Agenda

Public Hearing
Medical Cannabis Advisory Board (MCAB) to the New Mexico Department of Health Medical Cannabis Program (NMDOH MCP)
Tuesday, March 22, 2022, 9:00 a.m. to 12:00 p.m.
Conducted via web-based platform

1. Call to Order/Introductory Comments & Updates
2. Minutes from previous meeting
3. Introduction of Board Members
4. Medical Cannabis Program Update
    a. Adequate Supply Limit
    b. Streamlining Unit Increases
    c. Elimination of Background Check
5. Petitions from the Public:
    a. 2022-001 Add Anxiety Disorder
6. Public Comment
7. Set date for next MCAB meeting
8. Adjournment

Petition for Anxiety Disorder as qualifying medical condition under Lynn and Erin Compassionate Use Act
Dedam and Richmond

Index

Petition for Anxiety Disorder as qualifying medical condition under Lynn and Erin Compassionate Use Act
Dedam and Richmond

Introductory narrative of individuals requesting inclusion of new medical condition

Petition for Anxiety Disorder as qualifying medical condition under Lynn and Erin Compassionate Use Act

Stephanie Richmond
2400 Tucker Road NE
Albuquerque, NM 87131
505-385-5636

Jean-Paul Dedam
4901 Lang Avenue NE
Albuquerque, NM 87109
505-842-8171

PA-C Richmond and Dr. Dedam are both members of the New Mexico Medical Advisory Board and each have a professional interest as medical providers in the inclusion of this diagnosis for the Lynn and Erin Compassionate Use Act to assist their patients and provide quality healthcare with medical cannabis.

Petition for Anxiety Disorder as qualifying medical condition under Lynn and Erin Compassionate Use Act
Dedam and Richmond

Proposal and medical benefits

Generalized Anxiety Disorder (GAD), social anxiety disorder, medication-induced anxiety, panic disorder, specific phobias and anxiety disorder due to another medical condition incredibly common medical conditions affecting as much a 25% of the population in some meta-analyses (Baxter 2013). These conditions can cause significant disruption in the lives of those who suffer from them, as well as having severe negative consequences on that person's health.

While many good medical treatments exist, they don't always work for every patient, leaving as much at 40% of patients without relief (Bystrisky 2006). Some common treatments of anxiety, such as benzodiazepines, carry a significant risk for abuse, dependence and dangerous withdrawal syndromes. Mental health resources in this state are also severely limited and many patients lack access to care.

Self-medication and patient reports of cannabis use for treatment of anxiety disorders are also common and effective. One study tracking 1399 Medical Cannabis users found a 58% reduction in anxiety and stress symptoms that did not diminish overtime (Cuttler 2018).

While currently there have been no large randomized controlled trials, an observational study of 368 Medical Cannabis user again demonstrated improvement in anxiety symptoms as well as improved sleep (Martin 2021).

Despite the lack of studies, there is also amply basic science evidence to support the use of Medical Cannabis for anxiety. Activation of the Cannabinoid type 1 Receptor (the receptor stimulated by THC) in the brain has been for decades to have an anxiolytic effect at low and moderate does ( Lutz 2015). While high doses can actually cause a paradoxical increase in anxiety, with proper medical guidance and supervision this can be easily avoided with dosing and concomitant CBD administration. This biphasic effect has been used an argument against the use of Medical Cannabis for anxiety. However, since cannabis is now legalized recreationally and it makes far more sense to have this treatment under qualified medical supervision.

Medical cannabis has also been proven safe, is less addictive than benzodiazepines and is safer in both accidental and intentional overdose to all current conventional pharmacologic treatments.

To draw upon my own clinical experience in working with hundreds of cancer patients here in New Mexico, relief of stress and anxiety, be it from a pre-existing diagnosis or secondary to their cancer diagnosis or the treatment of their cancer, remains on the most consistent patient reported benefits.

Petition for Anxiety Disorder as qualifying medical condition under Lynn and Erin Compassionate Use Act
Dedam and Richmond

References:


Baxter AJ, Scott KM, Vos T, Whiteford HA. Global prevalence of anxiety disorders: a systematic review and meta-regression. Psychol Med. 2013 May;43(5):897-910. doi: 10.1017/S003329171200147X. Epub 2012 Jul 10. PMID: 22781489.

Bystritsky A. Treatment-resistant anxiety disorders. Mol Psychiatry. 2006 Sep;11(9):805-14. doi: 10.1038/sj.mp.4001852. Epub 2006 Jul 18. PMID: 16847460.

Cuttler C, Spradlin A, McLaughlin RJ. A naturalistic examination of the perceived effects of cannabis on negative affect. J Affect Disord. 2018 Aug 1;235:198-205. doi: 10.1016/j.jad.2018.04.054. Epub 2018 Apr 6. PMID: 29656267.

Martin EL, Strickland JC, Schlienz NJ, Munson J, Jackson H, Bonn-Miller MO, Vandrey R. Antidepressant and Anxiolytic Effects of Medicinal Cannabis Use in an Observational Trial. Front Psychiatry. 2021 Sep 9;12:729800. doi: 10.3389/fpsyt.2021.729800. PMID: 34566726; PMCID: PMC8458732.

Lutz B, Marsicano G, Maldonado R, Hillard CJ. The endocannabinoid system in guarding against fear, anxiety and stress. Nat Rev Neurosci. 2015 Dec;16(12):705-18. doi: 10.1038/nrn4036. PMID: 26585799; PMCID: PMC5871913.

# A Naturalistic Examination of the Perceived Effects of Cannabis on Negative Affect

**Article** *in* Journal of Affective Disorders · April 2018

DOI: 10.1016/j.jad.2018.04.054



**3 authors:**

Carrie Cuttler
Washington State University
**80** PUBLICATIONS **1,505** CITATIONS
SEE PROFILE

Alexander Spradlin
Washington State University
**19** PUBLICATIONS **352** CITATIONS
SEE PROFILE

Ryan Mclaughlin
Washington State University
**57** PUBLICATIONS **2,256** CITATIONS
SEE PROFILE

Journal of Affective Disorders 235 (2018) 198–205

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

# A naturalistic examination of the perceived effects of cannabis on negative affect

Carrie Cuttler[a,b,*], Alexander Spradlin[a], Ryan J. McLaughlin[a,b,c]

[a] Washington State University, Department of Psychology, P.O. Box 644820, Pullman, WA 99164-4820, USA
[b] Translational Addiction Research Center, Washington State University, USA
[c] Washington State University, Department of Integrative Physiology and Neuroscience, Pullman, WA 99164-7620, USA

ARTICLE INFO

*Keywords:*
Cannabis
Depression
Anxiety
Stress
Dose effects
Multilevel modeling

ABSTRACT

*Background:* Cannabis is commonly used to alleviate symptoms of negative affect. However, a paucity of research has examined the acute effects of cannabis on negative affect in everyday life. The current study provides a naturalistic account of perceived changes in symptoms of depression, anxiety, and stress as a function of dose and concentration of $\Delta^9$tetrahydrocannabinol (THC) and cannabidiol (CBD).

*Method:* Data from the app Strainprint™ (which provides medical cannabis users a means of tracking changes in symptoms as a function of different doses and chemotypes of cannabis) were analyzed using multilevel modeling. In total, 11,953 tracked sessions were analyzed (3,151 for depression, 5,085 for anxiety, and 3,717 for stress).

*Results:* Medical cannabis users perceived a 50% reduction in depression and a 58% reduction in anxiety and stress following cannabis use. Two puffs were sufficient to reduce ratings of depression and anxiety, while 10+ puffs produced the greatest perceived reductions in stress. High CBD (>9.5%)/low THC (<5.5%) cannabis was associated with the largest changes in depression ratings, while high CBD (>11%)/high THC (>26.5%) cannabis produced the largest perceived changes in stress. No changes in the perceived efficacy of cannabis were detected across time. However, baseline symptoms of depression (but not anxiety or stress) appeared to be exacerbated across time/tracked sessions.

*Limitations:* The primary limitations are the self-selected nature of the sample and the inability to control for expectancy effects.

*Conclusions:* Cannabis reduces perceived symptoms of negative affect in the short-term, but continued use may exacerbate baseline symptoms of depression over time.

## 1. Introduction

Cannabis is commonly used to alleviate depression, anxiety, and stress. Indeed, one of the most commonly reported motives for cannabis use is to cope with stress (Hyman and Sinha, 2009), with 72% of daily cannabis users reporting use of cannabis to relax or relieve tension (Johnston and O' Malley, 1986). Further, recent research by Sexton et al. (2016) revealed that the three most frequently endorsed reasons for medical cannabis use are for managing pain, anxiety, and depression, with over 58% of medical cannabis patients reporting use to manage anxiety and over 50% reporting use for depression. Consistent with these results, Webb and Webb (2014) found that 50% of medical cannabis patients – who were using cannabis to treat pain – reported that it provided relief from anxiety and stress. Nevertheless, only two of the 32 states, districts, and territories in the United States that permit medical cannabis use currently recognize anxiety as a qualifying condition, and none overtly recognize depression or high levels of perceived stress as qualifying conditions for a medical cannabis card. This is largely because of a paucity of evidence for the efficacy of cannabis in treating negative affect. As such, the primary purpose of the present study was to examine the perceived efficacy of cannabis in reducing symptoms of depression, anxiety, and stress in a naturalistic context.

While research on the acute effects of cannabis and its two primary constituents – $\Delta^9$tetrahydrocannabinol (THC) and cannabidiol (CBD) – on depression, anxiety, and stress is sparse, there is some limited evidence from double-blind, placebo-controlled studies indicating that oral CBD significantly reduced anxiety and discomfort during a simulated public speaking task in patients with social anxiety disorder (Bergamaschi et al., 2011) and in healthy students (Zuardi et al., 1993). Oral THC has similarly been shown to dose-dependently attenuate the

* Corresponding author at: Washington State University, Department of Psychology, P.O. Box 644820, Johnson Tower, Pullman, WA 99164-4820, USA.
*E-mail address:* carrie.cuttler@wsu.edu (C. Cuttler).

https://doi.org/10.1016/j.jad.2018.04.054
Received 12 February 2018; Received in revised form 2 April 2018; Accepted 4 April 2018
Available online 06 April 2018

subjective response to psychosocial stress (Childs et al., 2017). Further, in patients being treated for fibromyalgia, regular use of nabilone (an orally ingested synthetic cannabinoid receptor agonist) significantly decreased anxiety but had no effects on depression (Skrabek et al., 2008). Conversely, an oral dose of rimonabant (a cannabinoid receptor antagonist) has been shown to significantly increase depression (Christensen et al., 2007) and anxiety (Bergamaschi et al., 2014; Christensen et al., 2007).

It is important to note, however, that these effects have not always been consistent. Specifically, Pillard et al. (1974) found no significant effects of smoking low THC cannabis (1.4%) versus a placebo on anxiety following an anxiety-provoking film or public speaking task. Moreover, a handful of double-blind, placebo-controlled studies examining depression and anxiety as secondary outcomes in patients with other primary conditions (e.g., chronic pain, multiple sclerosis, cancer) found no significant effects of nabilone (Frank et al., 2008), dronabinol (another orally ingested synthetic cannabinoid receptor agonist) (Narang et al., 2008), or nabiximol (a cannabis-based oromucosal spray) (Portenoy et al., 2012; Rog et al., 2005; Wade et al., 2004) on secondary symptoms of anxiety and depression.

Some of these conflicting results may pertain to the use of varying doses and differences in THC vs. CBD content. For instance, a recent double-blind, placebo-controlled study with healthy adults revealed that low doses (7.5 mg) of oral THC attenuated the self-reported negative emotional effects of a psychosocial stressor, while high doses (12.5 mg) increased subjective distress, anxiety, and depression (Childs et al., 2017). Consistent with these results, Fusar-Poli et al. (2008) found that 10 mg of oral THC increased anxiety and other negative emotions relative to a placebo, while oral CBD (600 mg) led to a small decrease in anxiety that was not statistically significant in their small sample ($n = 15$, $p = .06$).

In the majority of studies described above, cannabinoids were administered orally. However, recent research indicates that only 8% of medical cannabis patients use oral administration (Sexton et al., 2016), while over 92% of medical and non-medical cannabis users report using combustion/inhalation methods of administration (Schauer et al., 2016). Moreover, in many of these studies cannabinoids were administered prior to an objectively stressful task and participants were asked to evaluate their affective state in response to that acute stressor. As such, we have a limited understanding of the perceived efficacy of cannabis in coping with feelings of negative affect in everyday life. Therefore, the primary objective of this study was to track the perceived efficacy of inhaled cannabis in coping with feelings of negative affect in medical cannabis users' naturalistic environment. Furthermore, given that symptoms of negative affect are more prevalent in women (American Psychiatric Association, 2013), and that women are more likely to use cannabis to cope with symptoms of anxiety (Cuttler et al., 2016), we further sought to examine potential gender differences in the perceived efficacy of cannabis in reducing symptoms of depression, anxiety, and stress.

The present study represents an attempt to complement the existing, internally valid, double-blind, placebo-controlled studies, with a more ecologically valid, naturalistic approach. To this end, we obtained the global back-data from the app Strainprint™, which offers medical cannabis users a means to track symptom severity before and after self-medicating with cannabis. One advantage of this approach is that it allows us to examine the perceived effects of cannabis over time. In doing so, we can address two important questions. First, does the perceived efficacy of cannabis in managing negative affect change over time? In other words, are the perceived effects of cannabis subject to tolerance? Second, does using cannabis to manage symptoms of depression, anxiety, and stress affect baseline symptoms of negative affect over time? Chronic cannabis use has been shown to cause down-regulation of CB1 receptors in areas such as the prefrontal cortex, anterior cingulate cortex, hippocampus, and parahippocampal gyrus (Hirvonen et al., 2012), which are known to be implicated in mood and emotionality (see Drevets et al., 2008 for review); therefore, regular use of cannabis may have longer-term effects on negative affect (that may be reversed following a period of abstinence). In the current study, we used archival data from Strainprint™ to specifically examine: 1) whether self-reported symptoms of depression, anxiety, and stress are significantly reduced after using cannabis, 2) whether there are gender differences in these putative effects, 3) whether interactions between THC and CBD predict symptom change, 4) whether symptom change varies according to dose, 5) whether perceived efficacy of cannabis changes over time, and 6) whether baseline (i.e., pre-cannabis use) symptoms of negative affect change over time.

## 2. Method

### 2.1. Procedure

To achieve our six aims, we obtained archival data from the medical cannabis app Strainprint™. Using this free app, medical cannabis users can track changes in the severity of their symptoms as a function of different chemotypes and doses of cannabis. Prior to using the app to track their medical cannabis use, individuals enter basic demographic information (i.e., gender and date of birth). Next, they enter all of their medical conditions and symptoms of those conditions by selecting from a list of 279 conditions and 46 symptoms. They are further given the opportunity to enter information about the cannabis that they use by selecting from a list of products sold by licensed medical cannabis distributors in Canada. The THC and CBD content for each of these products were obtained by analyses conducted by one of Health Canada's licensed dealers and is prepopulated within the app. It is important to note that Canada is somewhat unique to other countries in that Health Canada enforces strict production guidelines, quality control guidelines and mandatory lab testing from all ministry approved licensed dealers. This mandatory lab testing includes five stages of processing; preparation, chromatography, general spectrometry, heavy metal spectrometry, and microbial analysis. Users also have the opportunity to enter additional product names and cannabinoid content (% THC, % CBD) for products that are not prepopulated in the app. Users can subsequently track their medical cannabis sessions by: 1) selecting the symptom(s) they are experiencing at the time, 2) rating the severity of each symptom on a scale of 0 (none) to 10 (extreme), 3) selecting (or inputting) the product they will use, 4) indicating their method of administration (smoke, oil, vape, dab bubbler, dab portable, edible, pill, spray, transdermal, tincture), and 5) indicating the quantity of use (e.g., number of puffs ranging from 1 to 10 + ). Twenty minutes after use, individuals are prompted (via a push notification) to re-rate the severity of their symptom.

For the present study, we obtained anonymous data from medical cannabis users who used the app to treat symptoms of depression, anxiety, and stress. Specifically, we obtained data on these individuals' anonymous ID codes; gender; ages; medical conditions and symptoms; self-reported symptom severity before and after each session of medical cannabis use; duration of time between pre-and post-cannabis use symptom ratings; cannabinoid content (% THC, % CBD) for the cannabis used in each session; as well as the method and quantity of use for each session. The Office of Research Assurances determined that this anonymous archival study was exempt from the need for IRB review.

### 2.2. Inclusion/Exclusion criteria

We obtained data from 1,399 medical cannabis users who collectively used the app a total of 18,392 times to track changes in their symptoms of depression, anxiety, or stress. Individuals' use of the app to track their medical cannabis sessions ranged from 1–972 sessions, with a mean of 13.15 ($SD = 38.48$) tracked sessions. Given potential differences in efficacy and onset across different routes of administration (e.g., oral vs. inhaled), only tracked sessions in which individuals

indicated administering cannabis via one of five inhalation methods (smoking, vaping, concentrates, dab bubbler, dab portable) were selected ($n$ = 13,687; 74.42% of data). Tracked sessions that involved administration via other methods (e.g., tincture, edibles) were excluded. Given that the acute subjective effects of inhaled cannabis peak at about 10–30 minutes and taper off after 3–4 h (Grotenhermen, 2003; Menkes et al., 1991), only the 11,953 tracked inhalation sessions for those individuals who re-rated their symptoms within 4 h were included. The remaining inhalation sessions exceeded this time frame and were excluded.

### 2.3. Participants

The final sample comprised 11,953 tracked inhalation sessions. More specifically, 561 medical cannabis users (262 men, 299 women; age $M$ = 33, $SD$ = 10) used the app 3,151 times (Range 1–97; $M$ = 14.30, $SD$ = 17.38) to track changes in depression. A total of 770 users (363 men, 407 women; age $M$ = 33, $SD$ = 10) used the app 5,085 times (Range 1–197; $M$ = 23.38, $SD$ = 35.62) to track changes in anxiety. Finally, 726 people (323 men, 403 women; age $M$ = 34, $SD$ = 9) used the app 3,717 times (Range 1–173; $M$ = 18.93, $SD$ = 28.37) to track changes in stress.

### 2.4. Data analysis

Typically, in order to analyze data with both within- and between-subjects variability to consider, one would use a mixed-factorial analysis of variance (ANOVA) or a multiple regression analysis. However, each of these techniques requires equal numbers of within-subjects observations across participants. Given the differences in the number of sessions tracked using the app across individuals these approaches are not appropriate. However, multilevel modeling (also known as linear mixed models, hierarchical linear models, or mixed-effects models) is a technique that permits the examination of change considering both within- and between-subject variability despite differences in the number of observations across individuals. This technique involves estimating time-variant slope variables at the within-subject level that are then used to predict change at the between-subject level. The models tested in the present study were based on those presented in Finch and Bolin (2016) with additional modifications and settings specified according to the guidelines provided by Muthén and Muthén (2017).

Specifically, multilevel modeling was used to predict changes in symptom severity as a function of gender, dose, and % THC/CBD. We also used multilevel modeling to examine changes in efficacy (i.e., symptom reduction) and changes in baseline symptoms (i.e., pre-cannabis use symptom ratings) across tracked sessions. All models were tested with maximum likelihood estimation with robust standard errors using the latest version of Mplus (version 8; Muthén and Muthén, 2017). All predictor and outcome variables were modeled as functions of time/sessions at the within-subjects level, and the slopes of these regressions (i.e., regression coefficients) were used to test for the between-subjects level effects (e.g., for aim 3, time/sessions was used to predict % THC, % CBD, and symptom change at the within-subject levels, and the slope values produced at this level were used to test for the effects of % THC and % CBD on symptom change at the between-subjects level). All estimates of slopes and intercepts were allowed to vary randomly. For models that included interaction terms, the algorithm used to estimate model parameters was set to numerical integration with 7 integration points.

## 3. Results

### 3.1. Aim 1: overall change in symptom ratings

#### 3.1.1. Depression

Results of the first multilevel model revealed a significant reduction in ratings of depression from before ($M_{Before}$ = 6.02, $SE$ = 0.17) to after ($M_{After}$ = 3.06, $SE$ = 0.21) using cannabis, Wald $\chi^2$ (1, 560) = 364.08, $p$ < .001. Further analyses revealed that depression symptom ratings were reduced in 89.3% of tracked sessions, they were exacerbated in 3.2% of sessions, and there was no change in 7.5% of sessions.

#### 3.1.2. Anxiety

There was also a significant reduction in the ratings of anxiety ($M_{Before}$ = 5.98, $SE$ = 0.12 vs. $M_{After}$ = 2.50, $SE$ = 0.14), Wald $\chi^2$ (1, 769) = 659.50, $p$ < .001. Further, anxiety was reduced in 93.5% of tracked sessions, they were exacerbated in 2.1% of sessions, and there was no change in symptoms for 4.4% of sessions.

#### 3.1.3. Stress

Analysis of the stress model revealed a significant change in ratings from before ($M_{Before}$ = 5.99, $SE$ = 0.13) to after ($M_{After}$ = 2.52, $SE$ = 0.14) using cannabis, Wald $\chi^2$ (1, 725) = 620.08, $p$ < .001. Further, stress was reduced in 93.3% of tracked sessions, it increased in 2.7% of sessions, and there was no change in reported levels of stress for 4% of sessions.

### 3.2. Aim 2: gender differences in change in symptom ratings

#### 3.2.1. Depression

As depicted in Fig. 1 (panel A), results indicated that both women, Wald $\chi^2$ (1, 261) = 380.74, $p$ < .001, and men, Wald $\chi^2$ = (1, 299) = 137.52, $p$ < .001, reported a significant reduction in symptoms of depression following cannabis use. There was no significant difference in the magnitude of change between the genders, Wald chi-square (1, 261) = 0.02, $p$ = .88.

#### 3.2.2. Anxiety

Both women, Wald $\chi^2$ (1, 362) = 582.32, $p$ < .001 and men, Wald $\chi^2$ = (1, 407) = 244.61, $p$ < .001, reported a significant reduction in symptoms of anxiety following cannabis use. Comparisons of the genders indicated that women perceived a greater reduction in symptoms of anxiety than did men, Wald $\chi^2$ (1, 362) = 10.78, $p$ < .001 (see Fig. 1, panel B).

#### 3.2.3. Stress

As shown in Fig. 1 (panel C) both women, Wald $\chi^2$ (1, 322) = 274.45, $p$ < .001, and men, Wald $\chi^2$ = (1, 403) = 412.58, $p$ < .001, reported a significant reduction in stress after using cannabis. There was no significant difference in the magnitude of symptom change between the genders, Wald $\chi^2$ (1, 322) = 3.18, $p$ = .07.

### 3.3. Aim 3: THC × CBD effects on change in symptom ratings

#### 3.3.1. Depression

Models were also tested to examine whether the cannabinoid content (% THC, % CBD) could predict change in reported symptom severity. The results of the model tested, to examine whether THC, CBD, and their interaction predict change in symptoms of depression, revealed a significant THC x CBD interaction, $b$ = −0.03, $p$ = .03. As depicted in Fig. 2 (panel A) the greatest reduction in ratings of depression were reported after using cannabis with relatively low levels of THC (one standard deviation [$SD$] below the mean of THC) and relatively high levels of CBD (one $SD$ above the mean of CBD). See Table 1 for overall means and $SD$s for cannabis used to treat depression, anxiety, and stress.

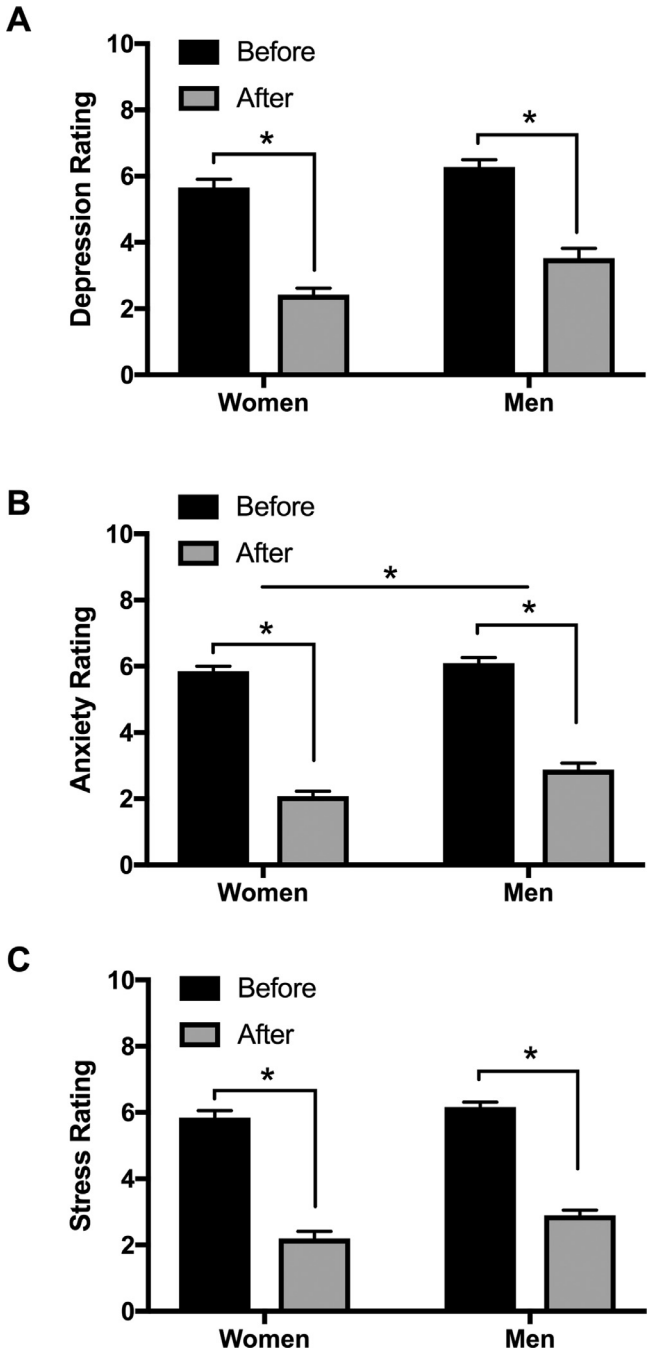



**Fig. 1.** Symptom ratings of depression (panel A), anxiety (panel B), and stress (panel C) before and after using cannabis in women and men with standard error bars.
*Note: * denotes significant difference with* $p < .01$.

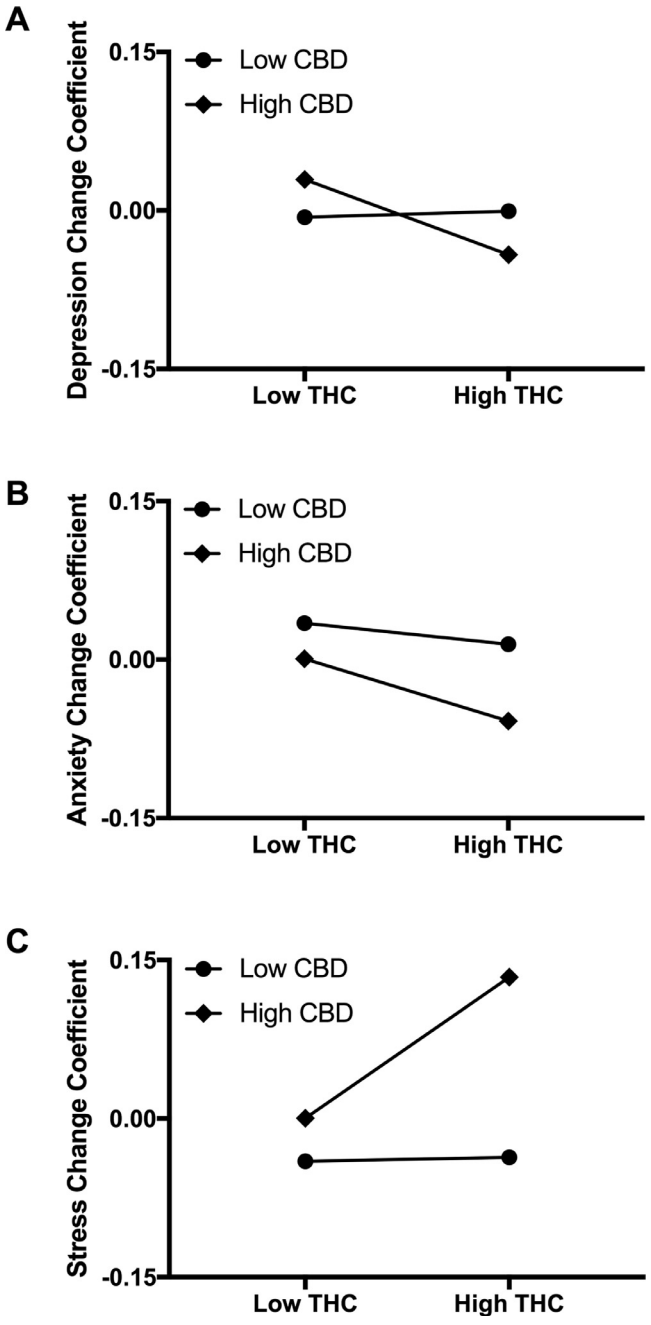



**Fig. 2.** THC x CBD interactions predicting change in depression (panel A), anxiety (panel B), and stress (panel C). *Note*: Low = one SD below the mean value; High = one SD above the mean value. Overall means and SDs are provided in Table 1.

