has so far not been investigated for painful sensations.

In the present pharm-fMRI study the hypothesis was tested whether cannabinoid effects on pain processing involve an inhibition of memory of sensory perception. For this purpose, pain stimuli of three different strengths were applied in a DSDT ("pain-DSDT"). To control whether the THC influence on discrimination performance is pain-specific, a second DSDT involving visual stimuli consisting of 5-, 6- or 7-edged polygons was conducted ("visual-DSDT"). The rationale is that an exclusive THC effect on pain-DSDT only, would support the analgesic properties of THC rather than an unspecific influence of memory.

Methods

Subjects, medications and study design

The present study was part of a complex research project addressing the effects of THC on the processing of different sensory stimuli, including nociceptive and olfactory stimuli (Walter et al., 2011). The observed effects of THC on the central processing of single nociceptive stimuli have been reported previously (Lötsch et al., 2013; Walter et al., 2016), as well as the effects on olfactory stimuli (Walter et al., 2017a). In the present report, the focus is on the DSDT experiment. The study followed the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Approval from the Ethics Committee of the Medical Faculty of the Goethe-University, Frankfurt, Germany (reference number 334/08), and written informed consent from each participating subject were obtained.

Twenty-two subjects (11 male; age 26.1 ± 2.9 years (mean \pm standard deviation); all within $\pm 10\%$ of their ideal body weight) were enrolled for the pain-DSDT experiment. Thirteen (6 male; age 25.5 ± 2.3 years (mean \pm standard deviation)) of these subjects additionally took part in control experiments involving visual stimuli

(visual-DSDT). The sample size was adapted from two studies on cannabis effects in human experimental settings (13 and 12 subjects (Naef et al., 2003; Roberts et al., 2006)) and in particular from a similarly designed two-way cross-over fMRI study on THC effects on pain (15 healthy subjects (Lee et al., 2013)). The described experiments were part of a broader project involving several tasks (Walter et al., 2016, 2015). The remaining nine subjects participated in a different task, thus leaving a smaller but still sufficient sample size for the control experiment. The subjects' status of good health was assessed via medical history, physical examination including vital signs, and routine clinical laboratory tests (red and white blood cell count and basic clinical chemistry parameters including creatinine, urea, albumin, total bilirubin, alanine aminotransferase, aspartate aminotransferase, and γ -glutamyl-transpeptidase). All subjects except one were non-smokers. The subjects confirmed no actual or past use or abuse of cannabis. On each study day, before starting the actual experiments, a urine drug screening was performed to detect carry-over effects or illicit cannabis consumption (THC, opiates, cocaine metabolites, amphetamines at baseline; Mahsan-Kombi/DOA 4-Test, MAHSAN Diagnostika Vertriebgesellschaft mbH, Reinbeck, Germany). Further screening methods such as analysing hair for traces of THC and its metabolites (Franz et al., 2018) were not applied.

Employing a randomized, placebo-controlled, double-blinded two-way crossover study design, subjects received 20 mg THC (two capsules containing each 10 mg THC dissolved in Adeps solidus, manufactured by the University Hospital Pharmacy Heidelberg, Germany) and placebo (mere Adeps solidus) orally, separated by a washout interval of at least four weeks. Prior to the experiments, drug intake (except contraceptives) was prohibited for one month, alcohol consumption for 24 h and food for 6 h. The actual fMRI measurements took place prior to (baseline) and 2 h after Δ^9 -THC or placebo administration (Fig. 1), when maximum THC effects were expected according to reported time courses of plasma concentrations in humans (Hollister et al., 1981), which was verified as part of the complex local research project and reported separately (Walter et al., 2013). Baseline measurements always started before 10 a.m. to account for possible circadian variability in regional cerebral blood flow (Hodkinson et al., 2014), pain perception (Glynn and Lloyd, 1976) and THC effects (Abel, 1973).

Stimulation procedures and DSDT paradigms

Nociceptive stimulation was achieved by using a chemosensory pain model (Kobal, 1985) which has a history of more than 30 years of continuously successful use in pharmacological pain studies, starting from 1985 (Kobal, 1985). It is based on the application of short pulses of gaseous CO₂ (500 ms, 75% v/v) to the subject's nostril by means of an olfactometer (OM/2, Burghart Messtechnik GmbH, Wedel, Germany). CO₂ stimuli were always applied to the right nostril as increased brain activity has been reported following stimulation of the right rather than the left nostril (Hari et al., 1997). The pulses were embedded in a constantly flowing airstream (8 l/min) with controlled temperature (36.5 °C) and humidity (80% relative humidity) to avoid concomitant excitation of thermal or mechanical sensors (Kobal, 1985). The olfactometer contains electronic mass flow controllers and electromagnetic valves to ensure stable stimulation conditions throughout a study. This was verified via weekly measurements of the CO₂ concentrations delivered by the device for each stimulus class, using a CO₂ meter (Siemens Ultramat 23, Siemens, Erlangen, Germany).

In the pain-DSDT experiment, 60 pairs of CO₂ stimuli were administered. One stimulus (either the first or the second) of each pair always had a CO₂ concentration of 65% v/v, the other was either identical (65% v/v), lower (55% v/v) or higher (75% v/v). All concentrations were above the pain thresholds which had previously been determined individually for each subject and were found to be below 50% v/v (Lötsch et al., 1997; Oertel et al., 2008). The resulting three conditions (i.e. stimulus 1 is weaker, equal, or stronger than stimulus 2)

