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REVIEW

# Anxiolytic effects of endocannabinoid enhancing compounds: A systematic review and meta-analysis

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Therapeutics;
Meta-analysis

**Abstract**
The endocannabinoid system is a promising candidate for anxiolytic therapy, but translation to the clinic has been lagging. We meta-analyzed the evidence for anxiety-reduction by compounds that facilitate endocannabinoid signaling in humans and animals. To identify areas of specific potential, effects of moderators were assessed. Literature was searched in Pubmed and Embase up to May 2021. A placebo/vehicle-control group was required and in human studies, randomization. We excluded studies that co-administered other substances. Risk of bias was assessed with SYRCLE's RoB tool and Cochrane RoB 2.0. We conducted three-level ran-

*Abbreviations:* AEA, Anandamide; CBD, Cannabidiol; FAAH, Fatty-acid amide hydrolase.

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dom effects meta-analyses and explored sources of heterogeneity using Bayesian regularized meta-regression (BRMA). The systematic review yielded 134 studies. We analyzed 120 studies (114 animal, 6 human) that investigated cannabidiol (CBD, 61), URB597 (39), PF-3845 (6) and AM404 (14). Pooled effects on conditioned and unconditioned anxiety in animals (with the exception of URB597 on unconditioned anxiety) and on experimentally induced anxiety in humans favored the investigational drugs over placebo/vehicle. Publication year was negatively associated with effects of CBD on unconditioned anxiety. Compared to approach avoidance tests, tests of repetitive-compulsive behavior were associated with larger effects of CBD and URB597, and the social interaction test with smaller effects of URB597. Larger effects of CBD on unconditioned anxiety were observed when anxiety pre-existed. Studies reported few side effects at therapeutic doses. The evidence quality was low with indications of publication bias. More clinical trials are needed to translate the overall positive results to clinical applications.
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## 1. Introduction

Cannabis has long been considered to have therapeutic potential (Cohen, 1978). Research on the cannabis constituent Δ9-THC and cannabimimetic compounds led to the discovery of cannabinoid receptors and, subsequently, of endogenous cannabinoids N-arachidonoylethanolamide (AEA; anandamide; Bisogno et al., 2001; Mlost et al., 2020) and 2-arachidonoylglycerol (2-AG; Devane et al., 1992; Mechoulam et al., 1998; Sugiura et al., 1995). Early studies with cannabidiol (CBD), a second major constituent of cannabis, demonstrated anxiolytic properties in animals (Guimarães et al., 1990; 1994; Onaivi et al., 1990) and humans (Zuardi et al., 1993).

In subsequent years, preclinical data in rodents accumulated suggesting that disruptions in endocannabinoid tone in brain regions including the amygdala, hippocampus and prefrontal cortex contribute to anxiety-like behavior induced by acute or repeated stress (for narrative reviews see Gorzalka et al., 2008; Hill et al., 2010; Morena et al., 2016; Patel et al., 2008). Several experiments in rodents used fear extinction (e.g., Chhatwal et al., 2005; Ganon-Elazar and Akirav, 2009; Marsicano et al., 2002), a widely used translational model for learning that takes place during exposure therapy (Craske et al., 2018). It was shown that endocannabinoid signaling in the amygdala and hippocampus mediates the stress and glucocorticoid-induced enhancement of fear extinction and fear memory consolidation, and impairment of fear memory retrieval (Morena et al., 2016). The clinical potential of this approach has spurred more mechanistic investigations in the endocannabinoid system (ECS) as a candidate target for anxiolytic drug development.

CBD is a prominent constituent of cannabis with a complex pharmacology, including as a mechanism of action inhibition of fatty acid amide hydrolase (FAAH), the primary metabolic enzyme of AEA. Although CBD's inhibition of FAAH is relatively weak (Bisogno et al., 2001; Mlost et al., 2020) subchronic CBD administration increased AEA levels in mouse hippocampal tissue (Campos et al., 2013) and in serum of patients with acute schizophrenia (Leweke et al., 2012).

In contrast to direct CB1R agonists such as Δ9-tetrahydrocannabinol (Δ9-THC), CBD does not induce psychomotor impairment (Dalton et al., 1976) or psychotomimetic effects (Dalton et al., 1976; Karniol et al., 1974). Further, CBD does not induce a change in heart rate (Dalton et al., 1976; Karniol et al., 1974), and seems to attenuate the anxiogenic effect of Δ9-THC in healthy volunteers (Karniol et al., 1974; Zuardi et al., 1982). These data suggest that CBD may indirectly exert CB1R mediated therapeutic actions, while circumventing unwanted side effects.

To overcome the lack of target selectivity of CBD (Bisogno et al., 2001; Mlost et al., 2020) and aiming to optimize a fear extinction enhancing effect, several classes of more selective inhibitors of FAAH have been developed. The *O*-aryl carbamate URB597 turned out to be a potent and irreversible inhibitor of FAAH (Kathuria et al., 2003). The transport inhibitor AM404 selectively attenuates breakdown of AEA (Bortolato et al., 2006) by inhibition of intracellular fatty acid binding proteins (FABS; Deutsch, 2016; Kaczocha et al., 2012). The irreversible FAAH inhibitor PF-3845 is more potent, more selective, and has a longer duration of action than URB597 (Ahn et al., 2009). URB597, PF-3845 and inhibitor of AEA cellular uptake AM404 are prototypical examples of the many compounds that were developed to increase CB1R activation by enhancing endocannabinoid levels (Paredes-Ruiz et al., 2021).

To the best of our knowledge, numerous narrative (Griebel and Holmes, 2013; Lutz et al., 2015; Morena et al., 2016) but no systematic review on preclinical research into anxiolytic effects of ECS manipulations has been published so far. One systematic review of animal studies of ECS manipulations including CBD, with a primary focus on inflammation and neurogenesis, included five studies that reported variable effects on anxiety outcomes (Giacobbe et al., 2021).

A previous systematic review and meta-analysis summarized the limited available evidence from controlled studies conducted in human patients suffering from anxiety disorders, which included only two randomized controlled studies in patients (Black et al., 2019). This meta-analysis demonstrated no benefit of single doses of CBD (up to 600 mg) over placebo (Black et al., 2019). These preliminary findings in humans raise questions about the often discussed potential of pharmacological enhancement of AEA levels for treating anxiety symptoms. Clearly, there is a need for a systematic review and meta-analysis of the large body of

mainly preclinical literature on this topic. This literature can provide an indication of clinical efficacy but is especially suitable for identifying potential moderators of clinical effects given the diversity in anxiety models used in these studies (Griebel and Holmes, 2013; Vesterinen et al., 2014).

The primary aim of the present systematic review and meta-analysis was to investigate anxiolytic effects of inhibitors of FAAH and AEA transport, by synthesizing all evidence from animal, human, preclinical and clinical studies. Behavioral, physiological, and subjective effects were investigated. In addition, theoretically relevant moderators and sources of heterogeneity of drug effects were explored. Part of the current literature examines acute anxiolytic effects, but a more recent approach is to develop treatments that aim to work synergistically with psychotherapeutic approaches by supporting adaptive learning, particularly fear extinction (cf., Davis et al., 2006). As discussed above, modulators of brain endocannabinoid levels have been shown to exert an effect on fear extinction and related learning mechanisms (for narrative reviews see Lafenêtre et al., 2007; Morena et al., 2016; Ruehle et al., 2012) and attempts have been made to translate these findings to potential use in psychotherapy (Kwee et al., 2022a). We therefore conducted separate meta-analyses for tests of conditioned versus unconditioned anxiety (Rodgers and Dalvi, 1997). Additionally, we explored whether drugs affected different aspects of fear conditioning and extinction, and investigated factors that are likely to moderate drug effects: 1) variables related to the drug regimen (single vs (sub)chronic administration, acute vs delayed effects); 2) species (Haller et al., 2007; Kwee et al., 2022b); 3) the pre-existing anxiety condition of the animal or human individual (Bach, 2022; Sams-Dodd, 2006); 4) type of anxiety test (Sams-Dodd, 2006); 5) sex differences with respect to the effects of AEA modulators, in light of the association between oestradiol and CB1 receptor density in amygdala and prefrontal cortex (Castelli et al., 2014); 6) Publication year (Shrout and Rodgers, 2018).

For our secondary research aim we summarized any information that was available in included studies on drug safety and tolerability. Several reviews are available for CBD (Chesney et al., 2020; Huestis et al., 2019; Iffland and Grotenhermen, 2017; Kwee et al., 2022b). Previous preclinical research shows divergent results with respect to safety and tolerability of FAAH inhibitors (Panlilio et al., 2016). We therefore evaluated adverse effects in included studies on a drug-by-drug basis.

## 2. Experimental procedures

This review was preregistered with PROSPERO (CRD42021236572) and conducted in line with Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (see Supplemental Tables 1 and 2).

### 2.1. Search strategy

Studies were searched in the electronic databases PubMed and Embase using both free text and underlying terms (MeSH and Emtree, respectively) up to 19-05-2021. The search was aimed at evidence on modulation of fear expression, anxiety symptoms and fear memory or extinction learning, by AEA hydrolysis and transport inhibitors in humans and non-human mammals (see Supplemental Table 3). Only peer-reviewed articles were included. No restrictions were placed on publication year or language. Preregistered but as of yet unpublished studies were searched in the EU Clinical Trials Register, the Australian and New Zealand Clinical Trials Registry, Animal Study Registry (German centre for the Protection of Laboratory Animals), ClinicalTrials.gov and Preclinicaltrials.eu, in order to get an indication of potential positive results bias.

### 2.2. In- and exclusion criteria

Table 1 lists in- and exclusion criteria for the selection of studies.

### 2.3. Study screening and selection

Titles and abstracts of articles retrieved using the search strategy were independently screened by a first (CK) and second reviewer (NL or RvdK) to identify studies that appeared to meet the inclusion criteria. They then independently screened the full text of these studies for eligibility. Disagreements were resolved through discussion, when no consensus was reached a third (LG) or fourth reviewer (JB) was consulted.

### 2.4. Data extraction

According to the PICO framework (Schardt et al., 2007) we recorded the details of the populations, interventions (including concomitant medication in human studies), and outcomes. The comparison group was always placebo/vehicle.