### 3.3.2. Anxiety

In contrast, results of the model predicting change in symptoms of anxiety, using THC, CBD and THC x CBD, revealed no significant interaction, $b = -0.26$, $p = .55$ (see Fig. 2, panel B). We then removed the interaction term from the model to test for main effects of THC and CBD. Results revealed that neither THC, $b = -0.05$, $p = .33$, nor CBD content, $b = -0.006$, $p = .80$, were significant predictors of change in anxiety ratings.

### 3.3.3. Stress

The multilevel model testing the effects of cannabinoid content on changes in ratings of stress revealed a significant THC x CBD interaction, $b = 1.47$, $p < .001$. As shown in Fig. 2 (panel C), ratings of stress were reduced the most after using cannabis with relatively high levels of THC (one *SD* above the mean of THC) and relatively high levels of CBD (one *SD* above the mean of CBD). In contrast, there was no appreciable differences in symptom change following use of cannabis with high THC/low CBD, low

**Table 1**
Overall means and standard deviations (SDs) of % THC and % CBD.

| | Depression | Anxiety | Stress |
|---|---|---|---|
| % THC Mean *(SD)* | 15.76 (10.35) | 15.29 (9.13) | 16.53 (9.97) |
| % CBD Mean *(SD)* | 2.90 (6.73) | 3.69 (7.93) | 2.97 (8.36) |

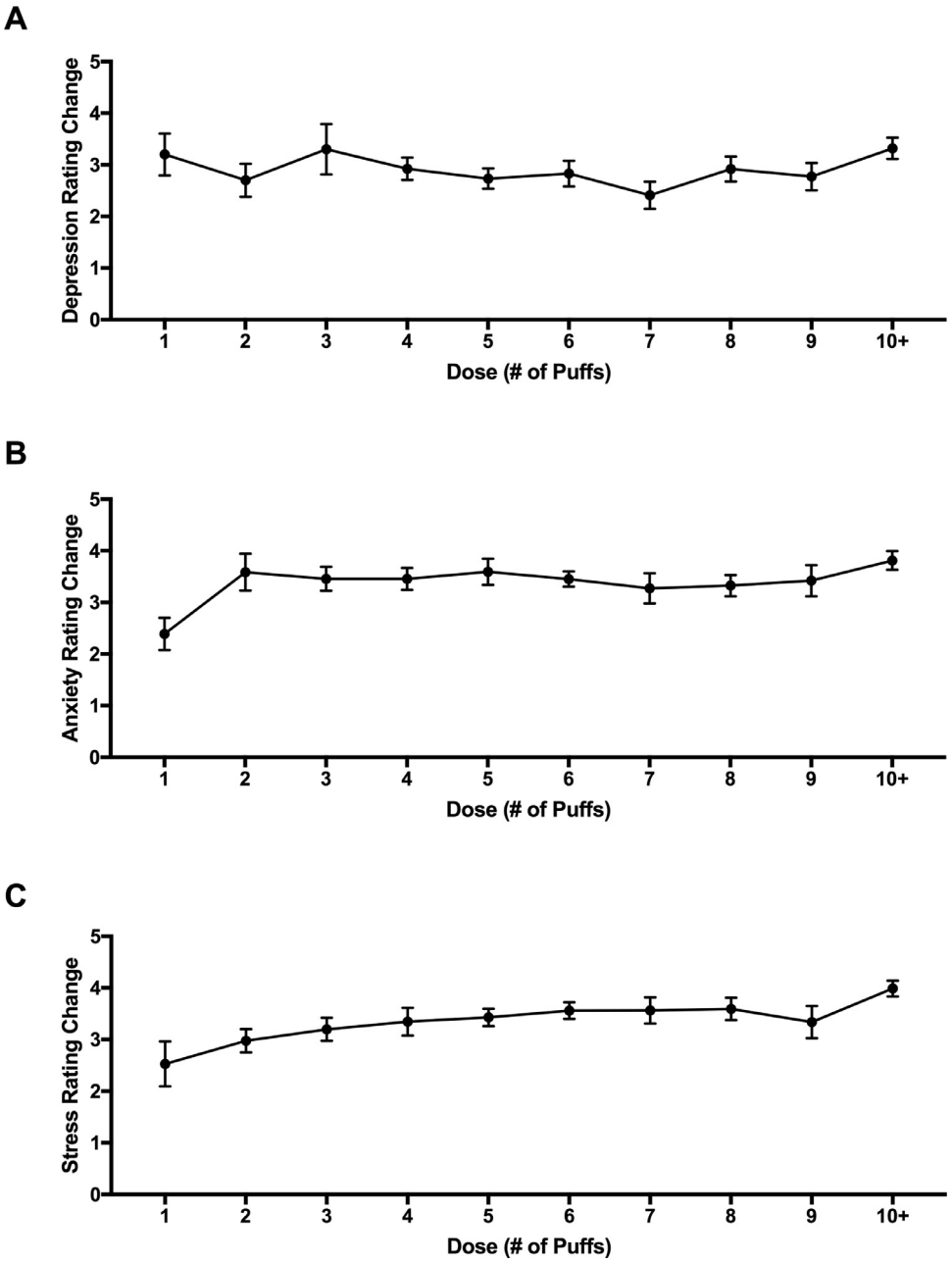



**Fig. 3.** Change in depression (panel A), anxiety (panel B), and stress (panel C) across different doses (1 to 10 + puffs) of cannabis with standard error bars.

**Table 2**
Multilevel models testing curvilinear relationships between dose and change in ratings of symptoms of anxiety.

| Model 1 (Dose$^2$) | | | | Model 2 (Dose$^3$) | | | | Model 3 (Dose$^4$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Predictors | *b* | *SE* | *p* | Predictors | *b* | *SE* | *p* | Predictors | *b* | *SE* | *p* |
| Dose | 0.17 | 0.15 | .26 | Dose | 0.17 | 0.14 | .24 | Dose | 0.17 | 0.15 | .25 |
| Dose$^2$ | 0.74 | 0.33 | .02 | Dose$^2$ | 0.31 | 0.14 | .04 | Dose$^2$ | 0.21 | 0.11 | .04 |
| | | | | Dose$^3$ | 0.40 | 0.18 | .03 | Dose$^3$ | 0.23 | 0.11 | .04 |
| | | | | | | | | Dose$^4$ | 0.29 | 0.13 | .03 |
| Intercept | −0.005 | 0.01 | .34 | Intercept | −0.005 | 0.01 | .34 | Intercept | −0.005 | 0.01 | .33 |

*Note:* b = unstandardized regression coefficient, SE = standard error of estimate.

THC/high CBD, or low THC/low CBD.

### 3.4. Aim 4: effects of dose on change in symptom ratings

#### 3.4.1. Depression

Several multilevel models were tested to examine the impact of dose on change in depression symptom ratings. Results revealed a non-significant linear effect of dose predicting change, $b = 0.06$, $p = .75$. We therefore tested for curvilinear relationships (i.e., dose$^2$, dose$^3$, dose$^4$); however, none of these models revealed significant effects of dose on change in symptoms of depression (see Fig. 3, panel A).

#### 3.4.2. Anxiety

Results of models testing change in ratings of anxiety across different doses also revealed a nonsignificant linear effect, $b = 0.15$, $p = .33$. We therefore tested several models to explore curvilinear relationships. First, a model was tested wherein we added the quadratic term for dose (i.e., dose$^2$), and we found a significant curvilinear relationship (see Table 2, Model 1). Another model was tested wherein we added a cubic term for dose (i.e., dose$^3$) to the previously tested model, and parameter estimates remained significant (see Table 2, Model 2). Lastly, a model was tested wherein we added dose to the fourth power (i.e., dose$^4$). Once again, results revealed a significant, curvilinear relationship (see Table 2, Model 3). Further contrasts revealed that 1 puff produced significantly smaller changes in ratings of anxiety than all other doses (2 to 10+), but no other differences across doses beyond 1 puff were detected (see Fig. 3, panel B).

#### 3.4.3. Stress

The model tested to examine the effect of varying doses on change in ratings of stress revealed a significant linear effect of dose, $b = 0.45$, $p = .03$. As depicted in Fig. 3 (panel C), further contrasts revealed the following significant differences in doses: 1 puff < 5, 6, 7, 8, and 10 + puffs; 2 puffs < 5, 6, 7, 8, and 10 puffs; 10 puffs > 9, 6, 5, 4, 3, 2, and 1 puffs.

### 3.5. Aim 5: changes in perceived efficacy of cannabis across tracked sessions

Results of the multilevel analyses examining changes in perceived efficacy of cannabis (i.e., tolerance effects) across time/sessions revealed no significant change in the perceived efficacy of cannabis on depression ($b = 0.003$, $p = .52$), anxiety ($b = 0.007$, $p = .07$), or stress ($b = 0.007$, $p = .32$) across tracked sessions.

### 3.6. Aim 6: changes in baseline symptom ratings across tracked sessions

Results of the multilevel analysis examining change in baseline symptom ratings (i.e., ratings of depression immediately before using cannabis) across tracked sessions indicated that baseline symptoms of depression significantly increased across time/sessions, $b = 0.008$, $p = .006$. In contrast, the analyses examining change in baseline symptom ratings of anxiety and stress across tracked sessions indicated no significant changes in baseline symptoms of anxiety ($b = 0.007$, $p = .09$), or stress, ($b = 0.01$, $p = .26$).

## 4. Discussion

The present study was conducted to provide a naturalistic account of perceived changes in symptoms of negative affect as a function of cannabis use. The results indicate that both women and men perceived a significant reduction in symptoms of depression, anxiety, and stress after inhaling cannabis. Despite comparable levels of anxiety before using cannabis, women reported a significantly greater decrease in anxiety following cannabis consumption compared to men. This is somewhat consistent with previous findings that women are more likely than men to report using cannabis to manage anxiety (Cuttler et al., 2016). Across both genders, self-reported symptoms of depression were reduced by approximately 50%, while symptoms of anxiety and stress were reduced by approximately 58%. Moreover, for the vast majority of tracked sessions, users reported a reduction in symptoms of depression (89%), anxiety (93%), and stress (93%) after inhaling cannabis. It is important to note that these percentages are likely inflated in the present sample, as individuals who regularly experience symptom exacerbation following use of cannabis may be less likely to continue to use cannabis to treat their symptoms and/or to track their symptom changes across time. Nevertheless, results from the present study are consistent with the reported anxiolytic, stress-alleviating effects of cannabis, and suggest that users experience significant and substantial reductions in symptoms of negative affect following cannabis use.

Results from the multilevel models tested to examine THC x CBD interactions revealed a significant cross-over interaction predicting change in ratings of depression. Low levels of THC combined with high levels of CBD predicted the greatest reductions in reported symptoms of depression, while high levels of THC combined with high levels of CBD predicted the lowest levels of reported symptom reduction. In contrast, varying levels of THC (high vs. low) appeared to have little influence on the degree of symptom reduction when CBD was low. Similarly, varying levels of THC (high vs. low) appeared to have little influence on change in stress ratings when CBD was low. However, when CBD was high, there was an effect of THC such that higher levels of THC predicted greater reductions in symptoms of stress relative to low levels of THC. These results suggest that cannabis with relatively high CBD (e.g., > 9.5%) and low THC (e.g., < 5.5%) is perceived to be more effective in reducing symptoms of depression, while cannabis with high CBD (e.g., > 11%) and high THC (e.g., > 26.5%) is perceived to be more effective in reducing stress. The non-medical cannabis market is currently dominated by the sales of high THC cannabis products (Smart et al., 2017) but these results suggest that CBD is an important component of cannabis and that medical cannabis users should seek out cannabis with CBD levels of 10% or higher. While intriguing, there are currently no controlled examinations of THC x CBD interactions in the treatment of depression or stress, and it is possible that anecdotal evidence propagated by bud-tenders and popular culture could be biasing medical cannabis users' expectations and experiences. That is, medical cannabis users' beliefs regarding the therapeutic efficacy of THC- and/or CBD-rich chemotypes could have contributed to a potential expectancy effect. Future studies should examine this possibility in a more controlled setting.

The results also indicate the presence of a positive, linear, relationship between dose and perceived changes in stress. 10+ puffs were associated with the greatest change in ratings of stress. In contrast, models tested to examine the effects of dose on perceived changes in symptoms of anxiety revealed the presence of significant curvilinear relationships. Follow-up tests revealed that 1 puff was perceived as less effective than any other dose; however, there were no other significant differences across any other doses (e.g., 2 puffs were perceived to be as effective as 10+ puffs). Finally, we found no evidence for dose effects on change in ratings of depression. In other words, a single puff resulted in the same magnitude of change in ratings of depression as 10+ puffs. These findings may support the notion of "micro-dosing" to alleviate symptoms of depression and anxiety. Nevertheless, it is important to note that different methods of administration (e.g., smoking, concentrate, dab bubbler) were included together in these analyses and these different methods of administration would affect the potency of the cannabis. Therefore, future research is needed to manipulate method of administration, and then contrast the relative effects of different doses of cannabis, to provide more precise guidance on optimal doses for different routes of administration.

Collectively, these results appear to suggest that in the short-term, cannabis effectively reduces perceived levels of depression, anxiety, and stress in both women and men. But what, if any, longer-term

consequences are associated with the repeated use of cannabis to manage states of negative affect? A major advantage of the multilevel modeling approach is that it affords exploration of change in perceived efficacy of cannabis in individuals over time. The results of these analyses revealed no apparent subjective tolerance effects, which is consistent with recent evidence of no objective tolerance to the psychomotor effects of THC (Ramaekers et al., 2016).

Finally, examination of whether repeated use of cannabis to manage states of negative affect results in any appreciable change in baseline (pre-cannabis use) symptoms over time indicated that baseline ratings of anxiety and stress remained fairly stable across tracked sessions, while baseline ratings of depression significantly increased over time/sessions. The value of the regression coefficient indicates that for every additional tracked session over time, one would predict a 0.008-unit increase in baseline ratings of depression (i.e., after 125 treatment sessions, one would predict a 1-unit increase in baseline depression ratings on a 0 to 10 scale). This is consistent with recent evidence indicating that using cannabis to cope with distress is associated with more cannabis-related problems and increased symptoms of depression (Bonn-Miller et al., 2014; Moitra et al., 2015). Chronic cannabis use decreases CB1 receptor availability in cortical areas implicated in mood disorders (Hirvonen et al., 2012), and a growing body of preclinical evidence indicates that genetic or pharmacological CB1 receptor blockade produces a phenotype that is strikingly reminiscent of the symptom profile of major depression (see Gorzalka and Hill, 2011 for review). Collectively these results suggest that chronic use of cannabis to cope with symptoms of depression may increase susceptibility for depression by altering the endocannabinoid system. Fortunately, alterations in CB1 receptor availability in chronic cannabis users are reversible after only a short (~2 day) period of abstinence, with no significant differences after 28 days of abstinence (D' Souza et al., 2016). Finally, it is worthwhile to note that there is evidence that antidepressant medications are effective in the short-term, but that longer duration of use may actually increase vulnerability to relapse upon discontinuation (Fava, 2003). Thus, similar to more conventional pharmacological treatments, cannabis may temporarily mask symptoms of negative affect but may not effectively reduce these symptoms in the long-term.

### 4.1. Limitations and strengths

One limitation of the present study is that the sample likely underrepresents individuals who do not find cannabis to be an effective means for reducing their symptoms of negative affect; such individuals would be unlikely to continue to use cannabis for this purpose. Another limitation is the lack of a placebo control group. Given that the data were obtained from medical cannabis users who were using their own cannabis in their own environment, it was not possible to obtain a comparison group. As such, it is possible – and likely – that at least some of the detected effects are driven by expectations individuals have about the efficacy of cannabis for treating states of negative affect. Therefore, it is vital that the results from the present study be further investigated under double-blind, placebo-controlled conditions.

While the majority of the data on THC and CBD levels were obtained from licensed producers who are held to strict testing standards by Health Canada, some of these data were entered by users of the app. As such some of these data may have more questionable reliability. Future more controlled research involving the testing of a select few products is therefore needed to further probe potential THC x CBD interactions. Finally, the failure to consider content of other phytocannabinoids and terpenoids found in cannabis represents a limitation of the present study. There are over 100 additional phytocannabinoids and at least 120 terpenes in cannabis that may contribute to its medicinal properties, either independently, or by exacerbating/mitigating the pharmacological effects of THC (Calvi et al., 2018). The effects of these other compounds will need to be explored in future research when these data become more readily available.

Nevertheless, these limitations are offset by several noteworthy strengths. Namely, since the data were obtained from a large sample of medical cannabis users who were using a variety of cannabis products in their natural environment, the study has very high ecological validity, and the results are likely to reflect the actual experiences of people who use cannabis to treat symptoms of negative affect. Moreover, medical cannabis users' motivation for using the app is predominantly to track their personal symptoms to better understand the products and doses of cannabis that produce the most beneficial effects for them. Although Strainprint's™ terms of use indicate that the data may be used for any purpose deemed appropriate, most users would be unaware that their data are being used for scientific investigations. Therefore, it is unlikely that the results are biased by users' explicit motivations to portray cannabis in a good light.

In summary, the internal validity of the findings of the present study may be threatened by implicit biases (i.e., expectancy effects), but the findings are unlikely to be threatened by explicit biases and have high ecological validity. As such, results from the present study provide an important complement to the more internally valid, controlled laboratory studies.

### 4.2. Conclusions

Results from the present study indicate that medical cannabis users report a substantial and significant reduction in symptoms of negative affect shortly after using cannabis. Importantly, while acute cannabis intoxication temporarily alleviates perceived states of depression, anxiety, and stress, the repeated use of cannabis does not appear to lead to any longer-term reductions in these symptoms.

## Contributors

Carrie Cuttler conceived of the idea and research questions, obtained the data, assisted with analyses, and prepared the first complete draft of the manuscript. Alexander Spradlin helped to conceive the research questions, conducted the analyses, and assisted in writing the results section. Ryan J. McLaughlin helped to conceive the research questions, created the figures, aided in the interpretation of the results, and contributed to the writing of the manuscript. All authors approved the final article.

## Funding

This work was supported by Washington State University's Alcohol and Drug Abuse Research Program (Dedicated Marijuana Account). The funder had no role in the conduct or results of the study.

## Declarations of interest

None.

## Acknowledgments

We would like to thank the creators of Strainprint™ for freely and openly providing the data used in this study.

## Conflict of interest

None.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2018.04.054.

## References


American Psychiatric Association., 2013. Diagnostic and Statistical Manual of Mental Disorders, fifth ed. American Psychiatric Publishing, Arlington, VA.

Bergamaschi, M.M., Queiroz, R.H.C., Chagas, M.H.N., de Oliveira, D.C.G., De Martinis, B.S., Kapczinski, F., Quevedo, J., Roesler, R., Schröder, N., Nardi, A.E., Martín-Santos, R., Hallak, J.E., Zuardi, A.W., Crippa, J.A.S., 2011. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naïve social phobia patients. Neuropsychopharmacology 36, 1219–1226. http://dx.doi.org/10.1038/npp.2011.6.

Bergamaschi, M.M., Queiroz, R.H.C., Chagas, M.H.N., Linares, I.M.P., Arrais, K.C., de Oliveira, D.C.G., Queiroz, M.E., Nardi, A.E., Huestis, M.A., Hallak, J.E.C., Zuardi, A.W., Moreira, F.A., Crippa, J.A.S., 2014. Rimonabant effects on anxiety induced by simulated public speaking in healthy humans: a preliminary report. Hum. Psychopharmacol. Clin. Exp. 29, 94–99. http://dx.doi.org/10.1002/hup.2374.

Bonn-Miller, M.O., Boden, M.T., Bucossi, M.M., Babson, K.A., 2014. Self-reported cannabis use characteristics, patterns and helpfulness among medical cannabis users. Am. J. Drug Alcohol Abuse 40, 23–30. http://dx.doi.org/10.3109/00952990.2013.821477.

Calvi, L., Pentimalli, D., Panseri, S., Giupponi, L., Gelmini, F., Beretta, G., Vitali, D., Bruno, M., Zilio, E., Pavlovic, R., Giorgi, A., 2018. Comprehensive quality evaluation of medical Cannabis sativa L. inflorescence and macerated oils based on HS-SPME coupled to GC-M and LC-HRMS (q-exactive orbitrap®) approach. J. Pharm. Biomed. Anal. 150, 208–219. http://dx.doi.org/10.1016/j.jpba.2017.11.073.

Childs., E., Lutz, A.A., de Wit, H., 2017. Dose-related effects of delta-9-THC on emotional responses to acute psychosocial stress. Drug Alcohol Depend. 177. , 136.144. doi:10.1016/j.drugalcdep.2017.03.030.

Christensen, R., Kristensen, P.K., Bartels, E.M., Bliddal, H., Astrup, A., 2007. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 370, 1706–1713. http://dx.doi.org/10.1016/S0140-6736(07)61721-8.

Cuttler, C., Mischley, L., Sexton, M., 2016. Sex differences in cannabis use and effects: a cross-sectional survey of cannabis users. Cannabis Cannabinoid Res. 1, 166–175. http://dx.doi.org/10.1089/can.2016.0010.

Drevets, W.C., Price, J.L., Furey, M.L., 2008. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. Brain Struct. Funct. 213, 93–118. http://dx.doi.org/10.1007/s00429-008-0189-x.

D'Souza, D.C., Cortes-Briones, J.A., Ranganathan, M., Thurnauer, H., Creatura, G., Surti, T., Planeta, B., Neumeister, A., Pittman, B., Normandin, M., Kapinos, M., Ropchan, J., Huang, Y., Carson, R.E., Skosnik, P.D., 2016. Rapid changes in CB1 receptor availability in cannabis-dependent males after abstinence from cannabis. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 1, 60–67. http://dx.doi.org/10.1016/j.bpsc.2015.09.008.

Fava, G.A., 2003. Can long-term treatment with antidepressant drugs worsen the course of depression. J. Clin. Psychiatry 64, 123–133. http://dx.doi.org/10.4088/JCP.v64n0204.

Finch, W.H., Bolin, J.E., 2016. Multilevel Modeling Using Mplus, first ed. Chapman Hall/CRC Statistics in the Social and Behavioral Sciences, Boca Raton, FL.

Frank, B., Serpell, M.G., Hughes, J., Matthews, J.N.S., Kapur, D., 2008. Comparisons of analgesic effects and patient tolerability of nabilone and dihydrocodeine for chronic neuropathic pain: randomized, crossover, double blind study. BMJ 336, 199–201. http://dx.doi.org/10.1136/bmj.39429.619653.80.

Fusar-Poli, P., Crippa, J.A., Bhattacharyya, S., Borgwardt, S.J., Allen, P., Martín-Santos, R., Seal, M., Surguladze, S.A., O'Carrol, C., Atakan, Z., Zuardi, A.W., McGuire, P.K., 2008. Distinct effects of $\Delta^9$-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. Arch. Gen. Psychiatry 66, 95–105. http://dx.doi.org/10.1001/archgenpsychiatry.2008.519.

Gorzalka, B.B., Hill, M.N., 2011. Putative role of endocannabinoid signaling in the etiology of depression and actions of antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1575–1585 doi: 10/1016/j.pnpbp.2010.11.021.

Grotenhermen, F., 2003. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin. Pharmacokinet. 42, 327–360. http://dx.doi.org/10.2165/00003088-200342040-00003.

Hirvonen, J., Goodwin, R.S., Li, C.T., Terry, G.E., Zoghbi, S.S., Morse, C., Pike, V.W., Volkow, N.D., Huestis, M.A., Innis, R.B., 2012. Reversible and regionally selective downregulation of brain cannabinoid CB1 receptors in chronic daily cannabis smokers. Mol. Psychiatry 17, 642–649. http://dx.doi.org/10.1038/mp.2011.82.

Hyman, S.M., Sinha, R., 2009. Stress-related factors in cannabis use and misuse: implications for prevention and treatment. J. Subst. Abuse Treat. 36, 400–413. http://dx.doi.org/10.1016/j.jsat.2008.08.005.

Johnston, L.D., O'Malley, P.M., 1986. Why do the nations' students use drugs and alcohol? Self-reported reasons from nine national surveys. J. Drug Issues 16, 29–66. http://dx.doi.org/10.1177/002204268601600103.

Menkes, D.B., Howard, R.C., Spears, G.F., Cairns, E.R., 1991. Salivary THC following cannabis smoking correlates with subjective intoxication and heart rate. Psychopharmacology 103, 277–279.

Moitra, E., Christopher, P.P., Anderson, B.J., Stein, M.D., 2015. Coping-motivated marijuana use correlates with DSM-5 cannabis use disorder and psychological distress among emerging adults. Psychol. Addict Behav. 29, 627–632. http://dx.doi.org/10.1037/adb0000083.

Muthén, L.K., Muthén, B.O., 2017. Mplus User's Guide, eighth ed. Muthén & Muthén, Los Angeles, CA.

Narang, S., Gibson, D., Wasan, A.D., Ross, E.L., Michna, E., Nedelijkovic, S.S., Jamison, R.N., 2008. Efficacy of dronabinol as an adjuvant treatment for chronic pain patients on opioid therapy. J. Pain 9. , 254-254. doi:10.1016/j.jpain.2007.10.018.

Pillard, R.C., McNair, D., Fisher, S., 1974. Does marijuana enhance experimentally induced anxiety? Psychopharmacologia 40, 205–210. http://dx.doi.org/10.1007/BF00429414.

Portenoy, R.K., Ganae-Motan, E.D., Allende, S., Yanagihara, R., Shaiova, L., Weinstein, S., McQuade, R., Wright, S., Fallon, M.T., 2012. Nabiximols for opioid-treated cancer patients with poorly-controlled chronic pain: a randomized placebo-controlled, graded-dose trial. J. Pain 13, 438–449. http://dx.doi.org/10.1016/j.pain.2012.01.003.

Ramaekers, J.G., van Wel, J.H., Spronk, D.B., Toennes, S.W., Kuypers, K.P.C., Theunissen, E.L., Verkes, R.J., 2016. Cannabis and tolerance: acute drug impairment as a function of cannabis use history. Sci. Rep. 6, 26843. http://dx.doi.org/10.1038/srep26843.

Rog, D.J., Nurmikko, T.J., Friede, T., Young, C.A., 2005. Randomized controlled trial of cannabis-based medicine in central pain in multiple sclerosis. Neurology 65, 812–819. http://dx.doi.org/10.1212/01.wnl.0000176753.45410.8b.

Schauer, G.L., King, B.A, Bunnell, R.E., Promoff, G., McAfee, T.A., 2016. Toking, vaping, and eating for health or fun: marijuana use patterns in adults, U.S. Am J Prev Med 50, 1–8. http://dx.doi.org/10.1016/j.amepre.2015.05.027.

Sexton, M., Cuttler, C., Finnell, J., Mischley, L., 2016. A cross-sectional survey of medical cannabis users: patterns of use and perceived efficacy. Cannabis Cannabinoid Res. 1, 131–138. http://dx.doi.org/10.1089/can.2016.0007.

Skrabek, R.Q., Galimova, L., Ethans, K., Perry, D, 2008. Nabilone for the treatment of pain in fibromyalgia. J. Pain 9, 164–173. http://dx.doi.org/10.1016/j.jpain.2007.09.002.

Smart, R., Caulkins, J.P., Kilmer, B., Davenport, S., Midgette, G., 2017. Variation in cannabis potency & prices in newly-legal market: evidence from 30 million cannabis sales in Washington State. Addiction 112, 2167–2177. http://dx.doi.org/10.1111/add.13886.

Wade, D.T., Makela, P., Robson, R., House, H., Bateman, C., 2004. Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients. Mult. Scler. 10, 434–441. http://dx.doi.org/10.1191/1352458504ms1082oa.

Webb, C.W., Webb, S.M., 2014. Therapeutic benefits of cannabis: a patient survey. Hawaii J. Med. Public Health 73, 109–111.