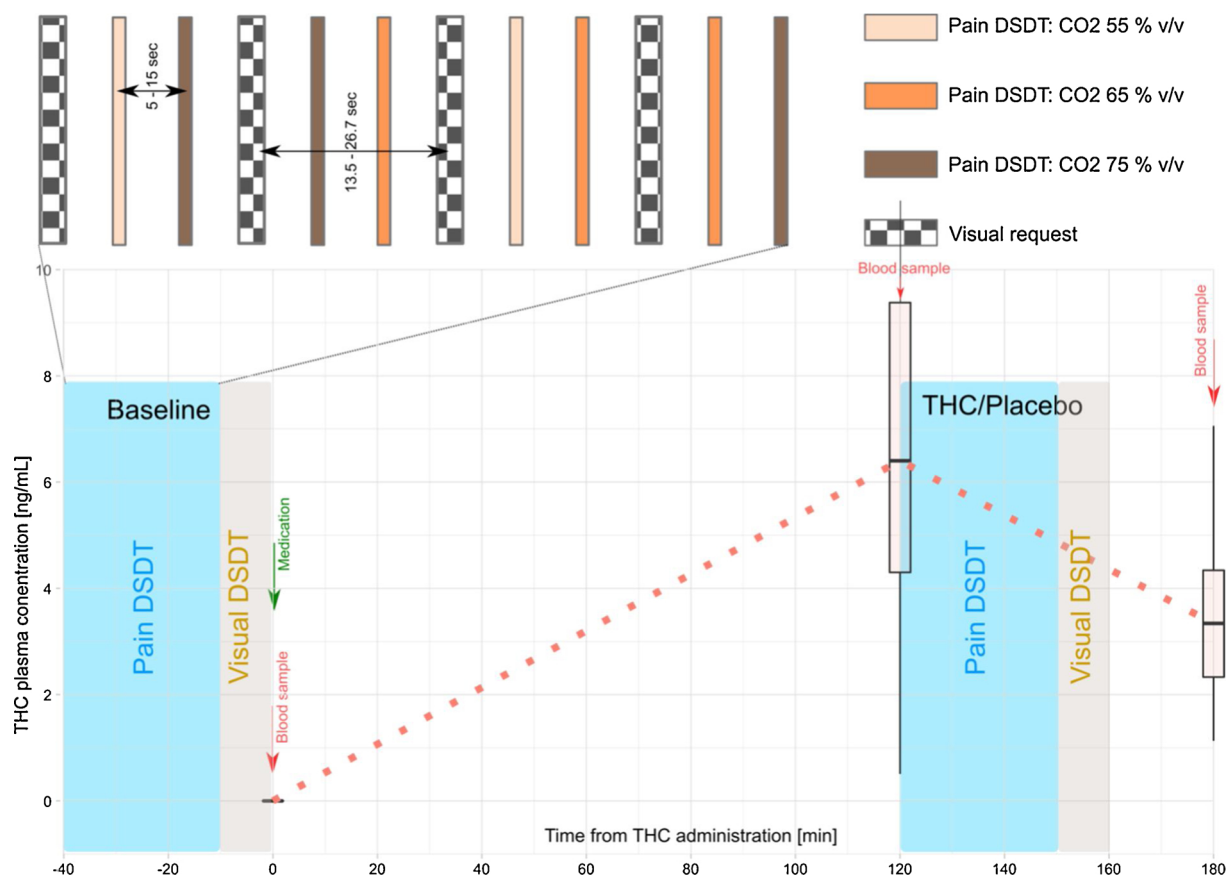


Fig. 1. Schematic representation of the experimental design during a study day. The time axis at the bottom relates the timing of the different study procedures to the time-point of medication administration (at time-point zero). A baseline measurement was performed before administration of the medication and the post-drug measurement at 2 h when effective plasma and brain concentration were expected based on prior observations (Hollister et al., 1981). The THC plasma concentrations observed in the present study cohort are shown as boxplots, connected with a dotted line for visual guidance. At the top of the figure a detail of the pain stimulation paradigm is shown. The visual control task was similar, except for the stimuli which consisted in the presentation of polygons with 5, 6 or 7 edges.

were equally often presented. The delay between the first and the second stimulus varied randomly between 5.54 s and 15.96 s (mean: 11.01 s), while the interval between pairs was ranged from 8.84 s to 25.6 s (mean: 15.63 s). The subjects were asked to rate the painfulness of the second stimulus of each pair in relation to the respective first stimulus by pressing one of three different buttons (stimulus 2 is more, equally or less painful than stimulus 1; displayed in random order). The question appeared about 5.4 s (mean value) after the second stimulus and disappeared with the subject's button press. The detailed experimental set-up is shown in Fig. 1.

A non-comparing condition was not included for the following reason: A non-comparing condition had been analyzed previously in the same laboratory setting (Lötsch et al., 2012) where results showed that it was virtually impossible to avoid comparing two successive stimuli in a follow-up experiment if a previous experiment had involved this task, even if subjects were explicitly requested to omit comparisons. Therefore, the inclusion of a non-comparing condition would have required a parallel-group study design as used previously (Lötsch et al., 2012), colliding with the present randomized cross-over design.

However, to control for effects of THC on the comparison of successive pain stimuli that are not pain specific, a visual DSDT was additionally performed after the nociceptive task. Visual stimuli consisted of white polygonal shapes with a different number of edges that were projected for 500 ms in the center of a black screen using the "Presentation" software (Neurobehavioral Systems, Albany, USA). Sixty pairs of polygons were shown at intervals of 2.54 – 4.48 s (mean: 3.51 s) between the two polygons of a pair. Intervals of 7.45 – 24.5 s (mean: 9.89 s) were observed between the pairs. One polygon within a

pair had always six edges while the other had 5, 6 or 7 edges. Approximately 5 s (mean value) after presentation of the second polygon of each pair, subjects were visually requested to rate whether polygon #2 had more, as many as or less edges than polygon #1.

Acquisition of functional magnetic resonance images

An event-related design was used for fMRI data acquisition. The blood oxygenation level-dependent (BOLD) response following each stimulus was recorded at a field strength of 3 T on a dedicated head scanner (Siemens Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany), equipped with a 4-channel transmit-receive head coil. To reduce motion artifacts, the subject's head was immobilized using foam pads. For acquisition of fMRI data, a T_2^* -weighted gradient-echo (GE) echo-planar imaging (EPI) sequence with the following parameters was used: TR = 2048 ms, TE = 30 ms, flip angle = 90°, echo spacing = 420 μ s, matrix size = 64 × 64, in-plane resolution = 3 × 3 mm². A total of 900 and 460 volumes for the pain- and visual DSDT, respectively, was acquired, each of which comprised 32 slices with 3 mm thickness and an inter-slice gap of 1 mm, acquired in descending order; the first five volumes of each scanning block were discarded to ensure fMRI steady-state conditions.

For subsequent off-line correction of distortions in the EPI images due to inhomogeneities of the static magnetic field B_0 (Andersson et al., 2001; Hutton et al., 2002), magnetic field mapping was performed via GE imaging with identical geometric parameters and two different TE values (4.89 and 7.35 ms) from which magnitude images and a phase difference map were calculated directly on the scanner. In addition, a

T₁-weighted anatomical data set with 1 mm isotropic resolution was acquired for each subject, using a three-dimensional (3D) magnetization prepared rapid acquisition of gradient echoes (MP-RAGE) (Mugler and Brookeman, 1991) sequence with the following parameters: TR = 2200 ms, TE = 3.93 ms, flip angle = 9°, TI = 900 ms, FOV = 256 × 256 mm², single slab with 160 sagittal slices, isotropic spatial resolution of 1 mm, using parallel acquisition (GeneRalized Autocalibrating Partial Parallel Acquisition, GRAPPA (Griswold et al., 2002)) with an acceleration factor of 2 in phase encoding direction and a duration of 4 min.

Data analysis

Analysis of THC effects on DSDT performance

Psychophysical data were analyzed using the software packages SPSS (version 19 for Windows/Linux, IBM Corp, Armonk, NY, USA; <https://www.ibm.com/analytics/spss-statistics-software>) and R (version 3.4.4 for Linux; <http://CRAN.R-project.org/> (R Development Core Team, 2008)). The percentage of correctly compared nociceptive and visual stimulus pairs per session was submitted to a repeated-measurement analysis of variance (rm-ANOVA), using a 2 × 2 design with the factors “drug” (i.e., THC or placebo; degrees of freedom (df) = 1) and “measurement” (i.e., baseline or following administration of THC/placebo; df = 1). The α level was set at 0.05 and corrected for post-hoc t-tests (Student, 1908) according to Bonferroni (Bonferroni, 1936).

The influence of THC on delay-dependent memory of nociceptive and visual discrimination was assessed using a so-called “forgetting function” (Lane et al., 2005) in which the performance in the pain discrimination task was calculated as $\text{logit}(p) = \log[p / (1 - p)]$, where p denotes the proportion of correct identifications of the stronger stimulus (Lane et al., 2005). Logit p values were calculated for four different interstimulus intervals (6.66, 8.67, 13.38, 15.42 s and 2.77, 3.27, 3.76, 4.24 s for the pain and visual discrimination task, respectively) in a stimulus pair and fitted to the equation (Lane et al., 2005), where t corresponds to time of the individual response delays and the parameters a and b represent an index of initial discriminability and the rate of forgetting, respectively. To avoid negative values of p , data were transformed as $\text{logit}(p) + 2$. After the fitting, the parameter b (delay-dependent discriminability) was submitted to rm-ANOVA as above.

Analysis of fMRI data

The acquired fMRI data were spatially preprocessed using the statistical parametric mapping software SPM8 (Wellcome Department of Imaging Neuroscience, London, UK; <http://www.fil.ion.ucl.ac.uk/spm/software/spm8/> (Friston et al., 1995; Worsley and Friston, 1995)), implemented in Matlab version 7.5.0.342 (2007b; Mathworks, Natick, MA, USA). Data were realigned to the first volume to correct for subject motion and unwarped, using the respective field map. The high-resolution T₁-weighted anatomical image was co-registered to the mean-EPI (created during the realign and unwarped process), segmented and normalized using 4th-degree B-spline interpolation to obtain image voxel sizes of 3 × 3 × 3 mm³. The resulting spatial normalization parameters were applied to the volumes of the EPI-sequence that were subsequently smoothed with an isotropic 9 mm full-width-half-

maximum Gaussian kernel.