#### 2.4.1. Primary research aim

For our first research aim of drug effects on anxiety outcomes within behavioral, physiological, and subjective outcome domains (see Supplemental Table 4), parameters of interest were means (Ms) and standard deviations (SDs) of the anxiety outcome in vehicle/placebo and active drug conditions. We used these parameters to calculate Hedge's g, an effect size that corrects for bias resulting from small sample sizes (Hedges, 1981). Higher scores on the effect size indicate an anxiolytic drug effect. Effect sizes were reverse-coded if higher values indicated less anxiety than lower values. Decision rules in case of unreported data, or multiple outcome measures or experimental drug-placebo comparisons are described in the Supplemental material, Section 2.2. If parameters were not fully reported we estimated them from graphs in the paper or requested the information from the authors.

We extracted theoretically relevant moderators dose, type of anxiety test, selected outcome parameter, publication year, information on frequency of drug administration and timing of effect measurement, pre-existing anxiety condition, sex, and species (ten moderators in total), of which the first three were selected as theoretically most relevant for exploratory follow-up analysis. To standardize 'dosages' across species human equivalent dose (HED) was calculated by using allometric scaling factors (Center for Drug Evaluation and Research, 2015). This dose-normalization approach is common in systematic overviews of preclinical study results across different species (Van Gerven and Cohen, 2018). Our semi-quantitative analyses on the relation between CBD dose and anxiety-reducing effects tentatively suggest an inverted U-shaped dose-response curve (Kwee et al., 2022b), modeled here with a quadratic trend for dose/HED.

**Table 1** Study in- and exclusion criteria.

| | Participants | Interventions | Comparison | Outcomes |
|---|---|---|---|---|
| Included | | | | |
| | 1. Healthy or anxious phenotype<br>2. Adult<br>3. Mammal | 1. FAAH inhibitor or AEA transport inhibitor | 1. Randomized placebo-controlled design | 1. Fear expression, fear or extinction memory, extinction learning or anxiety disorder symptoms<br>2. Outcome domain behavioral physiological, or subjective<br>3. Data type continuous |
| Excluded | | | | |
| | 1. Chronic users of cannabis compounds | 1. Compounds with catabolic pathways for AEA other than FAAH hydrolysis<br>2. Dual FAAH/ monoacylglycerol lipase inhibitors (Fowler, 2021)<br>3. Intracerebral/ intracere-broventricular/intravenous administration<br>4. Coadministration of other substances[b]<br>5. Time between drug administration and anxiety assay $\geq$ 24 h | 1. Studies without control group<br>2. Non-randomization<br><br>(studies in humans only)[a] | 1. Acquisition of fear |

*Note:* AEA: anandamide; FAAH: fatty acid amide hydrolase.

[a] The use of randomization is usually not reported in animal research (Muhlhausler et al., 2013) and it had not been empirically demonstrated whether the use of randomization would influence outcomes. Therefore, in animals we considered vehicle-controlled experiments without information about randomization and explicitly non-randomized but placebo/vehicle-controlled studies to be eligible as well.

[b] In humans, studies that allowed stable concomitant anxiolytic and/or antidepressant medication were included.

#### 2.4.2. Secondary research aim

The terms 'harm', 'adverse', 'side', 'unwanted', 'undesirable', 'safe*', 'toler*' were searched in included articles.

#### 2.4.3. Procedure

The majority of the data were extracted by CK, the remainder by a second reviewer (NL or one of the collaborators on the project. When one of the authors was in doubt about (categorization of) the data to be extracted, the issue was resolved through discussion (with a third (LG) and fourth reviewer (JB) when necessary). Generally, the outcomes extracted by the first and second reviewer matched (see Supplemental material, Section 2.3 for more information).

### 2.5. Data analysis

Meta-analyses were performed using R packages metafor (Viechtbauer, 2010) and pema (Van Lissa and Van Erp, 2021, Preprint). All models were three-level random effects models. A three-level random effects model accounts for three sources of variance: sampling error of the observed effect size (which is treated as known), within-experiment variance of true experiment-specific effect sizes, and variance of true experiment-specific effect sizes across experiments. Effect sizes from different papers were always categorized as independent; effect sizes from the same paper only if it was explicitly stated that effects were tested in independent experiments and/or independent sets of study subjects.

We conducted separate analyses per drug (within the class of AEA enhancing drugs), for unconditioned and conditioned anxiety in animals and experimentally induced anxiety in humans. Effect sizes per comparison and overall pooled effect size per meta-analysis were visualized in forest plots (Supplementary Figs. 2–9).

Statistical heterogeneity was assessed using $\tau^2$ (a measure of between-study variance) and $I^2$ (percent of variability in effect sizes not caused by sampling error; Higgins and Thompson, 2002; Vesterinen et al., 2014). We conducted sensitivity analyses to examine whether substantiated conclusions would change by excluding studies with high risk of bias or atypical route of drug administration.

For categorical moderators, we used dummy coding, treating the largest category of each variable as the reference category. We standardized continuous predictors only and not dummy variables. This may have given dummy variables a slight advantage, leading them to become significant sooner than continuous ones.

The number of effect sizes was small relative to the number of moderators. This introduces risks of model-nonidentification, overfitting, and multicollinearity (Van Lissa, 2020). A novel technique called Bayesian regularized meta-regression (BRMA) overcomes these risks by imposing a regularizing horseshoe prior to shrink the regression coefficients of irrelevant moderators towards zero (Van Lissa and Van Erp, 2021, Preprint). Thus, we used BRMA in all moderator analyses to select moderators that are important in predicting the effect size. The resulting regression coefficients are negatively biased by design, but simulation studies show that the estimate of residual heterogeneity $\tau^2$ is relatively unbiased (Van Lissa and Van Erp, 2021, Preprint). Supplementary classic meta-regression with the maximum likelihood approach (Supplemental Tables 11, 13, 16, 18, 20, 22, 24, 28, 30, 32, 34, 36, 38, 40, 42) indeed evidenced model non-convergence and high variance inflation factors (VIF) confirmed the expected problems caused by the high ratio of moderators to effect sizes.

**Table 2** Summary characteristics of included studies.

| | |
|---|---|
| **Population** | |
| Publication year | 1990–2021 |
| Species | 44% mouse, 50% rat, 5% human, 2% other |
| Pre-existing anxiety condition | in 17% of studies |
| Sample size per study* | 88 (109) |
| Sample size per effect | 20 (6) |
| Sex | 90% male |
| **Intervention** | |
| Drug | 52% CBD, 32% URB597, 11% AM404, 5% PF-3845 |
| HED*60 | 90.08 (143.65) |
| Administration route | 90% i.p., 10% oral |
| Frequency of administration | 68% single dose |
| Timing of effect measurement | 82% acute drug effects |
| **Outcome** | |
| Type of anxiety | 71% unconditioned |
| Type of anxiety test | See Supplemental Table 7 |
| Selected outcomes for tests of conditioned anxiety | See Supplemental Table 8 and Supplemental Fig. 1 |

*Note:* Numbers are mean (SD) or as otherwise stated.
* Sample sizes per tested effect can be found in the data files (doi:10.5281/zenodo.7829148).

We decided a priori to only perform the planned quantitative syntheses for each meta-analysis (separate per drug and conditioned/unconditioned/experimentally induced anxiety for humans and animals) if the number of included effect sizes in the meta-analysis exceeded the number of moderator variables + 1, which we considered the minimum for model identification. In addition to planned moderator analyses which included all moderators, we conducted exploratory moderator analyses on potential interactions of drug dose with a smaller number of key moderators.

To interpret these interaction effects, see plots with posterior predictive distribution of drug effects per moderator category, conditional upon the observed effects (Fig. 3).

The Workflow for Open Reproducible Code in Science (Van Lissa et al., 2021) was used to make analyses reproducible. A reproducible repository with all analysis codes and data are available at (doi:10.5281/zenodo.7829148).

### 2.6. Assessment of the quality of evidence

Assessment of the quality of the meta-analytic evidence with the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach (Schünemann and Santesso, 2010) was done by CK and checked by NL. GRADE criteria and are summarized in the Supplemental material, Section 2.4.

## 3. Results

### 3.1. Included studies and characteristics

A PRISMA flowchart is shown in Fig. 1. Study characteristics of included studies are summarized in Table 2. The majority of included studies ($n$ = 114 out of a total of $n$ = 120 studies; 95%) were conducted in non-human mammals. Only $n$ = 6 studies (5%) were conducted in humans. Types of anxiety tests in included studies are provided in Supplemental Table 7.

In Supplemental Table 8 the distribution is shown of outcomes in tests of conditioned anxiety, selected from the studies according to a-priori definitions (see Supplemental Fig. 1 for details). Outcomes were categorized as effects on fear memory reconsolidation when the drug was administered after memory retrieval, and as effects on extinction consolidation when administered after an extinction learning phase (before extinction retention was tested).

### 3.2. Effects of FAAH and AEA transport inhibitors on anxiety

#### 3.2.1. Overall summary of findings regarding drug effects

Across meta-analyses, the pooled effect size estimates indicated a lower level of anxiety after treatment with the investigational drug than after placebo/vehicle treatment (Fig. 2 and Table 3). This was true for all combinations of drug types and types of anxiety for humans and non-human mammals except one, the effect of URB597 on unconditioned anxiety in animals. The size of these drug effects was moderate-to-large. Note that CBD was the only compound for which sufficient studies in humans were available to analyze meta-analytically. For PF-3845 only studies with tests of unconditioned anxiety in animals were available. The illustrations of effect sizes of all studies from which the pooled effect sizes were derived can be found in Supplemental Figs. 2–9.