Zuardi, A.W., Cosme, R.A., Graeff, F.G., Guimarães, F.S., 1993. Effects of ipsapirone and cannabidiol on human experimental anxiety. J. Psychopharmacol. 7, 82–88. http://dx.doi.org/10.1177/026988119300700112.

# Global prevalence of anxiety disorders: a systematic review and meta-regression

Published online by Cambridge University Press: **10 July 2012**

A. J. Baxter, K. M. Scott, T. Vos and H. A. Whiteford



## Abstract

### Background

The literature describing the global prevalence of anxiety disorders is highly variable. A systematic review and meta-regression were undertaken to estimate the prevalence of anxiety disorders and to identify factors that may influence these estimates. The findings will inform the new Global Burden of Disease study.

### Method

A systematic review identified prevalence studies of anxiety disorders published between 1980 and 2009. Electronic databases, reference lists, review articles and monographs were searched and experts then contacted to identify missing studies. Substantive and methodological factors associated with inter-study variability were identified through meta-regression analyses and the global prevalence of anxiety disorders was calculated adjusting for study methodology.

### Results

The prevalence of anxiety disorders was obtained from 87 studies across 44 countries. Estimates of current prevalence ranged between 0.9% and 28.3% and past-year prevalence between 2.4% and 29.8%. Substantive factors including gender, age, culture, conflict and economic status, and urbanicity accounted for the greatest proportion of variability. Methodological factors in the final multivariate model (prevalence period, number of disorders and diagnostic instrument) explained an additional 13% of variance between studies. The global current prevalence of anxiety disorders adjusted for methodological differences was 7.3% (4.8–10.9%) and ranged from 5.3% (3.5–8.1%) in African cultures to 10.4% (7.0–15.5%) in Euro/Anglo cultures.

### Conclusions

Anxiety disorders are common and the substantive and methodological factors identified here explain much of the variability in prevalence estimates. Specific attention should be paid to cultural differences in responses to survey instruments for anxiety disorders.

## Keywords

Anxiety disorders epidemiology global burden of disease mental disorders prevalence psychiatric epidemiology

**Type**

Review Article

**Information**

Psychological Medicine, Volume 43, Issue 5, May 2013, pp. 897 - 910
DOI: https://doi.org/10.1017/S003329171200147X



# References

Ajakaiyea, O, Alib, AAG (2009). Managing post-conflict recovery in Africa: an overview. Journal of African Economies 18 (Suppl. 1), i3–i11.CrossRefGoogle Scholar

Andrews, G, Henderson, S, Hall, W (2001). Prevalence, comorbidity, disability and service utilisation: overview of the Australian National Mental Health Survey. British Journal of Psychiatry 178, 145–153.CrossRefGoogle ScholarPubMed

Antony, MM, Stein, MB (editors) (2009). Oxford Handbook of Anxiety and Related Disorders. Oxford University Press: New York.Google Scholar

Beesdo, K, Pine, DS, Lieb, R, Wittchen, H-U (2010). Incidence and risk patterns of anxiety and depressive disorders and categorization of generalized anxiety disorder. Archives of General Psychiatry 67, 47–57.CrossRefGoogle ScholarPubMed

Belsley, DA (1991). Conditioning Diagnostics: Collinearity and Weak Data in Regression. Wiley: New York.Google Scholar

Benjet, C, Borges, G, Medina-Mora, ME, Zambrano, J, Aguilar-Gaxiola, S (2009). Youth mental health in a populous city of the developing

worlds: results from the Mexican Adolescent Mental Health Survey. Journal of Child Psychology and Psychiatry 50, 386–395.CrossRefGoogle Scholar

Campbell, JC (2002). Health consequences of intimate partner violence. Lancet 359, 1331–1336.CrossRefGoogle ScholarPubMed

Chen, X, Ender, P, Mitchell, M, Wells, C (2003). Regression with Stata (http://www.ats.ucla.edu/stat/stata/webbooks/reg/default.htm). Accessed 30 November 2011.Google Scholar

Cheng, ATA (2001). Case definition and culture: are people all the same? British Journal of Psychiatry 179, 1–3.CrossRefGoogle ScholarPubMed

Costello, EJ, Egger, HL, Angold, A (2005). The developmental epidemiology of anxiety disorders: phenomenology, prevalence and comorbidity. Child and Adolescent Psychaitric Clinics of North America 14, 631–648.CrossRefGoogle ScholarPubMed

Das, J, Do, Q, Friedman, J, McKenzie, D, Scott, K (2007). Mental health and poverty in developing countries: revisiting the relationship. Social Science and Medicine 65, 467–480.CrossRefGoogle ScholarPubMed

de Graaf, R, Bijl, RV, Smit, F, Vollebergh, WAM, Spijker, J (2002). Risk factors for 12-month comorbidity of mood, anxiety, and substance use disorders: findings from the Netherlands Mental Health Survey and Incidence Study. American Journal of Psychiatry 159, 620–629.CrossRefGoogle ScholarPubMed

de Jong, JT, Komproe, IH, van Ommeren, M, El Masri, M, Araya, M, Khaled, N, van de Put, W, Somasundaram, D (2001). Lifetime events and posttraumatic stress disorder in 4 postconflict settings. Journal of the American Medical Association 286, 555–562.CrossRefGoogle ScholarPubMed

Degenhardt, L, Whiteford, H, Hall, W, Vos, T (2009). Estimating the burden of disease attributable to illicit drug use and mental disorders: what is ‘Global Burden of Disease 2005’ and why does it matter? Addiction 104, 1466–1471.CrossRefGoogle ScholarPubMed

Eaton, WW (1995). Progress in the epidemiology of anxiety disorders. Epidemiologic Reviews 17, 32–38.CrossRefGoogle ScholarPubMed

Espié, E, Gaboulaud, V, Baubet, T, Casas, G, Mouchenik, Y, Yun, O, Grais, RF , Moro, MR (2009). Trauma-related psychological disorders among Palestinian children and adults in Gaza and West Bank, 2005–2008. International Journal of Mental Health Systems 3, 21.CrossRefGoogle ScholarPubMed

Galea, S, Ahern, J, Tracy, M, Hubbard, A, Cerda, M, Goldmann, E, Vlahov, D ( 2008). Longitudinal determinants of posttraumatic stress in a population-based cohort study. Epidemiology 19, 47–54.CrossRefGoogle Scholar

Gigantesco, A, Palumbo, G, Mirabella, F, Pettinelli, M, Morosini, P (2006). Prev alence of psychiatric disorders in an Italian town: low prevalence confirmed with two different interviews. Psychotherapy and Psychosomatics 75, 170–176.CrossRefGoogle Scholar

Harris, RJ, Bradburn, M, Deeks, J, Harbord, R, Altman, D, Sterne, J (2008). Me tan: fixed- and random-effects meta-analysis. Stata Journal 8, 3–28.Google Scholar

Hinton, DE, Parl, L, Hsia, C, Hofmann, S, Pollack, MH (2009). Anxiety disorder presentations in Asian populations: a review. CNS Neuroscience and Therapeutics 15, 295–303.CrossRefGoogle ScholarPubMed

Homer, D, Lemeshow, S (1989). Applied Logistic Regression. Wiley: New York.Google Scholar

Hovens, JG, Giltay, EJ, Wiersma, JE, Spinhoven, P, Penninx, BW, Zitman, FG (2012). Impact of childhood life events and trauma on the course of depressive and anxiety disorders. Acta Psychiatrica Scandinavica. Published online 23 January 2012. doi:10.1111/j.1600-0447.2011.01828.x.CrossRefGoogle ScholarPubMed

Johnson, PB, Malgady, R (1999). Cultural/ethnic comparisons: a research agenda. Journal of Gender, Culture, and Health 4, 171–185.CrossRefGoogle Scholar

Kadri, N, Agoub, M, El
Gnaoui, S, Berrada, S, Moussaoui, D (2007). Prevalence of anxiety disorders: a population-based epidemiological study in metropolitan area of Casablanca, Morocco. Annals of General Psychiatry 6, 6.CrossRefGoogle ScholarPubMed

Kahn, RS, Wise, PH, Kennedy, BP, Kawachi, I (2000). State income inequality,

household income, and maternal mental and physical health: cross sectional national survey. British Medical Journal 321, 1311–1315.CrossRefGoogle ScholarPubMed

Kapiriri, L, Norheim, OF, Heggenhougen, K (2003). Using burden of disease information for health planning in developing countries: the experience from Uganda. Social Science and Medicine 56, 2433–2441.CrossRefGoogle ScholarPubMed

Kawakami, N, Abdulghani, EA, Alonso, J, Bromet, EJ, Bruffaerts, R, Caldas-de-Almeida, JM, Chiu, WT, de Girolamo, G, de Graaf, R, Fayyad, J, Ferry, F, Florescu, S, Gureje, O, Hu, C, Lakoma, MD, LeBlanc, W, Lee, S, Levinson, D, Malhotra, S, Matschinger, H, Medina-Mora, ME, Nakamura, Y, Oakley Browne, MA, Okoliyski, M, Posada-Villa, J, Sampson, NA, Viana, MC, Kessler, RC (2012). Early-life mental disorders and adult household income in the World Mental Health Surveys. Biological Psychiatry. Published online 20 April 2012. doi:10.1016/j.biopsych.2012.03.009.CrossRefGoogle ScholarPubMed

Kessler, RC, Amminger, GP, Aguilar-Gaxiola, S, Alonso, J, Lee, S, Ustun, TB (2007). Age of onset of mental disorders: a review of recent literature. Current Opinion in Psychiatry 20, 359–364.CrossRefGoogle ScholarPubMed

Kessler, RC, Berglund, P, Demler, O, Jin, R, Merikangas, KR, Walters, EE (2005). Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry 62, 593–602.CrossRefGoogle ScholarPubMed

Kessler, RC, Greenberg, PE (2002). The economic burden of anxiety and stress disorders. In Neuropsychopharmacology: The Fifth Generation of Progress (ed. Davis, K. L., Charney, D., Coyle, J. T. and Nemeroff, C.), pp 981–992. Lippencott, Williams and Wilkins: Philadelphia.Google Scholar

Kessler, RC, McLaughlin, KA, Koenen, KC, Petukhova, M, Hill, ED (2012). The importance of secondary trauma exposure for post-disaster mental disorder. Epidemiology and Psychiatric Sciences 21, 35–45.CrossRefGoogle ScholarPubMed

Kessler, RC, Wai, TC, Demler, O, Walters, EE (2005). Prevalence, severity,

and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. Archives of General Psychiatry 62, 617–627.CrossRefGoogle ScholarPubMed

Kruijshaar, ME, Barendregt, J, Vos, T, de Graaf, R, Spijker, J, Andrews, G (2005). Lifetime prevalence estimates of major depression: an indirect estimation method and a quantification of recall bias. European Journal of Epidemiology 20, 103–111.CrossRefGoogle Scholar

Lewis-Fernández, R, Hinton, D, Laria, A, Patterson, E, Hofmann, S, Craske, M, Stein, D, Asnaani, A, Liao, B (2010). Culture and the anxiety disorders: recommendations for DSM-V. Depression and Anxiety 27, 212–229.CrossRefGoogle ScholarPubMed

Marks, I (1986). Epidemiology of anxiety. Social Psychiatry 21, 167–171.CrossRefGoogle ScholarPubMed

Moffitt, TE, Caspi, A, Harrington, H, Milne, BJ, Melchior, M, Goldberg, D, Poulton, R (2007). Generalized anxiety disorder and depression: childhood risk factors in a birth cohort followed to age 32. Psychological Medicine 37, 441–452.CrossRefGoogle Scholar

Moffitt, TE, Caspi, A, Taylor, AJ, Kokaua, J, Milne, BJ, Polanczyk, G, Poulton, R (2010). How common are common mental disorders? Evidence that lifetime prevalence rates are doubled by prospective *versus* retrospective ascertainment. Psychological Medicine 40, 899–909.CrossRefGoogle ScholarPubMed

Moher, D, Liberati, A, Tetzlaff, J, Altman, DG, The PRISMA Group (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. PLoS Medicine 6, e1000097.CrossRefGoogle ScholarPubMed

Murray, CJL, King, G, Lopez, AD, Tomijima, N, Krug, EG (2002). Armed conflict as a public health problem. British Medical Journal 324, 346–349.CrossRefGoogle ScholarPubMed

Murray, CJL, Lopez, A (1996). Global Health Statistics: A Compendium of Incidence, Prevalence and Mortality Estimates for over 200

Conditions. Harvard School of Public Health on behalf of the World Health Organization and the World Bank: Cambridge, MA.Google Scholar

Murray, CJL, Lopez, AD, Black, R, Mathers, CD, Shibuya, K, Ezzati, M, Salomon, JA, Michaud, CM, Walker, N, Vos, T (2005). Global Burden of Disease 2005: call for collaborators. Lancet 370, 109–110.CrossRefGoogle Scholar

Overbeek, G, Vollebergh, W, de Graaf, R, Scholte, R, de Kemp, R, Engels, R (2006). Longitudinal associations of marital quality and marital dissolution with the incidence of DSM-III-R disorders. Journal of Family Psychology 20, 284–291.CrossRefGoogle ScholarPubMed

Phillips, MR, Zhang, J, Shi, Q, Song, Z, Ding, Z, Pang, S, Li, X, Zhang, Y, Wang, Z (2009). Prevalence, treatment, and associated disability of mental disorders in four provinces in China during 2001–05: an epidemiological survey. Lancet 373, 2041–2053.CrossRefGoogle ScholarPubMed

Pirkola, S, Isometsä, E, Survisaari, J, Aro, H, Joukamaa, M, Pöikolainen, K, Koshinen, S, Aromaa, A, Loennqvist, J (2005). DSM-IV mood-anxiety and alchohol disorders and their cormorbity in the Finnish general population. Results from the Health 2000 Study. Social Psychiatry and Psychiatric Epidemiology 40, 1–10.CrossRefGoogle Scholar

Prina, AM, Ferri, CP, Guerra, M, Brayne, C, Prince, M (2011). Prevalence of anxiety and its correlates among older adults in Latin America, India and China: cross-cultural study. British Journal of Psychiatry 199, 485–491.CrossRefGoogle ScholarPubMed

Regier, DA, Rae, DS, Narrow, WE, Kaelber, CT, Schatzberg, AF (1998). Prevalence of anxiety disorders and their comorbidity with mood and addictive disorders. British Journal of Psychiatry 173 (Suppl. 34), 24–28.Google Scholar

Somers, JM, Goldner, EM, Waraich, P, Hsu, L (2006). Prevalence and incidence studies of anxiety disorders: a systematic review of the literature. Canadian Journal of Psychiatry 51, 100–113.CrossRefGoogle ScholarPubMed

Steel, Z, Chey, T, Silove, D, Marnane, C, Bryant, RA, van Ommeren, M (2009 *a*). Association of torture and other potentially traumatic events with mental health outcomes among populations exposed to mass

conflict and displacement: a systematic review and meta-analysis. Journal of the American Medical Association 302, 537–549.CrossRefGoogle ScholarPubMed

Steel, Z, Silove, D, Giao, NM, Phan, TT, Chey, T, Whelan, A, Bauman, A, Brya nt, RA (2009 *b*). International and indigenous diagnoses of mental disorder among Vietnamese living in Vietnam and Australia. British Journal of Psychiatry 194, 326–333.CrossRefGoogle ScholarPubMed

Stein, DJ, Williams, SL, Jackson, PB, Seedat, S, Myer, L, Herman, A, Williams, DR (2009). Perpetration of gross human rights violations in South Africa: association with psychiatric disorders. South African Medical Journal 99, 390–395.Google ScholarPubMed

Stroup, DF, Berlin, JA, Morton, SC, Olkin, I, Williamson, GD, Rennie, D, Moher, D, Becker, BJ, Sipe, TA, Thacker, SB (2000). Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of Observational Studies in Epidemiology (MOOSE) group. Journal of the American Medical Association 283, 2008–2012.CrossRefGoogle ScholarPubMed

Tanios, CY, Abou-
Saleh, MT, Karam, AN, Salamoun, MM, Mneimneh, ZN, Karam, EG (2009). Th e epidemiology of anxiety disorders in the Arab world: a review. Journal of Anxiety Disorders 23, 409–419.CrossRefGoogle ScholarPubMed

Thompson, SG, Sharp, SJ (1999). Explaining heterogeneity in meta-analysis: a comparison of methods. Statistics in Medicine 18, 2693–2708.3.0.CO;2-V>CrossRefGoogle ScholarPubMed

United Nations Development Programme (2010). The Real Wealth of Nations: Pathways to Human Development. Human Development Report 2010. Palgrave Macmillan: New York.Google Scholar

Ventevogel, P, De
Vries, G, Scholte, WF, Shinwari, NR, Faiz, H, Nassery, R, van den Brink, W, Olff, M (2007). Properties of the Hopkins Symptom Checklist-25 (HSCL-25) and the Self-reporting Questionnaire (SRQ-20) as screening instruments used in primary care in Afghanistan. Social Psychiatry and Psychiatric Epidemiology 42, 328–335.CrossRefGoogle ScholarPubMed

Vorcaro, CMR, Rocha, FL, Uchoa, E, Lima-Costa, MF (2004). The burden of social phobia in a Brazilian community and its relationship with socioeconomic circumstances, health status and use of health services: The Bambui study. International Journal of Social Psychiatry 50, 216–226.CrossRefGoogle Scholar

Weiller, E, Bisserbe, JC, Maier, E (1998). Prevalence and recognition of anxiety syndromes in five European primary care settings. A report from the WHO study on Psychological Problems in General Health Care. British Journal of Psychiatry Supplement 34, 18–23.Google Scholar

Weissman, MM (1988). The epidemiology of anxiety disorders: rates, risks and familial patterns. Journal of Psychiatric Research 22 (Suppl. 1), 99–114.CrossRefGoogle ScholarPubMed

World Health Organization (2000). Cross-national comparisons of the prevalences and correlates of mental disorders. WHO International Consortium in Psychiatric Epidemiology. Bulletin of the World Health Organization 78, 413–426.Google Scholar

World Mental Health Survey Consortium (2008). Unpublished data provided by the World Mental Health Survey (WMHS) Consortium, December 2008 (http://www.hcp.med.harvard.edu/wmh/). Accessed June 2008.Google Scholar



## Related content

A. J. Baxter, T. Vos, K. M. Scott, A. J. Ferrari and H. A. Whiteford

Psychological Medicine

Published online: 22 January 2014

**Article**

**Global burden of depressive disorders in the year 2000**

T. B. Üstün, J. L. Ayuso-Mateos, S. Chatterji, C. Mathers and C. J. L. Murray

The British Journal of Psychiatry

Published online: 2 January 2018

**Article**

**The prevalence, age-of-onset and the correlates of DSM-IV psychiatric disorders in the Tianjin Mental Health Survey (TJMHS)**

H. Yin, G. Xu, H. Tian, G. Yang, K. J. Wardenaar and R. A. Schoevers

Psychological Medicine

Published online: 17 July 2017

**Article**

**Anxious and non-anxious major depressive disorder in the World Health Organization World Mental Health Surveys**

Epidemiology and Psychiatric Sciences

Published online: 27 February 2015

**Article**

**Delays in making initial treatment contact after the first onset of mental health disorders in the Argentinean Study of Mental Health Epidemiology**

Epidemiology and Psychiatric Sciences

Published online: 15 March 2018

Published in final edited form as:

*Nat Rev Neurosci*. 2015 December ; 16(12): 705–718. doi:10.1038/nrn4036.

# The endocannabinoid system in guarding against fear, anxiety and stress

**Beat Lutz**[1], **Giovanni Marsicano**[2,3], **Rafael Maldonado**[4], and **Cecilia J. Hillard**[5]

[1]Institute of Physiological Chemistry, University Medical Center Mainz, Duesbergweg 6, 55128 Mainz, Germany

[2]Institut national de la santé et de la recherche médicale (INSERM), U862 NeuroCentre Magendie, Group Endocannabinoids and Neuroadaptation, Bordeaux 33077, France

[3]University of Bordeaux, 146 rue Léo Saignat, Bordeaux 33077, France

[4]Laboratori de Neurofarmacologia, Facultat de Ciències de la Salut i de la Vida, Universitat Pompeu Fabra, Dr. Aiguader 88, 08003 Barcelona, Spain

[5]Department of Pharmacology and Toxicology, Neuroscience Research Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA

## Abstract

The endocannabinoid (eCB) system has emerged as a central integrator linking the perception of external and internal stimuli to distinct neurophysiological and behavioural outcomes (such as fear reaction, anxiety and stress-coping), thus allowing an organism to adapt to its changing environment. eCB signalling seems to determine the value of fear-evoking stimuli and to tune appropriate behavioural responses, which are essential for the organism's long-term viability, homeostasis and stress resilience; and dysregulation of eCB signalling can lead to psychiatric disorders. An understanding of the underlying neural cell populations and cellular processes enables the development of therapeutic strategies to mitigate behavioural maladaptation.

If asked for the main reason why they use this largely illicit drug, the majority of cannabis users in the world would probably answer "it relaxes me" (REF. 1). This indicates that cannabinoid signalling in the brain and the body has a central role in the control of stress, fear and anxiety. Recently, the molecular, cellular and circuit mechanisms underlying these functions have started to be deciphered.

Appropriate behavioural responses to external (such as sensory inputs) and internal stimuli (such as endocrine, paracrine, metabolic and neuronal signals) are vital for an organism's survival. Ideally, the consequent reactivity of the organism to stimuli is intrinsically regulated in an optimal manner, to avoid excessive or insufficient reactions, both of which can jeopardize the organism's survival. A large body of data has emerged in recent years

Correspondence to B.L. beat.lutz@uni-mainz.de.

**Competing interests statement**

The authors declare no competing interests.

pointing to a crucial role of the endocannabinoid (eCB) system in the regulation of the behavioural domains of acquired fear, anxiety and stress-coping[2–7]. The eCB system modulates synaptic transmission processes[8,9], thereby regulating behavioural outputs.

Despite the fact that the eCB system is widely distributed in the CNS[9,10], its activity is highly specific and localized. To understand this specificity in the context of fear, anxiety and stress-coping, one needs an integrated view of the eCB-mediated control of relevant brain regions (mainly the hippocampus, prefrontal cortex (PFC), amygdala and hypothalamus) and their interregional connectivity, and of the communication of these brain regions with peripheral organs (via the hypothalamic–pituitary–adrenal (HPA) axis and sympathetic nervous system). Within distinct brain regions, eCB signalling can differentially modulate the activity of multiple cell types (neuronal subtypes[9], astrocytes[11] and microglia[12]), and in turn can execute context-related alterations in synaptic transmission, resulting in fine-tuned patterns of neuronal activity.

The eCB system classically includes cannabinoid receptor type 1 (CB1R) and CB2R, their endogenous lipid ligands (the eCBs; the most-studied of which are 2-arachidonoyl glycerol (2-AG) and *N*-arachidonoylethanolamine (AEA; also known as anandamide)), and eCB-synthesizing and -degrading enzymes[9] (FIG. 1).

Here, we discuss recent progress in understanding how the eCB system is an integral part of the interface between stimulus input and executive responses at the synaptic and behavioural levels, and how it is involved in feedback mechanisms leading to adapted neuronal and behavioural reactions. Our discussion also includes pathophysiological states, as observed in anxiety- and stress-related dysfunctions, such as anxiety disorders. We evaluate whether the activity of the eCB system is altered in these disease states and whether these observations might lead to possible approaches for therapeutic intervention.

## Modulation of synaptic processes

The eCB system is widely distributed in the CNS[10], constituting a complex signalling system[9] that subserves multiple modes of synaptic transmission modulation[8,13–15]. The specific outcome of eCB-mediated modulation of synaptic transmission is dependent on the synapse-specific expression of the protein components of the eCB system (FIG. 1). Although the eCB system is highly abundant in the CNS[10], not all synapses contain a functional eCB system. As CB1R is the major constituent of the eCB system, the expression of CB1R is highly indicative of the presence of eCB signalling at that particular synapse. The eCB system is expressed at some synapses in all brain regions that are important for the processing of anxiety, fear and stress, including the hippocampus[16,17], the PFC[18], the bed nucleus of the stria terminalis (BNST)[19], the basolateral amygdala (BLA)[16,20], the central amygdala (CeA)[21,22] and various hypothalamic nuclei[23]. In cortical areas (including the cerebral cortex, hippocampus and cortical parts of the amygdala), CB1R is highly expressed in cholecystokinin (CCK)-positive GABAergic interneurons, whereas CB1R expression is largely absent from other interneuronal subtypes (for example, calretinin-and parvalbumin-positive interneurons)[16,17]. Much lower levels of CB1R expression are present in glutamatergic neurons of cortical regions[9,10,16]. However, cortical glutamatergic CB1R has

been shown to have important functional roles, including the control of synaptic transmission and neuronal excitability[15,24,25]. CB1R is also present in the cholinergic, serotonergic and noradrenergic system, suggesting that the eCB system is involved in the suppression of the release of these neurotransmitters, although direct electrophysiological evidence is mostly lacking[26–29]. Furthermore, CB1R is present at very low levels in astrocytes[30]. CB2R is expressed in microglia, particularly in activated microglia, but the question of CB1R expression in these cells needs further investigation[12].

At the synapse, eCBs function as retrograde messengers, binding to presynaptic CB1R, which in turn mediates the suppression of neurotransmitter release, leading to either transient eCB-mediated short-term depression (eCB-STD) or eCB-mediated long-term depression (eCB-LTD) of synaptic transmission (FIG. 2a). In addition, arachidonic acid (AA), which is both a precursor (in a lipid-esterified form) and a degradation product of eCBs, has recently been found to also act as a retrograde messenger, potentiating excitatory transmission in a process called depolarization-induced potentiation of excitation (DPE)[31] (FIG. 2b). Interestingly, many years ago, it was reported that AA can modulate synaptic transmission by various mechanisms[32,33]. DPE has to be taken into consideration, as the genetic and pharmacological modulation of eCB-synthesizing and -degrading enzymes can lead to considerable changes in AA levels[34–36], thereby presumably also influencing synaptic transmission. Owing to diffusion in the extracellular space of eCBs at the synapses, eCBs can also modulate neurotransmitter release at neighbouring synaptic terminals, leading to heterosynaptic suppression of neurotransmitter release[14] (FIG. 3a). The eCB system and CB1R are also present and functional in astrocytes, thus eCB signalling is integrated into the concept of the ‘tripartite synapse’, including pre-and postsynaptic elements and surrounding astroglial processes[11,37] (FIG. 3b).

A central feature of the eCB system is that eCBs are synthesized on demand from cellular membrane lipids following various stimuli[14]. This is well documented for 2-AG, whose synthesis was shown to be stimulated after increased postsynaptic intracellular $Ca^{2+}$ concentration or increased activity of phospholipase Cβ (PLCβ). The on-demand generation of AEA is not well characterized yet, owing to the lack of a detailed understanding of the mechanisms of AEA synthesis. The concept of on-demand eCB synthesis represents an attractive construct for understanding the roles of the eCB system in neuronal-network modulation and behaviours. In this construct, the eCB system is thought to be transiently activated at distinct synapses where the different cellular elements involved have been stimulated beyond a certain threshold. According to this view, the eCB system constitutes a brake mechanism used to fine-tune the network activity of specific brain circuits[9,38]. This mechanism, which seems to be mediated mainly by 2-AG, is an activity-driven process: eCB signalling is mostly silent when activity is low. Other molecules that induce eCB synthesis have also been identified, including corticosteroids[39,40] and estradiol[41], leading to the hypothesis that eCB signalling is the effector by which these hormones alter synaptic activity. Conversely, there are convincing data that AEA is involved in the tonic suppression of neurotransmitter release[14].

Thus, the eCB system has emerged as a modulator of synaptic activity via a multitude of different mechanisms, resulting in either enhancement or suppression of general network

activity. The eCB system is present throughout brain areas and neuronal circuits controlling anxiety[4,42], fear[4,42] and stress[43], from sensory circuits to output nuclei.

## Anxiety behaviour

Anxiety is an innate behavioural state associated with the anticipation of potential future threats that allows an organism to avoid potentially dangerous or harmful situations. Inputs from multiple senses are evaluated to assess these potential dangers and to initiate appropriate behavioural responses. The physiological and emotional state of the organism at the moment of this perception plays an important part in the evaluation of threat and determines the intensity of the autonomic, hormonal and behavioural outputs. Anxiety-like behaviours (for example, avoidance, decreased motion, increased heart rate and hypervigilance) occurring within the normal range of intensity are important for survival. However, when anxiety behaviours chronically exceed the normal range and become disproportionate to the actual level of danger, deleterious physical and psychological consequences ensue, eventually leading to anxiety-related neuropsychiatric disorders[44,45]. Increasing insights into the brain regions and neuronal circuits regulating anxiety have been gained during the last years[42].