A general linear model was used to partition the observed neurophysiological responses into components of interest, confounds, and errors. Events were sorted into several regressors as a function of the trial component with all four scanning sessions of the two study days included in one model. An event-related analysis estimated the BOLD-response evoked by the pain and visual stimuli by modeling them as separate delta functions convolved with the canonical hemodynamic response function as implemented in SPM8. Each stimulus was included as an event with duration 0. The visual request for stimulus rating and the subsequent button-press were modeled within the design matrix but omitted from flexible factorial second-level analysis according to the instructions given in (Gläscher and Gitelman, 2008). Furthermore, the six rotational and translational parameters from the rigid body transformation, obtained during image realignment and unwarping, were modeled as covariates of no interest. Low-frequency fluctuations of the MR signal were removed with a high pass filter with a cut-off at 128 s.

The resulting parametrical maps of T statistics from the second-level analyses were interpreted regarding the probabilistic behavior of Gaussian random fields (Worsley, 1994). Results were deemed significant at a p-value < 0.05 (FWE-corrected; p-value chosen according to the default value of the SPM MATLAB toolbox) at cluster-level with a cluster size threshold of 5 voxels. The localization of brain activation was aided by the Anatomical Automatic Labeling toolbox (Tzourio-Mazoyer et al., 2002). Significant cluster activations are reported as Montreal Neurological Institute (MNI) coordinates, specifying the distance (in mm) from the anterior commissure in x (right to left), y (anterior to posterior) and z (top to bottom) directions.

The sensory stimuli were analyzed by adding two first-order parametric regressors to the model, each modulating the stick function and orthogonalized with respect to the prior to account either for the CO₂ concentration (55, 65, 75% v/v) or for polygon edge count (5, 6, 7) according to the sensory conditions. In addition, the stimulus number within a stimulus pair (#1, #2) was included as a further parameter. This implemented a step-down regression that allowed categorizing the stimulus-related effects (i) in activations and deactivations occurring with each stimulus irrespective of its intensity and stimulus number, (ii) activations or deactivations depending on the stimulus intensity or edge count irrespective of stimulus number, (iii) activations or deactivations exclusively related to the stimulus number within a stimulus pair.

To explore unspecific modulations of the memory tasks by THC induced side effects, the percentage signal change associated with the nociceptive and visual stimuli, respectively, were calculated for a 5 mm spherical search volume around peak coordinates of the THC effects, using the “rfxplot” MATLAB toolbox (Gläscher, 2009). The extracted differences in brain activation due to THC administration, controlled for the placebo effect, were non-parametrically correlated with the corresponding differences in side effect ratings by calculating Spearman’s ρ (Spearman, 1904).

Results

All 22 participants completed the study without experiencing side effects requiring medical intervention. Following THC administration,

Table 1

Results of the rm-ANOVA tests of the VAS ratings of side effects. A 2 × 2 design was used, with the factors “drug” (i.e., THC or placebo; degrees of freedom (df) = 1) and “measurement” (i.e., baseline or following administration of the medication; df = 1).

Side effect	Main rm-ANOVA effect “drug”			Main rm-ANOVA effect “measurement”			Interaction “drug” by “measurement”		
	df	F-values	p-value	df	F-values	p-value	df	F-values	p-value
Tiredness	1,21	8.089	0.01	1,21	3.092	0.093	1,21	3.608	0.071
Sickness	1,21	10.738	0.004	1,21	7.64	0.012	1,21	8.987	0.007
Drowsiness	1,21	56.378	2.24 · 10 ⁻⁷	1,21	31.624	0.000014	1,21	52.529	3.86 · 10 ⁻⁷
Euphoria	1,21	5.135	0.034	1,21	6.254	0.021	1,21	4.133	0.055

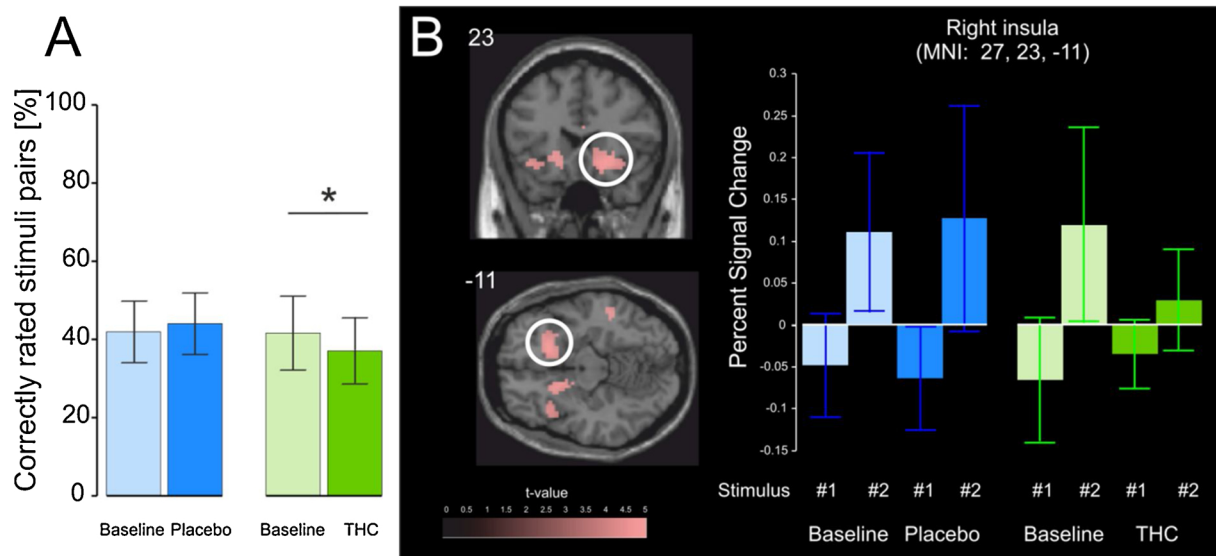


Fig. 2. THC effect on the subjects' performance in a delayed discrimination task of pain stimuli and the corresponding stimulus-related brain activations. **A:** Bar plot showing means and standard deviations of the number of correctly rated stimulus pairs, which decreased significantly after THC administration (rm-ANOVA interaction for "drug" by "measurement": $p = 0.042$). **B:** Differences in brain activations between stimulus #2 and stimulus #1 (SPM interaction "drug" by "measurement" in a 2×2 factorial design, contrast -1 1 1 -1, in the succession placebo-baseline, placebo post-drug, THC baseline, THC post-drug session, respectively). Reduced activations following THC administration included the right insula, right inferior frontal cortex and caudate nucleus and putamen bilaterally. The localizations of differences in brain activation are superimposed on the canonical MR template implemented in SPM8. Voxels are shown at a threshold of $p < 0.001$ (FWE-uncorrected, $t > 3.22$). The bar plots (right) indicate the signal change (mean and standard deviation) for the activation intensities of the parameter "stimulus number" (split into stimulus 1 and stimulus 2) at coordinates with highest voxel level t value within the largest cluster. Coordinates are presented in MNI space. Lateral (x), anterior (y) and superior (z) stereotactic coordinates (in millimeters) are relative to midline, anterior commissure and commissural line, respectively (positive values are right, anterior and superior). * $p < 0.05$ (interaction "drug" by "measurement").

significant increases in fatigue, nausea, drowsiness, and euphoria were observed as reported previously (Walter et al., 2017b). Detailed results of the rm-ANOVA tests are shown in Table 1. Plot of the changes in VAS ratings during the experiments are shown as profile plots in the Supplemental Figures.