For most analyses, both within- and between-experiment variance were significant, which indicates heterogeneity be-

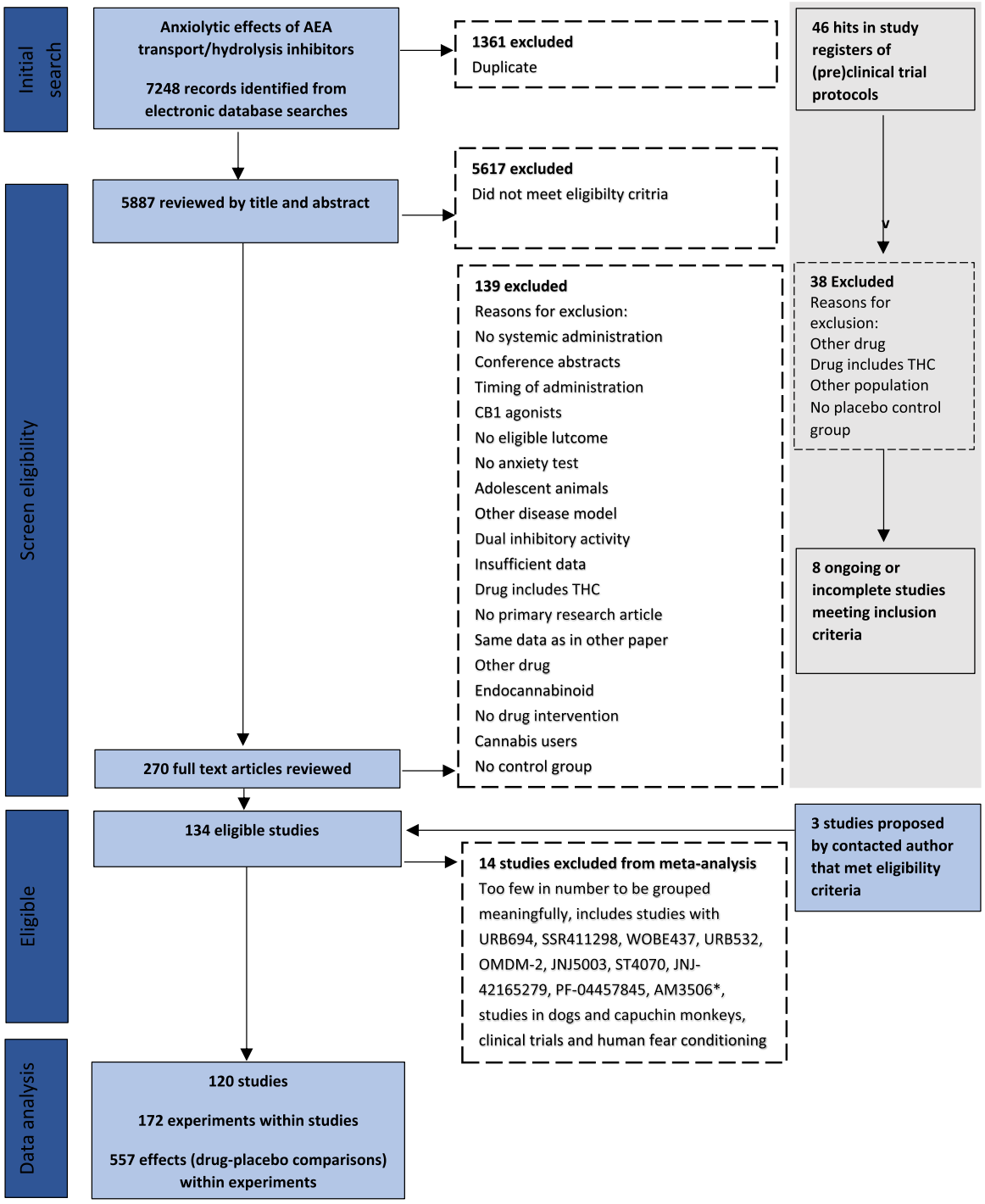


**Fig. 1** PRISMA flowchart. *Note:* References of eligible studies are listed in Supplemental Table 5. Supplemental Table 6 describes ongoing or incomplete studies that meet inclusion criteria.

tween effect sizes both within and across experiments (see Sections 3.2.2 and 3.2.5 for results of moderator analyses).

### 3.2.2. Planned moderator analyses

Moderator analyses with theoretically relevant moderators were conducted to identify sources of heterogeneity of drug effects and to generate hypotheses on which circumstances and for whom the tested drugs could be beneficial. Supplemental Table 9 presents the applicable moderators per meta-analysis. Relevant predictors selected with BRMA are listed in the Supplemental Tables 10, 12, 14, 15, 17, 19, 21, 23. In the text below, only moderator effects whose 95% credible interval excluded zero are discussed. This interval contains the population effect size with 95% probability and is the Bayesian counterpart of statistical significance.

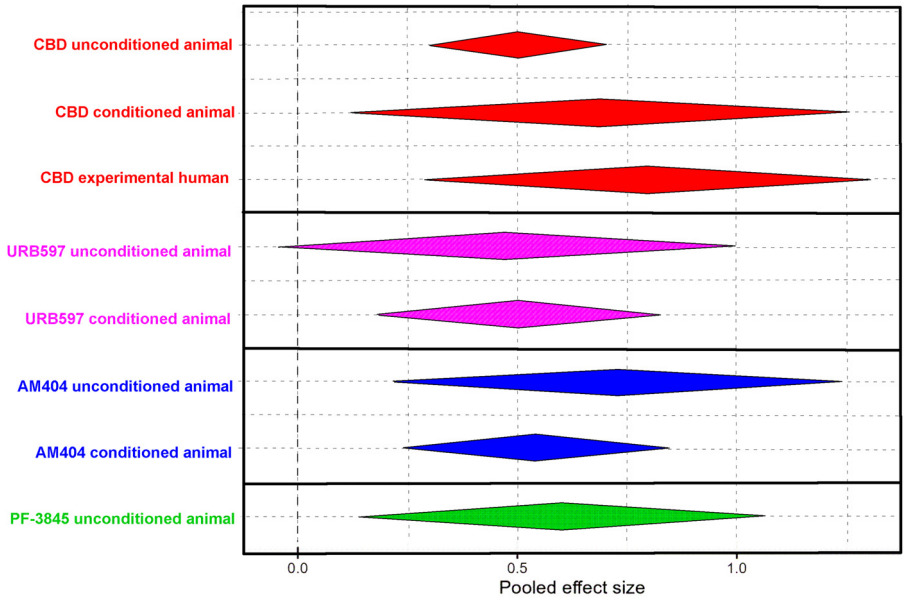


**Fig. 2** Pooled effects per drug for unconditioned and conditioned anxiety in animals and experimentally induced anxiety in humans. *Note:* Diamonds illustrate point estimates plus 95% confidence intervals for each meta-analysis, see Table 3 for further details. Negative values indicate effects in favor of the placebo group; positive values indicate effects in favor of the experimental group that received the drug. Supplementary Fig.s 2–9 provide forest plots of the distributions of observed effect sizes.

**Table 3** Summary of findings of anxiolytic effects of FAAH and AEA transport inhibitors.

| Type of anxiety | Drug | Participants (experiments) | Hedge's G [95%CI] | $\sigma_w^2$, $\sigma_b^2$ [95%CI] | $I_w^2$, $I_b^2$ | Favors | QoE |
|---|---|---|---|---|---|---|---|
| Unconditioned in animals | Cannabidiol | 4859 (61) | 0.50 [0.29, 0.70]* | 0.20 [0.12, 0.31] 0.44 [0.23, 0.82] | 23.49 52.23 | Cannabidiol | Low |
| | URB597 | 2153 (50) | 0.47 [-0.06, 1.00] | 0.12 [0.01, 0.31]* 3.31 [2.13, 5.35] | 3.29 89.18 | Neither | Low |
| | AM404 | 743 (12) | 0.73 [0.21, 1.24]* | 0.62 [0.27, 1.32]* 0.46 [0.08, 1.74]* | 46.02 34.04 | AM404 | Low |
| | PF-3845 | 726.5 (7) | 0.60 [0.13, 1.07]* | 0.15 [0.00, 0.47]* 0.28 [0.01, 1.57]* | 23.80 45.30 | PF-3845 | Low |
| Conditioned in animals | Cannabidiol | 1125 (16) | 0.68 [0.11, 1.26]* | <0.01 [<0.01, 0.10]* 1.22 [0.50, 3.17] | < 0.01 83.57 | Cannabidiol | Low |
| | URB597 | 787 (13) | 0.50 [0.17, 0.83]* | 0.01 [0.00, 0.23]* 0.23 [0.04, 0.83] | 2.46 49.31 | URB597 | Low |
| | AM404 | 351 (7) | 0.54 [0.24, 0.85]* | 0.14 [0.00, 0.66] <0.01 [<0.01, 0.70] | 47.40 <0.01 | AM404 | Low |
| Experimentalin humans | Cannabidiol | 442 (6) | 0.79 [0.28, 1.31]* | <0.01 [<0.01,0.25]* 0.28 [0.03, 2.07] | 0.09 60.62 | Cannabidiol | Moderate |

*Note:* QoE: Quality of evidence; $\sigma_w^2$, $\sigma_b^2$, $I_w^2$, $I_b^2$: heterogeneity statistics.
* $p < 0.05$.

Publication year, presence or absence of a pre-existing anxiety condition, and anxiety test moderated CBD effects on unconditioned anxiety. Effects of CBD were larger in the presence of pre-existing anxiety (Fig. 3, panel A) and in tests of repetitive compulsive-like behavior (RCLB) than in approach avoidance tests (Fig. 3, panel B). Conversely, the effects of CBD were smaller in more recent compared to older publications. In URB597, anxiety test moderated drug effects on unconditioned anxiety. The social interaction test was associated with smaller anxiolytic effects compared to approach avoidance tests (Fig. 3, panel C).