A large number of pharmacological and genetic studies support the role of the eCB system as an important regulator of anxiety-like behaviours[4,46]. Analysis of global CB1R-deficient mice revealed increased anxiety-like behaviour under highly aversive conditions but not under less aversive conditions[47]. This could occur because eCB signalling is mobilized only when the stimulus is very strong or because eCB synthesis has been sensitized by a previous negative event[48]. The conditional deletion of the gene encoding CB1R in cortical glutamatergic neurons, which interconnect several brain areas of the anxiety circuits, did not result in differences in behavioural responses in standard anxiety tests (for example, the elevated plus maze, which is a model of mild stress) under aversive[47] or non-aversive conditions[47,49]. However, these mice exhibited decreased exploratory behaviour[50,51] and increased thigmotaxis in the Morris water maze (a model of spatial learning that also places the animal under mild stress)[52]. These phenotypes might be seen as a neophobic behaviour, and thus as increased anxiety-like behaviour. Complementary to these loss-of-function studies, specific genetic-rescue experiments showed that re-expression of CB1R in cortical glutamatergic neurons is sufficient to almost completely restore wild-type anxiety-like behaviour in mice lacking CB1R expression in all cells of the body except cortical glutamatergic neurons[53].

Conversely, the loss of CB1R in forebrain GABAergic neurons leads to increased exploratory behaviours in mildly aversive conditions[50,51], which can be interpreted as reduced anxiety-like or neophobic behaviour. However, in the elevated plus maze, the exploratory behaviour of these conditional-knockout mice was the same as that of controls[49]. Thus, CB1Rs on cortical glutamatergic and GABAergic neurons exert opposing control on anxiety-like behaviours, but only when the environmental aversiveness exceeds a certain threshold. This is in agreement with the notion that the eCB system exerts a ‘buffering’ effect on neuronal activity in specific circuits. This function seems to operate within specific limits of neuronal activity, whereby a certain minimal strength of stimulus is

needed to engage eCB signalling, and when this activity overcomes certain limits, the buffering capacity is exhausted[9].

Numerous pharmacological studies support the notion of bidirectional regulation of anxiety circuits and behaviour by CB1R. It is well known that exogenous cannabinoids influence anxiety-like behaviour in a biphasic manner, with low and high doses exerting anxiolytic and anxiogenic states, respectively, in both animals and humans[54]. These pharmacological effects are mediated by CB1R. Cell-type-specific CB1R-deficient mice have enabled the identification of the underlying mechanisms of these biphasic effects[49]. The anxiolytic effect of cannabinoids at low doses depends on the presence of CB1R on cortical glutamatergic neurons, whereas the anxiogenic effect of higher doses is mediated by CB1R on forebrain GABAergic neurons. This observation is consistent with previous experiments in which the cannabinoid receptor agonist $\Delta^9$-tetrahydrocannabinol (THC) was locally injected into the ventral hippocampus or PFC: a low THC dose evoked an anxiolytic response, whereas a high THC dose led to an anxiogenic response[55].

Taken together, these genetic and pharmacological experiments suggest a mechanism for the processing of anxiety-related stimuli in which CB1R on glutamatergic neurons and GABAergic neurons decreases excitatory and inhibitory drive, respectively, thereby explaining the opposing effects of manipulation of these two transmitter systems on anxiety[49,51]. Consistent with these behavioural data and the notion of opposing functions depending on cellular expression, mice with CB1R deficiency in cortical glutamatergic neurons were shown to exhibit overexcited hippocampal circuits (that is, increased long-term potentiation (LTP), spine density and dendritic branching in pyramidal neurons). By contrast, mice with CB1R deficiency in forebrain GABAergic neurons displayed decreased excitability of these circuits (that is, decreased LTP, spine density and dendritic branching in pyramidal neurons) (FIG. 4). These results indicate that the loss of CB1R in either neuronal population induced an allostatic shift and long-term dysregulation of the functions of hippocampal pyramidal neurons[56]. Interestingly, this bimodal control of excitability exerted by glutamatergic and GABAergic CB1R correlates well with the behavioural alterations observed.

Genetic and pharmacological interference with eCB-synthesizing and -degrading enzymes, leading to alterations in eCB levels, has also revealed the importance of eCBs in the regulation of anxiety. Genetic deletion of fatty acid amide hydrolase (FAAH), the primary AEA-degrading enzyme in the CNS, leads to increased AEA levels in the brain and to decreased anxiety-like behaviour in the elevated plus maze and the light–dark test (animal models that measure anxiety-like behaviour)[57]. Similar effects occur with pharmacological blockade of FAAH under basal conditions[57–59] and after chronic unpredictable stress (CUS)[60]. Currently, the analysis of conditional inactivation of FAAH is still pending but it should provide additional information to clarify the exact sites where this enzyme controls anxiety-related behaviours. Some years ago, a polymorphism in human FAAH was identified (rs324420; in which cysteine 385 is changed to alanine)[61]. This alteration leads to a destabilized FAAH enzyme and, consequently, to an increase in AEA signalling. Humans and mice homozygous in this allele ($FAAH^{A/A}$) show decreased anxiety-like behaviour and increased fear-extinction learning[62]. Functional MRI (fMRI) investigations revealed

increased functional connectivity between the ventromedial PFC (vmPFC) and amygdala in these people. Remarkably, the presence of this polymorphism results in comparable phenotypes in mice[62].

With regard to 2-AG signalling, it has recently been reported that deficiency of the 2-AG-synthesizing enzyme, diacylglycerol lipase-α (DAGLα), leads to markedly decreased 2-AG brain levels and to increased anxiety-like behaviour[34,36]. The anxiety-like behaviour was rescued by pharmacological blockade of the 2-AG-degrading enzyme monoacylglycerol lipase (MAGL) with JZL184 (REF. 34). In agreement with these observations, impairment of 2-AG signalling in hippocampal glutamatergic neurons by viral overexpression of MAGL also led to decreased 2-AG levels and increased anxiety-like behaviour[63]. Conversely, pharmacological inhibition of MAGL in wild-type mice by JZL184 had anxiolytic effects under basal conditions[60,64,65], under increased aversive conditions[66] and after CUS[60,67].

## Non-CB1R actions in anxiety

The influence of eCBs on anxiety is made more complex by their activity at receptors other than CB1R. Postsynaptic transient receptor potential cation channel subfamily V member 1 (TRPV1) can be activated by AEA, thereby enhancing postsynaptic currents. Decreased anxiety-like behaviour has been observed in mice lacking TRPV1 (REF. 68). As TRPV1 is expressed and functional in both GABAergic and glutamatergic neurons[23,69–72], the analysis of conditional TRPV1-deficient mice will be important and might reveal a behavioural dichotomy similar to that observed with CB1R. Furthermore, CB2R has been implicated in the eCB-dependent regulation of anxiety-like behaviours[73,74]. However, owing to the enigmatic expression of this receptor in neurons (BOX 1), the mechanistic basis of these observations is far from being understood.

The importance of CB1R-and TRPV1-mediated signalling in anxiety-like behaviour has also been investigated by using local applications of pharmacological agents that modulate eCB-system activity. These experiments revealed direct roles for CB1R-and TRPV1-mediated signalling within the ventral hippocampus[55], PFC[55,75], BLA[55] and periaqueductal grey (PAG)[76,77]. Although they have a common ligand, AEA, the involvement of CB1R and TRPV1 in anxiety is opposite, constituting an intriguing antagonistic regulatory mode between both signalling systems[78].

In conclusion, the eCB system controls anxiety-related brain regions at many different levels. Based on present knowledge, it seems that the general role of this system is to control excessive activation (thereby exerting anxiolytic functions). However, it is interesting to note that additional mechanisms (for example, CB1R signalling at GABAergic synapses) seem to mediate opposite (that is, anxiogenic) functions under certain conditions (FIG. 4). From an evolutionary point of view, the presence of CB1R and TRPV1, and the eCB signalling on both antagonizing neurotransmitter systems (that is, glutamatergic and GABAergic neurons), seems to be highly beneficial for the appropriate regulation of anxiety-like behaviour.

## Fear behaviour

Anxiety is elicited by potentially dangerous but unspecified future threats, whereas fear is the response to specific and actual threatening stimuli. Thus, we will probably be anxious if we are walking in an area known to contain poisonous snakes (a potential but unspecific threat), but we feel fear when we encounter a poisonous snake directly (an actual and specific threat)[79].

In a similar manner to anxiety, fear perception, elaboration and response involves neuronal, autonomic and hormonal responses. The behavioural reactions to specific threats can be passive in nature (that is, aimed at hiding from or passively avoiding the source of threat; for example, by freezing) or active in nature (that is, aimed at escaping and actively avoiding the danger). Fear can be innate (such as human fear of snakes and/or other animals) or acquired (when the individual learns that a certain stimulus represent a specific threat to well-being or life). All these modalities of fear and fear responses have been studied in experimental settings, and there is scientific literature linking these aspects to the eCB system[2–5]. Cued fear conditioning is the most widely used model to study fear circuits[42,80]. In this protocol, an animal learns to associate an initially neutral stimulus (called a conditioned stimulus (CS); for example, an acoustic, visual, tactile, gustatory or olfactory cue) with a simultaneous fear-inducing stimulus (known as an unconditioned stimulus (US); for example, a mild electric shock). After one or more pairings of the US with the CS, the subject associates the two stimuli, and the presentation of the CS alone is able to evoke a fear response[79]. This association of the two stimuli (the ‘fear learning’) is typically consolidated into long-term memory within 6–8 hours. In rodents, the strongest and most-immediate fear reaction is freezing[79]. Scoring of freezing behaviour during presentations of the CS alone is used to evaluate the strength of the fear elicited by the specific CS. However, the re-exposure of the subject to the fear stimulus triggers additional neuronal processes, aimed at adapting the behavioural response to changing environmental conditions[81,82]. Thus, short re-exposure to the CS triggers a second round of memory consolidation (called reconsolidation), whereby new information can be integrated into the original memory[81]. Conversely, prolonged or repeated exposure to the CS in the absence of the US triggers extinction, resulting in a decline of CS-evoked fear expression[82,83]. Clinically, extinction is thought to be impaired in patients suffering from specific fear-related disorders, such as phobias or post-traumatic stress disorder (PTSD). Thus, enhanced understanding of the mechanisms involved in fear extinction can lead to novel treatment options for these patients.

Brain regions and neural circuits regulating fear have been investigated intensively[42]. The eCB system is present in these fear-related brain areas and is centrally involved in the regulation of fear-memory processing[3–5]. Global genetic deletion and pharmacological blockade of CB1R consistently induces marked impairment in the decrease of fear responses (that is, freezing) after repeated or prolonged CS-alone presentations, but less in acquisition and consolidation of fear memory[84,85]. Subsequent experiments using mice lacking CB1R in cortical glutamatergic neurons revealed that CB1R in these cells is necessary for proper reduction of the fear response[86]. However, genetic-rescue experiments revealed that CB1R in cortical glutamatergic neurons is not sufficient to guarantee this behaviour[53]. This is in

strong contrast to the CB1R-dependent control of anxiety behaviour, which was in large part rescued when CB1R was re-expressed (see above). CB1R deficiency in forebrain GABAergic neurons does not seem to have an essential role in the reduction of conditioned freezing responses[86], although a recent study reported decreased freezing in GABAergic-specific CB1R mutants on the first re-exposure to the conditioned stimulus[87]. Further support for a role of CB1R in GABAergic interneurons comes from evidence that fear extinction can cause specific remodelling of perisomatic inhibitory synapses in the basal amygdala, including alterations in the localization of the CB1R on CCK-positive neurons in this region[88]. Furthermore, an interaction between the eCB system and CCK signalling has been demonstrated, as the decrease in fear extinction that is normally induced by CB1R antagonism was blunted in CCK-B receptor-deficient mice. This effect is possibly linked to CB1R expressed on GABAergic neurons in the amygdala[89]. CB1R can also control the expression of aversive memories in different brain regions. For instance, CB1R in the synaptic terminals of neurons of the medial habenula projecting to the interpeduncular nucleus was shown to promote the expression of aversive memories in fear conditioning and conditioned odour aversion experiments[90]. Interestingly, these recent results suggest that CB1R can increase or decrease aversive responses, depending on the specific brain circuits and cell types that are involved.

The role of 2-AG in fear extinction was shown in mice deficient in DAGLα, which have reduced 2-AG brain levels. These mutant mice exhibit no impairments in fear acquisition but show impaired fear extinction[36]. These data are in agreement with the requirement of the eCB system for proper fear extinction[84]. Interestingly, pharmacological enhancement of 2-AG with the MAGL inhibitor JZL184 promotes fear expression and impairs fear extinction, an effect that requires CB1R in forebrain GABAergic neurons[87]. In fact, genetic and pharmacological blockade of MAGL enhances hippocampal depolarization-induced suppression of inhibition (DSI; a form of eCB-STD at GABAergic synapses)[91,92], leading to insufficient GABAergic transmission, which is consistent with increased fear expression. These results suggest that an optimal level of 2-AG is required for appropriate processing of fear responses and that having 2-AG levels that are too high or too low impairs the decrease in fear expression.

Pharmacological experiments that applied CB1R antagonists to specific brain regions revealed that eCB signalling in the BLA and CeA are important for different phases of fear extinction[21]. CB1R blockade in the BLA led to an impairment of long-term extinction, whereas CB1R antagonism in the CeA reduced within-session extinction[21]. In addition, it was reported that the magnitude of depolarization-induced suppression of excitation (DSE) and DSI in the CeA was increased on the day after fear conditioning, showing that CB1R-mediated synaptic plasticity in the CeA is a consequence of fear conditioning[21]. Pharmacological blockade of CB1R in the infralimbic subregion of the medial PFC also impairs fear extinction[93].

Pharmacological enhancement of AEA signalling by inhibition of FAAH in the BLA facilitates fear extinction via activation of the CB1R[94]. In these pharmacological experiments, CB1R on both afferent synaptic terminals and local GABAergic interneurons is

likely to be activated. Thus, although these experiments reveal the importance of CB1R signalling in the BLA, they do not give information on the specific neuronal circuits.

## Reduction of fear responses

How does the eCB system modulate the fear reaction and the extinction process? Several theories have been proposed to explain the reduction of fear responses on repeated or prolonged exposure to a CS[82], but the two primary theories refer to overlapping processes of 'extinction' and of 'habituation' (REFS 3,82). Both these processes are learning phenomena by which experience modifies future behavioural responses. Extinction is considered an active associative-learning process, in which a new association is formed, predicting the absence of the US after CS presentations[82]. Conversely, habituation is one of the simplest forms of memory and relies on the non-associative reduction of responses to repeated stimuli occurring even in very simple neuronal systems (for example, aplysia[95]). The eCB system has been proposed to participate in both of these processes. One possibility is that the local, CB1R-dependent control of neuronal transmission and plasticity might regulate associative properties of extinction, by favouring the activation of putative 'no-fear' neurons at the expense of 'fear' neurons in amygdalar circuits[2]. However, another hypothesis is that eCB signalling might potentiate or activate non-associative habituation processes to dampen the fear reaction after repeated CS-alone exposures[96]. The decrease of freezing response elicited by repeated tone presentations to mice previously exposed to a footshock not associated to the tone (non-associative sensitization) was shown to be strongly impaired in global CB1R-deficient mice and in conditional mutant mice lacking CB1R in cortical glutamatergic neurons[3,96,97]. Remarkably, this concept of habituation is congruent with the notion of how the eCB system works in stress-coping (see also below), in which it is activated by repeated exposure to a homotypic stress (that is, an identical repeated sensory input) and is required for habituation[98]. Therefore, the available data support the hypothesis that the eCB system is required for appropriate and efficient fear relief[3], whereby the activity of the eCB system is increased with each re-exposure to a stimulus associated with a threat that is no longer present, and the fear response (for example, the freezing behaviour) is reduced in a manner inversely related to the elevation of eCB-mediated CB1R activation. This model still has to be verified experimentally by cell-type-specific analyses of eCB system activity under conditions of repeated exposures to the threatening stimuli.

The extinction of conditioned fear is inhibited by stress exposure, which can be problematic in the application of extinction-like procedures to treat PTSD in humans. In the inhibitory-avoidance paradigm, in which the animal (for example, a rat) learns to avoid places previously associated with punishments, exposure to stress enhances conditioning and reduces extinction. Both of these effects of stress are inhibited by CB1R agonist injection into the BLA[99]. Similarly, the effect of a single prolonged stress (SPS) to inhibit contextual fear extinction 1 week later is reduced by CB1R activation in BLA or hippocampus immediately after the SPS[100]. Furthermore, CB1R activation prevents SPS-induced upregulation of the glucocorticoid receptor in the BLA and hippocampus, suggesting that high CB1R activity at the time of trauma reduces the glucocorticoid receptor's ability to hyperactivate the fear circuit. Chronic social-defeat stress also impairs contextual fear extinction, an effect that is alleviated by the treatment with the FAAH inhibitor URB597

(REF. 101). These data are consistent with the hypothesis that chronic stress creates a 'hypocannabinergic state' that results in impaired fear extinction and can be alleviated by CB1R agonists and indirect agonists (see below).

## Fear-coping strategies

Several theories of fear refer to the coexistence of different coping strategies, or 'styles', which induce specific types of responses to threatening situations; these are usually classified as passive (or reactive) and active (or proactive)[102,103]. Another distinct feature of the eCB system is its role in the regulation of switching between these different strategies, that is, between a passive fear response (such as freezing) and active behaviours (such as escape attempts and risk assessment). In classical fear conditioning, after prolonged exposure to the threatening stimuli, a switch occurs from passive to active behaviour. Global CB1R deficiency disrupts this pattern and favours passive responses[104]. This phenotype seems to depend on CB1R expressed on cortical glutamatergic neurons, as mutants lacking CB1R in these neurons display longer freezing responses in fear conditioning and slower learning of avoidance behaviour in active-avoidance paradigms. Conversely, loss of CB1R on forebrain GABAergic neurons leads to the opposite phenotype, decreasing freezing and favouring active behaviours (in this case, digging and rearing) in fear conditioning, and promoting more efficient active avoidance. Interestingly, when all CS-induced responses (freezing, rearing and digging, which could all be considered rationally as 'fear responses') were summed over a sufficiently long period of CS presentation, there was no difference in the patterns displayed by either of the conditional mutants compared with wild-type controls; that is, there is no difference in total fear-related behaviours. These data provokingly suggest that the CB1R expressed on either cortical glutamatergic or forebrain GABAergic neurons does not strongly affect the 'memory' of the conditioning event and the consequent levels of perceived 'fear' by the individuals during CS exposition, but merely determines the individual coping style towards specific threats[104].

## Stress-coping

Stress can be defined as a reaction of the body to an internal or external challenge to prepare its response to possible dangers or injuries. Physical and psychological stress induces a pattern of responses that allow for coping with the immediate threat followed by recovery to homeostasis. The earliest responses to stress are neural and occur within seconds of the stress. Several neurotransmitters are involved in this process, including noradrenaline, serotonin, GABA, glutamate and the fast-reacting stress hormone adrenaline. Endocrine responses, mediated by activation of the HPA axis, with the ultimate release of adrenal glucocorticoids, occur minutes to hours after the stress. Preclinical data strongly support the hypothesis that eCB signalling is altered by stress (BOX 2) and represents a central mechanism by which stress alters synaptic plasticity in many brain regions.

Acute stress produces changes in the brain concentrations of the two major eCBs, AEA and 2-AG, and thereby alters CB1R signalling. Acute stress exposure reduces the concentration of AEA in the amygdala and PFC; these changes are accompanied by an increase in the activity of FAAH[105] and are mediated by effects of corticotropin-releasing hormone (CRH)

that alter FAAH activity[106]. In the amygdala, reduced AEA concentrations enable the activation of the HPA axis, and inhibition of FAAH reduces the glucocorticoid response[105]. Both stress and glucocorticoids increase the concentrations of 2-AG in the hypothalamus, hippocampus, PFC and raphe nuclei. In the hypothalamus, activation of a plasma membrane-associated glucocorticoid receptor rapidly increases levels of 2-AG, which acts to inhibit glutamate release[107]. In the PFC, the mechanism by which glucocorticoids elevate 2-AG levels is not clear, but this increase results in the inhibition of GABA release[108]. Both in the hypothalamus[109] and in the PFC[108], activation of CB1R signalling is required for glucocorticoid-mediated feedback inhibition of the HPA axis. Interestingly, recent data indicate that restraint stress is also able to switch eCB-dependent plasticity from LTD to LTP in the BNST, suggesting that the eCB system can regulate stress responses in several different brain regions[110]. Food deprivation was also shown to convert an eCB-dependent LTD of inhibitory transmission to a nitric oxide (NO)-dependent, CB1R-independent LTP of inhibitory transmission in the hypothalamus[111], indicating that eCB signalling is centrally involved in plastic adaptations induced by different types of stress.

Chronic stress exposure also alters the eCB system throughout the brain. Exposure to non-habituating, chronic stress downregulates CB1R signalling in many brain regions involved in emotional processing, including the hippocampus[112], striatum[113], nucleus accumbens[114], PFC[115], dorsal raphe nucleus[116], amygdala[117] and hypothalamus[118]. In the hippocampus, hypothalamus and striatum, chronic stress reduces signalling by downregulating CB1R[112,113,118]. However, in the PFC, chronic stress increases CB1R mRNA expression but clearly reduces CB1R responsivity at GABAergic terminals[115]. Yet another mechanism is at play in the amygdala, where chronic stress increases FAAH activity and decreases AEA concentrations, which would be expected to decrease eCB signalling at the level of ligand availability[117]. Different neuronal types, such as cortical glutamatergic, forebrain GABAergic and serotonergic neurons, are differentially involved in the responses to acute and chronic stress in mice, again indicating multilevel control of stress responses by the eCB system[28,86,119,120].

Repeated exposures to the same stress result in habituation of HPA axis activation and of the behavioural stress response. Repeated homotypic stress exposures sensitize the eCB system, which contributes to the habituation to stress[98,121]. The mechanism involves increased concentrations of 2-AG, possibly owing to reduced catabolism by MAGL[48]. The ability to habituate to repeated exposure to a non-threatening stimulus is protective, as it avoids the consequences of chronic stress. The ability of eCB-mediated synaptic plasticity to facilitate habituation could be one of the most critical roles of this process in the context of human psychopathology.

In summary, the brain's eCB system links stress exposure to changes in synaptic plasticity, contributing to activation and feedback regulation of the HPA axis. More importantly for the understanding of human psychopathology, chronic stress can downregulate CB1R signalling in brain regions vital for the regulation of affective processes, whereas habituation to stress, which reduces its effect, is accompanied by enhanced eCB system activity. In more general terms, the hypothesis can be put forward that the eCB system facilitates the activation of resilience factors[122,123] during and/or after stress exposure.

## Future directions

### Which specific neuronal circuits are regulated by the eCB system?

The eCB system is present in many brain circuits that are known to regulate anxiety, fear and stress-coping processes. Together with anatomical descriptions, substantial functional evidence corroborates the idea that eCB signalling modulates synaptic activity at many 'nodes' of these circuits. However, direct evidence of these functions of eCB signalling in freely behaving animals challenged with specific tasks to measure anxiety, fear and stress-coping behaviours is mostly missing and will require the manipulation of the activity of well-identified circuits in behaving animals. Therefore, most of the presently available evidence is correlative in nature and based on parallel mechanisms between *in vitro*, *ex vivo* and *in vivo* data, which are powerful and consistent but cannot be used to demonstrate causality. This limitation, which has negatively affected the progress of behavioural neurosciences in general, is being addressed by the advent of new technological approaches. For instance, experimental approaches such as optogenetics and pharmacogenetics[124,125] will allow the examination of the direct causal relationship between the activity of specific circuits and behaviour in freely moving animals. The application of these techniques to the field of the eCB system, in combination with cell-type genetic manipulation of eCB system components using the Cre–*loxP* system and viral techniques, will allow the direct causal relationships between the function of, for example, CB1R in specific circuits and behavioural outputs to be uncovered[126]. Similarly, causal links between eCB system-meditated electrophysiological and/or synaptic modulations and behavioural outputs need to be established.

### The eCB system and CNS–periphery crosstalk

The eCB system is also centrally involved in the crosstalk between central and peripheral processes regulating behaviour. This is well known in the control of energy balance and feeding, in which CB1R expression in the brain and in the periphery synergizes to regulate both metabolic activity and behavioural outputs[127]. This potential crosstalk has been extended to anxiety-and fear-related behaviours[128]. The anxiogenic effect in the elevated plus maze test and the freezing-promoting effect in fear-conditioning settings exerted by the CB1R antagonist rimonabant were blocked by the administration of peripherally restricted β-adrenergic receptor antagonists. Interestingly, this blockade also occurred when rimonabant was administered directly into the brain, suggesting that centrally mediated hyperactivation of the sympathetic nervous system is a primary consequence of CB1R blockade[128]. There is still much to be learned about eCB-mediated modulation of the crosstalk between the CNS and the periphery and how this can influence behavioural outputs (including in anxiety-and fear-related dimensions).

### Astroglial CB1R in anxiety, fear and stress-coping

By secreting 'gliotransmitters' (for example, glutamate, GABA, ATP and d-serine)[129] and providing energy supply and protection to neurons[130], astrocytes can profoundly influence synaptic activity and brain function, including anxiety-and fear-related behaviours. Astrocytes and other glial cell types produce eCBs in response to activity-related ATP release[131] and express low, but functionally important, levels of cannabinoid receptors[11,132].

Recent data indicate that physiological synaptic functions are regulated by astroglial cannabinoid receptors[30,133–135]. Interestingly, whereas the CB1R expressed at presynaptic terminals seems to reduce neurotransmitter release, the astroglial CB1R seems to potentiate synaptic glutamatergic signalling[133,134]. Considering that astroglial cells have been suggested to participate in anxiety, fear and stress-coping[136,137], it will be interesting to assess whether similar astroglial CB1R-dependent mechanisms operate in the effect of cannabinoids and endocannabinoid signalling on these processes.

### Brain bioenergetics in fear, anxiety and stress-coping: a role for CB1R?

The brain, with a weight of about 2% of the entire body, consumes up to 20% of the body's energy[138], presumably because bioenergetic processes in the brain are highly active and go beyond mere cell 'housekeeping' and survival. This has been demonstrated both biochemically[139] and by fMRI[140], and recent studies have revealed the profound effect of even limited alterations of energy supply (in the form of ATP) on synaptic functions[141]. Anxiety, fear and stress elicit high neuronal activity in distinct brain regions accompanied by high energy requirements, which mean a great demand for ATP. Mitochondria, which are the main cellular 'power plants' producing the large majority of ATP, are therefore crucial for efficient brain function, including the regulation of mood and anxiety[142]. The ability of cannabinoids to control mitochondrial activity was first reported in the 1970s[143] and is now thought to be a potentially important way in which eCBs influence cellular and brain functions[144,145]. Recently, the presence of functional CB1R at mitochondrial membranes was demonstrated by different groups[146,147] (see REFS 148,149 for methodological discussions). The brain mitochondrial CB1R (mtCB1R) directly regulates respiration and ATP production and, at the synaptic level, participates in eCB-dependent synaptic plasticity. The role of mtCB1R in anxiety-, fear-and stress-related circuits is not known yet, but this represents an interesting new field for future research.

### Functions of peptide eCBs

The eCB family is typically represented by lipid fatty acid derivatives linked to glycerol (sn-2 acyl glycerol) or amines (*N*-acyl amides). However, evidence has recently accumulated that a family of peptide derivatives of α-haemoglobin, called peptide eCBs (pepcans)[150], modulates CB1R activity. In particular, pepcan-12 acts as a negative allosteric modulator of CB1R[150,151] and was identified in noradrenergic/adrenergic cells in the brain and in the adrenal glands[152]. Considering the importance of the regulation of the noradrenergic and adrenergic systems in stress regulation, further studies on pepcan-12 are keenly awaited.

## Concluding remarks

The effects of phytocannabinoids on fear, anxiety and stress-coping have been appreciated for a long time, and the discovery of the active components of the plant *Cannabis sativa* — which is celebrated by this series of Review articles on endocannabinoid function in the brain[15,153–155] — has fuelled the search for underlying mechanisms. Future studies will need to integrate new discoveries into the larger picture of the eCB-dependent regulation of anxiety, fear and stress responses. Based on its multiple and diverse mechanisms of action, the eCB system can also be considered as a suitable tool to shape and fine-tune diverse and

complex behaviours. Indeed, in a bottom-up approach, starting from any of the elements of the eCB system (receptors, different types of ligands, triggers and enzymes involved in ligand synthesis and degradation), researchers are asked to follow several different pathways involving diverse elements of the mechanisms underlying complex behaviours, such as neuronal circuits, synaptic plasticity, astroglial functions, eCB biochemistry and bioenergetics. In the end, these studies raise strong hopes not only for a better understanding of basic behavioural processes but also for future therapeutic interventions to tackle their dysfunctions, which are particularly warranted in affective disorders (BOX 3). These reasons make the study of the eCB system a highly fascinating aspect of neuroscience, and the next decades of research will surely bring new and exciting discoveries and concepts.