THC effects in a pain discrimination task

The percentage of correct ratings (stimulus 2 is more, equally or less painful than stimulus 1) of the 60 stimulus pairs was reduced following THC administration, amounting to 37.05% ($\pm 8.45\%$), as compared to 41.61% ($\pm 9.46\%$) correct identifications before THC administration. The percentage increased from 41.92% ($\pm 7.88\%$) to 44.01% ($\pm 7.88\%$) after placebo administration. This decrease in pain stimulus intensity discrimination performance was statistically significant (rm-ANOVA interaction "drug" by "measurement": $F(1,21) = 4.685$, $p = 0.042$; main effect "drug": $F(1,21) = 5.943$, $p = 0.024$; main effect "measurement": $F(1,21) = 0.639$, $p = 0.433$; Fig. 2). The same analysis was performed separately for the subgroup of 13 subjects who participated in the visual discrimination task. Significance was not reached in this separate group (rm-ANOVA performed as above, interaction "drug" by "measurement": $df = 1,12$, $F = 2.349$, $p = 0.151$; main effect "drug": $F(1,12) = 0.316$, $p = 0.449$; main effect "measurement": $F(1,12) = 4.68$, $p = 0.051$). The THC induced reduction of the number of correct identifications did not depend on the length of the interval between the two stimuli as indicated by the lack of a significant statistical interaction "drug" by "measurement" for the delay-dependent parameter b , as obtained by fitting the equation ($p > 0.898$).

The THC-induced performance decrease in discriminating pairs of CO₂ stimuli with different strengths was accompanied by changes in the brain activation patterns observed during the discrimination task. Overall, the administration of THC was associated with a general reduction of the brain activations observed during the delayed sensory discrimination task (Fig. 3). Specifically, the stronger brain activations following the second as compared to the first stimulus while performing

the task were reduced in a similar way as in a previous study under the non-comparing condition, i.e., when a separate cohort of subjects received two successive CO₂ stimuli without being requested to compare their intensity (Lötsch et al., 2012). The THC effects were mainly located in two clusters comprising the right insula, right inferior frontal cortex and the caudate nucleus and putamen bilaterally (peak MNI coordinates $x = 24$, $y = 23$, $z = -8$; $t > 4.64$, $p = 0.032$, cluster-threshold FWE-corrected, for the factorial SPM analysis of the 2×2 matrix; Fig. 2, Table 1). However, no significant changes in activations were observed in association with single pain stimuli (SPM interaction "drug" by "measurement" for pain stimulus) or related to intensity (SPM interaction "drug" by "measurement" for stimulus intensity).

The percentage signal change associated with the nociceptive stimuli was calculated at peak coordinates of the two main clusters showing a THC effect on the difference in brain activations between the second and the first stimulus of a pair (MNI coordinates $x = 24$ mm, $y = 23$ mm, $z = 8$ mm and $x = -12$ mm, $y = 11$ mm, $z = -11$ mm) (Table 1). The extracted differences in brain activation due to THC administration, controlled for the placebo effect, were not correlated with the corresponding differences in side effect ratings. Significant correlations (Fig. 4) were only obtained between the signal change in the brain ($\rho = 0.867$, $p = 3.57 \cdot 10^{-7}$) and between euphoria and drowsiness ($\rho = -0.439$, $p = 0.041$).

THC effects in a visual DSDT

The percentage of correct identifications of the more complex polygon in the visual delayed discrimination task was reduced from 69.87% ($\pm 9.04\%$) at baseline to 54.35% ($\pm 13.08\%$) 2 h after THC administration, whereas performance increased from 65.38% ($\pm 15.0\%$) at baseline to 67.41% ($\pm 9.96\%$) after placebo administration (rm-ANOVA interaction of "drug" by "measurement": $F(1,12) = 13.478$, $p = 0.003$, main effect "measurement": $F(1,12) = 14.179$, $p = 0.003$; main effect "drug": $F(1,12) = 3.505$, $p = 0.086$; Table 2 and Fig. 5). The reduced percentage of correct identifications of the

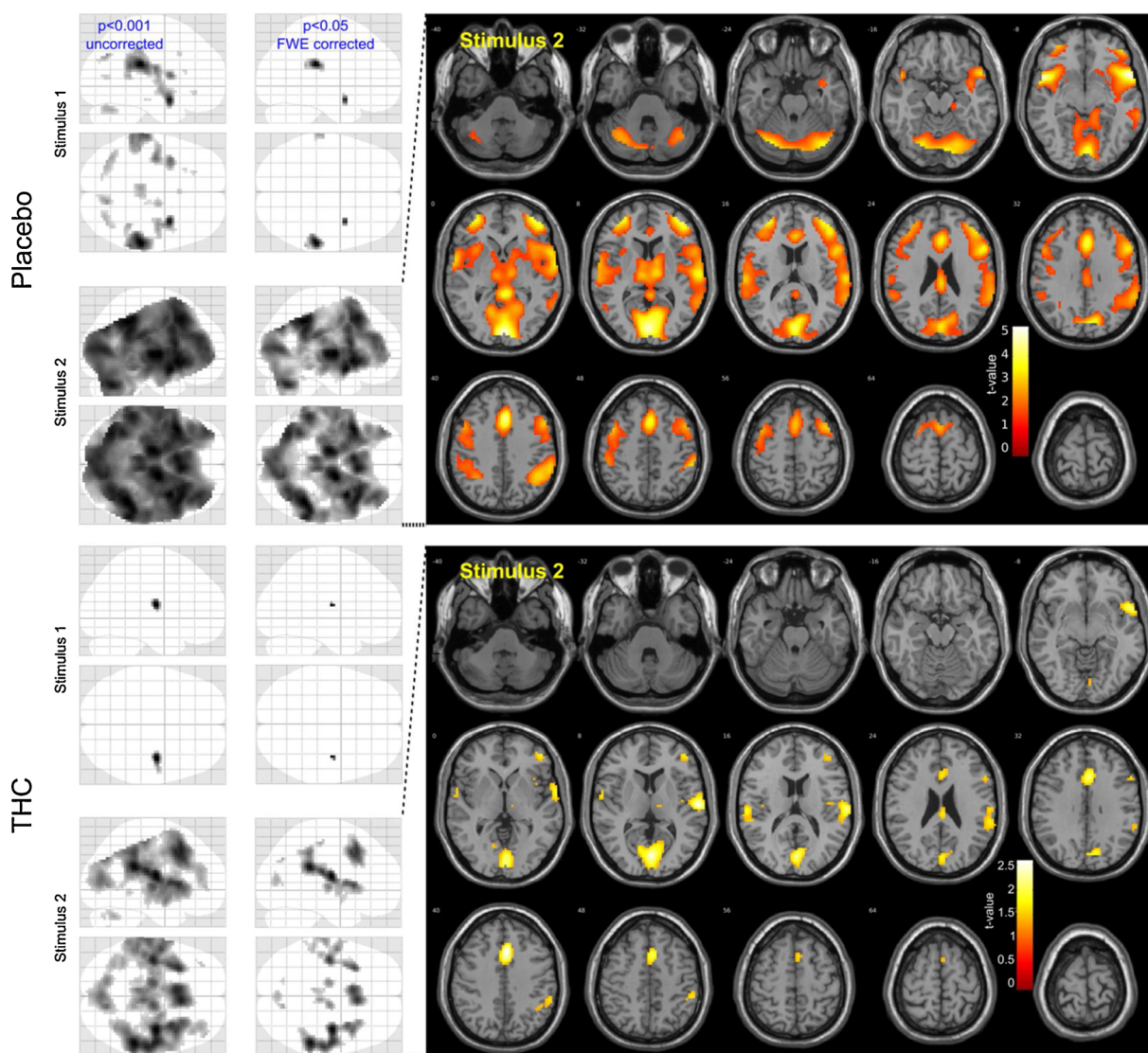


Fig. 3. Brain regions with significant activations associated with pain stimulus 1 or 2. More extended activations associated with stimulus #2 as compared to those associated with stimulus #1 were observed during the placebo condition (upper part). This difference was considerably reduced during the THC condition where the second stimulus evoked comparatively less enhanced activations than during the placebo condition. The results of a *t*-test are shown as a glass brain representation (left). Activations are shown both at a threshold of $p < 0.001$ with uncorrected α level and at a threshold of $p < 0.05$ with FWE-corrected α level. Right: the activations associated with the second stimulus are shown superimposed on axial slices of a canonical MR template. The significance at voxel level is color coded from red to light yellow with increasing *t* values, at a threshold of $p < 0.05$ (FWE-corrected). The figure was created using the SPM12 Matlab toolbox (<http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>; Wellcome Department of Imaging Neuroscience, London, UK (Friston et al., 1995; Worsley and Friston, 1995)) and the xjView Matlab toolbox (<http://www.alivelearn.net/xjview>).

more complex polygon in pairs of visual stimuli after THC administration was independent of the length of the interval between two successive stimuli. Specifically, when calculating the performance in the discrimination task as $\text{logit } p = \log [p / (1 - p)]$, with p denoting the proportion of correct identifications, for different interstimulus intervals t and fitting them to the exponential function, there was no significant effect of THC on the delay-dependent parameter b (interaction “drug” by “measurement” $p > 0.65$).