### 3.2.3. Quality of evidence

Assessments of the quality of evidence using the GRADE approach (Schünemann and Santesso, 2010) are summarized in Supplemental Table 25. Risk of bias assessments for anxiety outcomes for individual studies are provided in Supplemental Fig. 10. Our ratings of quality of the body of evidence were low for all combinations of drug (CBD, URB597, AM404, PF-3845) in unconditioned and conditioned anxiety in animals and experimentally induced anxiety in humans. Quality of evidence was impacted negatively by:

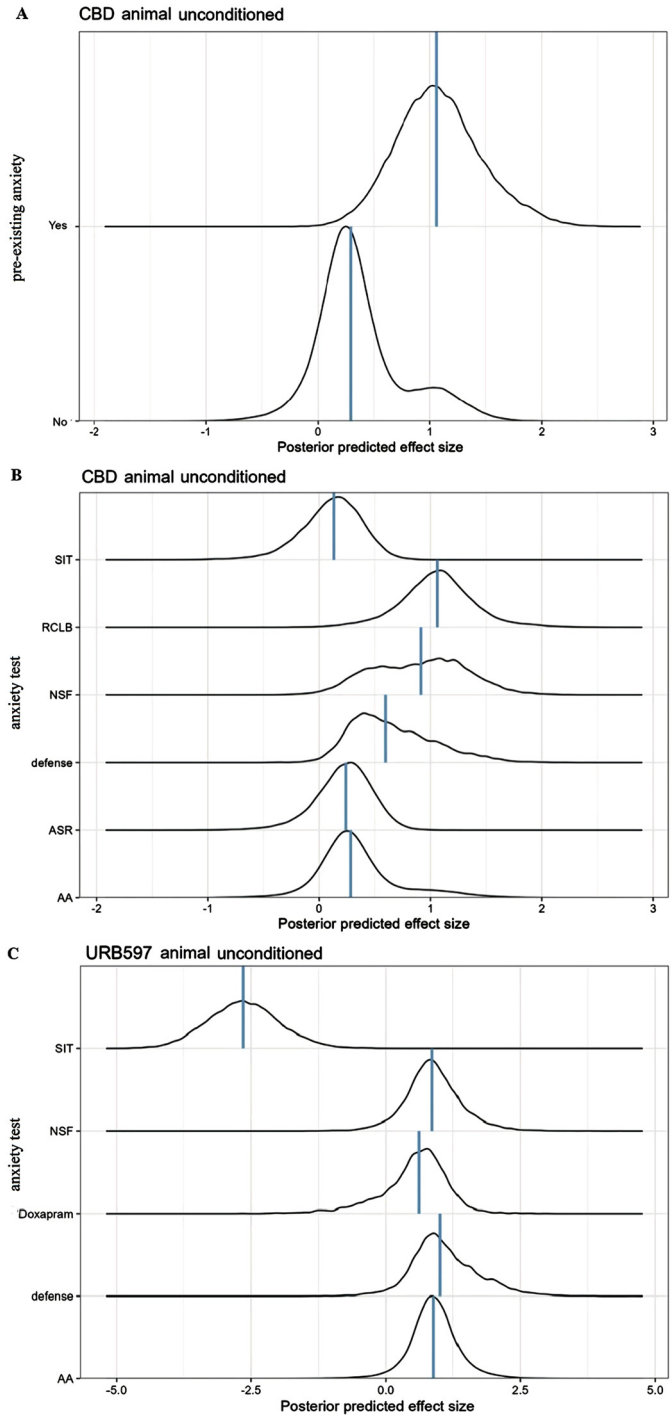


**Fig. 3** Plots of posterior predictive distributions of effect sizes for the levels of significant categorical moderator variables. *Note:* Break-down is presented of the different levels of significant moderators of unconditioned anxiety in animals: pre-existing anxiety condition (Panel A, CBD) and anxiety test (Panel B, CBD; Panel C, URB597). Blue lines represent median effect sizes. CBD: Cannabidiol; SIT: social interaction test; RCLB: repetitive compulsive-like behavior; NSF: novelty suppressed feeding; ASR: acoustic startle response; AA: approach avoidance. Please note that all anxiety tests investigated per drug are plotted.

1) Unclear to high risk of bias for reported effects. Risk of bias was considered serious across all animal studies due to underreporting of this information, and competing financial interests. Risk of bias was also considered serious for the effect of CBD on experimentally induced anxiety in humans, as 3 out of 6 studies were assessed as high risk of bias because of 1) increased mental sedation in the CBD condition and, as a potential consequence, unsuccessful blinding (Crippa et al., 2004); 2) highly variable CBD plasma concentrations (4.7 (7) and 17 (29) ng/mL 1 and 2 h after administration (Fusar-Poli et al., 2009), that led to con-

cerns about failures in implementing the intervention; 3) unclear bias due to missing outcome data and concerns about selective outcome reporting (Zuardi et al., 1993).

2) Publication bias which was (very) strongly suspected for all drugs and types of anxiety. Visual inspection of funnel plots (see Supplemental Fig.s 11–18) and significant results (ps $\leq$.02) on Egger's test for funnel plot asymmetry indicated an overrepresentation of publications with large and beneficial compared to smaller or adverse drug effects across smaller studies, relative to a more balanced mix of findings across larger studies (Peters et al., 2008);
3) Significant unexplained heterogeneity. High heterogeneity in our included animal studies renders interpretation of an overall effect rather difficult (Vesterinen et al., 2014);
4) Indirect evidence by the use of healthy subjects and no pre-existing anxiety in most preclinical studies, which may lower the level of face and predictive validity (Bach, 2022; Sams-Dodd, 2006), the use of conventional rather than ethological measures of anxiety (e.g., Carobrez and Bertoglio, 2005), and test conditions that were not always optimized to measure anxiolytic effects (Seillier and Giuffrida, 2017);
5) Imprecision of URB597 effects on unconditioned anxiety, indicated by a large range of drug effects, from anxiolytic to anxiety increasing;

The moderate to large overall effect sizes, despite the fact that within many studies (52%) different drug doses were tested, led to quality of evidence upgrades.

#### 3.2.4. Sensitivity analyses

The robustness of the findings regarding our primary research aim was evaluated in sensitivity analyses (see Supplemental Table 26 for excluded effects). Results of the sensitivity analyses are available in supplementary online material (doi:10.5281/zenodo.7829148). After excluding studies with a high risk of bias, the pooled effect of CBD on human experimentally induced anxiety became smaller and non-significant, Hedge's g [95% CI] = 0.50 [-0.05, 1.05], $p = 0.07$. The pooled effect of URB597 on unconditioned anxiety became significant, Hedge's g [95% CI] = 0.55 [0.11, 1.00], $p = 0.01$, but direction and magnitude of the effect were unaltered. For the other compounds and types of anxiety, direction, magnitude, and significance of pooled effects remained unchanged in the sensitivity analyses. The moderators identified as having a non-zero effect with BRMA in the planned moderator analyses (Section 3.2.2) remained the same in the sensitivity analyses. This indicates that the meta-analytic findings are largely robust to excluding studies assessed as high risk of bias or otherwise strongly affecting the overall results.

#### 3.2.5. Exploratory moderator analyses

Exploratory moderator analyses were planned with a subset of theoretically most important study characteristics: anxiety test, drug dose (human equivalent dose (HED)*60 across drugs in included studies ranged between 0.05 and 900 mg) and type of outcome for tests of conditioned anxiety. See Supplemental Tables 27, 29, 31, 33, 35, 37, 39, 41 for all selected predictors with BRMA. Interaction effects between anxiety test and type of outcome, and dose and dose$^2$ (or HED and HED$^2$ for animal studies) were included in these models to explore dose-response relationships. The moderator analyses showed that tests of repetitive-compulsive behavior were associated with larger CBD effects and the social interaction test was associated with smaller URB597 effects compared to approach avoidance tests. Further, only effects of AM404 in tests of repetitive compulsive-like behavior were dependent on dose. Within the range of tested doses (HED 0.0081–1.62), higher HED was associated with larger drug effects (Supplemental Fig. 19).

### 3.3. Safety and tolerability of FAAH hydrolysis and AEA transport inhibitors

Harm-related information was a secondary outcome, and our literature search did not include terms related to safety and tolerability. Our qualitative summary of harm-related information from the included studies with harm-related objectives ($n = 17$) is therefore non-systematic.

#### 3.3.1. Safety and tolerability of CBD

Included studies employing CBD, in which side effects were either noted when mentioned spontaneously by human participants (Masataka, 2019) or were monitored as part of the study in humans (Fusar-Poli et al., 2009) or dogs (Morris et al., 2020), reported no significant adverse events. Self-rating of subjective states yielded no particularities (Crippa et al., 2004, 2011; Fusar-Poli et al., 2009), except from increased mental sedation in healthy individuals with 400 mg CBD, 60 and 75 min after oral drug intake (Crippa et al., 2004), that was not observed in patients with social anxiety disorder (Crippa et al., 2011). This is in line with previous reviews (Chesney et al., 2020; Huestis et al., 2019; Iffland and Grotenhermen, 2017; Kwee et al., 2022b).

No undesirable effects of the drug on learning and memory were observed when repeatedly administered in mice (Myers et al., 2019; Schleicher et al., 2019) and rats (Kajero et al., 2020).

Differential effects of repeated CBD administration, including no effect on motor activity in mice (Schleicher et al., 2019; Todd et al., 2017) and rats (Kajero et al., 2020) and weight gain in rats (Kajero et al., 2020) and dogs (Morris et al., 2020) underline the difficulties of interspecies translation.

#### 3.3.2. Safety and tolerability of FAAH inhibitors

Sub-chronic treatment with irreversible FAAH inhibitors PF-04457845 (Mayo et al., 2020) and JNJ-42165279 (Paulus et al., 2021) in experimental studies with healthy human volunteers, and JNJ-42165279 in a clinical trial with patients with social anxiety disorder (Schmidt et al., 2021) yielded no serious adverse events.

Doses of PF-3845 sufficient to induce an anxiolytic effects in acute (Bedse et al., 2018; Duan et al., 2017) and chronically (Duan et al., 2017) stressed mice exerted no effect on working memory (Duan et al., 2017), locomotor activity, body temperature, and tests of learning and memory (Bedse et al., 2018).

Six weeks of treatment with the irreversible FAAH inhibitor URB597 unexpectedly led to chemical alterations in the cingulate cortex in mice (Lomazzo et al., 2017). The reversible FAAH inhibitor SSR411298 elicited in mice hyperlocomotion, hypothermia, antinociception, and catalepsy at doses higher than needed to produce an anxiolytic effect (Griebel et al., 2018).

#### 3.3.3. Safety and tolerability of AEA transport inhibitors

The endocannabinoid transport inhibitor WOBE437 (Chicca et al., 2017) elicited in mice a full cannabinoid tetrad response at doses higher than needed to produce an anxiolytic effect.

#### 3.3.4. Risk of bias for harm-related outcomes

All studies ($n = 17$) with information on safety and tolerability were assessed as unclear risk of bias, see Supplemental material, section 3.7 for grading per criterion. Risk of bias for individual studies and summary risk of bias assessments are displayed in Supplemental Fig. 20.

## 4. Discussion

The endocannabinoid system has gathered a lot of interest in relation to its potential role in (the alleviation of) anxiety. The potential of pharmacological enhancement of AEA levels for treating anxiety symptoms has often been discussed. However, a comprehensive systematic review and meta-analysis into the effectiveness of this strategy, potential moderators, and side effects, had not yet been conducted, which was the aim of this paper.