**Box 1**

### Enigmatic neuronal CB2R and its role in anxiety

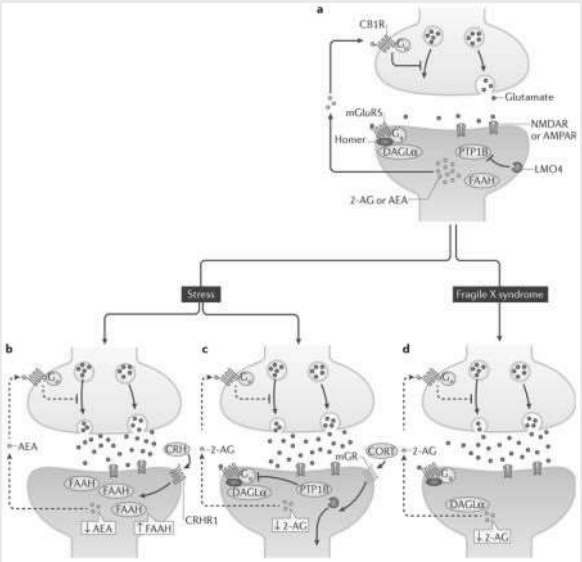
The presence of neuronal cannabinoid receptor type 2 (CB2R) was recently revealed in several brain structures[156,157], although the low expression levels and technical aspects have continuously raised questions about the validity of these results[157–159]. Recent behavioural studies using genetically modified mice and pharmacological tools have provided evidence that CB2R is involved in several behavioural responses, including anxiety. Indeed, chronic pharmacological blockade of CB2R produced anxiolytic effects mediated by an alteration of $GABA_A$ receptor function[160]. Furthermore, transgenic mice overexpressing the CB2R in CNS neurons showed decreased anxiety-like behaviour and impaired anxiolytic effects of benzodiazepines[161]. CB2R was shown to be involved in the anxiolytic-like responses induced by 2-arachidonoyl glycerol (2-AG)[64], and blockade of CB2R normalized the anxiety-like phenotype of fragile X mental retardation 1 (*Fmr1*)-knockout (*Fmr1*$^{-/y}$) mice[74]. Despite these accumulating data, the molecular and cellular mechanisms by which the neuronal CB2R may influence behaviour have remained enigmatic. In this context, it is important to note that the CB2R is clearly expressed in brain immune cells (microglia). Thus, an immune cell–neuron interaction might be accountable for the behavioural phenotypes observed in CB2R-deficient mice, and the 'enigmatic' CB2R expression in neurons may not be functionally relevant in anxiety behaviours. To this end, further investigations using cell-type-specific deletions of the gene encoding CB2R are required.

**Box 2**

### eCB-based synaptopathies

A major function of the endocannabinoid (eCB) system is its ability to suppress neurotransmitter release in a retrograde manner (see the figure, part **a**). Many studies have investigated whether dysregulation of eCB signalling contributes to synaptopathies. Stress has a strong effect on eCB system functions. For example, acute stress results in increased activity of fatty acid amide hydrolase (FAAH) in the basolateral amygdala (BLA), via a corticotropin-releasing hormone (CRH) receptor type 1 (CRHR1)-mediated mechanism[106] (see the figure, part **b**). Increased FAAH activity results in reduced concentrations of *N*-arachidonoylethanolamine (AEA) and thus in increased excitability

of principal neurons in the BLA because AEA is not available for the retrograde suppression of glutamate release; eventually leading to increased anxiety-like behaviour. Chronic stress (see the figure, part **c**) has recently been shown to cause an impairment of 2-arachidonoyl glycerol (2-AG) synthesis, through collapse of a signalling cascade in glutamatergic neurons in the BLA, a process involving the activation of a metabotropic glucocorticoid receptor (mGR), leading to increased activity of protein tyrosine phosphatase 1B (PTP1B) via decreased palmitoylation and cytoplasmic activity of its inhibitor, LIM domain only 4 (LMO4; translocation out of dendrite). In consequence, PTP1B shows enhanced inhibition of metabotropic glutamate receptor 5 (mGluR5; also known as GRM5) phosphorylation, resulting in decreased diacylglycerol lipase-α (DAGLα) activity and 2-AG production[162]. Pharmacological inhibition of PTP1B rescues the insufficient 2-AG production and the anxiety-like phenotype after chronic stress[162]. Likewise, mutations in the gene fragile X mental retardation 1 (*FMR1*) in the fragile X syndrome (see the figure, part **d**) result in an uncoupling of DAGLα from the mGluR5–Homer complex[163–165]. This leads to impaired 2-AG production and decreased retrograde suppression of both GABAergic and glutamatergic transmission, and coincides with increased anxiety and cognitive impairments[74]. AMPAR, AMPA receptor; CB1R, cannabinoid receptor type 1; CORT, corticosterone; $G_q$, $G_q$ family G protein; NMDAR, NMDA receptor.




## Box 3

### Therapeutic targeting

**Stimulation of cannabinoid receptors**

Clinical findings suggest a negative correlation between endocannabinoid (eCB) system activity and anxiety[166]. Cannabinoid receptor type 1 (CB1R) agonists can have unacceptable side effects[54], whereas increasing eCB levels by inhibiting eCB-degrading enzymes could be more selective. Enhancement of *N*-arachidonoylethanolamine (AEA)

and 2-arachidonoyl glycerol (2-AG) by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) blockade, respectively, attenuates anxiety in rodents[6,67,167]. The anxiolytic effect of AEA depends on CB1R and is associated with cognitive impairments, whereas the anxiolytic effect of 2-AG is CB2R-dependent and not associated with cognitive disruption[64]. However, other studies reported a CB1R involvement in the 2-AG anxiolytic effect using different behavioural models[65] and animal species[66]. As these indirect agonists have not yet been approved for use in humans, the option of direct CB1R stimulation has yet to be explored, whereby CB1R stimulation with $\Delta^9$-tetrahydrocannabinol (THC) enhances fear extinction in humans[168], which warrants further investigation in post-traumatic stress disorders (PTSD)[169].

Other strategies have been proposed to minimize cannabinoid side effects. Blockade of the mammalian target of rapamycin (mTOR) pathway prevents THC-induced cognitive impairment in mice, without modifying its anxiolytic effects[170]. COX2 (also known as PTGS2) inhibition increases eCB brain levels[171], reduces anxiety in rodents[172] and blocks THC-induced cognitive impairment[173]. Positive allosteric modulators regulating orthosteric ligand activity can open new perspectives for reducing side effects[174,175]. Alternatively, interruption of heterodimers between CB1R and serotonin 5-hydroxytryptamine receptor 2A (5-HT2AR) using cell-penetrable peptides selectively abrogates THC-induced memory impairments[176].

**Inhibition of cannabinoid receptors**

An interesting therapeutic approach has recently been suggested for fragile X syndrome, which is caused by a mutation in the fragile X mental retardation 1 (*FMR1*) gene. An eCB system dysregulation seems to be responsible for the imbalance between excitatory and inhibitory inputs in the hippocampus, leading to the behavioural fragile X phenotype of *Fmr1*-knockout ($Fmr1^{-/y}$) mice. CB1R blockade normalized the main cognitive and hippocampal neurological alterations in these mice, whereas CB2R blockade alleviated their anxiolytic phenotype[74]. The use of synthetic[174,175] and endogenous[150,177] negative allosteric modulators of cannabinoid receptors is another possible avenue to achieve better therapeutic effects with reduced side-effects.

## Acknowledgments

B.L. was supported by the German Research Foundation (SFB TRR 58, CRC 1080 and FOR 926); G.M. by the Institut national de la santé et de la recherche médicale (INSERM), the European Commission Seventh Framework Programme (REPROBESITY, HEALTH-F2-2008-223713, PAINCAGE and HEALTH-2014-603191), the European Research Council (Endofood, ERC–2010–StG-260515, CannaPreg and ERC-2014-PoC-640923), the Fondation pour la Recherche Medicale (DRM20101220445), the Human Frontiers Science Program, Region Aquitaine, Agence Nationale de la Recherche (ANR Blanc NeuroNutriSens ANR-13-BSV4-0006 and BRAIN ANR-10-LABX-0043); R.M. by the grants SAF2014-59648P, RETICS-RTA#RD12/0028/0023, AGAUR#2014-SGR-1547 and Health-F2-2013-602891; and C.J.H. by the US National Institutes of Health grants DA038663, DA026996 and MH102838.

## Glossary

**Endocannabinoid (eCB)**

A type of lipid signalling molecule derived from arachidonic acid. The eCBs are the endogenous counterparts of the cannabinoids

**Microglia**
Immune cells of the brain that are involved in defence

**Anxiety disorders**
Mental disorders involving feelings of anxiety and fear, caused by physical or psychological harm. There are different forms, such as general anxiety disorders and specific phobias

**Thigmotaxis**
Movement of an organism towards an object (for example, a wall), giving them a sense of increased safety

**Neophobic behaviour**
Fear of anything new; unwillingness to try new things and break from routine

**Polymorphism**
A genetic variant of a gene, with possible emergence of distinct phenotypes

**Habituation**
A form of learning in which an organism reduces its response to a stimulus after repeated presentations of the stimulus

## References


1. Tart CT. Marijuana intoxication common experiences. Nature. 1970; 226:701–704. [PubMed: 5443246]
2. Lafenetre P, Chaouloff F, Marsicano G. The endocannabinoid system in the processing of anxiety and fear and how $CB_1$ receptors may modulate fear extinction. Pharmacol Res. 2007; 56:367–381. [PubMed: 17951068]
3. Riebe CJ, Pamplona FA, Kamprath K, Wotjak CT. Fear relief—toward a new conceptual frame work and what endocannabinoids gotta do with it. Neuroscience. 2012; 204:159–185. [PubMed: 22173015]
4. Ruehle S, Rey AA, Remmers F, Lutz B. The endocannabinoid system in anxiety, fear memory and habituation. J Psychopharmacol. 2012; 26:23–39. [PubMed: 21768162]
5. Akirav I. The role of cannabinoids in modulating emotional and non-emotional memory processes in the hippocampus. Front Behav Neurosci. 2011; 5:34. [PubMed: 21734875]
6. Gunduz-Cinar O, Hill MN, McEwen BS, Holmes A. Amygdala FAAH and anandamide: mediating protection and recovery from stress. Trends Pharmacol Sci. 2013; 34:637–644. [PubMed: 24325918]
7. McLaughlin RJ, Hill MN, Gorzalka BB. A critical role for prefrontocortical endocannabinoid signaling in the regulation of stress and emotional behavior. Neurosci Biobehav Rev. 2014; 42:116–131. [PubMed: 24582908]
8. Castillo PE, Younts TJ, Chavez AE, Hashimotodani Y. Endocannabinoid signaling and synaptic function. Neuron. 2012; 76:70–81. [PubMed: 23040807]
9. Katona I, Freund TF. Multiple functions of endocannabinoid signaling in the brain. Annu Rev Neurosci. 2012; 35:529–558. [PubMed: 22524785]
10. Marsicano, G., Kuner, R. Anatomical Distribution of Receptors, Ligands and Enzymes in the Brain and in the Spinal Cord: Circuitries and Neurochemistry in Cannabinoids in the Brain. Köfalvi, A., editor. Springer; 2008. p. 161-201.
11. Metna-Laurent M, Marsicano G. Rising stars: modulation of brain functions by astroglial type-1 cannabinoid receptors. Glia. 2015; 63:353–364. [PubMed: 25452006]

12. Stella N. Endocannabinoid signaling in microglial cells. Neuropharmacology. 2009; 56:244–253. [PubMed: 18722389]
13. Alger BE, Kim J. Supply and demand for endocannabinoids. Trends Neurosci. 2011; 34:304–315. [PubMed: 21507493]
14. Ohno-Shosaku T, Kano M. Endocannabinoid-mediated retrograde modulation of synaptic transmission. Curr Opin Neurobiol. 2014; 29:1–8. [PubMed: 24747340]
15. Soltesz I, et al. Weeding out bad waves: towards selective cannabinoid circuit control in epilepsy. Nat Rev Neurosci. 2015; 16:264–277. [PubMed: 25891509]
16. Marsicano G, Lutz B. Expression of the cannabinoid receptor $CB_1$ in distinct neuronal subpopulations in the adult mouse forebrain. Eur J Neurosci. 1999; 11:4213–4225. [PubMed: 10594647]
17. Katona I, et al. Presynaptically located $CB_1$ cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci. 1999; 19:4544–4558. [PubMed: 10341254]
18. Lafourcade M, et al. Molecular components and functions of the endocannabinoid system in mouse prefrontal cortex. PLoS ONE. 2007; 2:e709. [PubMed: 17684555]
19. Puente N, et al. Localization and function of the cannabinoid $CB_1$ receptor in the anterolateral bed nucleus of the stria terminalis. PLoS ONE. 2010; 5:e8869. [PubMed: 20111610]
20. Katona I, et al. Distribution of $CB_1$ cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J Neurosci. 2001; 21:9506–9518. [PubMed: 11717385]
21. Kamprath K, et al. Short-term adaptation of conditioned fear responses through endocannabinoid signaling in the central amygdala. Neuropsychopharmacology. 2011; 36:652–663. [PubMed: 20980994]
22. Ramikie TS, et al. Multiple mechanistically distinct modes of endocannabinoid mobilization at central amygdala glutamatergic synapses. Neuron. 2014; 81:1111–1125. [PubMed: 24607231]
23. Cristino L, et al. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. Neuroscience. 2006; 139:1405–1415. [PubMed: 16603318]
24. Marsicano G, et al. $CB_1$ cannabinoid receptors and on-demand defense against excitotoxicity. Science. 2003; 302:84–88. [PubMed: 14526074]
25. Marinelli S, Pacioni S, Cannich A, Marsicano G, Bacci A. Self-modulation of neocortical pyramidal neurons by endocannabinoids. Nat Neurosci. 2009; 12:1488–1490. [PubMed: 19915567]
26. Kirilly E, Hunyady L, Bagdy G. Opposing local effects of endocannabinoids on the activity of noradrenergic neurons and release of noradrenaline: relevance for their role in depression and in the actions of $CB_1$ receptor antagonists. J Neural Transm. 2013; 120:177–186. [PubMed: 22990678]
27. Häring M, Marsicano G, Lutz B, Monory K. Identification of the cannabinoid receptor type 1 in serotonergic cells of raphe nuclei in mice. Neuroscience. 2007; 146:1212–1219. [PubMed: 17383106]
28. Häring M, et al. Cannabinoid type-1 receptor signaling in central serotonergic neurons regulates anxiety-like behavior and sociability. Front Behav Neurosci. 2015; 9:235. [PubMed: 26388750]
29. Nyiri G, et al. GABAB and $CB_1$ cannabinoid receptor expression identifies two types of septal cholinergic neurons. Eur J Neurosci. 2005; 21:3034–3042. [PubMed: 15978014]
30. Han J, et al. Acute cannabinoids impair working memory through astroglial $CB_1$ receptor modulation of hippocampal LTD. Cell. 2012; 148:1039–1050. [PubMed: 22385967]
31. Carta M, et al. Membrane lipids tune synaptic transmission by direct modulation of presynaptic potassium channels. Neuron. 2014; 81:787–799. [PubMed: 24486086]
32. Piomelli D, et al. Dopamine activation of the arachidonic acid cascade as a basis for D1/D2 receptor synergism. Nature. 1991; 353:164–167. [PubMed: 1909771]
33. Meves H. Arachidonic acid and ion channels: an update. Br J Pharmacol. 2008; 155:4–16. [PubMed: 18552881]

34. Shonesy BC, et al. Genetic disruption of 2-arachidonoylglycerol synthesis reveals a key role for endocannabinoid signaling in anxiety modulation. Cell Rep. 2014; 9:1644–1653. [PubMed: 25466252]
35. Schlosburg JE, et al. Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system. Nat Neurosci. 2010; 13:1113–1119. [PubMed: 20729846]
36. Jenniches, I., et al. Anxiety, stress and fear response in mice with reduced endocannabinoid levels. Biol Psych. 2015. http://dx.doi.org/10.1016/j.biopsych.2015.03.033
37. Navarrete M, Diez A, Araque A. Astrocytes in endocannabinoid signalling. Phil Trans R Soc B. 2014; 369:20130599. [PubMed: 25225093]
38. Lutz B. Endocannabinoid signals in the control of emotion. Curr Opin Pharmacol. 2009; 9:46–52. [PubMed: 19157983]
39. Di S, et al. Activity-dependent release and actions of endocannabinoids in the rat hypothalamic supraoptic nucleus. J Physiol. 2005; 569:751–760. [PubMed: 16239276]
40. Hill MN, Karatsoreos IN, Hillard CJ, McEwen BS. Rapid elevations in limbic endocannabinoid content by glucocorticoid hormones *in vivo*. Psychoneuroendocrinology. 2010; 35:1333–1338. [PubMed: 20399021]
41. Huang GZ, Woolley CS. Estradiol acutely suppresses inhibition in the hippocampus through a sex-specific endocannabinoid and mGluR-dependent mechanism. Neuron. 2012; 74:801–808. [PubMed: 22681685]
42. Tovote P, Fadok JP, Luthi A. Neuronal circuits for fear and anxiety. Nat Rev Neurosci. 2015; 16:317–331. [PubMed: 25991441]
43. Bains JS, Cusulin JI, Inoue W. Stress-related synaptic plasticity in the hypothalamus. Nat Rev Neurosci. 2015; 16:377–388. [PubMed: 26087679]
44. Graham BM, Langton JM, Richardson R. Pharmacological enhancement of fear reduction: preclinical models. Br J Pharmacol. 2011; 164:1230–1247. [PubMed: 21175588]
45. Sylvers P, Lilienfeld SO, LaPrairie JL. Differences between trait fear and trait anxiety: implications for psychopathology. Clin Psychol Rev. 2011; 31:122–137. [PubMed: 20817337]
46. Mechoulam R, Parker LA. The endocannabinoid system and the brain. Annu Rev Psychol. 2013; 64:21–47. [PubMed: 22804774]
47. Jacob W, et al. Endocannabinoids render exploratory behaviour largely independent of the test aversiveness: role of glutamatergic transmission. Genes Brain Behav. 2009; 8:685–698. [PubMed: 19563475]
48. Sumislawski JJ, Ramikie TS, Patel S. Reversible gating of endocannabinoid plasticity in the amygdala by chronic stress: a potential role for monoacylglycerol lipase inhibition in the prevention of stress-induced behavioral adaptation. Neuropsychopharmacology. 2011; 36:2750–2761. [PubMed: 21849983]
49. Rey AA, Purrio M, Viveros MP, Lutz B. Biphasic effects of cannabinoids in anxiety responses: $CB_1$ and $GABA_B$ receptors in the balance of GABAergic and glutamatergic neurotransmission. Neuropsychopharmacology. 2012; 37:2624–2634. [PubMed: 22850737]
50. Häring M, Kaiser N, Monory K, Lutz B. Circuit specific functions of cannabinoid $CB_1$ receptor in the balance of investigatory drive and exploration. PLoS ONE. 2011; 6:e26617. [PubMed: 22069458]
51. Lafenetre P, Chaouloff F, Marsicano G. Bidirectional regulation of novelty-induced behavioral inhibition by the endocannabinoid system. Neuropharmacology. 2009; 57:715–721. [PubMed: 19607846]
52. Legler A, Monory K, Lutz B. Age differences in the role of the cannabinoid type 1 receptor on glutamatergic neurons in habituation and spatial memory acquisition. Life Sci. 2015; 138:63.
53. Ruehle S, et al. Cannabinoid $CB_1$ receptor in dorsal telencephalic glutamatergic neurons: distinctive sufficiency for hippocampus-dependent and amygdala-dependent synaptic and behavioral functions. J Neurosci. 2013; 33:10264–10277. [PubMed: 23785142]
54. Moreira FA, Grieb M, Lutz B. Central side-effects of therapies based on $CB_1$ cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab. 2009; 23:133–144. [PubMed: 19285266]

55. Rubino T, et al. CB1 receptor stimulation in specific brain areas differently modulate anxiety-related behaviour. Neuropharmacology. 2008; 54:151–160. [PubMed: 17692344]
56. Monory K, Polack M, Remus A, Lutz B, Korte M. Cannabinoid $CB_1$ receptor calibrates excitatory synaptic balance in the mouse hippocampus. J Neurosci. 2015; 35:3842–3850. [PubMed: 25740514]
57. Moreira FA, Kaiser N, Monory K, Lutz B. Reduced anxiety-like behaviour induced by genetic and pharmacological inhibition of the endocannabinoid-degrading enzyme fatty acid amide hydrolase (FAAH) is mediated by $CB_1$ receptors. Neuropharmacology. 2008; 54:141–150. [PubMed: 17709120]
58. Kathuria S, et al. Modulation of anxiety through blockade of anandamide hydrolysis. Nat Med. 2003; 9:76–81. This landmark publication shows the high efficacy of drugs that inhibit AEA hydrolysis and their potential use in the treatment of anxiety-related disorder. [PubMed: 12461523]
59. Patel S, Hillard CJ. Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. J Pharmacol Exp Ther. 2006; 318:304–311. [PubMed: 16569753]
60. Lomazzo E, et al. Therapeutic potential of inhibitors of endocannabinoid degradation for the treatment of stress-related hyperalgesia in an animal model of chronic pain. Neuropsychopharmacology. 2015; 40:488–501. [PubMed: 25100669]
61. Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci USA. 2002; 99:8394–8399. [PubMed: 12060782]
62. Dincheva I, et al. FAAH genetic variation enhances fronto-amygdala function in mouse and human. Nat Commun. 2015; 6:6395. [PubMed: 25731744]
63. Guggenhuber, S., et al. Impaired 2-AG signaling in hippocampal glutamatergic neurons: aggravation of anxiety-like behavior and unaltered seizure susceptibility. Int J Neuropsychopharmacol. 2015. http://dx.doi.org/10.1093/ijnp/pyv091
64. Busquets-Garcia A, et al. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol Psychiatry. 2011; 70:479–486. This study shows that anxiolytic-like effects of AEA are mediated by CB1R and associated with memory disruption, whereas 2-AG induces an anxiolytic effect via CB2R without affecting cognitive functions. [PubMed: 21684528]
65. Kinsey SG, O'Neal ST, Long JZ, Cravatt BF, Lichtman AH. Inhibition of endocannabinoid catabolic enzymes elicits anxiolytic-like effects in the marble burying assay. Pharmacol Biochem Behav. 2011; 98:21–27. [PubMed: 21145341]
66. Sciolino NR, Zhou W, Hohmann AG. Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. Pharmacol Res. 2011; 64:226–234. [PubMed: 21600985]
67. Zhong P, et al. Monoacylglycerol lipase inhibition blocks chronic stress-induced depressive-like behaviors via activation of mTOR signaling. Neuropsychopharmacology. 2014; 39:1763–1776. [PubMed: 24476943]
68. Marsch R, et al. Reduced anxiety, conditioned fear, and hippocampal long-term potentiation in transient receptor potential vanilloid type 1 receptor-deficient mice. J Neurosci. 2007; 27:832–839. [PubMed: 17251423]
69. Sun FJ, et al. Increased expression of TRPV1 in the cortex and hippocampus from patients with mesial temporal lobe epilepsy. J Mol Neurosci. 2013; 49:182–193. [PubMed: 22936245]
70. Puente N, et al. The transient receptor potential vanilloid-1 is localized at excitatory synapses in the mouse dentate gyrus. Brain Struct Funct. 2015; 220:1187–1194. [PubMed: 24487914]
71. Chavez AE, Hernandez VM, Rodenas-Ruano A, Chan CS, Castillo PE. Compartment-specific modulation of GABAergic synaptic transmission by TRPV1 channels in the dentate gyrus. J Neurosci. 2014; 34:16621–16629. [PubMed: 25505315]
72. Chavez AE, Chiu CQ, Castillo PE. TRPV1 activation by endogenous anandamide triggers postsynaptic long-term depression in dentate gyrus. Nat Neurosci. 2010; 13:1511–1518. [PubMed: 21076423]

73. Bahi A, et al. β-caryophyllene, a $CB_2$ receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiol Behav. 2014; 135:119–124. [PubMed: 24930711]
74. Busquets-Garcia A, et al. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat Med. 2013; 19:603–607. [PubMed: 23542787]
75. Aguiar DC, Terzian AL, Guimaraes FS, Moreira FA. Anxiolytic-like effects induced by blockade of transient receptor potential vanilloid type 1 (TRPV1) channels in the medial prefrontal cortex of rats. Psychopharmacology (Berl). 2009; 205:217–225. [PubMed: 19387617]
76. Almeida-Santos AF, et al. Modulation of anxiety-like behavior by the endocannabinoid 2-arachidonoylglycerol (2-AG) in the dorsolateral periaqueductal gray. Behav Brain Res. 2013; 252:10–17. [PubMed: 23714073]
77. Terzian AL, Aguiar DC, Guimaraes FS, Moreira FA. Modulation of anxiety-like behaviour by transient receptor potential vanilloid type 1 (TRPV1) channels located in the dorsolateral periaqueductal gray. Eur Neuropsychopharmacol. 2009; 19:188–195. [PubMed: 19064314]
78. Moreira FA, Aguiar DC, Terzian AL, Guimaraes FS, Wotjak CT. Cannabinoid type 1 receptors and transient receptor potential vanilloid type 1 channels in fear and anxiety-two sides of one coin? Neuroscience. 2012; 204:186–192. [PubMed: 21906661]
79. LeDoux JE. Emotion circuits in the brain. Annu Rev Neurosci. 2000; 23:155–184. [PubMed: 10845062]
80. Pape HC, Pare D. Plastic synaptic networks of the amygdala for the acquisition, expression, and extinction of conditioned fear. Physiol Rev. 2010; 90:419–463. [PubMed: 20393190]
81. Nader K, Hardt O. A single standard for memory: the case for reconsolidation. Nat Rev Neurosci. 2009; 10:224–234. [PubMed: 19229241]
82. Myers KM, Davis M. Mechanisms of fear extinction. Mol Psychiatry. 2007; 12:120–150. [PubMed: 17160066]
83. Maren S. Seeking a spotless mind: extinction, deconsolidation, and erasure of fear memory. Neuron. 2011; 70:830–845. [PubMed: 21658578]
84. Marsicano G, et al. The endogenous cannabinoid system controls extinction of aversive memories. Nature. 2002; 418:530–534. This was the first study to demonstrate the effect of the eCB system on acquired fear response. [PubMed: 12152079]
85. Plendl W, Wotjak CT. Dissociation of within- and between-session extinction of conditioned fear. J Neurosci. 2010; 30:4990–4998. [PubMed: 20371819]
86. Dubreucq S, et al. Genetic dissection of the role of cannabinoid type-1 receptors in the emotional consequences of repeated social stress in mice. Neuropsychopharmacology. 2012; 37:1885–1900. [PubMed: 22434220]
87. Llorente-Berzal A, et al. 2-AG promotes the expression of conditioned fear via cannabinoid receptor type 1 on GABAergic neurons. Psychopharmacology (Berl). 2015; 232:2811–2825. [PubMed: 25814137]
88. Trouche S, Sasaki JM, Tu T, Reijmers LG. Fear extinction causes target-specific remodeling of perisomatic inhibitory synapses. Neuron. 2013; 80:1054–1065. [PubMed: 24183705]
89. Bowers ME, Ressler KJ. Interaction between the cholecystokinin and endogenous cannabinoid systems in cued fear expression and extinction retention. Neuropsychopharmacology. 2015; 40:688–700. [PubMed: 25176168]
90. Soria-Gomez E, et al. Habenular CB receptors control the expression of aversive memories. Neuron. 2015; 88:306–313. [PubMed: 26412490]
91. Pan B, et al. Blockade of 2-arachidonoylglycerol hydrolysis by selective monoacylglycerol lipase inhibitor 4-nitrophenyl 4-(dibenzo[*d*][1,3] dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate (JZL184) enhances retrograde endocannabinoid signaling. J Pharmacol Exp Ther. 2009; 331:591–597. [PubMed: 19666749]
92. Pan B, et al. Alterations of endocannabinoid signaling, synaptic plasticity, learning, and memory in monoacylglycerol lipase knock-out mice. J Neurosci. 2011; 31:13420–13430. [PubMed: 21940435]
93. Lin HC, Mao SC, Su CL, Gean PW. The role of prefrontal cortex $CB_1$ receptors in the modulation of fear memory. Cereb Cortex. 2009; 19:165–175. [PubMed: 18477688]