The fMRI data related to the visual stimuli were analyzed in a similar way as the fMRI data related to the pain stimuli, replacing the three CO_2 concentrations with the number of edges of the three different polygons. The cortical correlates of the THC effects in the visual-DSDT (Table 3) consisted in a reduction of stimulus associated deactivations in two clusters, comprising bilaterally the angular gyrus, middle occipital gyrus, middle temporal gyrus, the right supramarginal gyrus and the inferior parietal lobule (peak MNI coordinates $x = -42$, $y = -64$, $z = 22$; $t > 4.83$, $p = 0.048$ and $x = 48$, $y = -70$, $z = 28$;

$t > 4.73$, $p = 0.018$, cluster-threshold FWE-corrected, for the flexible factorial SPM analysis of the 2×2 matrix with the contrast 1 -1 -1 1; denoting interaction “drug” by “measurement”; Fig. 5). This THC effect applied equally to activations associated with the first and second stimulus of one pair, while no significant changes in activations were observed in association with stimulus complexity (SPM interaction “drug” by “measurement” for edge count) and stimulus number (SPM interaction “drug” by “measurement” for stimulus number).

Discussion

In this study, a delayed stimulus discrimination task involving nociceptive stimuli (pain-DSDT) was employed to address a memory component of the effects of THC on the perception and processing of nociceptive input. To control for a pain-specific effect, a second DSDT with visual stimuli (visual-DSDT) was performed. THC influenced the performance in a task where pain intensities in pairs of pain stimuli

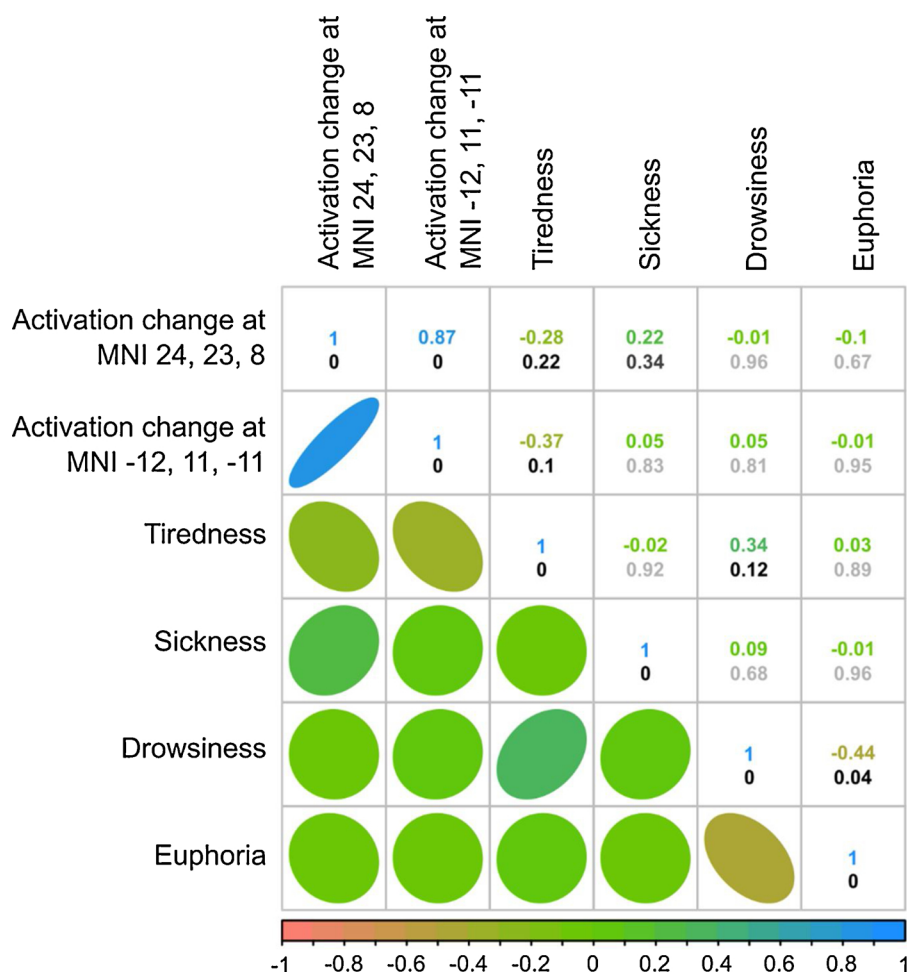


Fig. 4. Explorative analysis of the correlations between brain activations in the center of the two main clusters showing a THC effect on the difference in brain activations between the second and the first stimulus of a pair and THC induced side effects. Bottom left: Correlations are shown as ellipses, with the direction toward positive (upwards) or negative (downwards) correlations, and colored according to the color code of Spearman's ρ (Spearman, 1904) shown at the bottom of the panel. The narrower the ellipses are, the higher was the correlation. Top right: Correlations are provided numerically as values of Spearman's ρ (colored). The p-values are shown in grey to black numbers below the correlation coefficients; "0" indicates $p < 1 \cdot 10^{-5}$. The figure has been created using the R software package (version 3.4.4 for Linux; <http://CRAN.R-project.org/> (R Development Core Team, 2008)) and the library "corrplot" (<https://cran.r-project.org/package=corrplot> (Wei and Simko, 2017)).

were compared. While the percentage of correct ratings without THC was similar to a previous DSDT based study (Lötsch et al., 2012), reflecting the intended difficulty of the task, it significantly dropped by 4.56% to 37.05% after THC administration. This deterioration in performance was accompanied by a reduction in pain stimulus-associated brain activations. When considering that the DSDT paradigm is frequently used to study working memory (Pasternak and Greenlee, 2005; Sahgal and Iversen 1978; Terry et al., 1996), the presented results indicate that the working memory contributes to the effects of THC on the processing and perception of pain stimuli. However, effects of THC on memory encoding and recall have been reported previously for visual stimuli (Bossong et al., 2012), which challenges the assumption that the

presented observations are pain-specific. In spite of a smaller sample size, THC had a significant effect on discrimination performance of the visual stimuli, similar to the observed THC effect in the pain stimulus experiment. Both in pain and in visual sensory perceptions, the THC effects observed in this study exceeded in strength results reported in a previous study where THC was shown to alter the brain activations related to a memory task while the task performance itself remained unchanged (Bossong et al., 2012). A possible explanation may be a dose-effect, as in the cited study the oral THC dose was only 9 mg in total.

During DSDT tasks, changes in brain activations have been observed both inside and outside of the sensory areas primarily involved in the

Table 2

Clusters of brain regions where the parameter stimulus number (i.e. the contrast stimulus 2 > stimulus 1) was specifically associated with less activation during the THC condition in the post-drug session. Interaction "drug" by "measurement" in a 2 x 2 flexible factorial design, contrast -1 1 1 -1 in the succession placebo baseline session, placebo post-drug session and THC baseline session, THC post-drug session, respectively. Results reflect a 22-subject analysis. Voxels are given at a threshold of $P < 0.001$ (uncorrected; $t > 3.22$, Cluster size threshold 5 voxel). Coordinates are reported in MNI space [mm].