### 4.1. Overall drug effects

Our results showed significant anxiety reduction across drugs for conditioned and unconditioned anxiety in rats, mice and Cricetidae, and for experimentally induced anxiety in humans, with moderate to large effect sizes (Hedge's g between 0.47–0.79) and anxiety-reducing effects with all compounds (CBD, URB597, AM404, PF-3845). The only exception to these positive meta-analytic results was a lack of significant effect of the selective and irreversible FAAH inhibitor URB597 on unconditioned anxiety in animals. These findings provide broad evidence for the often discussed potential of AEA augmentation for treating symptoms of anxiety and related disorders.

### 4.2. Moderators of drug effects

We identified several moderators of drug effects on anxiety outcomes, as expected given the large diversity in study procedures. As explained in the introduction, a theoretical distinction can be made between unconditioned and conditioned anxiety. For animal studies we conducted meta-analyses for both classes of anxiety for CBD, URB597 and AM404. For PF-3845, only tests of unconditioned anxiety were available. Overall, the meta-analytic analyses demonstrated evidence of beneficial effects of CBD, AM404, and PF-3845 on unconditioned anxiety and of CBD, URB597 and AM404 on conditioned anxiety.

Moderators analyses were conducted using Bayesian regularized meta-regression (BRMA, Van Lissa and Van Erp, 2021, Preprint). Firstly, we found drug effects of CBD and URB597 on unconditioned anxiety to be dependent on type of anxiety test. More than half (56%) of the effects on anxiety outcomes in this meta-analysis were measured using approach avoidance tests in animals. Interestingly, approach avoidance tests yielded relatively low effect sizes, and in comparison larger beneficial effects of CBD were found in tests of repetitive compulsive-like behavior. The marble burying test is an established and often used model of repetitive behavior (Thomas et al., 2009). Attenuating effects of CBD on marble burying are not likely a consequence of sedation. Motor functioning was not affected by CBD in included studies that measured both marble burying and motor activity (Casarotto et al., 2010; Murphy et al., 2017; Nardo et al., 2014).

The dose effect-relation for AM404 on repetitive compulsive-like behavior, identified in exploratory moderator analyses, strengthens the evidence for beneficial effects of AEA enhancement for this type of behavior. However, beneficial effects of CBD and AM404 on repetitive compulsive-like behavior have mostly been demonstrated in studies using the marble burying test. Single test results have limited predictive validity for drug effects in patients. These preclinical findings therefore warrant more extensive testing in other models of repetitive behavior as well as in humans.

While URB597 was anxiolytic in other anxiety tests, our moderator analysis showed that overall, it decreased time in social interaction across studies (Matricon et al., 2016; Seillier et al., 2010, 2013, 2018). An explanation for this finding may be that the social interaction test is not aversive enough to detect beneficial URB597 effects on anxiety (Bambico et al., 2016; Haller et al., 2009). Some effects in the opposite direction may result from a curvilinear relation between amygdalar AEA levels and time in social interaction (Seillier et al., 2013). That is, normal physiological AEA levels in the amygdala during the test were associated with maximum time in social interaction, and URB597 could only improve interaction time in rats with pharmacologically reduced amygdalar AEA levels. Administration of URB597 to healthy animals increased AEA levels above the optimum and led to social withdrawal (Seillier et al., 2013, 2018).

Next to type of anxiety test, a second moderator with respect to the effects of CBD on unconditioned anxiety in animals was pre-existing anxiety condition, which increased effects compared to no such condition. Anxiety conditions were generated by exposure to a single stressor (Campos et al., 2012; Rock et al., 2017; Shallcross et al., 2019), or to chronic unpredictable stressors (Campos et al., 2013; Fogaça et al., 2018). All procedures had in common that they induced anxiogenic behavior by stress, compared to control animals. From the stress literature it is known that the ECS acts mediates stress effects on behavior (for a review, see Morena et al., 2016). Further, within single studies, anxiolytic effects of inhibitors of FAAH in rats seemed to depend on the stressfulness of experimental conditions (Haller et al., 2009; Song et al., 2016).

A third moderator of CBD effects on unconditioned anxiety was publication year. Our sample was characterized by a large range in publication years (1990–2021). Effects of CBD were smaller in more recent compared to older publications. This result is in line with a phenomenon called the decline effect: over time, the number of controlled studies increases and scientifically discovered effects tend to become smaller (Schooler, 2011). Statistical power is very important to the decline effect: When studies are underpowered, the chance increases that positive (non-null) effects are not indicative of a true effect in the population, or are an overestimations of this true effect (Button et al., 2013). Unfortunately, most studies in neuroscience are grossly underpowered (Button et al., 2013). When initial underpowered studies that report an effect are being followed up, it is very likely to find smaller effects over time.

Further, differences between the sexes might partly explain the effect of publication year on effect size, given that only in publications from recent years the effect of CBD on female animals has been studied. Sex differences were not identified in the analysis. However, this might be a consequence of females still being poorly represented compared to males. Dependent on type of anxiety test, female animals show differences in anxiety-like behavior compared to their male counterparts (Krokas and Dalla, 2014). In addition, differences in the endocannabinoid system in male and female rodents have been observed (Reich et al., 2009). These differences may influence the effect of CBD in males and females.

No moderator effects related to different types of outcomes in conditioned anxiety tests in animals were identified. This may partly be due to the duration of drug effects that can overlap different phases unless they are carefully separated experimentally. No other moderators of drug effects on conditioned anxiety in animals, and of the effect of CBD on experimentally induced anxiety in humans were identified, whereas significant statistical heterogeneity suggests variation in effect sizes. Some categories in the moderator analyses included only few studies, and therefore these levels of moderator variables were relatively poorly represented.

### 4.3. Quality of the evidence

Notwithstanding our positive results, the quality of the evidence was assessed as low. Importantly, publication bias was strongly or very strongly suspected across all drug types and types of anxiety. To date no procedures are yet available to estimate the extent of this bias for multilevel meta-analysis. Nevertheless, we caution that the reported pooled effect sizes likely overestimate the true effect sizes. Furthermore, our findings provide only indirect evidence of clinical efficacy, since the vast majority of included studies (95%) was conducted in non-human mammals. Given the diversity in study procedures in preclinical research (Vesterinen et al., 2014), the available body of evidence is suitable for identifying potential moderators of clinical effects, while conclusions about overall clinical efficacy are premature.

Our sensitivity analyses demonstrated lack of robustness of our findings with respect to the effect of URB597 on unconditioned anxiety in animals and of CBD on experimentally induced anxiety in humans. We excluded studies based on our assessment of bias that was, in retrospect, rather stringent. For example, concerns about blinding success given sedative effects of CBD led to a high risk of bias rating in one human study, while blinding may have been unsuccessful in other studies as well. However, this remains obscure because blinding success was rarely assessed across studies. Yet, the results of these sensitivity analyses indicate that more high quality evidence is paramount to further substantiate our findings regarding beneficial effects of AEA augmentation for treating symptoms of anxiety and related disorders (Guyatt et al., 2008).

### 4.4. Safety and tolerability

We described data from the $n = 17$ included papers with harm-related objectives, each with unclear risk of bias for harm-related outcomes. In most of these studies no functional or behavioral side-effects were reported that could be attributed to the drugs under study. Side effects typically induced by CB1 receptor agonists were reported in two studies with drugs that were not studied enough to warrant meta-analysis (SSR411298; Griebel et al., 2018 and WOBE437; Chicca et al., 2017). In line with the overall favorable picture that emerges from previous reviews (Chesney et al., 2020; Huestis et al., 2019; Iffland and Grotenhermen, 2017; Kwee et al., 2022b), the studies we reviewed reported no severe adverse events after CBD administration. A systematic investigation of relations between drug concentrations and desirable and undesirable drug effects is needed to elucidate whether undesirable effects also occur at doses needed for anxiolytic effects. As we argue in Kwee et al. (2022b), more studies that also include integrated pharmacokinetic and anxiety assessments are needed to answer this question for repeated CBD dosing.

### 4.5. Limitations of the review

A primary critical note concerns the assumption that the effects of the studied compounds are associated with an increase in AEA levels. Most studies have relied on single dosing, whereas available evidence with CBD suggests significant increases in AEA levels after continuous dosing during several weeks (Leweke et al., 2012). Moreover, some compounds exert additional effects next to enhancement of AEA availability. Specifically, FAAH inhibitors do not only elevate AEA levels, but also elevate levels of oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) (Bortolato et al., 2006; Fegley et al., 2005; Schmidt et al., 2021. Nevertheless, AM404, an AEA transport inhibitor that does not affect PEA and OEA levels (Bortolato et al., 2006) also exerted beneficial effects on anxiety outcomes in our meta-analysis. This strengthens the assumption that the anxiolytic effects of the drugs under study are set about via pharmacological enhancement of AEA levels. For CBD, the mechanistic route for anxiety reduction is even less clear. Although CBD is a weak inhibitor of FAAH (Bisogno et al., 2001; Mlost et al., 2020) its action may also be partly explained by

its binding to intracellular AEA transporters. In fact, 76 different molecular targets of CBD were identified, including ionotropic, non-cannabinoid targets (Mlost et al., 2020).

Several methodological limitations affect the generalizability of our results. First, the number of studies in our meta-analysis did not allow testing a plethora of moderator variables. That is, although BRMA limits overfitting, generalizability can still be low if the sample of studies is small and idiosyncratic (Van Lissa and Van Erp, 2021, Preprint). With this in mind, the data of different types of non-human mammals were analyzed together and we only investigated main effects of species. Although the 95% credible interval of species on itself included zero in our planned moderator analyses, an interaction between species and other variables, such as dose (Kwee et al., 2022b) cannot be excluded.

Second, our findings regarding safety and tolerability of tested compounds do not result from a systematic literature search and evaluation of these parameters. For a translation of wanted and undesirable drug effects in preclinical models to substantiated and safe dose selection for clinical trials we recommend using the IB-de-risk tool (Van Gerven and Cohen, 2018) see, for example Cohen et al. (2022) and Kwee et al. (2022b) for a dose response analysis of CBD. Such a structured approach for dose-rationale, as well as FAAH inhibition assays and measurement of AEA plasma concentrations (e.g., Russo et al., 2007) are required to identify what constitutes unnecessarily high and perhaps riskful dosing.