94. Gunduz-Cinar O, et al. Convergent translational evidence of a role for anandamide in amygdala-mediated fear extinction, threat processing and stress-reactivity. Mol Psychiatry. 2013; 18:813–823. This study provides evidence for the conserved role of AEA in rodents and humans regarding amygdala function and threat processing. [PubMed: 22688188]

95. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. Science. 2001; 294:1030–1038. [PubMed: 11691980]

96. Kamprath K, et al. Cannabinoid $CB_1$ receptor mediates fear extinction via habituation-like processes. J Neurosci. 2006; 26:6677–6686. [PubMed: 16793875]

97. Kamprath K, et al. Endocannabinoids mediate acute fear adaptation via glutamatergic neurons independently of corticotropin-releasing hormone signaling. Genes Brain Behav. 2009; 8:203–211. [PubMed: 19077175]

98. Patel S, Roelke CT, Rademacher DJ, Hillard CJ. Inhibition of restraint stress-induced neural and behavioural activation by endogenous cannabinoid signalling. Eur J Neurosci. 2005; 21:1057–1069. [PubMed: 15787710]

99. Ganon-Elazar E, Akirav I. Cannabinoid receptor activation in the basolateral amygdala blocks the effects of stress on the conditioning and extinction of inhibitory avoidance. J Neurosci. 2009; 29:11078–11088. [PubMed: 19741114]

100. Ganon-Elazar E, Akirav I. Cannabinoids and traumatic stress modulation of contextual fear extinction and GR expression in the amygdala-hippocampal-prefrontal circuit. Psychoneuroendocrinology. 2013; 38:1675–1687. [PubMed: 23433741]

101. Laricchiuta D, Centonze D, Petrosini L. Effects of endocannabinoid and endovanilloid systems on aversive memory extinction. Behav Brain Res. 2013; 256:101–107. [PubMed: 23948212]

102. Gozzi A, et al. A neural switch for active and passive fear. Neuron. 2010; 67:656–666. [PubMed: 20797541]

103. Koolhaas JM, de Boer SF, Coppens CM, Buwalda B. Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Front Neuroendocrinol. 2010; 31:307–321. [PubMed: 20382177]

104. Metna-Laurent M, et al. Bimodal control of fear-coping strategies by $CB_1$ cannabinoid receptors. J Neurosci. 2012; 32:7109–7118. [PubMed: 22623656]

105. Hill MN, et al. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic–pituitary–adrenal axis. Neuropsychopharmacology. 2009; 34:2733–2745. [PubMed: 19710634]

106. Gray JM, et al. Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. J Neurosci. 2015; 35:3879–3892. [PubMed: 25740517]

107. Di S, Malcher-Lopes R, Marcheselli VL, Bazan NG, Tasker JG. Rapid glucocorticoid-mediated endocannabinoid release and opposing regulation of glutamate and γ-aminobutyric acid inputs to hypothalamic magnocellular neurons. Endocrinology. 2005; 146:4292–4301. This study shows how hypothalamic eCBs and CB1R are involved in the negative feedback mechanism of the HPA axis. [PubMed: 15994343]

108. Hill MN, et al. Recruitment of prefrontal cortical endocannabinoid signaling by glucocorticoids contributes to termination of the stress response. J Neurosci. 2011; 31:10506–10515. [PubMed: 21775596]

109. Evanson NK, Tasker JG, Hill MN, Hillard CJ, Herman JP. Fast feedback inhibition of the HPA axis by glucocorticoids is mediated by endocannabinoid signaling. Endocrinology. 2010; 151:4811–4819. [PubMed: 20702575]

110. Glangetas C, et al. Stress switches cannabinoid type-1 ($CB_1$) receptor-dependent plasticity from LTD to LTP in the bed nucleus of the stria terminalis. J Neurosci. 2013; 33:19657–19663. [PubMed: 24336729]

111. Crosby KM, Inoue W, Pittman QJ, Bains JS. Endocannabinoids gate state-dependent plasticity of synaptic inhibition in feeding circuits. Neuron. 2011; 71:529–541. [PubMed: 21835348]

112. Hill MN, et al. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology. 2005; 30:508–515. This study provides strong evidence that the eCB system is dysregulated under chronic stress. [PubMed: 15525997]

113. Rossi S, et al. Chronic psychoemotional stress impairs cannabinoid-receptor-mediated control of GABA transmission in the striatum. J Neurosci. 2008; 28:7284–7292. [PubMed: 18632932]
114. Wang W, et al. Deficiency in endocannabinoid signaling in the nucleus accumbens induced by chronic unpredictable stress. Neuropsychopharmacology. 2010; 35:2249–2261. [PubMed: 20664582]
115. Hillard, C. Cannabinoids, Endocannabinoids and Stress in Cannabinoids. Di Marzo, V., editor. Wiley-Blackwell; 2014. p. 139-174.
116. Haj-Dahmane S, Shen RY. Chronic stress impairs alpha1-adrenoceptor-induced endocannabinoid-dependent synaptic plasticity in the dorsal raphe nucleus. J Neurosci. 2014; 34:14560–14570. [PubMed: 25355210]
117. Hill MN, et al. Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol Psychiatry. 2013; 18:1125–1135. [PubMed: 22776900]
118. Wamsteeker JI, Kuzmiski JB, Bains JS. Repeated stress impairs endocannabinoid signaling in the paraventricular nucleus of the hypothalamus. J Neurosci. 2010; 30:11188–11196. This paper demonstrates the mechanistic underpinnings for how repeated stress interferes with eCB system activity. [PubMed: 20720126]
119. Häring M, Grieb M, Monory K, Lutz B, Moreira FA. Cannabinoid $CB_1$ receptor in the modulation of stress coping behavior in mice: the role of serotonin and different forebrain neuronal subpopulations. Neuropharmacology. 2013; 65:83–89. [PubMed: 23000076]
120. Steiner MA, Marsicano G, Wotjak CT, Lutz B. Conditional cannabinoid receptor type 1 mutants reveal neuron subpopulation-specific effects on behavioral and neuroendocrine stress responses. Psychoneuroendocrinology. 2008; 33:1165–1170. [PubMed: 18653287]
121. Patel S, Kingsley PJ, Mackie K, Marnett LJ, Winder DG. Repeated homotypic stress elevates 2-arachidonoylglycerol levels and enhances short-term endocannabinoid signaling at inhibitory synapses in basolateral amygdala. Neuropsychopharmacology. 2009; 34:2699–2709. [PubMed: 19675536]
122. Russo SJ, Murrough JW, Han MH, Charney DS, Nestler EJ. Neurobiology of resilience. Nat Neurosci. 2012; 15:1475–1484. [PubMed: 23064380]
123. Franklin TB, Saab BJ, Mansuy IM. Neural mechanisms of stress resilience and vulnerability. Neuron. 2012; 75:747–761. [PubMed: 22958817]
124. Tye KM, Deisseroth K. Optogenetic investigation of neural circuits underlying brain disease in animal models. Nat Rev Neurosci. 2012; 13:251–266. [PubMed: 22430017]
125. Masseck OA, et al. Vertebrate cone opsins enable sustained and highly sensitive rapid control of $G_{i/o}$ signaling in anxiety circuitry. Neuron. 2014; 81:1263–1273. [PubMed: 24656249]
126. Soria-Gomez E, et al. The endocannabinoid system controls food intake via olfactory processes. Nat Neurosci. 2014; 17:407–415. [PubMed: 24509429]
127. Quarta C, et al. CB1 signaling in forebrain and sympathetic neurons is a key determinant of endocannabinoid actions on energy balance. Cell Metab. 2010; 11:273–285. [PubMed: 20374960]
128. Bellocchio L, et al. Activation of the sympathetic nervous system mediates hypophagic and anxiety-like effects of CB1 receptor blockade. Proc Natl Acad Sci USA. 2013; 110:4786–4791. [PubMed: 23487769]
129. Araque A, et al. Gliotransmitters travel in time and space. Neuron. 2014; 81:728–739. [PubMed: 24559669]
130. Belanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. Cell Metab. 2011; 14:724–738. [PubMed: 22152301]
131. Walter L, Dinh T, Stella N. ATP induces a rapid and pronounced increase in 2-arachidonoylglycerol production by astrocytes, a response limited by monoacylglycerol lipase. J Neurosci. 2004; 24:8068–8074. [PubMed: 15371507]
132. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. Glia. 2010; 58:1017–1030. [PubMed: 20468046]
133. Navarrete M, Araque A. Endocannabinoids potentiate synaptic transmission through stimulation of astrocytes. Neuron. 2010; 68:113–126. [PubMed: 20920795]

134. Min R, Nevian T. Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nat Neurosci. 2012; 15:746–753. [PubMed: 22446881]

135. Gomez-Gonzalo M, et al. Endocannabinoids induce lateral long-term potentiation of transmitter release by stimulation of gliotransmission. Cereb Cortex. 2015; 25:3699–3712. [PubMed: 25260706]

136. Ostroff LE, Manzur MK, Cain CK, LeDoux JE. Synapses lacking astrocyte appear in the amygdala during consolidation of Pavlovian threat conditioning. J Comp Neurol. 2014; 522:2152–2163. [PubMed: 24338694]

137. Hagemann TL, Paylor R, Messing A. Deficits in adult neurogenesis, contextual fear conditioning, and spatial learning in a *Gfap* mutant mouse model of Alexander disease. J Neurosci. 2013; 33:18698–18706. [PubMed: 24259590]

138. Clark, DD., Sokoloff, L. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. Siegel, GJ., et al., editors. Lippincott; 1999. p. 637-670.

139. Suzuki A, et al. Astrocyte-neuron lactate transport is required for long-term memory formation. Cell. 2011; 144:810–823. [PubMed: 21376239]

140. Phelps EA. Emotion and cognition: insights from studies of the human amygdala. Annu Rev Psychol. 2006; 57:27–53. [PubMed: 16318588]

141. Rangaraju V, Calloway N, Ryan TA. Activity-driven local ATP synthesis is required for synaptic function. Cell. 2014; 156:825–835. [PubMed: 24529383]

142. Marazziti D, et al. Mitochondrial alterations and neuropsychiatric disorders. Curr Med Chem. 2011; 18:4715–4721. [PubMed: 21864278]

143. Chari-Bitron A, Bino T. Effect of 1-tetrahydrocannabinol on ATPase activity of rat liver mitochondria. Biochem Pharmacol. 1971; 20:473–475. [PubMed: 4260786]

144. Nunn A, Guy G, Bell JD. Endocannabinoids in neuroendopsychology: multiphasic control of mitochondrial function. Phil Trans R Soc B. 2012; 367:3342–3352. [PubMed: 23108551]

145. Zaccagnino P, et al. The endocannabinoid 2-arachidonoylglicerol decreases calcium induced cytochrome *c* release from liver mitochondria. J Bioenerg Biomembr. 2012; 44:273–280. [PubMed: 22437740]

146. Benard G, et al. Mitochondrial $CB_1$ receptors regulate neuronal energy metabolism. Nat Neurosci. 2012; 15:558–564. This study includes the first description of mitochondrial CB1R and the mechanisms by which it regulates energy metabolism. [PubMed: 22388959]

147. Koch M, et al. Hypothalamic POMC neurons promote cannabinoid-induced feeding. Nature. 2015; 519:45–50. [PubMed: 25707796]

148. Morozov YM, et al. Antibodies to cannabinoid type 1 receptor co-react with stomatin-like protein 2 in mouse brain mitochondria. Eur J Neurosci. 2013; 38:2341–2348. [PubMed: 23617247]

149. Hebert-Chatelain E, et al. Cannabinoid control of brain bioenergetics: Exploring the subcellular localization of the $CB_1$ receptor. Mol Metab. 2014; 3:495–504. [PubMed: 24944910]

150. Bauer M, et al. Identification and quantification of a new family of peptide endocannabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. J Biol Chem. 2012; 287:36944–36967. [PubMed: 22952224]

151. Straiker A, Mitjavila J, Yin D, Gibson A, Mackie K. Aiming for allosterism: evaluation of allosteric modulators of CB in a neuronal model. Pharmacol Res. 2015; 99:370–376. [PubMed: 26211948]

152. Hofer, SC., et al. Localization and production of peptide endocannabinoids in the rodent CNS and adrenal medulla. Neuropharmacology. 2015. http://dx.doi.org/10.1016/j.neuropharm.2015.03.021

153. Mechoulam R, Hanus LO, Pertwee R, Howlett AC. Early phytocannabinoid chemistry to endocannabinoids and beyond. Nat Rev Neurosci. 2014; 15:757–764. [PubMed: 25315390]

154. Maccarrone M, Guzman M, Mackie K, Doherty P, Harkany T. Programming of neural cells by (endo)cannabinoids: from physiological rules to emerging therapies. Nat Rev Neurosci. 2014; 15:786–801. [PubMed: 25409697]

155. Di Marzo V, Stella N, Zimmer A. Endocannabinoid signalling and the deteriorating brain. Nat Rev Neurosci. 2015; 16:30–42. [PubMed: 25524120]

156. Van Sickle MD, et al. Identification and functional characterization of brainstem cannabinoid $CB_2$ receptors. Science. 2005; 310:329–332. [PubMed: 16224028]

157. Zhang HY, et al. Cannabinoid $CB_2$ receptors modulate midbrain dopamine neuronal activity and dopamine-related behavior in mice. Proc Natl Acad Sci USA. 2014; 111:5007–5015. This study demonstrates the presence of CB2Rs in dopaminergic neurons.

158. Brusco A, Tagliaferro PA, Saez T, Onaivi ES. Ultrastructural localization of neuronal brain $CB_2$ cannabinoid receptors. Ann NY Acad Sci. 2008; 1139:450–457. [PubMed: 18991892]

159. Dhopeshwarkar A, Mackie K. $CB_2$ cannabinoid receptors as a therapeutic target— What does the future hold? Mol Pharmacol. 2014; 86:430–437. [PubMed: 25106425]

160. Garcia-Gutierrez MS, Garcia-Bueno B, Zoppi S, Leza JC, Manzanares J. Chronic blockade of cannabinoid $CB_2$ receptors induces anxiolytic-like actions associated with alterations in $GABA_A$ receptors. Br J Pharmacol. 2012; 165:951–964. [PubMed: 21838753]

161. Garcia-Gutierrez MS, Manzanares J. Overexpression of $CB_2$ cannabinoid receptors decreased vulnerability to anxiety and impaired anxiolytic action of alprazolam in mice. J Psychopharmacol. 2011; 25:111–120. [PubMed: 20837564]

162. Qin Z, et al. Chronic stress induces anxiety via an amygdalar intracellular cascade that impairs endocannabinoid signaling. Neuron. 2015; 85:1319–1331. [PubMed: 25754825]

163. Jung KM, et al. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun. 2012; 3:1080. [PubMed: 23011134]

164. Zhang L, Alger BE. Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. J Neurosci. 2010; 30:5724–5729. [PubMed: 20410124]

165. Tang AH, Alger BE. Homer protein-metabotropic glutamate receptor binding regulates endocannabinoid signaling and affects hyperexcitability in a mouse model of fragile X syndrome. J Neurosci. 2015; 35:3938–3945. [PubMed: 25740522]

166. Dlugos A, Childs E, Stuhr KL, Hillard CJ, de Wit H. Acute stress increases circulating anandamide and other *N*-acylethanolamines in healthy humans. Neuropsychopharmacology. 2012; 37:2416–2427. [PubMed: 22763622]

167. Blankman JL, Cravatt BF. Chemical probes of endocannabinoid metabolism. Pharmacol Rev. 2013; 65:849–871. [PubMed: 23512546]

168. Rabinak CA, et al. Cannabinoid modulation of prefrontal-limbic activation during fear extinction learning and recall in humans. Neurobiol Learn Mem. 2014; 113:125–134. [PubMed: 24055595]

169. Roitman P, Mechoulam R, Cooper-Kazaz R, Shalev A. Preliminary, open-label, pilot study of add-on oral $\Delta^9$-tetrahydrocannabinol in chronic post-traumatic stress disorder. Clin Drug Investig. 2014; 34:587–591.

170. Puighermanal E, et al. Dissociation of the pharmacological effects of THC by mTOR blockade. Neuropsychopharmacology. 2013; 38:1334–1343. This study identifies the mammalian target of rapamycin (mTOR) pathway as a novel strategy to dissociate beneficial effects and side effects of cannabinoid agonists. [PubMed: 23358238]

171. Hermanson DJ, et al. Substrate-selective COX-2 inhibition decreases anxiety via endocannabinoid activation. Nat Neurosci. 2013; 16:1291–1298. This study shows that the inhibition of eicosanoid synthesis by COX2 blockade could represent a novel strategy to tackle anxiety. [PubMed: 23912944]

172. Hermanson DJ, Gamble-George JC, Marnett LJ, Patel S. Substrate-selective COX-2 inhibition as a novel strategy for therapeutic endocannabinoid augmentation. Trends Pharmacol Sci. 2014; 35:358–367. [PubMed: 24845457]

173. Chen R, et al. $\Delta^9$-THC-caused synaptic and memory impairments are mediated through COX-2 signaling. Cell. 2013; 155:1154–1165. [PubMed: 24267894]

174. Shore DM, et al. Allosteric modulation of a cannabinoid G protein-coupled receptor: binding site elucidation and relationship to G protein signaling. J Biol Chem. 2014; 289:5828–5845. [PubMed: 24366865]

175. Ross RA. Allosterism and cannabinoid $CB_1$ receptors: the shape of things to come. Trends Pharmacol Sci. 2007; 28:567–572. [PubMed: 18029031]

176. Vinals X, et al. Cognitive impairment induced by Δ-9-tetrahydrocannabinol occurs through heteromers between cannabinoid $CB_1$ and serotonin 5-HT2A receptors. PLoS Biol. 2015;

13:1002194. This paper demonstrates that the formation of a heteromer between cannabinoid CB1Rs and serotonin 5-hydroxytryptamine 2A (5-HT2A) receptors is responsible for the memory-impairment effects of cannabis, and that prevention of this interaction could allow selective harnessing of the beneficial effects of cannabinoids without the detrimental effects.

177. Vallee M, et al. Pregnenolone can protect the brain from cannabis intoxication. Science. 2014; 343:94–98. This study includes a demonstration of pregnenolone as an endogenous allosteric and signal-specific inhibitor of CB1R that regulates the effect of (endo)cannabinoids on behaviour. [PubMed: 24385629]
178. Gibson HE, Edwards JG, Page RS, Van Hook MJ, Kauer JA. TRPV1 channels mediate long-term depression at synapses on hippocampal interneurons. Neuron. 2008; 57:746–759. [PubMed: 18341994]
179. Puente N, et al. Polymodal activation of the endocannabinoid system in the extended amygdala. Nat Neurosci. 2011; 14:1542–1547. [PubMed: 22057189]
180. Sigel E, et al. The major central endocannabinoid directly acts at $GABA_A$ receptors. Proc Natl Acad Sci USA. 2011; 108:18150–18155. [PubMed: 22025726]
181. Yoshida T, et al. Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid $CB_1$ receptor. J Neurosci. 2006; 26:4740–4751. [PubMed: 16672646]
182. Katona I, et al. Molecular composition of the endocannabinoid system at glutamatergic synapses. J Neurosci. 2006; 26:5628–5637. [PubMed: 16723519]
183. Suarez J, et al. Distribution of diacylglycerol lipase alpha, an endocannabinoid synthesizing enzyme, in the rat forebrain. Neuroscience. 2011; 192:112–131. [PubMed: 21756982]
184. Gao Y, et al. Loss of retrograde endocannabinoid signaling and reduced adult neurogenesis in diacylglycerol lipase knock-out mice. J Neurosci. 2010; 30:2017–2024. [PubMed: 20147530]
185. Tanimura A, et al. The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. Neuron. 2010; 65:320–327. [PubMed: 20159446]
186. Gulyas AI, et al. Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. Eur J Neurosci. 2004; 20:441–458. [PubMed: 15233753]
187. Uchigashima M, et al. Molecular and morphological configuration for 2-arachidonoylglycerol-mediated retrograde signaling at mossy cell-granule cell synapses in the dentate gyrus. J Neurosci. 2011; 31:7700–7714. [PubMed: 21613483]
188. Marrs WR, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci. 2010; 13:951–957. [PubMed: 20657592]
189. Naydenov AV, et al. ABHD6 blockade exerts antiepileptic activity in PTZ-induced seizures and in spontaneous seizures in R6/2 mice. Neuron. 2014; 83:361–371. [PubMed: 25033180]
190. Viader A, et al. Metabolic interplay between astrocytes and neurons regulates endocannabinoid action. Cell Rep. 2015; 12:808.
191. Nyilas R, et al. Enzymatic machinery for endocannabinoid biosynthesis associated with calcium stores in glutamatergic axon terminals. J Neurosci. 2008; 28:1058–1063. [PubMed: 18234884]
192. Egertova M, Simon GM, Cravatt BF, Elphick MR. Localization of *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) expression in mouse brain: A new perspective on N-acylethanolamines as neural signaling molecules. J Comp Neurol. 2008; 506:604–615. [PubMed: 18067139]
193. Tsuboi K, et al. Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: a possible involvement in bioactive *N*-acylethanolamine biosynthesis. Biochim Biophys Acta. 2015; 1851:537–548. [PubMed: 25596343]
194. Rahman IA, Tsuboi K, Uyama T, Ueda N. New players in the fatty acyl ethanolamide metabolism. Pharmacol Res. 2014; 86:1–10. [PubMed: 24747663]
195. Witting A, Walter L, Wacker J, Moller T, Stella N. P2X7 receptors control 2-arachidonoylglycerol production by microglial cells. Proc Natl Acad Sci USA. 2004; 101:3214–3219. [PubMed: 14976257]

196. Steindel F, et al. Neuron-type specific cannabinoid-mediated G protein signalling in mouse hippocampus. J Neurochem. 2013; 124:795–807. [PubMed: 23289830]

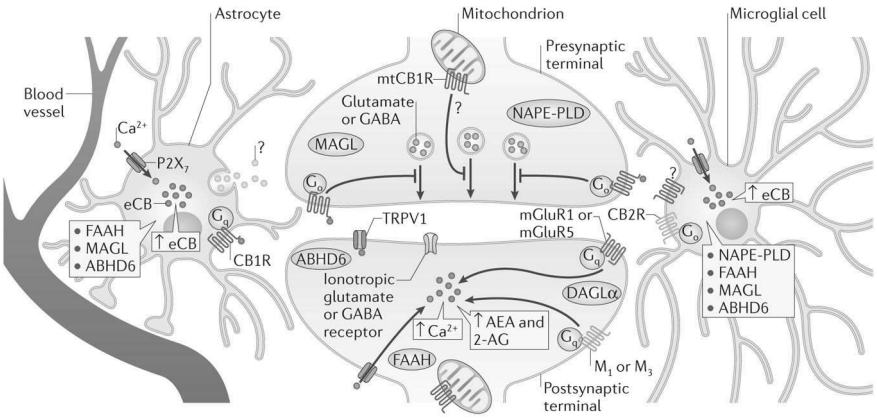



**Figure 1. Architecture of eCB system components in neurons and glia**

In the CNS, endocannabinoid (eCB) system components show a distinct anatomical distribution. The $G_{i/o}$-coupled protein cannabinoid receptor type 1 (CB1R) is typically present at the presynaptic terminal. Its stimulation by 2-arachidonoyl glycerol (2-AG) or *N*-arachidonoylethanolamine (AEA) leads to the suppression of neurotransmitter release from the presynaptic terminal[8,14]. CB1R is also present in the outer mitochondrial membrane at both pre-and postsynaptic sites (mitochondrial CB1R (mtCB1R))[146]. Stimulation of the mtCB1R leads to inhibition of mitochondrial oxidative phosphorylation and ATP production in the mitochondria and can modulate neurotransmitter release through mechanisms that are still unknown (indicated by the question mark)[146]. AEA can also activate the postsynaptic non-selective cation channel transient receptor potential cation channel subfamily V member 1 (TRPV1)[71,72,178,179], leading to an increase in postsynaptic current, whereas 2-AG can also stimulate postsynaptic $GABA_A$ receptors[180]. On depolarization of the postsynaptic terminal, for example, by activation of metabotropic receptors (metabotropic glutamate receptor 1 (mGluR1; also known as GRM1), mGluR5, muscarinic receptor type 1 ($M_1$) or $M_2$)[8,14], 2-AG is postsynaptically synthesized 'on-demand' by diacylglycerol lipase-α (DAGLα) in dendritic spines of excitatory synapses[181–183]. 2-AG then travels to the presynaptic CB1R in a retrograde manner to inhibit neurotransmitter release[184,185], thus acting as a negative-feedback mechanism to tune-down synaptic transmission, especially when the postsynaptic terminal is strongly activated. The major 2-AG degrading enzyme monoacylglycerol lipase (MAGL) is located at the presynaptic terminal[186] or in astrocytes[187], whereas α-β-hydrolase domain 6 (ABHD6), another 2-AG degrading enzyme, can limit 2-AG availability at the site of production[188,189]. Astrocytic MAGL seems to enable astrocyte–neuron transcellular shuttling and metabolism of 2-AG and arachidonic acid[190]. Several pathways are involved in AEA synthesis. One of the enzymes involved in AEA synthesis, *N*-acyl phosphatidyl ethanolamine-phospholipase D (NAPE-PLD), is predominantly expressed in the presynaptic terminal[191,192], although it might also be synthesized postsynaptically[41]. Other AEA-synthesizing enzymes have been described but are not fully characterized[193,194]. The AEA-degrading enzyme fatty acid amide hydrolase (FAAH) is present at the postsynaptic terminal[186]. Thus, AEA can act in both an autocrine and a retrograde manner (an anterograde AEA-signalling mechanism awaits description). CB2R and possibly CB1R (indicated by a question mark) are also present on microglial cells and are involved in immune reactions[12]. Furthermore, whereas presynaptic CB1R is coupled

to $G_o$, CB1R on astrocytes is $G_q$-coupled[11,37]. Thus, agonist stimulation of the receptor leads to an increase in intracellular $Ca^{2+}$ concentration, possibly with a concomitant release stimulation of 'gliotransmitters' (whose exact nature is not yet known, indicated by the question mark), finally modulating synaptic transmission[11,37]. eCB synthesis in microglia and astrocytes can be stimulated by the activation of P2X purinoreceptor 7 ($P2X_7$) by ATP[131,195].

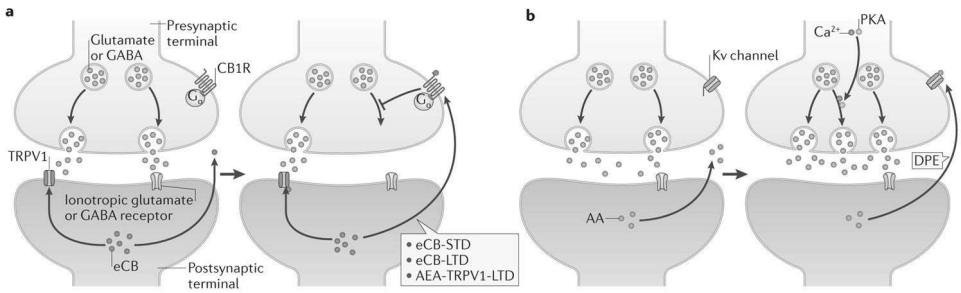



**Figure 2. Regulation of synaptic excitatory and inhibitory transmission**

**a |** Schematic representation of endocannabinoid (eCB)-mediated suppression of synaptic transmission[8,14]; the mechanisms shown apply to both excitatory and inhibitory synapses. At excitatory synapses, afferent stimulation evokes increased glutamate release and subsequent activation of the postsynaptic terminal. This stimulates the synthesis of eCBs (such as *N*-arachidonoylethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG)), which travel through the synaptic cleft to activate presynaptic cannabinoid receptor type 1 (CB1R), leading to the suppression of glutamate release. eCB-mediated short-term depression (eCB-STD, also termed depolarization-induced suppression of excitation (DSE)) or eCB-mediated long-term depression (eCB-LTD) can occur. A similar mechanism occurs at GABAergic synapses, in which postsynaptic activation by excitatory inputs can stimulate the production of eCBs, the inhibition of presynaptic CB1R and the retrograde suppression of GABA release. This form of eCB-STD is termed depolarization-induced suppression of inhibition (DSI). Both DSE and DSI require the synthesis of 2-AG by diacylglycerol lipase-α (DAGLα)[184,185]. AEA can also mediate LTD, although at a slower rate than 2-AG. AEA can act both through CB1R, to produce eCB-LTD, and through transient receptor potential cation channel subfamily V member 1 (TRPV1), to generate AEA-TRPV1-LTD (in an autocrine manner in which AEA activates postsynaptic TRPV1). AEA-TRPV1-LTD can occur at both glutamatergic and GABAergic synapses[14,19,70–72,178,179]. **b |** Schematic presentation of the modulation of excitatory transmission by the eCB precursor and degradation product arachidonic acid (AA). Postsynaptic AA acts in a retrograde manner via inhibition of presynaptic voltage-gated potassium (Kv) channels and potentiation of excitatory neurotransmission, a process called depolarization-induced potentiation of excitation (DPE)[31]. PKA, protein kinase A.