Brain regions within the cluster	Number of voxel in cluster	Peak coordinates			t value of peak coordinates
		x	y	z	
Right caudate/putamen/insula/inferior orbital frontal gyrus/rectal gyrus/superior frontal orbitalgyrus/olfactory bulb	130	24	23	-8	4.64*
		15	17	1	3.79
Left putamen/caudate/pallidum/rectal gyrus/olfactory bulb/superior frontal orbital gyrus	119	-12	11	-11	3.84*
		-15	8	4	3.34
Left inferior frontal orbita gyrus/insula	22	-36	20	-11	3.57
Right middle temporal gyrus/inferior temporal gyrus	16	54	-37	-11	3.50
Left/right anterior cingulate	43	6	32	16	3.33
		-6	26	22	3.21

* $p < 0.05$ FWE-corrected at cluster-level.

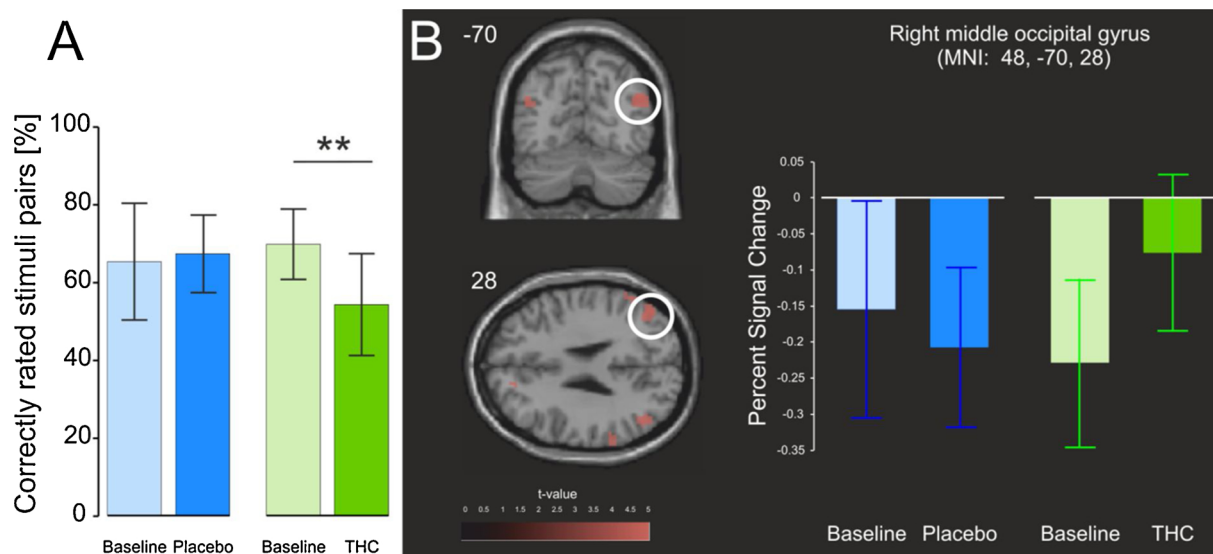


Fig. 5. THC effect on the subjects’ performance in a delayed discrimination task of polygons with different numbers of edges and the corresponding stimulus-related brain deactivations obtained from 13 subjects. A: The number of correctly rated visual stimulus pairs (means and standard deviations) decreased significantly after THC application (interaction “drug” by “measurement”: $p = 0.003$). B: THC evoked significantly less deactivation following visual stimulus presentation in brain regions consistently showing decreased activity in response to external stimuli (Default Mode Network) (interaction “drug” by “measurement” in a 2×2 factorial design, contrast 1 -1 -1 1, in the succession placebo-baseline, placebo post-drug, THC baseline, THC post-drug session, respectively). The localization of differences in brain activation are superimposed upon the canonical MR template implemented in SPM8 (red). Voxels are shown at a threshold of $p < 0.001$ (FWE-uncorrected, $t > 3.33$). The percentage of signal change for the visual stimuli shows the deactivation induced by polygon presentation, irrespective of the number of edges and sequence number. Coordinates are presented in MNI space. Lateral (x), anterior (y) and superior (z) stereotactic coordinates (in millimeters) are relative to midline, anterior commissure and commissural line, respectively (positive values are right, anterior and superior). The significance at voxel level is color coded from black to red for decreased deactivation with increasing t-values.

processing of particular sensory input such as visual, acoustic or tactile stimuli (Pasternak and Greenlee, 2005). In the present study, this is reflected by the observation of extended brain activations associated with the second stimulus of a pair, during the placebo condition, similar to previous observations of comparatively more extended brain activations when nociceptive input is associated with a comparison task (Albanese et al., 2007; Lobanov et al., 2013; Lötsch et al., 2012; Oshiro et al., 2007, 2009). Evidence from recent fMRI studies in humans support the assumption of distinct pathways being activated during pain discrimination tasks, in contrast to brain activation patterns during the mere perception of pain stimuli without a comparison context

(Lobanov et al., 2013; Oshiro et al., 2007, 2009). As an example, the orbitofrontal cortex has been shown to be involved in pain processing, being rather engaged in decision-making about pain and attentional processing than in the evaluation of sensory aspects (Lorenz et al., 2003; Winston et al., 2014). Moreover, altered functional connectivity of the dorsolateral prefrontal cortex was shown to be associated with THC induced analgesia in neuropathic pain patients, demonstrating that THC alters pain perception (Weizman et al., 2018).

THC reduced the increased brain activations associated with the second stimulus in a pair of nociceptive stimuli. This effect was distributed across several brain areas. From a physiological point of view,

Table 3

Clusters of brain regions where the polygon-induced deactivation was specifically less pronounced in the post-medication session after THC administration. Interaction “drug” by “measurement” in a 2×2 factorial design, contrast 1 -1 -1 1 in the succession placebo baseline session, placebo post-drug session and THC baseline session, THC post-drug session, respectively. Results reflect a 13-subject analysis. Voxels are given at a threshold of $P < 0.001$ (uncorrected; $t > 3.33$, Cluster size threshold 5 voxel). Coordinates are reported in MNI space [mm].

Brain regions within the cluster	Number of voxel in cluster	Peak coordinates			t value of peak coordinates
		x	y	z	
Right insula/inferior frontal orbital gyrus/superior frontal orbital gyrus/ rectal gyrus	25	24	20	-17	5.15
Left angular gyrus/middle temporal gyrus/middle occipital gyrus	61	-42	-64	22	4.83*
Right angular gyrus/middle occipital gyrus/middle temporal gyrus/supramarginal gyrus/ inferior parietal lobule	80	48	-70	28	4.73*
Left parahippocampal gyrus/ hippocampus/olfactory bulb/ hippocampus	31	-9	5	-20	4.52
Left fusiform gyrus /parahippocampal gyrus	8	-33	-28	-20	4.14
Left anterior cingulate/medial superior frontal gyrus	19	-12	41	16	3.96
Left inferior frontal orbital gyrus	13	-54	26	-8	3.88
Right/Left middle cingulate	12	9	-37	34	3.71
		-3	-43	34	3.47
Left supramarginal gyrus	9	-60	-37	28	3.68
Right superior temporal pole	6	42	11	-26	3.62
Left middle cingulate	9	-6	-31	40	3.61
Left superior frontal gyrus	6	-12	44	28	3.58
Left middle frontal orbital gyrus	8	-6	44	-11	3.52

* $p < 0.05$ FWE-corrected at cluster-level.

a contribution of the insular cortex is plausible in a memory-related task as the insular region is regarded as a relay in a functional cortical network, processing saliency, task switching, attention and executive control (Menon and Uddin, 2010). It has extensive connections with other brain regions including the prefrontal cortex, cingulate cortex, amygdala, parahippocampal gyrus, and secondary somatosensory cortex, all being involved in the conscious perception of pain (Friedman et al., 1986; Mesulam and Mufson, 1982; Mufson et al., 1981). Moreover, it has been suggested that the posterior insula plays a role in the sensory discrimination of pain stimuli (Coghill et al., 1999; Derbyshire et al., 1997), including decision-making processes (Craig and Prkachin, 1978; Malow et al., 1987, 1989). Involvement in sensory pain perception has also been described for the putamen and caudate, which show reduced activity in pain stimulus comparison, as observed in the presented study. The striatum, comprising putamen and caudate nucleus, has been assumed to be involved primarily in pain-related motor processing. However, a recent study demonstrated the involvement of the striatum in the judgment of behavioral relevance and saliency of nociceptive information (Starr et al., 2011), which corresponds to the present observation of a reduced ability to compare pain stimuli correctly.