Third, literature was searched up to May 2021 and at that time, only two clinical trials with inhibitors of FAAH and AEA transport were published. The first randomized controlled trial reported a positive effect of four weeks of 300 mg CBD in social anxiety disorder ($n = 37$; Masataka, 2019), whereas the second observed no effect of 12 weeks of JNJ-42,165,279 ($n = 134$; Schmidt et al., 2021). More recent publications including two clinical trials were not included in this meta-analysis. The first entailed an open-label study in which 300 mg oral CBD plus standard care ($n = 61$) was compared to standard care alone ($n = 59$) in frontline health care professionals working with patients with COVID-19 (Crippa et al., 2021). In this study, CBD induced anxiolytic effects. The second double-blind clinical, trial augmentation of eight therapist-assisted exposure in vivo sessions (weekly, outpatient) with 300 mg oral CBD yielded no differences in treatment outcome over time between CBD ($n = 39$) and placebo ($n = 41$; Kwee et al., 2022a).

### 4.6. Recommendations for future work

This promising field of research has room for improvement. More systematic reporting of methods and study design can aid in interpreting each other's work and assessment of research quality. A structured approach to reporting for human research has been available in the form of the CONSORT statement (Begg et al., 1996). Standards for reporting are now also available for animal research in the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020). More uniformity across anxiety tests in the parameters that are studied may aid in synthesizing findings from multiple studies. The definitions for outcomes of tests of conditioned anxiety that we established for this meta-analysis (see Supplemental Fig. 1) may help specify (reporting of) endpoints in conditioned anxiety research.

In the past two decades FAAH inhibitors have been developed at a rapid pace. These compounds have greater selectivity than the 'old' FAAH inhibitor URB597, for example with respect to off-target carboxylesterases that may limit therapeutic applicability (Clapper et al., 2009; Hill et al., 2013). Keeping in mind the serious adverse events in the BIAL phase 1 trial (Kerbrat et al., 2016) and given the divergent results with respect to safety and tolerability of FAAH inhibitors (Panlilio et al., 2016), a structured approach for dose-rationale (e.g., Cohen et al., 2022; Kwee et al., 2022) should be employed on a drug-by-drug basis before proceeding to first in-human trials.

### 4.7. Conclusions

This systematic review and meta-analysis provides extensive evidence for the beneficial effects of FAAH inhibitors and inhibitors of AEA transport in preclinical tests of anxiety. The beneficial drug effects on conditioned anxiety are especially relevant to clinical practice, because fear conditioning paradigms model the learning that takes place during psychotherapy. Furthermore, a pre-existing anxiety condition in animals predicted larger effects of CBD on unconditioned anxiety. It is therefore tempting to conclude from our meta-analytic results that effective application in patients is feasible. However, the quality of the evidence was low and human studies are still scarce. Therefore, definitive conclusions will have to await more high quality evidence. The analyses we present here indicate that anxiety-reducing effects of the studied compounds can be demonstrated across-the-board but may also depend on the specific facets of anxiety that are studied. They suggest that anxious animals and repetitive behavior seem most susceptible to pharmacological AEA enhancement. An increased focus on the specific aspects of stress and anxiety that are under endocannabinoid control will narrow down potential clinical applications. At the same time, investigation of drug efficacy in patients remains paramount to allow the flow of information back and forth between preclinical and clinical research.

## Role of the funding source

Only the team of researchers had a role in the design and execution of the study, the analyses and interpretation of the data, and decision to submit for publication. There was no influence from the funding organizations in any of these aspects.

## Contributors

All authors contributed to the design of the study. CK, LG and JB were responsible for the study protocol. CK, NL and RvdK screened and selected studies, CK, NL and collaborators IdV and AM extracted data, and CK and NL graded the quality of the evidence, under the supervision of LG and

JB. CvL analyzed the data. CK wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

## Conflict of interest

The authors have no conflicting interests to declare.

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## Supplementary materials

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## References

Ahn, K., Johnson, D.S., Mileni, M., Beidler, D., Long, J.Z., McKinney, M.K., Weerapana, E., Sadagopan, N., Liimatta, M., Smith, S.E., Lazerwith, S., Stiff, C., Kamtekar, S., Bhattacharya, K., Zhang, Y., Swaney, S., Van Becelaere, K., Stevens, R.C., Cravatt, B.F., 2009. Discovery and characterization of a highly selective FAAH inhibitor that reduces inflammatory pain. Chem. Biol. 16, 411–420.

Bach, D.R., 2022. Cross-species anxiety tests in psychiatry: pitfalls and promises. Mol. Psychiatry. 27, 154–163.

Bambico, F.R., Duranti, A., Nobrega, J.N., Gobbi, G., 2016. The fatty acid amide hydrolase inhibitor URB597 modulates serotonin-dependent emotional behaviour, and serotonin1A and serotonin2A/C activity in the hippocampus. Eur. Neuropsychopharmacol. 26, 578–590.

Bedse, G., Bluett, R.J., Patrick, T.A., Romness, N.K., Gaulden, A.D., Kingsley, P.J., Plath, N., Marnett, L.J., Patel, S., 2018. Therapeutic endocannabinoid augmentation for mood and anxiety disorders: comparative profiling of FAAH, MAGL and dual inhibitors. Transl. Psychiatry 8, 92.

Begg, C., Cho, M., Eastwood, S., Horton, R., Moher, D., Olkin, I., Pitkin, R., Rennie, D., Schulz, K.F., Simel, D., Stroup, D.F., 1996. Improving the quality of reporting of randomized controlled trials: the CONSORT statement. JAMA 276, 637–639.

Bisogno, T., Hanuš, L., De Petrocellis, L., Tchilibon, S., Ponde, D.E., Brandi, I., Moriello, A.S., Davis, J.B., Mechoulam, R., Di Marzo, V., 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134, 845–852.

Black, N., Stockings, E., Campbell, G., Tran, L.T., Zagic, D., Hall, W.D., Farrell, M., Degenhardt, L., 2019. Cannabinoids for the treatment of mental disorders and symptoms of mental disorders: a systematic review and meta-analysis. Lancet Psychiatry 6, 995–1010.

Bortolato, M., Campolongo, P., Mangieri, R.A., Scattoni, M.L., Frau, R., Trezza, V., La Rana, G., Russo, R., Calignano, A., Gessa, G.L., Cuomo, V., Piomelli, D., 2006. Anxiolytic-like properties of the anandamide transport inhibitor AM404. Neuropsychopharmacology 31, 2652–2659.

Button, K.S., Ioannidis, J.P.A., Mokrysz, C., Nosek, B.A., Flint, J., Robinson, E.S.J., Munafò, M.R., 2013. Power failure: why small sample size undermines the reliability of neuroscience. Nat Rev Neurosci 14, 365–376.

Campos, A.C., Ferreira, F.R., Guimarães, F.S., 2012. Cannabidiol blocks long-lasting behavioral consequences of predator threat stress: possible involvement of 5HT1A receptors. J. Psychiatr. Res. 46, 1501–1510.

Campos, A.C., Ortega, Z., Palazuelos, J., Fogaça, M.V., Aguiar, D.C., Díaz-Alonso, J., Ortega-Gutiérrez, S., Vázquez-Villa, H., Moreira, F.A., Guzmán, M., Galve-Roperh, I., Guimarães, F.S., 2013. The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int. J. Neuropsychopharmacol. 16, 1407–1419.

Carobrez, A.P., Bertoglio, L.J., 2005. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. Neurosci. Biobehav. Rev. 29, 1193–1205.

Casarotto, P.C., Gomes, F.V., Resstel, L.B., Guimarães, F.S., 2010. Cannabidiol inhibitory effect on marble-burying behaviour: involvement of CB1 receptors. Behav. Pharmacol. 21, 353–358.

Castelli, M.P., Fadda, P., Casu, A., Spano, M.S., Casti, A., Fratta, W., Fattore, L., 2014. Male and female rats differ in brain cannabinoid CB1 receptor density and function and in behavioural traits predisposing to drug addiction: effect of ovarian hormones. Curr. Pharm. Des. 20, 2100–2113.

Center for Drug Evaluation and Research. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers; 2015. Available from: http://www.fda.gov/downloads/Drugs/Guidance/UCM078932.pdf [Last accessed 13 December 2021].

Chesney, E., Oliver, D., Green, A., Sovi, S., Wilson, J., Englund, A., Freeman, T.P., Mcguire, P., 2020. Adverse effects of cannabidiol: a systematic review and meta-analysis of randomized clinical trials. Neuropsychopharmacology 45, 1799–1806.

Chhatwal, J.P., Davis, M., Maguschak, K.A., Ressler, K.J., 2005. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30, 516–524.

Chicca, A., Nicolussi, S., Bartholomäus, R., Blunder, M., Aparisi Rey, A., Petrucci, V., Reynoso Moreno, I.D.C., Viveros-Paredes, J.M., Dalghi Gens, M., Lutz, B., Schiöth, H.B., Soeberdt, M., Abels, C., Charles, R.P., Altmann, K.H., Gertsch, J., 2017. Chemical probes to potently and selectively inhibit endocannabinoid cellular reuptake. Proc. Natl. Acad. Sci. U S A 114, E5006–e5015.

Clapper, J.R., Vacondio, F., King, A.R., Duranti, A., Tontini, A., Silva, C., Sanchini, S., Tarzia, G., Mor, M., Piomelli, D., 2009. A second generation of carbamate-based fatty acid amide hydrolase inhibitors with improved activity in vivo. ChemMedChem 24 (9), 1505–1513.

Cohen, S., 1978. Marijuana. Does it have a possible therapeutic use? JAMA 240, 1761–1763.

Cohen, A.F., van Smeden, J., Webb, D.J., 2022. De-risking clinical trials: the BIAL phase I trial in foresight. Clin. Pharmacol. Ther. 111, 362–365.

Craske, M.G., Hermans, D., Vervliet, B., 2018. State-of-the-art and future directions for extinction as a translational model for fear and anxiety. Phil. Trans. R. Soc. B. 373, 20170025.

Crippa, J.A., Derenusson, G.N., Ferrari, T.B., Wichert-Ana, L., Duran, F.L., Martin-Santos, R., Simões, M.V., Bhattacharyya, S., Fusar-Poli, P., Atakan, Z., Santos Filho, A., Freitas-Ferrari, M.C.,

McGuire, P.K., Zuardi, A.W., Busatto, G.F., Hallak, J.E., 2011. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. J. Psychopharmacol. 25, 121–130.