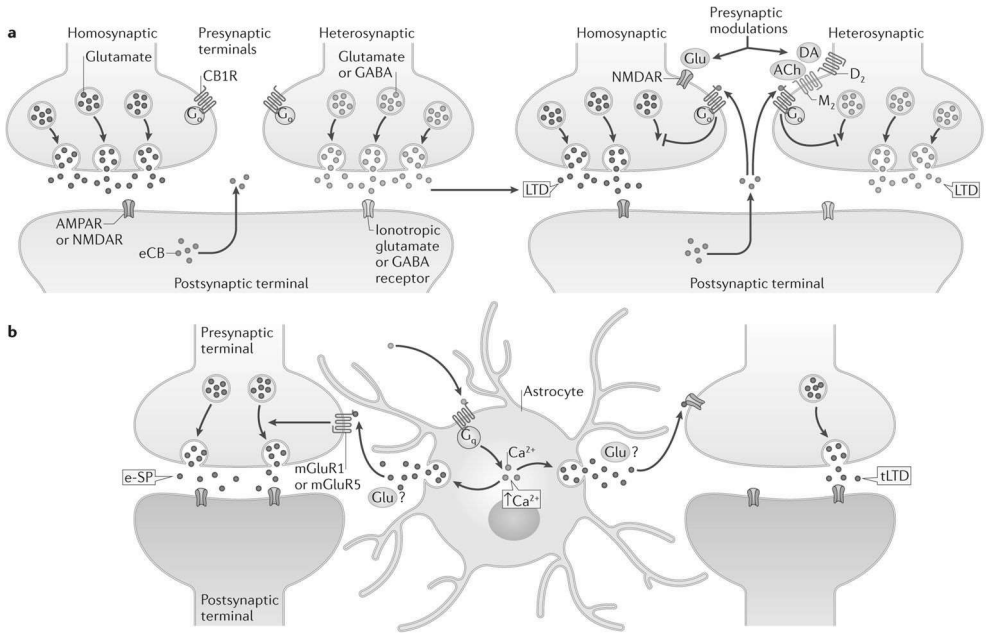



**Figure 3. Heterosynaptic effects and eCB function in the tripartite synapse**

**a |** Schematic representation of homosynaptic and heterosynaptic effects of eCB signalling on neurotransmitter release. Typically, repetitive afferent stimulation causes glutamate (Glu) release from excitatory presynaptic sites, activating the postsynaptic terminal and inducing the generation and release of 2-arachidonoyl glycerol (2-AG), which then activates cannabinoid receptor type 1 (CB1R) on the same presynaptic terminal (a homosynaptic effect) and on the nearby synaptic terminal (a heterosynaptic effect). For long-term depression (LTD) to be produced, concomitant activation of other presynaptic receptors is required. For example, activation of NMDA receptor (NMDAR), dopamine (DA) receptor type 2 ($D_2$) or muscarinic receptor type 2 ($M_2$) by Glu, DA or acetycholine (ACh), respectively, is required. These associative mechanisms may ensure the selectivity of the synapses to be regulated by endocannabinoids (eCBs)[14]. **b |** Integration of the eCB system into the 'tripartite synapse' concept and modulation of synaptic transmission. Activation of CB1R on astrocytes leads to increased intracellular levels of $Ca^{2+}$, promoting the release of 'gliotransmitters' (although this remains subject to debate, as indicated by the question mark), possibly including Glu. These gliotransmitters could then promote heterosynaptic excitatory potentiation (e-SP)[133] or time-spiking-dependent LTD (tLTD) of glutamatergic transmission via presynaptic NMDAR[134]. AMPAR, AMPA receptor; mGluR, metabotropic glutamate receptor.

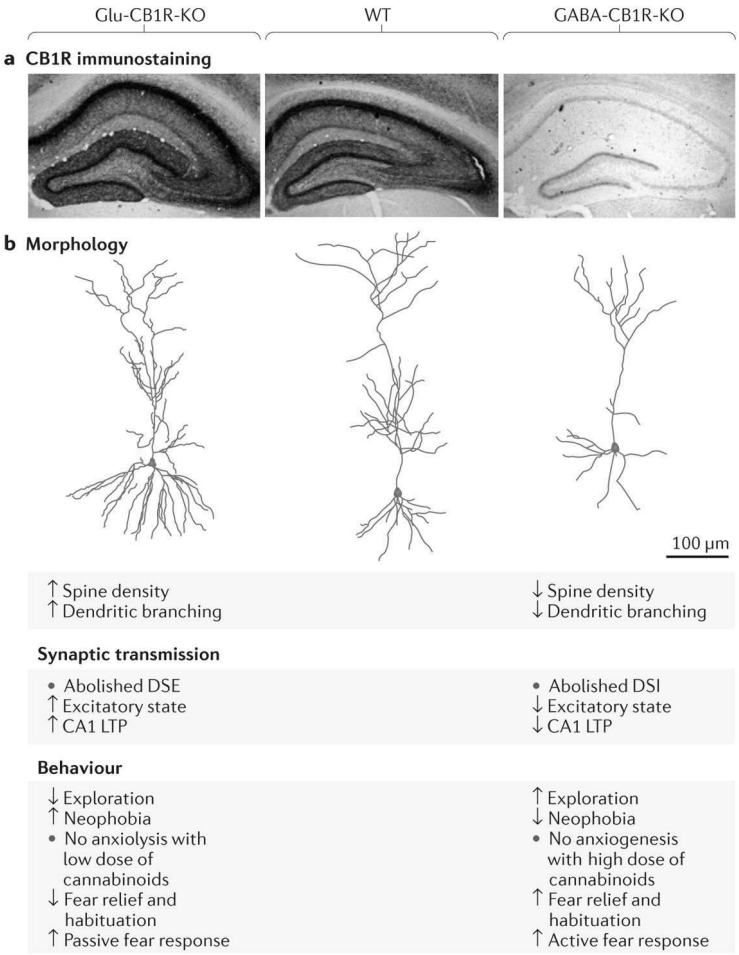



**Figure 4. Dichotomic CB1R function in glutamatergic and GABAergic neurons**

**a |** A prominent feature of the endocannabinoid (eCB) system in the forebrain is its distinct distribution in glutamatergic and GABAergic neurons, with low cannabinoid receptor type 1 (CB1R) expression in glutamatergic neurons and high CB1R expression in GABAergic neurons[16,196]. This is evident when immunostaining for CB1R of hippocampi in mice with CB1R deficiency in glutamatergic (Glu-CB1R-KO; left panel) and GABAergic (GABA-CB1R-KO; right panel) neurons; in comparison with wild-type controls (WT; middle panel). **b |** In principal neurons of the hippocampal CA1 formation, spine density and dendritic branching are increased in Glu-CB1R-KO mice (left panel) and decreased in GABA-CB1R-KO mice (right panel), as compared with these neurons in wild-type mice (middle panel)[56]. This coincides with increased and decreased hippocampal CA1 long-term potentiation (LTP) formation, respectively[56]. Moreover, the two mutant-mouse lines display opposing phenotypes in behaviours such as neophobia, exploration, fear relief and habituation. Thus, CB1R in cortical glutamatergic and forebrain GABAergic neurons calibrates excitatory synaptic balance and consequently regulates fear and anxiety-like behaviours. DSE, depolarization-induced suppression of excitation; DSI, depolarization-induced suppression of inhibition. Part **a** adapted with permission from REF. 196, Copyright ©1999–2015 John

Molecular Psychiatry (2006) 11, 805–814

www.nature.com/mp

FEATURE REVIEW

# Treatment-resistant anxiety disorders

A Bystritsky

*UCLA, Department of Psychiatry, Los Angeles, CA, USA*

**Several epidemiological studies confirmed that Anxiety Disorders as a group are the most prevalent psychiatric conditions in the United States. The importance of these conditions is underlined by the fact that they cause significant disability, poor quality of life, alcohol and drug abuse. Anxiety disorders are treatable conditions and respond to the front-line interventions such as serotonin reuptake inhibitors and cognitive behavioral therapy. However, only about 60% of patients respond to those treatments to any significant degree. Many still have residual symptoms or stay treatment refractory. The group of anxiety patients that is resistant to the treatment has been shown to have very poor quality of life and have highest rate of suicidal attempts than any other disorders. Many biological, treatment specific and social factors are affecting treatment resistance. In this paper, we are attempting to review reasons for the treatment resistance. In addition, we would like to review current strategies that could be helpful in reducing treatment resistance and aiding people chronically suffering from these severe and disabling conditions.**
*Molecular Psychiatry* (2006) **11,** 805–814. doi:10.1038/sj.mp.4001852; published online 18 July 2006

**Keywords:** anxiety; treatment resistance; psychopharmacology; review

## Introduction

Anxiety disorders, which include Obsessive Compulsive Disorder, Panic Disorder, Social Phobia, and Generalized Anxiety Disorder, are the largest and the most prevalent group of psychiatric disorders.[1–3] They are also least recognized compared with other major psychiatric syndromes such as mood or psychotic disorders. In fact, although the Epidemiological Catchments Area study first revealed the prevalence of this group of disorders over 20 years ago, they remain poorly understood, understudied, and inadequately treated. Nevertheless, this group is responsible for decreasing productivity, and increasing morbidity, mortality, and alcohol and drug abuse in a large segment of the population.[4–6]

A listing of each anxiety disorder and the prevalence rate over 12 months is listed in Table 1.[7] The lifetime prevalence estimated without an adjustment for clinical significance is twice the annual prevalence rate indicating that 28.8% or roughly one out of three people has a risk of meeting criteria for an anxiety disorder sometime at some point in their life. In addition, there is a large co-morbidity and overlap with other disorders, specifically with major depression. Furthermore, more mild forms of anxiety disorders can result in permanent disability and even death.[8,9]

Correspondence: Dr A Bystritsky, UCLA, Department of Psychiatry, Box 956968, Suite 2200, 300 Medical Plaza, Los Angeles, CA 90095-6968, USA.
E-mail: abystritsky@mednet.ucla.edu
Received 28 April 2006; accepted 8 May 2006; published online 18 July 2006

Anxiety disorders have a serious impact on the health care. That impact is explained not by the cost of treatments of the disorder but by the high cost of frequent medical evaluations and treatment of physical manifestations of the disorder (i.e., muscle pains, aches). Unlike other serious mental conditions where cost is measured by complete disability and inpatient care, in anxiety disorders patients have decrease of productivity and quality of life that are more difficult to measure. However, some studies report that the decrease in productivity and quality of life of severely ill and/or treatment-resistant anxiety patients was comparable to those of schizophrenics.[10,11] Anxiety Disorders Association of America estimates the costs to be over 42 billion dollars per year comparable to those of stroke and cardiovascular disorders.[12]

## Standard treatment of anxiety disorders

Over the last two decades, significant progress has been made in the area of treatment for anxiety disorders. Evidence-based treatments are available for each anxiety disorder with the efficacy of psychological and biological treatment between 60 and 85%.[13–17] Table 2 details the first line pharmacological treatments that are available and FDA approved for the treatment of the anxiety disorder. The selective serotonin reuptake inhibitors (SSRIs) are prescribed as first-line treatments according to most commonly used algorithms and physician guidelines.[18] Patients who show immediate intolerance to SSRIs are tried on serotonin norepinephrine reuptake inhibitors (SNRIs) or tricyclic antidepressants and MAOIs that used to be used in practice and

**Table 1** Anxiety disorders according to DSM IIIR 1 year prevalence in US (adjusted for clinical significance)[a]

| | *Prevalence (%)* | *Population (millions)* |
|---|---|---|
| Any anxiety disorder | 13.3 | 23.9 |
| Panic disorder (PD) | 1.8 | 2.8 |
| Agoraphobia (AG, PDA) | 2.2 | 4.3 |
| Social phobia (SP) | 3.7 | 6.5 |
| Simple phobias (SPP) | 4.4 | 8.7 |
| Generalized anxiety disorder (GAD | 2.8 | 4.0 |
| Post-traumatic stress disorder (PTSD) | 3.6 | 5.2 |
| Obsessive compulsive disorder (OCD)[b] | 2.4 | 4.3 |
| Acute traumatic stress | Unk | Unk |
| Adjustment disorder with anxious mood | Unk | Unk |
| Anxiety disorders due to | Unk | Unk |

[a]National Co-morbidity Survey Data (1994).
[b]Epidemiological Catchments Area Survey Data (1987).

research prior to introduction of SSRIs with approximately the same success but with less favorable tolerance (Table 3). The second large group of medications includes benzodiazepines such as alprazolam (Xanax), clonazepam (Klonopin) and lorazepam (Ativan). Benzodiazepines have proven efficacy (over 80% response) and FDA approval for use on generalized anxiety and panic disorder.[19] These agents, however, have potential to cause tolerance and dependence, which currently limits their use.[20]

Cognitive-behavioral treatment of anxiety disorders has also been accepted as a first-line treatment showing response rates in the range of 60–90%.[21] However, 10–40% of patients do not respond to psychological treatments and many more have residual symptoms.[22] This situation is rather unacceptable taking into account the high prevalence of the disorder, which means that many millions of people continue suffering from anxiety even if they received the best possible treatment. There is a great need to study treatment resistance in anxiety patients. In this article, we will review factors that appear to contribute to treatment resistance in anxiety and review the ways clinicians and researchers address this problem.

## Definition of the treatment resistance

First of all we need to define the treatment resistance. The definition of treatment resistance is reversely related to the definition of remission and recovery that has been explored and debated in the field of depression.[23,24] In the field of anxiety, this issue is more complicated.[25] The absence of anxiety does not always mean recovery. It frequently does not even mean improvement since a phobic patient can have no anxiety when they can successfully avoid a phobic

**Table 2** Use of selective serotonin reuptake inhibitors (SSRIs) in anxiety disorders

| *Drug* | *Indicated by the FDA for use in:* | | | | | *Randomized clinical trials conducted for use to treat:* | | | | | *Case reports and/or open trials documenting use for:* | | | | | *Drug type* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *PD* | *SD* | *GAD* | *PTSD* | *OCD* | *PD* | *SAD* | *GAD* | *PTSD* | *OCD* | *PD* | *SAD* | *GAD* | *PTSD* | *OCD* | |
| Fluoxetine (Prozac) | ✓ | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | SSRIs |
| Sertraline (Zoloft) | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | |
| Fluvoxamine (Luvox) | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Escitalopram (Lexapro) | | | ✓ | | | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | ✓ | |
| Paroxetine (Paxil) | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |

**Table 3** Use of other antidepressants in anxiety disorders

| Drug | *Indicated by the FDA for use* | | | | | *Randomized clinical trials conducted for use to treat* | | | | | *Case reports and/or Open trials documenting use for* | | | | | *Drug type* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *PD* | *SD* | *GAD* | *PTSD* | *OCD* | *PD* | *SAD* | *GAD* | *PTSD* | *OCD* | *PD* | *SAD* | *GAD* | *PTSD* | *OCD* | |
| Nortriptyline (Pamelor) | | | | | | | | ✓ | | ✓ | ✓ | | ✓ | ✓ | | Tricyclic antidepressants |
| Amitriptyline (Elavil) | | | | | | | | ✓ | ✓ | | ✓ | | | ✓ | ✓ | |
| Imipramine (Tofranil) | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | |
| Clomipramine (Anafranil) | | | | | ✓ | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | | ✓ | ✓ | |
| Duloxetine (Cymbalta) | | | | | | | | | | | ✓ | | | | | SNRIs |
| Venlafaxine (Effexor) | | | ✓ | | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Phenelzine sulfate (Nardil) | | | | | | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | MAOIs |
| Tranylcypromine sulfate (Parnate) | | | | | | | | | | | | ✓ | ✓ | | ✓ | |
| Moclobemide | | | | | | ✓ | ✓ | | | | ✓ | | ✓ | ✓ | ✓ | |
| Trazodone (Desyrel) | | | | | | | | ✓ | | | | | | ✓ | ✓ | Atypical |
| Nefazodone (Serzone) | | | | | | ✓ | | | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Mirtazapine (Remeron) | | | | | | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | |
| Cognitive-behavioral therapy (CBT) | | | | | | ✓ | ✓ | ✓ | ✓ | ✓ | | | | | | |

situation. On the other hand, the presence of anxiety does not always indicate pathology and could be a normal response to an ongoing stress. The assessment of remission and recovery in anxiety patients should be multidimensional and should always include functional parameters. If we apply this criterion, the recovery from anxiety states becomes a relatively rare event due to chronic and waxing and waning course.[26,27] We need to probably apply a different and more lax criterion, which is restoration or near restoration of functional status in the presence (absence) of tolerable treatment. With this lax criterion, one can assume that approximately 30% of patients would be considered recovered from the standard treatments and 30–40% of patients would be considered improved. Still 30% of the patients would be barely touched by the contemporary treatments.[28]

## Mechanisms of resistance

*Diagnostic factors participating in treatment resistance*
Many studies have attempted to analyze predictors of response or conversely nonresponse in anxiety disorders. The factors participating in treatment resistance can be roughly classified as pathology related, environment related, patient related and clinician related (see Table 4). Several factors may be participating in the confusion within this area of research. The diagnostic criteria of anxiety disorder have been changed several times over the last 20 years.[29] Current diagnostic categories are essentially statistically validated lists of symptoms characteristic for a given condition. This categorization leads to several problems. For example, the disorders as described in the DSM-IV rarely exist in their pure form, at least in clinically significant cases. There is large overlapping among anxiety disorders themselves and with other disorders that interfere with specificity of clinical management and research.[30] Attempts to resolve this issue lead to dimensional or symptomatic or to spectrum approach that leads to other set of problems such as overgeneralization. For example, a widely accepted obsessive-compulsive disorder (OCD) spectrum includes a very diverse group of disorders ranging from autism to kleptomania.[31] One of the issues is that symptoms elicited on a cross-sectional interview do not provide us with the full presentation of the disorder. It has been noted that symptoms such as obsessions and compulsions are functionally related to each other but this notion is rarely used in other disorders. Thus, current cross-sectional diagnosis may be one of the factors complicating our ability to effectively treat the anxiety disorders since most of the biological treatments are developed as diagnosis specific (which they are not.)

Additional diagnostic factors of treatment resistance include the presence of personality disorders. This could include the personality disorders that could be confused with anxiety disorders. The examples are OCD personality disorder that could be confused with OCD and borderline personality that is frequently

**Table 4** Outline of reasons for poor response to the treatment of anxiety

| |
|---|
| *Pathology related* |
| 1. Exact underlying pathophysiology is unknown (Birth defects? Infections? Genetic? Autoimmune?) |
| 2. Multiple neurotransmitters participation and interaction |
| 3. Complex receptor and feedback structure of every single transmitter system. |
| 4. Diagnosis – dimension approach |
| 5. Genetics of the disorders is overlapping and unclear what is inherited |
| 6. Our current biological treatments are empirical and have limitations |
| 7. Cognitive Behavioral Theory is disconnected from biological substrate |
| *Environment related* |
| 1. Severe stressors |
| 2. Childhood stressors |
| 3. Long-term persistent stressors |
| 4. Lifecycles |
| *Patient related* |
| 1. Severity |
| 2. Medical co-morbidity |
| 3. Psychiatric co-morbidity |
| 4. Noncompliance |
| 5. Cultural factors |
| *Clinician related* |
| 1. Lack of knowledge in primary care |
| 2. Lack of CBT training |
| 3. Cost leading to limited doctor–patient relationship |

present with panic attacks. Those disorders need to be recognized early in the treatment so that appropriate psychotherapeutic treatments could be administered.

*Biopsychosocial models of anxiety and treatment resistance*

The exact biological mechanisms of anxiety disorders are unknown.[32] Multiple theories exist on different levels of science ranging from molecular all the way to the psychosocial. None of the theories can fully explain the complexity of the anxiety disorders. Biological theories attempt to postulate anxiety as an alarm reaction mediated by specific brain circuits.[33] These circuits include amygdale and other limbic structures.[34] Activation of these circuits are most often found in animal models and human neuroimaging studies of Panic Disorder and Generalized Anxiety.[35] Some other anxiety disorders, such as OCD, have a disturbance of circuits responsible for emotional information processing and integration. These circuits include striatum, cingulum and prefrontal and orbito-frontal connection.[36] These circuits are responsible for gating, ordering and integration information about the threat.

Cognitive scientists base their theories of anxiety on a specific way of thinking that is excessive, dichotomized (i.e. back or white) and anxiety provoking.[37] Behaviorists explain anxiety disorders as a set of maladaptive coping safety strategies that lead to not testing the validity of the threat and as a result increase anxiety and apprehension. While the theories do not contradict each other, we are yet to see the integration of biological and psychological mechanisms within the framework of united theory of anxiety disorders.

One of the ways to look at the interaction between the psychological and biological is to understand anxiety disorders as three interrelated processes. The first process involves the neuronal circuits responsible for the initial detection and reaction to the threat (i.e. alarm). These circuits well described by several scientists play an important role in all anxiety disorders and specifically in Panic. The amygdale and adjunct limbic system play the central role. The second process involves more extensive threat information analysis. This process is most characteristic of Obsessive Compulsive Disorder. The cortico-striatum-cortical circuit is involved in multiple functions including gating, stop-and-go, and coordination between emotional and thought processing. The disturbance of these processes leads to excessively detailed view of threat information leading in turn to the excessive perception of threat. Patient frequently focuses on a particular aspect of threat rather than all the evidence. This may lead to cognitive distortions typically described in the literature (i.e. overestimation of probability, overgeneralization and all or nothing thinking). The third process is coping with the threat. Normally everyone reacts to a threat with series of safety behaviors such as exploration of the threat, safety behaviors directed to elimination of the threat and avoidance of the threat.

The anxiety patients engage in the same behaviors but due to heightened alarm and faulty information processing their behaviors become excessive and interfere with their function instead of helping it. Excessive security behaviors (i.e. washing in OCD patients) could lead to resetting the alarm to even higher level because those behaviors invariably fail to protect 100% while taking a long time. Avoidance of threat, which is another coping strategy frequently, prevents patients from assessing the threat and as such increases the informational distortions.

Psychosocial models of anxiety underscore interplay between biological and environmental factors. Even catastrophic stressors are not always recognized by patients and their physicians. Severe persistent stressors for most part go undetected and impact the treatment response. The patient who is in the midst of a severe stress would less likely respond to the treatment.[38] Unfortunately, research in assessment of environmental factors is lagging despite their importance. Especially it is true about the research directed to measure the degree of severe persistent stress.

Another major factor of treatment resistance is alcohol and drug abuse. Frequently, co-morbid in anxiety patients it is also frequently unnoticed.

It can effect resistance through non-compliance and through interaction between medications and alcohol or drugs. In addition, use of alcohol to reduce anxiety could interfere with the behavioral strategies.

## Predictors of nonresponse in clinical literature

The best information about patient-related factors is usually derived from analysis of predictors of response/nonresponse. Usually the factors identified by these methods related to severity of illness, comorbidity and presence of personality disorders and noncompliance with the treatment.[39,40]

Studies analyzing usual care delivery in primary care produced some insight on treatment resistance in 'care as usual'.[41] The studies indicated that inadequate recognition, inadequate training, incorrect use of antidepressants and lack of understanding and the use of CBT are among the main reasons for patient's non-response. For example, Katon determined that many patients in primary care administered medications for very short period of time.[42] This is particularly important for OCD where higher than usual doses of SSRIs usually require (i.e. more than 100 mg of fluoxetine per day) usually for at least 10 weeks before one sees an adequate response. The titration could be too rapid or the doses are inadequate. Frequently, Panic patients who usually require smaller than usual doses and slower titration (i.e. 5 mg of fluoxetine initially with increases every 2 weeks) are started on 20 mg of fluoxetine causing excessive anxiety and treatment discontinuation. Patients in primary care as well in general psychiatry clinics most often do not receive correct psychological treatments.[43] Patients are frequently not educated about medication response and have incorrect expectations. The literature also notes the inadequacy in training of many psychologists in contemporary methods of the treatment of anxiety.[44]

## Strategies for improvement of treatment resistance

One of the strategies in improving outcomes and diminishing treatment resistance is reevaluation and optimization of the treatment. Patient who failed or insufficiently responded to at least two SSRI and one SNRI and a behavior therapy should be reevaluated by a psychiatrist who is familiar with the treatment of anxiety for identification of the reasons for the treatment failure. Multiple factors mentioned above should be explored. The presence of co-morbidity, personality disorder and environmental factors should be assessed. Motivation for treatment and treatment compliance needs to be explored. Adequacy of medication treatment needs to be assessed. Once the assessment is performed, the clinician may try a previously attempted treatment but in adequate dose and for an adequate duration of time. If noncompliance is an issue then better patient education and motivational techniques could be employed.[45]

Augmentation strategies have been tried for the treatment-resistant cases. These include adding buspirone, or lithium, combining two SSRI or SSRI with SNRI. Using tricyclic antidepressants with SSRI could be very helpful especially in case of clomipramine–SSRI combination for OCD. However, this combination needs to be well monitored with blood levels of a tricyclic to avoid complications that may include seizures.

The use of long-term benzodiazepines for the long-term treatment of resistant anxiety is controversial due to large comorbidity of anxiety disorders with addictions. However, some long-term studies indicated that these medications could be used in chronic anxiety patients with a great degree of success and that those who do not have comorbid addictive disorders actually decrease their medications over time. These are powerful medications and their cognitive side effects should be taken into consideration especially in elderly populations.

In case of co-morbidity, one may target the co-morbid conditions such as bipolar disorder or psychosis first and then attempt to treat anxiety disorder. This could lead to the use of multiple pharmacological treatments at the same time. However, polypharmacy is considered to be a rule rather than exception in complicated co-morbid cases. Recent surge of co-administration of mood stabilizers (lithium, gabaergic antiepileptics and atypical antipsychotics) may be explained by very high prevalence of bipolar disorders and psychoses in anxiety patients (Table 5).

Combining CBT and medications for patients resistant to either treatment alone deserves further scientific exploration. Several studies conducted in anxiety patients including panic disorders OCD and

**Table 5** Treatment of resistant anxiety

| | *Case-series open trials* | *RTC* |
|---|---|---|
| *Gabaergic antiepileptics* | | |
| Gabapentin | Y | N |
| Tiagabine | Y | N |
| Pregabalin | Y | Y (GAD) |
| Topiramine | Y | N |
| *Atypical neuroleptics (combined with SSRIs)* | | |
| Risperidone | Y | Y (OCD) |
| Olanzapine | Y | Y (OCD) |
| Ziprazidone | Y | N |
| Quetiapine | Y | Y (OCD) |
| Aripiprazole | Y | N |
| *Other treatments (mostly for OCD)* | | |
| IV Anafranil | Y | Y |
| ECT | N | N |
| Deep brain stimulation | Y | N |
| VNS | Y | N |
| rTMS | Y | N |
| Psychosurgery | Y | N |

Social Phobias did not reveal clear superiority of combination treatment over either treatment strategy administered alone.[46–49] However, combined algorithms administered in primary care are clearly more effective than treatment as usual.

One has to explore the targets of these treatments to understand the nature of the treatment failure. Medication such as an SSRI is likely to suppress the increased alarm reactivity by suppression of the alarm system (i.e. amygdale and related areas). In larger doses, they may improve information processing by slowing transmission in the cortico-striato-cortical circuits. However, it is unlikely that medications can affect complex behavioral coping strategies such as safety behaviors and avoidance directly. Improvement in those behaviors occurs, most likely, secondary to reduction in fears and takes several weeks. Patients with OCD frequently perceive their medications nonworking even though they felt calmer on the medication. They were, however, still continuing to perform their rituals because they were not instructed otherwise. Severity of rituals and avoidance was one of the most reliable predictors of nonresponse in a meta-analysis of a large sample of OCD patients treated with SSRIs.[50] Convergent, behavioral interventions most likely do not affect alarm reactivity and information processing directly. Cognitive therapy may improve thinking by making the patients test alternative hypotheses related to fear response, but it is not clear that cognitive strategies are effective alone in majority of anxiety patients. Choosing alternative coping behaviors most likely secondary 'resets' the alarm and improves their processing of the threat information. Using excessive medication could be counterproductive because it could fully suppress anxiety, affect information processing and slow down the extinction processes. Keeping this theory in mind one may combine both treatment strategies rationally to achieve a greater success. However, that strategy is more difficult to implement in a controlled studies because it requires flexibility in medication administration. Most of the controlled trials, however, used a set dose schedule for the medication treatments.