The reduced discrimination ability after THC consumption is compatible with THC effects shown previously on stimulus memorizing in mice (Marsicano et al., 2002; Ranganathan and D'Souza, 2006). However, the present experiments are limited with respect to the stage of memory formation which was influenced by THC, i.e., initial learning (encoding), storage, or retrieval (Ranganathan and D'Souza, 2006). Previous assessments addressing pictorial memory had employed a design where encoding and retrieval had been separated by an additional task (Bossong et al., 2012). The respective results showed that THC caused reductions in activity during memory encoding in the right insula, the right inferior frontal gyrus, and the left middle occipital gyrus. While the reduced activations in the present study agree with these results, suggesting that in the present study the THC effects affected memory encoding, this observation was only made for the second stimulus. In contrast, a THC-effect on brain activation with regard to the first stimulus was not observed, although it can be assumed that also in this case encoding processes had taken place. With respect to retrieval, previous observations of a network-wide increase in activity, mainly in the bilateral cuneus and precuneus (Bossong et al., 2012), were suggested to possibly reflect an impaired recall function. In the present study, increased stimulus associated activations have not been observed, discouraging the interpretation that the observations reflect THC effects on memory retrieval. Moreover, since the performance in the discrimination task was independent of the interval between the two successive stimuli, the results do not support an effect on memory storage, which has been suggested previously as an effect of cannabis in humans (Lane et al., 2005). Finally, since recent results have shown that THC alters the perception of olfactory stimuli (i.e. the hedonic perception of vanillin stimuli changed from pleasant to neutral (Walter et al., 2016)), the present study design does not allow to determine whether an altered incoming information leads to deteriorated rating performance, or whether memory retrieval of stimulus characteristics is rather influenced by THC. To enhance the understanding of the neural mechanism underlying the effects of THC on memory, future studies might apply paradigms involving a learning phase of, e.g., numerical figures (Nie et al., 2019) prior to the experiment, and the retrieval of memorized material after THC application. This would allow to decide whether the processes of encoding or retrieval are impaired. Thus, although present results probably show an effect of THC on memory involving the sensation of pain, further research is needed to study the effects on nociceptive sensory memory in more detail.

Nevertheless, the lack of a correlation between side effects and brain activations makes it unlikely that the presently observed effects are a mere consequence of THC induced tiredness or drowsiness, which could have unspecifically reduced the DSDT performance in experiments

using different sensory stimuli. This would also conflict with the above-mentioned memory effects of THC, considering the wide acceptance of DSDT as a working-memory test paradigm (Pasternak and Greenlee, 2005; Sahgal and Iversen, 1978; Terry et al., 1996). A further point involves the known influences of circadian rhythms on neuronal activity of the brain (Hodkinson et al., 2014; Toth et al., 2007). Although study sessions were always performed at the same time of the day, starting before 10 a.m., inter-individual variability of the biological clock was not accounted for. This may imply that the performed task was not necessarily synchronized for each participant as it is known that circadian rhythmicity varies inter-individually (Gobbo and Falciati, 2014). However, as THC produced a clear group effect and both placebo and the active drug had always been administered at the same time of the day, lack of synchronization with the individual biological clock does not provide a better alternative explanation of the present observations than the factors discussed so far. Finally, THC has been discussed to influence the blood flow, raising the question whether changes in activation reflect drug-induced changes in cerebral blood flow rather than changes in neural activity (Bhattacharyya et al., 2015; Bossong et al., 2013). However, substances known to have a vascular effect such as cocaine did not alter the shape of the hemodynamic response that is used to estimate effects in fMRI (Gollub et al., 1998; Murphy et al., 2006). Moreover, even if such effects had occurred, it seems unlikely that they would have been restricted to specific brain regions as observed in the present data.

When studying memory-mediated effects of THC on pain, a doubt about the pain specificity is justified when considering that memory effects of cannabinoids have been widely discussed in many contexts other than pain. In the present study, the specificity of THC on memory involving nociceptive perception has been controlled for by performing an experiment including visual stimuli which provided basically the same results as observed with pain stimuli. In this subgroup of 13 subjects involved in the visual task, the observed effects related to the pain stimuli showed a similar trend as in the whole group comprising 22 subjects, but significance was not reached in this subgroup. This lack of significance in a pain-related but not in a visual task may simply be due to the different degrees of difficulty when estimating a pain stimulus or a simple visual stimulus. The effect of more consistent (i.e. less variable) activations evoked by visual rather than by nociceptive stimuli in the same subjects has been reported two decades ago (e.g. (Grosser et al., 2000; Oelkers et al., 1999)). Thus, the problem of statistical power impedes a clearer discrimination between a pain-specific and a non-specific effect. More relevant in this respect is a report on cannabinoid effects on stimulus memorizing in rodents, where aversive memories were impeded (Marsicano et al., 2002). Moreover, in a sub-project of the present investigations, THC had been found to reduce the subjects' performance in an odor discrimination task. In detail, 16 triplets of odors had been presented at intervals of approximately 10 s within triplets and of 30 s between the different triples. Two odors were identical, and subjects had to identify the odor that had been presented only once. Results showed that task performance was reduced after THC administration (Walter et al., 2014). In addition, a memory-unspecific effect due to a possible influence on attention seems plausible, considering the known effect of THC on cognitive performance, although a recent study performing two measures on neuropsychological functioning failed to show a significant deterioration of test performance after three doses of smoked cannabis (Wallace et al., 2007). Thus, present observation with the visual DSDT and further reports about reduced memory performance in humans (Ranganathan and D'Souza, 2006) raise doubts about a pain-specificity of THC effects. However, considering the high dosage of 20 mg applied in this experiment, future studies involving lower doses could examine whether pain relief is still achieved while influencing cognition to a lesser extent. Moreover, it would be of interest to determine whether participants who develop tolerance to the memory effects still experience pain relief.