Crippa, J.A.S., Zuardi, A.W., Guimarães, F.S., Campos, A.C., de Lima Osório, F., Loureiro, S.R., dos Santos, R.G., Souza, J.D.S., Ushirohira, J.M., Pacheco, J.C., Ferreira, R.R., Costa, K.C.M., Scomparin, D.S., Scarante, F.F., Pires-Dos-Santos, I., Mechoulam, R., Kapczinski, F., Fonseca, B.A.L., Esposito, D.L.A., Pereira-Lima, K., Sen, S., Andraus, M.H., Hallak, J.E.C., 2021. Efficacy and safety of cannabidiol plus standard care vs standard care alone for the treatment of emotional exhaustion and burnout among frontline health care workers during the COVID-19 pandemic: a randomized clinical trial. JAMA Netw. Open 4, e2120603.

Crippa, J.A., Zuardi, A.W., Garrido, G.E., Wichert-Ana, L., Guarnieri, R., Ferrari, L., Azevedo Marques, P.M., Hallak, J.E., McGuire, P.K., Filho Busatto, G., 2004. Effects of cannabidiol (CBD) on regional cerebral blood flow. Neuropsychopharmacology 29, 417–426.

Dalton, W.S., Martz, R., Lemberger, L., Rodda, B.E., Forney, R.B., 1976. Influence of cannabidiol on delta-9 tetrahydrocannabinol effects. Clin. Pharmacol. Ther. 19, 300–309.

Davis, M., Ressler, K., Rothbaum, B.O., Richardson, R., 2006. Effects of D-cycloserine on extinction: translation from preclinical to clinical work. Biol. Psychiatry 60, 369–375.

Deutsch, D.G., 2016. A Personal Retrospective: elevating Anandamide (AEA) by Targeting Fatty Acid Amide Hydrolase (FAAH) and the Fatty Acid Binding Proteins (FABPs). Front. Pharmacol. 13, 370.

Devane, W.A., Hanuš, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Duan, T., Gu, N., Wang, Y., Wang, F., Zhu, J., Fang, Y., Shen, Y., Han, J., Zhang, X., 2017. Fatty acid amide hydrolase inhibitors produce rapid anti-anxiety responses through amygdala long term depression in male rodents. J. Psychiatry Neurosci. 42, 230–241.

Fegley, D., Gaetani, S., Duranti, A., Tontini, A., Mor, M., Tarzia, G., Piomelli, D., 2005. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′ carbamoyl-biphenyl 3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. J. Pharmacol. Exp. Ther. 313, 352–358.

Fogaça, M.V., Campos, A.C., Coelho, L.D., Duman, R.S., Guimarães, F.S., 2018. The anxiolytic effects of cannabidiol in chronically stressed mice are mediated by the endocannabinoid system: role of neurogenesis and dendritic remodeling. Neuropharmacology 135, 22–33.

Fowler, C.J., 2021. The endocannabinoid system - current implications for drug development. J. Intern. Med..

Fusar-Poli, P., Crippa, J.A., Bhattacharyya, S., Borgwardt, S.J., Allen, P., Martin-Santos, R., Seal, M., Surguladze, S.A., Carrol, C., Atakan, Z., Zuardi, A.W., McGuire, P.K., 2009. Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch. Gen. Psychiatry 66, 95–105.

Ganon-Elazar, E., Akirav, I., 2009. Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. J. Neurosci. 29, 11078–11088.

Giacobbe, J., Marrocu, A., Di Benedetto, M.G., Pariante, C.M., Borsini, A., 2021. A systematic, integrative review of the effects of the endocannabinoid system on inflammation and neurogenesis in animal models of affective disorders. Brain. Behav. Immun. 93, 353–367.

Gorzalka, B.B., Hill, M.N., Hillard, C.J., 2008. Regulation of endocannabinoid signaling by stress: implications for stress-related affective disorders. Neurosci. Biobehav. Rev. 32, 1152–1160.

Griebel, G., Holmes, A., 2013. 50 years of hurdles and hope in anxiolytic drug discovery. Nat. Rev. Drug. Discov. 12, 667–687.

Griebel, G., Stemmelin, J., Lopez-Grancha, M., Fauchey, V., Slowinski, F., Pichat, P., Dargazanli, G., Abouabdellah, A., Cohen, C., Bergis, O.E., 2018. The selective reversible FAAH inhibitor, SSR411298, restores the development of maladaptive behaviors to acute and chronic stress in rodents. Sci. Rep. 8, 2416.

Guimaraes, F.S., Chiaretti, T.M., Graeff, F.G., Zuardi, A.W., 1990. Antianxiety effect of cannabidiol in the elevated plus-maze. Psychopharmacology (Berl) 100, 558–559.

Guimaraes, F.S., De Aguiar, J.C., Mechoulam, R., Breuer, A., 1994. Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. Gen. Pharmacol. 25, 161–164.

Guyatt, G.H., Oxman, A.D., Vist, G.E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., Schunemann, H.J., 2008. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ 336, 924–926.

Haller, J., Barna, I., Barsvari, B., Gyimesi Pelczer, K., Yasar, S., Panlilio, L.V., Goldberg, S., 2009. Interactions between environmental aversiveness and the anxiolytic effects of enhanced cannabinoid signaling by FAAH inhibition in rats. Psychopharmacology (Berl) 204, 607–616.

Haller, J., Mátyás, F., Soproni, K., Varga, B., Barsy, B., Németh, B., Mikics, É., Freund, T.F., Hájos, N., 2007. Correlated species differences in the effects of cannabinoid ligands on anxiety and on GABAergic and glutamatergic synaptic transmission. Eur. J. Neurosci. 25, 2445–2456.

Hedges, L.V., 1981. Distribution theory for Glass's estimator of effect size and related estimators. J. Educ. Statistics 6, 107–128.

Higgins, J.P., Thompson, S.G., 2002. Quantifying heterogeneity in a meta-analysis. Stat. Med. 21, 1539–1558.

Hill, M.N., Kumar, S.A., Filipski, S.B., Iverson, M., Stuhr, K.L., Keith, J.M., Cravatt, B.F., Hillard, C.J., Chattarji, S., McEwen, B.S., 2013. Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol Psychiatry 18 (10), 1125–1135.

Hill, M.N., McEwen, B.S., 2010. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. Prog. Neuropsychopharmacol. Biol. Psychiatry. 34, 791–797.

Huestis, M.A., Solimini, R., Pichini, S., Pacifici, R., Carlier, J., Busardò, F.P., 2019. Cannabidiol adverse effects and toxicity. Curr. Neuropharmacol. 17, 974–989.

Iffland, K., Grotenhermen, F., 2017. An update on safety and side effects of cannabidiol: a review of clinical data and relevant animal studies. Cannabis Cannabinoid Res 2, 139–154.

Kaczocha, M., Vivieca, S., Sun, J., Glaser, S.T., Deutsch, D.G., 2012. Fatty acid-binding proteins transport N acylethanolamines to nuclear receptors and are targets of endocannabinoid transport inhibitors. J. Biol. Chem. 287, 3415–3424.

Kajero, J.A., Seedat, S., Ohaeri, J., Akindele, A., Aina, O., 2020. Investigation of the effects of cannabidiol on vacuous chewing movements, locomotion, oxidative stress and blood glucose in rats treated with oral haloperidol. World J. Biol. Psychiatry 21, 612–626.

Karniol, I.G., Shirakawa, I., Kasinski, N., 1974. Cannabidiol interferes with the effects of $\Delta$9 tetrahydrocannabinol in man. Eur. J. Pharmacol. 28, 172–177.

Kathuria, S., Gaetani, S., Fegley, D., Valiño, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., Piomelli, D., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. Nat. Med. 9, 76–81.

Kerbrat, A., Ferré, J.C., Fillatre, P., Ronzière, T., Vannier, S., Carsin-Nicol, B., Lavoué, S., Vérin, M., Gauvrit, J.Y., Le