## Experimental treatment strategies

Non-response to single treatments and their combinations calls for the development of new treatments of anxiety disorders. A few of the have been recently tested.

*Herbal*

Herbal preparations are extensively used by anxiety disorders patients.[51] They frequently take the herbals surreptitiously, that is, without knowledge of the physician administering pharmacological treatment. One has to remember that despite the general belief that herbals are safer that may not be so. Some of the most potent poisons and mind altering drugs could be herbals. The surreptitious use for the herbals needs to be further explored in anxiety patients since it may contribute to the treatment resistance. There are also possible interactions between the herbal preparations and SSRIs, which clinicians need to pay attention to.

*Pharmacological*

One of the most fruitful areas of research was recently the use of combined SSRI–antipsychotic treatments for non-psychotic anxiety disorders including OCD, agoraphobia and Social Anxiety disorders. Nonpsychotic OCD patients seem to show moderate response to atypical antipsychotics that has been documented in multiple reports, case studies and some of the controlled studies, although the information is still scarce (Table 5). The use of antipsychotic is complicated by wide range of side effects they bring into the clinical picture. Their usefulness long term reminds to be documented in anxiety patients.[52]

The use of Gaba-ergic antiepileptics seems to be growing. This is prompted by multiple reports involving gabapentin, pregabalin and tiagabin among others.[53,54] While these medications are less dependency forming than benzodiazepines they are also less effective. Some newer agents such as pregabaline seem to have more antianxiety properties, but this remains to be documented in large controlled clinical trials.

Multiple pharmacological medications with novel mechanisms of action have been recently tested. Those include medication with peptide mechanisms of action, that is, substance P, NK, CRF antagonists.[55,56] None of these novel medications are yet approved on the US market and most of the recent experiments failed to prove their efficacy. It seems that while acting on more specific systems the medications losing their efficacy.

Conversely, medications with multiple mechanisms of action or 'poly-pharmacy cocktails' seem to be most effective in the treatment-resistant population.[57] The scientific literature does not contain any good efficacy data for polypharmacy. However, it is apparent that the use of multiple medications with different indications is a rule rather exception in the treatment-resistant anxiety patients. Some of the best teachers of contemporary psychopharmacology are actively teaching a rational polypharmacy.[58] In practice, experienced psychopharmacologists arrive to those complex regimens by trial and error in the attempt to decrease the suffering of this population which is often immense. The logic behind the polypharmacy is understandable. Treatment-resistant patients usually suffer from several syndromes that may include, for example, OCD, Panic, Bipolar Disorder and some form of psychosis. If one attempt to use a single agent in this kind of a patient they usually get worse. For example, high doses of an SSRI required to treat the OCD may trigger mania or psychotic reaction in bipolar or psychotic patient with OCD as primary presentation. The ultimate cocktail found in some patients could include: an SSRI, sometimes in a mixture with an SNRI, a

GABA-ergic mood stabilizer, an atypical antipsychotic and a benzodiazepine.

For same of the patients, this regimen could be appropriate and even life saving. For some of them it could mask an underlying problem by numbing the feeling and not addressing abnormal coping of these patients. The examples of this could be an oversedated OCD patient, who continues his compulsive behaviors or a PTSD patient where the core traumatic even has never been addressed in psychotherapy. In my opinion, the extensive polypharmacy in patients should be periodically reevaluated and a second opinion should be obtained. It is especially important when the patient is treated with a complicated regimen for more than 2 years without clear improvement. Sometimes a 'subtraction' of medications from a polypharmacy regimen could lead to an improvement.

Some of the prospective treatments, even experienced psychopharmacologists may be reluctant to administer. A once a week opioid receptor agonist trial in OCD patients has shown some success and is under investigation.[59] Since potential adversities of these treatments are high they should probably still be conducted only in specialized centers under scrutiny of researchers and with explicit informed consents until more evidence is gathered.

There is some evidence for the efficacy and safety of intravenous clomipramine, which may become the optimal strategy in treatment-resistant cases. Researchers have suggested that the ratio of clomipramine to its metabolite desmethylclomipramine (which also inhibits noradrenaline reuptake) is increased with parenteral treatment through reduction of first-pass hepatic metabolism, and that this explains the greater tolerability and efficacy of the intravenous form of the drug.[60] In a double-blind, randomized, controlled trial in patients with treatment-refractory OCD, Fallon and Mathew[61] found that nine of 21 patients treated with 14 days of clomipramine infusions and 7 days of oral treatment were responders, compared with none of 18 in the placebo group. Improvement was maintained to the end of blind ratings at 3 weeks, and the regimen was well tolerated.

*Behavioral and other psychotherapies*

Anxiety disorder patients who do not respond to ordinary behavioral strategies could utilize more extensive CBT treatment. This treatment is usually provided as an intensive outpatient, partial hospitalization or residential treatment.[62,63] Many of these programs specifically targeting OCD are currently available around the country. The programs generally offered different length of treatment ranging from several weeks to several months and different degree of intensity.

Many authors recognized limitations of narrow behavioral approach in the treatment-resistant population. Other psychotherapeutic modalities including focused cognitive, mindfulness, meditation, interpersonal and psychodynamic have recently been tried in anxiety populations with various degree of successes.[64–66] It is clear that a complex patient may require a long-term complex psychotherapeutic approach rather than a brief behavioral strategy.

*Nonpharmacological strategies*

Electroconvulsive therapy has a role in cases of treatment-refractory anxiety complicated by severe comorbid depression, but it is not believed to be consistently effective for primary treatment-refractory OCD or Panic Disorder.[67,68] In one uncontrolled case series, the majority of patients with treatment-refractory OCD improved considerably for a year following such therapy.[69] Although the response was associated with improved depression ratings, the authors suggested an independent effect on obsessional symptoms. Use of ECT in treatment-resistant PD is also controversial since some clinicians suggest that panic attacks worsen in this population and only depression improves.

Several nonpharmacological experimental treatment strategies are under development and testing. This includes Deep Brain Stimulation (DBS), Vagus Nerve Stimulation (VNS), and Repetitive Transcranial Magnetic Stimulation (rTMS).

*Deep brain stimulation.* Bilateral DBS has been used successfully for essential tremor and Parkinson's disease since about 1995.[70] Significant adverse events from the DBS procedure have included equipment failure or lead wire breakage, intracranial hemorrhage, infection, seizures, and paresis.[71] Since 1999 when Netherlands's neurosurgeon discovered OCD response to DBS, there have been multiple publications on the use of DBS in treatment refractory OCD.[72,73] Initial results seem to be promising but need to be confirmed in larger trials using sham surgeries and treatments.

*Vagus nerve stimulation (VNS)*

The vagus nerve (10th cranial nerve) is best known for its efferent function with parasympathetic inervation to organs such as the heart and gut. However, approximately 80% of vagal nerve fibers are afferent sensory fibers and relay information from the body to the brain. These afferent fibers project via the nucleus tractus solitarii (NTS) to the locus ceruleus (LC) and parabracial nucleus (PB). The LC and PB project to all levels of the forebrain including the hypothalamus, orbital frontal cortex, amygdala, and bed nucleus of the stria terminalis. In theory, direct stimulation of the vagus afferent fibers could affect sensory input to limbic, brain stem and cortical areas known to be involved in mood and anxiety disorders. VNS has had an excellent safety record in seizure patients.[74] It has also been recently approved by FDA as an adjunct treatment for treatment-resistant depression.[75] Many of treatment refractory depressed patients in pivotal studies were also suffering from anxiety, which improved simultaneously with depression. However,

true efficacy of this treatment in refractory anxiety populations remains to be explored. The most common adverse event related to implantation is mild pain at the incision site that typically resolves over the 2 weeks following surgery. There are currently seven patients with OCD, two patients with PTSD and one panic disorder patient implanted with the device. Acute and long-term data are not available on these patients yet.[76]

*Repetitive transcranial magnetic stimulation.* Introduced in mid-1980s, transcranial magnetic stimulation is a noninvasive mean of stimulating the cerebral cortex. It involves placing an electromagnetic coil on the scalp and passing a rapidly alternating high-intensity current through the coil. This sets up a magnetic field, which passes through the cranium and induces local electrical changes on the surface of the cortex. Therapeutically, rTMS has received the most attention with treatment-resistant depression.[77] Greenberg *et al*[78] found that rTMS may be helpful in OCD whereas Alonso *et al.*[79] who randomly assigned 18 patients with OCD to real or sham rTMS did not find any difference between the treatment groups. Overall review of the field produced mixed results.[80] However, a recent study opens the possibility that a different set of rTMS parameters may need to be used for the treatment of anxiety and OCD and that research needs to be continued.[81]

*Neurosurgery*

OCD was the only anxiety disorder where the neurosurgical approach has been explored. With the failure to find effective therapies for OCD over the past three decades, psychosurgery has become an intervention of last resort.[82] It is important to balance the risks of nonintervention (social, physical and psychological complications, including suicide) against those of surgery (frontal lobe dysfunction and psychological complications including personality alteration, substance abuse and suicide), which are not excessive with current techniques. Unfortunately, in the absence of a controlled comparison with 'sham' surgery, efficacy remains unproven. Recent retrospective and prospective studies have reported response in 30–60%[83]. A 'gamma knife' using cobalt 60 has been used in some centers to create surgical lesions without opening the skull, making a controlled comparison with sham surgery feasible. The procedures favored across various centers include cingulotomy, subcaudate tractotomy, capsulotomy, and limbic leucotomy (cingulotomy plus subcaudate tractotomy). No conclusive data exist on comparative efficacy or safety. Further research is needed to identify the best target sites. For these procedures, a 'stereotactic' frame is used, and target sites are visualized with magnetic resonance imaging. It is hypothesized that such lesions disrupt dysfunctional neural circuits by severing connections between the orbitomedial frontal lobes and limbic or thalamic structures. However, the observation that most patients take weeks or months to improve suggests that secondary effects such as nerve degeneration may be important.

## Conclusion

Treatment resistance is a significant problem in anxiety patients affecting approximately one out of three patients with diagnosis of anxiety disorder. Due to high prevalence of AD, this problem translates into significant mortality, morbidity and decrease in quality of life. There also significant cost to society associated with high disability and high health care utilization. The treatment resistance occurs due to multiple factors and clinicians need better ways to study and address them. A careful assessment of treatment-resistant anxiety patients by an experienced clinician who is aware of the current psychobiological treatments of anxiety is very important. Development of the new treatment modalities is the task of future generations of researchers in this important field of science.

## Acknowledgments

I like to thank Lauren Kerwin and Mary Grace Savella for their help with the preparation of this manuscript. This work was supported in part by Saban Family Foundation.

## References

1 Kessler RC, McGonagle KA, Zhao S, Nelson CB, Hughes M, Eshleman S *et al*. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. Results from the National Comorbidity Survey. *Arch Gen Psychiatry* 1994; **51**: 8–19.
2 Kessler RC, Demler O, Frank RG, Olfson M, Pincus HA, Walters EE *et al*. Prevalence and treatment of mental disorders, 1990 to 2003. *N Engl J Med* 2005; **352**: 2515–2523.
3 Kessler RC, Chiu WT, Demler O, Merikangas KR, Walters EE. Prevalence severity, and comorbidity of 12-month DSM-IV disorders in the National Comorbidity Survey Replication. *Arch Gen Psychiatry* 2005; **62**: 617–627.
4 Leon AC, Portera L, Weissman MM. The social costs of anxiety disorders. *Br J Psychiatry Suppl* 1995; **27**: 19–22.
5 Wittchen HU, Fehm L. Epidemiology, patterns of comorbidity, and associated disabilities of social phobia. *Psychiatr Clin N Am* 2001; **24**: 617–641.
6 Wittchen HU, Kessler RC, Beesdo K, Krause P, Hofler M, Hoyer J. Generalized anxiety and depression in primary care: prevalence, recognition, and management. *J Clin Psychiatry* 2002; **63**: 24–34.
7 *Diagnostic and Statistical Manual for Mental Disorders (DSM-IV).* American Psychiatric Association: Washington, DC, 2000.
8 Olfson M, Broadhead WE, Weissman MM, Leon AC, Farber L, Hoven C *et al*. Subthreshold psychiatric symptoms in a primary care group practice. *Arch Gen Psychiatry* 1996; **53**: 880–886.
9 Pini S, Dell'Osso L, Mastrocinque C, Marcacci G, Papasogli A, Vignoli S *et al*. Prevalence and 12-month outcome of threshold and subthreshold mental disorders in primary care. *J Affect Disord* 1999; **56**: 37–48.
10 Lochner C, Mogotsi M, du Toit PL, Kaminer D, Niehaus DJ, Stein D. Quality of life in anxiety disorders: a comparison of obsessive-compulsive disorder, social anxiety disorder, and panic disorder. *Psychopathology* 2003; **36**: 255–262.

11 Bystritsky A, Saxena S, Maidment K, Vapnik T, Tarlow G, Rosen R. Quality-of-life changes among patients with obsessive-compulsive disorder in a partial hospitalization program. *Psychiatr Serv* 1999; **50**: 412–414.
12 ADAA. Anxiety Disorders Association of America Website (ADAA.org). 2005.
13 Barlow DH, Lehman CL. Advances in the psychosocial treatment of anxiety disorders. Implications for national health care. *Arch Gen Psychiatry* 1996; **53**: 727–735.
14 Ballenger JC, Davidson JR, Lecrubier Y, Nutt DJ, Baldwin DS, den Boer JA *et al.* Consensus statement on panic disorder from the International Consensus Group on Depression and Anxiety. *J Clin Psychiatry* 1998; **59**: 47–54.
15 Ballenger JC, Davidson JR, Lecrubier Y, Nutt DJ, Bobes J, Beidel DC *et al.* Consensus statement on social anxiety disorder from the International Consensus Group on Depression and Anxiety. *J Clin Psychiatry* 1998; **59**: 54–60.
16 Ballenger JC, Davidson JR, Lecrubier Y, Nutt DJ, Borkovec TD, Rickels K *et al.* Consensus statement on generalized anxiety disorder from the International Consensus Group on Depression and Anxiety. *J Clin Psychiatry* 2001; **62**: 53–58.
17 Bandelow B, Zohar J, Hollander E, Kasper S, Moller HJ. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and posttraumatic stress disorders. *World J Biol Psychiatry* 2002; **3**: 171–199.
18 Roy-Byrne P, Stein M, Bystrisky A, Katon W. Pharmacotherapy of panic disorder: proposed guidelines for the family physician. *J Am Board Fam Pract* 1998; **11**: 282–290.
19 Klein E. The role of extended-release benzodiazepines in the treatment of anxiety: a risk–benefit evaluation with a focus on extended-release alprazolam. *J Clin Psychiatry* 2002; **63**: 27–33.
20 Otto MW, Pollack MH, Sachs GS, Reiter SR, Meltzer-Brody S, Rosenbaum JF. Discontinuation of benzodiazepine treatment: efficacy of cognitive-behavioral therapy for patients with panic disorder. *Am J Psychiatry* 1993; **150**: 1485–1490.
21 Barlow DH, Gorman JM, Shear MK, Woods SW. Cognitive-behavioral therapy, imipramine, or their combination for panic disorder: a randomized controlled trial. *JAMA* 2000; **283**: 2529–2536.
22 Cowley DS, Ha DE, Roy-Byrne PP. Determinants of pharmacologic treatment failure in panic disorder. *J Clin Psychiatry* 1997; **58**: 555–561.
23 Bakish D. New standard of depression treatment: remission and full recovery. *J Clin Psychiatry* 2001; **62**: 5–9.
24 Cuffel BJ, Azocar F, Tomlin M, Greenfield SF, Busch AB, Croghan TW. Remission, residual symptoms, and nonresponse in the usual treatment of major depression in managed clinical practice. *J Clin Psychiatry* 2003; **64**: 397–402.
25 Nemeroff CB. Remission: today's target in the treatment of mood and anxiety disorders. *Psychopharmacol Bull* 2002; **36**: 5.
26 Pollack MH, Meoni P, Otto MW, Simon N, Hackett D. Predictors of outcome following venlafaxine extended-release treatment of DSM-IV generalized anxiety disorder: a pooled analysis of short- and long-term studies. *J Clin Psychopharmacol* 2003; **23**: 250–259.
27 Brown C, Schulberg HC, Madonia MJ, Shear MK, Houck PR. Treatment outcomes for primary care patients with major depression and lifetime anxiety disorders. *Am J Psychiatry* 1996; **153**: 1293–1300.
28 Brown C, Schulberg HC, Madonia MJ, Shear MK, Houck PR. Treatment outcomes for primary care patients with major depression and lifetime anxiety disorders. *Am J Psychiatry* 1996; **153**: 1293–1300.
29 Kessler RC, Wittchen HU. Anxiety and depression: the impact of shared characteristics on diagnosis and treatment. Introduction. *Acta Psychiatr Scand Suppl* 2000; **406**: 5–6.
30 Bystritsky A. Diagnosis and treatment of anxiety. *Focus* 2004; **2**: 333–342.
31 Hollander E, Kwon JH, Stein DJ, Broatch J, Rowland CT, Himelein CA. Obsessive-compulsive and spectrum disorders: overview and quality of life issues. *J Clin Psychiatry* 1996; **57**: 3–6.
32 Gorman JM, Kent JM, Sullivan GM, Coplan JD. Neuroanatomical hypothesis of panic disorder, revised. *Am J Psychiatry* 2000; **157**: 493–505.
33 LeDoux J. The emotional brain, fear, and the amygdala. *Cell Mol Neurobiol* 2003; **23**: 727–738.
34 Rauch SL. Neuroimaging and neurocircuitry models pertaining to the neurosurgical treatment of psychiatric disorders. *Neurosurg Clin N Am* 2003; **14**: 213–223.
35 Baxter LR. Neuroimaging studies of obsessive compulsive disorder. *Psychiatr Clin North Am* 1992; **15**: 871–884.
36 Saxena S, Brody AL, Schwartz JM, Baxter LR. Neuroimaging and frontal-subcortical circuitry in obsessive-compulsive disorder. *Br J Psychiatry Suppl* 1998; **35**: 26–37.
37 Craske MG, Barlow DH (eds). Contributions of Cognitive Psychology To Assessment and Treatment of Anxiety. In: *Handbook of behavior therapy and psychological science: an integrative approach*. Pergamon Press, Inc.: Elmsford, NY, US, 1991, pp 151–168.
38 Connor KM, Hidalgo RB, Crockett B, Malik M, Katz RJ, Davidson JR. Predictors of treatment response in patients with posttraumatic stress disorder. *Prog Neuropsychopharmacol Biol Psychiatry* 2001; **25**: 337–345.
39 Pollack MH, Meoni P, Otto MW, Simon N, Hackett D. Predictors of outcome following venlafaxine extended-release treatment of DSM-IV generalized anxiety disorder: a pooled analysis of short- and long term studies. *J Clin Psychopharmacol* 2003; **23**: 250–259.
40 Katerndahl DA. Predictors and outcomes in people told that they have panic attacks. *Depress Anxiety* 2003; **17**: 98–100.
41 Roy-Byrne PP, Craske MG, Stein MB, Sullivan G, Bystritsky A, Katon W *et al.* A randomized effectiveness trial of collaborative care for patients with panic disorder in primary care. *Arch Gen Psychiatry* 2001; **58**: 869–876.
42 Katon W. Panic disorder: epidemiology, diagnosis, and treatment in primary care. *J Clin Psychiatry* 1986; **47**: 21–30.
43 Craske MG, Edlund MJ, Sullivan G, Roy-Byrne P, Sherbourne C, Bystritsky A *et al.* Perceived unmet need for mental health treatment and barriers to care among patients with panic disorder. *Psychiatr Serv* 2005; **56**: 988–994.
44 Craske MG, Roy-Byrne P, Stein MB, Donald-Sherbourne C, Bystritsky A, Katon W *et al.* Treating panic disorder in primary care: a collaborative care intervention. *Gen Hosp Psychiatry* 2002; **24**: 148–155.
45 Nickel C, Tritt K, Kettler C, Lahmann C, Loew T, Rother W *et al.* Motivation for therapy and the results of inpatient treatment of patients with a generalized anxiety disorder: a prospective study. *Wien Klin Wochenschr* 2005; **117**: 359–363.
46 Barlow DH, Gorman JM, Shear MK, Woods SW. Cognitive-behavioral therapy, imipramine, or their combination for panic disorder: A randomized controlled trial. *JAMA* 2000; **283**: 2529–2536.
47 Craske MG, Golinelli D, Stein MB, Roy-Byrne P, Bystritsky A, Sherbourne C. Does the addition of cognitive behavioral therapy improve panic disorder treatment outcome relative to medication alone in the primary-care setting? *Psychol Med* 2005; **35**: 1645–1654.
48 Foa EB, Kozak MJ, Steketee GS, McCarthy PR. Treatment of depressive and obsessive-compulsive symptoms in OCD by imipramine and behavior therapy. *Br J Clin Psychol* 1992; **31**: 279–292.
49 Hambrick JP, Weeks JW, Harb GC, Heimberg RG. Cognitive-behavioral therapy for social anxiety disorder: supporting evidence and future directions. *CNS Spectr* 2003; **8**: 373–381.
50 Ackerman DL, Greenland S. Multivariate meta-analysis of controlled drug studies for obsessive-compulsive disorder. *J Clin Psychopharmacol* 2002; **22**: 309–317.
51 Roy-Byrne PP, Bystritsky A, Russo J, Craske MG, Sherbourne CD, Stein MB. Use of herbal medicine in primary care patients with mood and anxiety disorders. *Psychosomatics* 2005; **46**: 117–122.
52 Hollander E, Bienstock CA, Koran LM, Pallanti S, Marazziti D, Rasmussen SA *et al.* Refractory obsessive-compulsive disorder: state-of-the-art treatment. *J Clin Psychiatry* 2002; **63**: 20–29.
53 Blanco C, Raza MS, Schneier FR, Liebowitz MR. The evidence-based pharmacological treatment of social anxiety disorder. *Int J Neuropsychopharmacol* 2003; **6**: 427–442.
54 Lauria-Horner BA, Pohl RB. Pregabalin: a new anxiolytic. *Expert Opin Invest Drugs* 2003; **12**: 663–672.

55 McDougle CJ, Barr LC, Goodman WK, Price LH. Possible role of neuropeptides in obsessive compulsive disorder. *Psychoneuroendocrinology* 1999; **24**: 1–24.
56 Nemeroff CB. New vistas in neuropeptide research in neuropsychiatry: focus on Corticotrophin releasing factor. *Neuropsychopharmacology* 1992; **6**: 69–75.
57 Pollack MH, Smoller JW. The longitudinal course and outcome of panic disorder. *Psychiatr Clin N Am* 1995; **18**: 785–801.
58 Goodman WK. Obsessive-compulsive disorder: diagnosis and treatment. *J Clin Psychiatry* 1999; **60**: 27–32.
59 Koran LM, Aboujaoude E, Bullock KD, Franz B, Gamel N, Elliott M. Double-blind treatment with oral morphine in treatment-resistant obsessive compulsive disorder. *J Clin Psychiatry* 2005; **66**: 353–359.
60 Pallanti S, Hollander E, Goodman WK. A qualitative analysis of nonresponse: management of treatment-refractory obsessive-compulsive disorder. *J Clin Psychiatry* 2004; **65**: 6–10.
61 Fallon BA, Mathew SJ. Biological therapies for obsessive-compulsive disorder. *J Psychiatr Pract* 2000; **6**: 113–128.
62 Saxena S, Maidment KM, Vapnik T, Golden G, Rishwain T, Rosen RM *et al.* Obsessive-compulsive hoarding: symptom severity and response to multimodal treatment. *J Clin Psychiatry* 2002; **63**: 21–27.
63 Bystritsky A, Liberman RP, Hwang S, Wallace CJ, Vapnik T, Maindment K *et al.* Social functioning and quality of life comparisons between obsessive-compulsive and schizophrenic disorders. *Depress Anxiety* 2001; **14**: 214–218.
64 Shear MK, Houck P, Greeno C, Masters S. Emotion-focused psychotherapy for patients with panic disorder. *Am J Psychiatry* 2001; **158**: 1993–1998.
65 Borkovec TD, Ruscio AM. Psychotherapy for generalized anxiety disorder. *J Clin Psychiatry* 2001; **62**: 37–42.
66 Krisanaprakornkit T, Krisanaprakornkit W, Piyavhatkul N, Laopaiboon M. Meditation therapy for anxiety disorders. *Cochrane Database Syst Rev* 2006; **1**: CD004998.
67 Hughes JR. A review of the usefulness of the standard EEG in psychiatry. *Clin Electroencephalogr* 1996; **27**: 35–39.
68 Jenike MA. An update on obsessive-compulsive disorder. *Bull Menninger Clin* 2001; **65**: 4–25.
69 Beale MD, Kellner CH, Pritchett JT, Burns CM. ECT for OCD. *J Clin Psychiatry* 1995; **56**: 81–82.
70 Benabid AL, Pollak P, Gross C, Hoffmann D, Benazzouz A, Gao DM *et al.* Acute and long-term effects of subthalamic nucleus stimulation in Parkinson's disease. *Stereotact Funct Neurosurg* 1994; **62**: 76–84.
71 Gross RE, Lozano AM. Advances in neurostimulation for movement disorders. *Neurol Res* 2000; **22**: 247–258.
72 Nuttin BJ, Gabriels L, van Kuyck K, Cosyns P. Electrical stimulation of the anterior limbs of the internal capsules in patients with severe obsessive-compulsive disorder: anecdotal reports. *Neurosurg Clin N Am* 2003; **14**: 267–274.
73 Abelson JL, Curtis GC, Sagher O, Albucher RC, Harrigan M, Taylor SF *et al.* Deep brain stimulation for refractory obsessive-compulsive disorder. *Biol Psychiatry* 2005; **57**: 510–516.
74 Morris GL, Mueller WM. Long-term treatment with vagus nerve stimulation in patients with refractory epilepsy. The Vagus Nerve Stimulation Study Group E01-E05. *Neurology* 1999; **53**: 1731–1735.
75 Rush AJ, Marangell LB, Sackeim HA, George MS, Brannan SK, Davis SM *et al.* Vagus nerve stimulation for treatment-resistant depression: a randomized, controlled acute phase trial. *Biol Psychiatry* 2005; **58**: 347–354.
76 Groves DA, Brown VJ. Vagal nerve stimulation: a review of its applications and potential mechanisms that mediate its clinical effects. *Neurosci Biobehav Rev* 2005; **29**: 493–500.
77 Martin JL, Barbanoj MJ, Schlaepfer TE, Clos S, Perez V, Kulisevsky J *et al.* Transcranial magnetic stimulation for treating depression. *Cochrane Database System Rev* 2002; **2**: CD003493.
78 Greenberg BD, Ziemann U, Cora-Locatelli G, Harmon A, Murphy DL, Keel JC *et al.* Altered cortical excitability in obsessive-compulsive disorder. *Neurology* 2000; **54**: 142–147.
79 Alonso P, Menchon JM, Pifarre J, Mataix-Cols D, Torres L, Salgado P *et al.* Right prefrontal repetitive transcranial magnetic stimulation in obsessive-compulsive disorder: a double-blind, placebo-controlled study. *Am J Psychiatry* 2001; **158**: 1143–1145.
80 Martin JL, Barbanoj MJ, Perez V, Sacristan M. Transcranial magnetic stimulation for the treatment of obsessive-compulsive disorder. *Cochrane Database System Rev* 2003; **3**: CD003387.
81 Mantovani A, Lisanby SH, Pieraccini F, Ulivelli M, Castrogiovanni P, Rossi S. Repetitive transcranial magnetic stimulation (rTMS) in the treatment of obsessive-compulsive disorder (OCD) and Tourette's syndrome (TS). *Int J Neuropsychopharmacol* 2006; **9**: 95–100.
82 Greenberg BD, Price LH, Rauch SL, Friehs G, Noren G, Malone D *et al.* Neurosurgery for intractable obsessive compulsive disorder and depression: critical issues. *Neurosurg Clin N Am* 2003; **14**: 199–212.
83 Cosgrove GR, Rauch SL. Stereotactic cingulotomy. *Neurosurg Clin N Am* 2003; **14**: 225–235.