In the present study, THC administration seemed to have the same

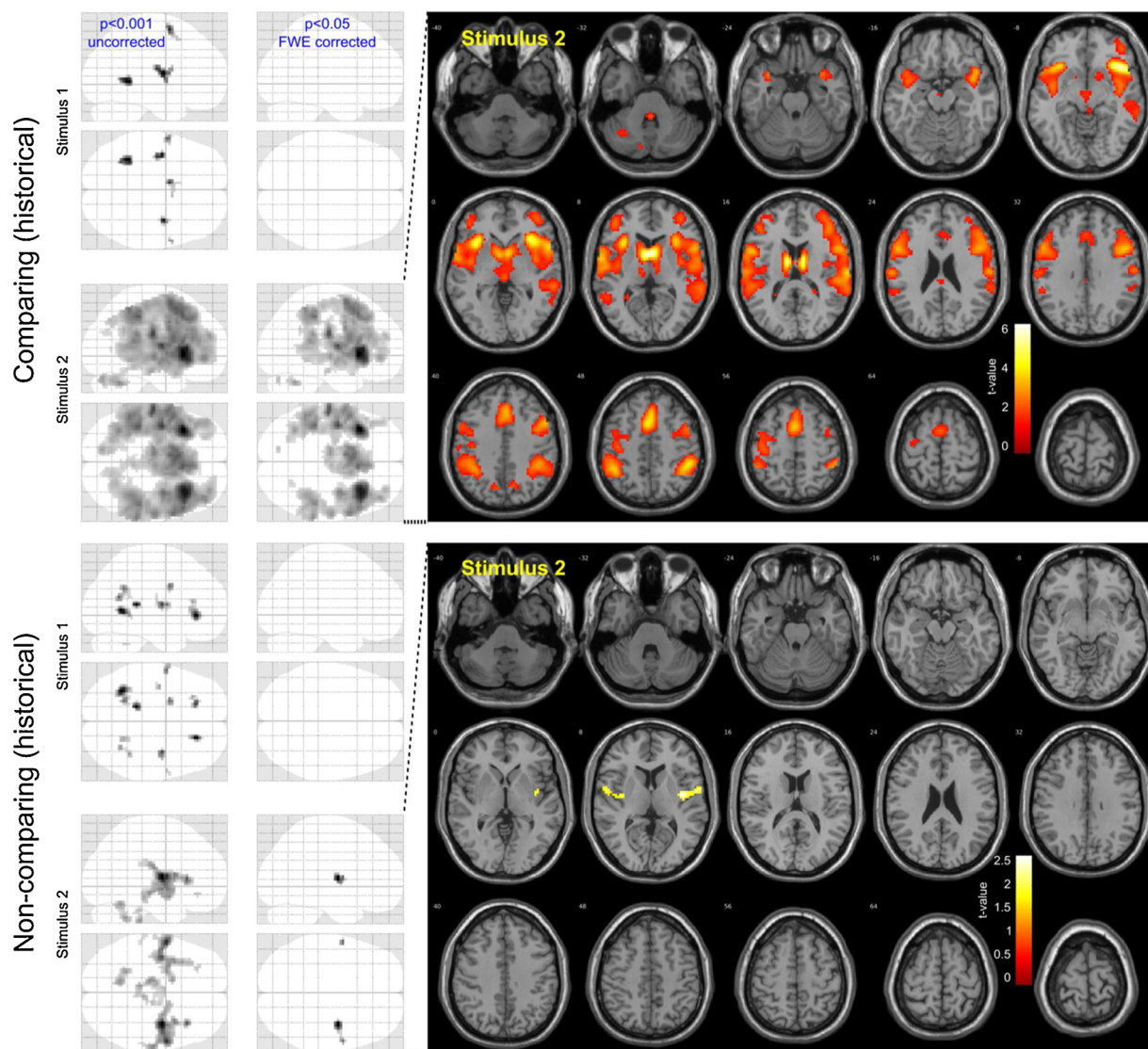


Fig. 6. Previous observation (Lötsch et al., 2012) of more extended brain activations associated with stimulus #2 as compared to those associated with stimulus #1 (glass brains, left), which was not observed when 2 CO₂ pain stimuli were administered without an accompanying comparison task. The results of t-tests are shown as a glass brain representation (left). Activations are shown both at a threshold of $p < 0.001$ with uncorrected α level and at a threshold of $p < 0.05$ with FWE-corrected α level. Right: the activations associated with the second stimulus are shown superimposed on axial slices of a canonical MR template. The significance at voxel level is color coded from red to light yellow with increasing t values, at a threshold of $p < 0.05$ (FWE-corrected). The figure was created using the SPM12 Matlab toolbox (<http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>; Wellcome Department of Imaging Neuroscience, London, UK (Friston et al., 1995; Worsley and Friston, 1995)) and the xjView Matlab toolbox (<http://www.alivelearn.net/xjview/>).

effect on the pain-DSDT as previously observed when, instead of THC administration, the task to discriminate successive pain stimuli had been omitted (Fig. 6) (Lötsch et al., 2012). Specifically, masking of the brain regions displaying THC-induced activity reductions with areas involved in pain stimulus comparison revealed overlaps with areas that had previously been reported to be differently activated when omitting the comparison task, in particular the insular cortex (Lötsch et al., 2012). Indeed, the THC effects were located in two clusters comprising mainly the right insula, right inferior frontal cortex and the caudate nucleus and putamen bilaterally. These regions, in particular the insular cortex, correspond to areas that had previously been demonstrated to be differently activated during the comparing and the non-comparing conditions (Lötsch et al., 2012). Thus, THC mimicked an omission of the comparison task, which agrees with the observed reduced performance in this task. In the previous study, the comparison between the two conditions had been obtained by evaluating data from two separate cohorts. This was due to the problem mentioned above that a cross-over

design had to be dismissed, due to the apparent impossibility of avoiding comparisons, once a subject had participated in the comparison task. Therefore, data obtained on the non-comparing cohort of the previous study (Lötsch et al., 2012) may be included in the interpretation of present results as a substitute for the missing control condition in the present study, in particular as the experiments had been performed by the same researchers in the same laboratory using the same equipment (Lötsch et al., 2012). It should be noted that the involved brain regions, in particular the insular cortex, resembled areas that had been reported in the cited study as showing differences between the comparing and non-comparing conditions (Lötsch et al., 2012) (inclusive masking with an anatomical mask including bilateral insular and left postcentral cortex, $p < 0.001$, uncorrected).

Finally, THC effects on pain seem to differ between preclinical, human-experimental and clinical settings (Lötsch et al., 2017). As the present study was conducted in an experimental pain setting involving healthy subjects, the translation of its results to patients experiencing

non-experimental pain may be limited. Although discussions have been raised about the validity of results obtained in non-clinical settings to predict clinical analgesia, analyses suggested an overall satisfactory prediction performance (Lötsch et al., 2014; Oertel and Lötsch, 2013): depending on the selection of the model for a particular clinical setting, experimental pain models have been shown to correctly predict drug efficacy in a number of clinically relevant pain settings (Lötsch et al., 2014). This includes the presently chosen pain model, which emerged from a computational analysis of several different pain models among those with the best record of correct predictions of clinical analgesia (Lötsch et al., 2014). The findings of the presented study have to be interpreted with a certain care, given the reduced statistical power. It was not possible to determine a suitable sample size in advance, as the expected and relevant effect sizes and the variance of the effects were unknown during study planning. While post-hoc power calculations are discouraged (Goodman and Berlin, 1994) and should therefore be avoided (Zhang et al., 2019), the statistical software package SPSS nevertheless provided these values, stating a power of 0.643 to detect the observed effects of “drug” on the correctness of the ratings of the stronger pain stimulus. For the interaction “drug” by measurement”, the post-hoc power estimate was 0.542. This should be considered when interpreting the presently reported THC effects and their relevance for a clinical setting related to pain therapy.

Conclusions

Employing a delayed stimulus discrimination task (DSDT) paradigm, the results of the present study demonstrate an effect of THC on the memory of nociceptive sensory perceptions in humans. The reduction of the subjects’ performance in identifying the stronger of two successive pain stimuli was accompanied by reductions in brain activations associated with the second stimulus of a pair. Thus, THC seems to influence pain when memorizing its intensity is involved, which is in line with preclinical findings that cannabinoids inhibit aversive memories (Marsicano et al., 2002). However, the effects of THC on the working memory of nociceptive sensory stimuli were not pain-specific. Experimental and reported evidence suggests that these effects are part of a broader pharmacological action of THC on the working memory which involves pain, among other sensory systems. Nevertheless, the memory effects of THC found in this study could help to predict in which clinical settings THC based analgesic treatments may be useful.

Author contributions

Conceived and designed the experiments: JL, CW, RD. Performed the study, collected the data: CW, UN, BGO, LF. Analyzed the data: CW, JL. Wrote the paper: JL, CW, RD, UN. Revised the manuscript: CW, JL, RD, UN.

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Conflicts of interest

The authors have declared that no conflicts of interest exist.

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Parts of the overall project have been reported separately with a different focus and in a non-redundant manner in J Clin

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.ibror.2019.11.004>.

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