Tulzo, Y., Edan, G., 2016. Acute neurologic disorder from an inhibitor of fatty acid amide hydrolase. N Engl. J. Med. 375 (18), 1717–1725.
Kokras, N., Dalla, C., 2014. Sex differences in animal models of psychiatric disorders. Br J Pharmacol 171 (20), 4595–4619.
Kwee, C.M.B., Baas, J.M.P., Van der Flier, F.E., Groenink, L., Duits, P., Eikelenboom, M., van der Veen, D.C., Moerbeek, M., Batelaan, N.M., van Balkom, A.J.L.M., Cath, D.C., 2022a. Cannabidiol enhancement of exposure therapy in treatment refractory patients with social anxiety disorder and panic disorder with agoraphobia: a randomised controlled trial. Eur. Neuropsychopharmacol. 59, 58–67.
Kwee, C.M.B., Van Gerven, J.M.A., Bongaerts, F.L.P., Cath, D.C., Jacobs, G., Baas, J.M.P, Groenink, L., 2022b. Cannabidiol in clinical and preclinical anxiety research. A systematic review into concentration-effect relations using the IB-de-risk tool. J. Psychopharmacol. 36, 1299–1314.
Lafenêtre, P., Chaouloff, F., Marsicano, G., 2007. The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. Pharmacol. Res. 56, 367–381.
Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., Klosterkotter, J., Hellmich, M., Koethe, D., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl. Psychiatry 2, e94.
Lomazzo, E., König, F., Abassi, L., Jelinek, R., Lutz, B., 2017. Chronic stress leads to epigenetic dysregulation in the neuropeptide-Y and cannabinoid CB1 receptor genes in the mouse cingulate cortex. Neuropharmacology 113, 301–313.
Lutz, B., Marsicano, G., Maldonado, R., Hillard, C.J., 2015. The endocannabinoid system in guarding against fear, anxiety and stress. Nat. Rev. Neurosci. 16, 705–718.
Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M.G., Hermann, H., Tang, J., Hofmann, C., Zieglgänsberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.
Masataka, N., 2019. Anxiolytic effects of repeated cannabidiol treatment in teenagers with social anxiety disorders. Front. Psychol. 10, 2466.
Matricon, J., Seillier, A., Giuffrida, A., 2016. Distinct neuronal activation patterns are associated with PCP-induced social withdrawal and its reversal by the endocannabinoid enhancing drug URB597. Neurosci. Res. 110, 49–58.
Mayo, L.M., Asratian, A., Lindé, J., Morena, M., Haataja, R., Hammar, V., Augier, G., Hill, M.N., Heilig, M., 2020. Elevated Anandamide, Enhanced Recall of Fear Extinction, and Attenuated Stress Responses Following Inhibition of Fatty Acid Amide Hydrolase: a Randomized. Controlled Experimental Medicine Trial. Biol. Psychiatry 87, 538–547.
Mechoulam, R., Fride, E., Di Marzo, V., 1998. Endocannabinoids. Eur. J. Pharmacol. 359, 1–18.
Mlost, J., Bryk, M., Starowicz, K., 2020. Cannabidiol for Pain Treatment: focus on Pharmacology and Mechanism of Action. Int. J. Mol. Sci. 21, 8870.
Morena, M., Patel, S., Bains, J.S., Hill, M.N., 2016. Neurobiological Interactions Between Stress and the Endocannabinoid System. Neuropsychopharmacology 41, 80–102.
Morris, E.M., Kitts-Morgan, S.E., Spangler, D.M., McLeod, K.R., Costa, J.H.C., Harmon, D.L., 2020. The impact of feeding cannabidiol (CBD) containing treats on canine response to a noise-induced fear response test. Front. Vet. Sci. 7.
Muhlhausler, B.S., Bloomfield, F.H., Gillman, M.W., 2013. Whole animal experiments should be more like human randomized controlled trials. PLoS Biol 11, e1001481.
Murphy, M., Mills, S., Winstone, J., Leishman, E., Wager-Miller, J., Bradshaw, H., Mackie, K., 2017. Chronic adolescent Δ9-tetrahydrocannabinol treatment of male mice leads to long term cognitive and behavioral dysfunction, which are prevented by concurrent cannabidiol treatment. Cannabis Cannabinoid Res. 2, 235–246.
Myers, A.M., Siegele, P.B., Foss, J.D., Tuma, R.F., Ward, S.J., 2019. Single and combined effects of plant-derived and synthetic cannabinoids on cognition and cannabinoid-associated withdrawal signs in mice. Br. J. Pharmacol. 176, 1552–1567.
Nardo, M., Casarotto, P.C., Gomes, F.V., Guimarães, F.S., 2014. Cannabidiol reverses the mCPP induced increase in marble-burying behavior. Fundam. Clin. Pharmacol. 28, 544–550.
Onaivi, E.S., Green, M.R., Martin, B.R., 1990. Pharmacological characterization of cannabinoids in the elevated plus maze. J. Pharmacol. Exp. Ther. 253, 1002–1009.
Panlilio, L.V., Thorndike, E.B., Nikas, S.P., Alapafuja, S.O., Bandiera, T., Cravatt, B.J., Makriyannis, A., Piomelli, D., Goldberg, S.R., Justinova, Z., 2016. Effects of fatty acid amide hydrolase (FAAH) inhibitors on working memory in rats. Psychopharmacology (Berl) 233, 1879–1888.
Paredes-Ruiz, K.J., Chavira-Ramos, K., Orozco-Morales, M., Karasu, C., Tinkov, A.A., Aschner, M., Santamaria, A., Colin-Gonzalez, A.L., 2021. On the biomedical properties of endocannabinoid degradation and reuptake inhibitors: pre-clinical and clinical evidence. Neurotox. Res. 39, 2072–2097.
Patel, S., Hillard, C.J., 2008. Adaptations in endocannabinoid signaling in response to repeated homotypic stress: a novel mechanism for stress habituation. Eur. J. Neurosci. 27, 2821–2829.
Paulus, M.P., Stein, M.B., Simmons, A.N., Risbrough, V.B., Halter, R., Chaplan, S.R., 2021. The effects of FAAH inhibition on the neural basis of anxiety-related processing in healthy male subjects: a randomized clinical trial. Neuropsychopharmacology 46, 1011–1019.
Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Wurbel, H., 2020. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. Exp. Physiol. 105, 1459–1466.
Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R., Rushton, L., 2008. Contour-enhanced meta- analysis funnel plots help distinguish publication bias from other causes of asymmetry. J. Clin. Epidemiol. 61 991e996.
Reich, C.G., Taylor, M.E., McCarthy, M.M., 2009. Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. Behav. Brain Res. 203 (2), 264–269.
Rock, E.M., Limebeer, C.L., Petrie, G.N., Williams, L.A., Mechoulam, R., Parker, L.A., 2017. Effect of prior foot shock stress and Δ9-tetrahydrocannabinol, cannabidiolic acid, and cannabidiol on anxiety-like responding in the light-dark emergence test in rats. Psychopharmacology (Berl) 234, 2207–2217.
Rodgers, R.J., Dalvi, A., 1997. Anxiety, defence and the elevated plus-maze. Neurosci. Biobehav. Rev. 21, 801–810.
Ruehle, S., Rey, A.A., Remmers, F., Lutz, B., 2012. The endocannabinoid system in anxiety, fear memory and habituation. J. Psychopharmacol. 26, 23–39.
Russo, R., LoVerme, J., La Rana, G., Compton, T.R., Parrott, J., Duranti, A., Tontini, A., Mor, M., Tarzia, G., Calignano, A., Piomelli, D., 2007. The fatty acid amide hydrolase inhibitor URB597 (cyclohexylcarbamic acid 3′-carbamoylbiphenyl-3-yl ester) reduces neuropathic pain after oral administration in mice. J. Pharmacol. Exp. Ther. 322 (1), 236–242.
Sams-Dodd, F., 2006. Strategies to optimize the validity of disease models in the drug discovery process. Drug. Discov. Today. 11, 355–363.
Schardt, C., Adams, M.B., Owens, T., Keitz, S., Fontelo, P., 2007. Utilization of the PICO framework to improve searching PubMed for clinical questions. BMC Med. Inform. Decis. Mak. 7, 16.

Schleicher, E.M., Ott, F.W., Müller, M., Silcher, B., Sichler, M.E., Löw, M.J., Wagner, J.M., Bouter, Y., 2019. Prolonged cannabidiol treatment lacks on detrimental effects on memory, motor performance and anxiety in C57BL/6J mice. Front. Behav. Neurosci. 13, 94.

Schmidt, M.E., Liebowitz, M.R., Stein, M.B., Grunfeld, J., Van Hove, I., Simmons, W.K., Van Der Ark, P., Palmer, J.A., Saad, Z.S., Pemberton, D.J., Van Nueten, L., Drevets, W.C., 2021. The effects of inhibition of fatty acid amide hydrolase (FAAH) by JNJ-42165279 in social anxiety disorder: a double-blind, randomized, placebo-controlled proof-of-concept study. Neuropsychopharmacology 46, 1004–1010.

Schooler, J., 2011. Unpublished results hide the decline effect. Nature 470, 437.

Schünemann H, Santesso N. Introduction to GRADE; 2010. Available from: https://training.cochrane.org/introduction-grade [Last accessed 14 February 2022].

Seillier, A., Advani, T., Cassano, T., Hensler, J.G., Giuffrida, A., 2010. Inhibition of fatty-acid amide hydrolase and CB1 receptor antagonism differentially affect behavioural responses in normal and PCP treated rats. Int. J. Neuropsychopharmacol. 13, 373.

Seillier, A., Giuffrida, A., 2017. Anxiety does not contribute to social withdrawal in the subchronic phencyclidine rat model of schizophrenia. Behav. Pharmacol. 28, 512–520.

Seillier, A., Giuffrida, A., 2018. The cannabinoid transporter inhibitor OMDM-2 reduces social interaction: further evidence for transporter-mediated endocannabinoid release. Neuropharmacology 130, 1–9.

Seillier, A., Martinez, A.A., Giuffrida, A., 2013. Phencyclidine-induced social withdrawal results from deficient stimulation of cannabinoid CB 1 receptors: implications for schizophrenia. Neuropsychopharmacology 38, 1816–1824.

Shallcross, J., Hámor, P., Bechard, A.R., Romano, M., Knackstedt, L., Schwendt, M., 2019. The Divergent Effects of CDPPB and Cannabidiol on Fear Extinction and Anxiety in a Predator Scent Stress Model of PTSD in Rats. Front. Behav. Neurosci. 13, 91.

Shrout, P.E., Rodgers, J.L., 2018. Psychology, science, and knowledge construction: broadening perspectives from the replication crisis. Annu. Rev. Psychol. 69, 487–510.

Song, C., Stevenson, C.W., Guimaraes, F.S., Lee, J.L., 2016. Bidirectional Effects of Cannabidiol on Contextual Fear Memory Extinction. Front. Pharmacol. 7, 493.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L.A., Paylor, R., 2009. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology (Berl) 204, 361–373.

Todd, S.M., Zhou, C., Clarke, D.J., Chohan, T.W., Bahceci, D., Arnold, J.C., 2017. Interactions between cannabidiol and $\Delta$(9)-THC following acute and repeated dosing: rebound hyperactivity, sensorimotor gating and epigenetic and neuroadaptive changes in the mesolimbic pathway. Eur. Neuropsychopharmacol. 27, 132–145.

Van Gerven, J., Cohen, A., 2018. Integrating data from the Investigational Medicinal Product Dossier/investigator's brochure. A new tool for translational integration of preclinical effects. Br. J. Clin. Pharmacol. 84, 1457–1466.

Van Lissa, C.J., 2020. Small sample meta-analyses. In: van de Schoot, R., Miočević, M. (Eds.), Small Sample Size Solutions. Routledge, London, pp. 186–202.

Van Lissa, C.J., Brandmaier, A.M., Brinkman, L., Lamprecht, A.L., Peikert, A., Struiksma, M.E., Vreede, B.M., 2021. WORCS: a workflow for open reproducible code in science. Data Science 4, 29–49.

Van Lissa, C.J., Van Erp, S., Select relevant moderators using Bayesian regularized meta- regression. Preprint at https://psyarxiv.com/6phs5 (2021).

Vesterinen, H.M., Sena, E.S., Egan, K.J., Hirst, T.C., Churolov, L., Currie, G.L., Antonic, A., Howells, D.W., Macleod, M.R., 2014. Meta-analysis of data from animal studies: a practical guide. J. Neurosci. Methods. 221, 92–102.

Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Softw. 36, 1–48.

Zuardi, A.W., Cosme, R.A., Graeff, F.G., Guimaraes, F.S., 1993. Effects of ipsapirone and cannabidiol on human experimental anxiety. J. Psychopharmacol. 7, 82–88.

Zuardi, A.W., Shirakawa, I., Finkelfarb, E., Karniol, I.G., 1982. Action of cannabidiol on the anxiety and other effects produced by $\Delta$9-THC in normal subjects. Psychopharmacology (Berl) 76, 245–250.