UNIVERSITY OF SÃO PAULO

SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO

Subjective effects of cannabidiol in anxiety disorder and cannabinoid excretion in chronic daily cannabis smokers during sustained abstinence

Mateus M. Bergamaschi

Ribeirão Preto
2012

UNIVERSITY OF SÃO PAULO

SCHOOL OF PHARMACEUTICAL SCIENCES OF RIBEIRÃO PRETO

Subjective effects of cannabidiol in anxiety disorder and cannabinoid excretion in chronic daily cannabis smokers during sustained abstinence

Tese de Doutorado apresentada ao Programa de Pós-Graduação em Toxicologia para obtenção do Título de Doutor em Ciências

Área de Concentração: Toxicologia.

Dissertation submitted to the Graduate Program in Toxicology in fulfillment of the requirements for the degree of Doctor of Philosophy

Field of Study: Toxicology

PhD Candidate: Mateus M. Bergamaschi

Advisor: Regina Helena C. Queiroz, PhD

Co-Advisor: José A. S. Crippa, MD, PhD

Ribeirão Preto
2012

I HEREBY AUTHORIZE THE TOTAL OR PARTIAL REPRODUCTION AND PUBLISHING OF THIS WORK FOR STUDY AND RESEARCH PURPOSES, BY DIGITAL OR CONVENTIONAL SOURCE, SINCE THIS WORK IS PROPERLY REFERENCED.

Bergamaschi, Mateus M.
Subjective effects of cannabidiol in anxiety disorder and cannabinoid excretion in chronic daily cannabis smokers during sustained abstinence. Ribeirão Preto, 2012.
114p. : il. ; 30cm.

Dissertation submitted to School of Pharmaceutical
Sciences of Ribeirão Preto / USP – Field of Study: Toxicology

Advisor: Queiroz, Regina H. C.
Co- Advisor: Crippa, José A. S.

1. Social anxiety disorder. 2. Cannabidiol. 3. Cannabis

DISSERTATION APPROVAL SHEET

Mateus M. Bergamaschi

Subjective effects of cannabidiol in anxiety disorder and cannabinoid excretion during sustained abstinence in chronic daily cannabis smokers

Dissertation submitted to the Graduate Program in Toxicology in fulfillment of the requirements for the degree of Doctor of Philosophy

Field of Study: Toxicology

Advisor: Regina Helena C. Queiroz, PhD

Co-Advisor: José A. S. Crippa, MD, PhD

Date Approved:

Committee

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Prof. Dr. _____

Institution: _____ Signature:_____

Acknowledgments

First, I would like to thank my family. Mom, Dad, brothers, sisters-in-law, and Franciele. Thank you all for your support and sacrifices made during this journey and especially during the past two years.

I would like to thank my advisors, Dr. Regina Helena Costa Queiroz, Dr. José Alexandre de Souza Crippa, and Dr. Marilyn A. Huestis. I have learned so much over the past four years from you about clinical research, data analysis, writing proposals, manuscripts, and publishing. Your guidance and support through this journey is much appreciated. Dr. Huestis, I am grateful for the opportunity you gave me to work in your lab and to have you as a co-advisor for my work done at NIDA.

To Dr. Antônio Cardozo dos Santos, Dr. Eliane Candiani Arantes Braga, Dr. Fernando Barbosa Júnior, and all FCFRP Graduate Program staff for your dedication to the Graduate Program in Toxicology and willingness to help.

I also would like to thank Dr. Maria Eugênia Queiroz and Dr. Bruno Spinosa de Martinis for providing me the opportunity to work with GCMS; Dr. Sérgio Akira Uyemura and Dr. Andréia Machado Leopoldino for giving me the opportunity to work in molecular biology and mentoring undergraduate students during the teaching improvement program.

Dr. Jaime Eduardo Cecílio Hallak, Dr. Flávia de Lima Osório, and Dr. Leonardo Régis Leira Pereira deserve special mention for their contribution on my dissertation proposal as committee members.

Past and current graduate students Daniel Valério, Greyce Steinhorst, Flavia Isaura de Santi Ferreira, Marina Salviato Balbão Santiago Fonseca, Ricardo Augusto de Pádua, Camila Matsumoto, Fernanda Gomes Cardoso, Cristiana Bernadelli, Renata Vilela Rodrigues, Silveli Suzuki, Natalia Valadares de Moraes, Nathalie Desrosiers, Rebecca Hartman, Sarah Himes, Marisol Castaneto and Dayong Lee, Dr. Ariane Wohlfarth, Dr. Emeline Chauchard, and Dr. Sebastien Anizan for being good friends. Marcos Hortes, Kátia Cruvinel Arrais, Danielle Gomes de Oliveira and Ila Linares

deserve special acknowledgement for their assistance during the clinical study and Dr. Garry Milman for teaching me the basics of a 2d-GCMS system.

Sandra Bernardo and Selma Pontes were fundamental in helping the study to run smoothly. Sandra, I cannot thank you enough for your willingness to help and sacrifices made through the study, even during holidays and weekends. Your contribution in capsule preparation and blood collection is much appreciated. Sônia Dreossi, thank you for teaching me the first steps of working with liquid and gas chromatography. Maria Aparecida Buzeto, your help on teaching me how to work with rodents was always appreciated. I would also like to thank Gilda Alves Carvalho Gatto for her help on drug analysis when needed.

The entire Chemistry and Drug Metabolism Section (NIDA/NIH), especially Megan Taylor, David Darwin, Dr. Karl Scheidweiler, Dr. Marta Concheiro-Guisan, and Allan Barnes for their fundamental assistance with paperwork, clinical study, chromatography troubleshooting and training during my time at NIDA.

All ‘Baltimorean’ friends, Eva Cunha, Fábio Cruz, Ana Rita Nunes, Leonel Maldonado, Mariana Brait, Miguel Bastos, Nuno Pinto, Mario Inacio, Andreia Ribeiro and Recep Özgün, for being good friends and present when most needed. My experience in Baltimore would not have been good without you all.

To the School of Pharmaceutical Sciences of Ribeirão Preto, School of Medicine of Ribeirão Preto, and National Institute on Drug Abuse for the support during my PhD training.

Finally, I would like to thank National Institute on Drug Abuse, Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior (CAPES) and Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP) for the financial support to conduct this research.

RESUMO

BERGAMASCHI, M. M. **Efeitos comportamentais do cannabidiol na ansiedade e eliminação de canabinóide durante abstinência em usuários crônicos de cannabis**. 2012. 114f. Tese (Doutorado). Faculdade de Ciências Farmacêuticas de Ribeirão Preto – Universidade de São Paulo, Ribeirão Preto, 2012.

Esta tese é dividida em três partes. A primeira parte consiste em investigar o efeito ansiolítico do canabidiol na ansiedade social através do teste de simulação de falar em público. Vinte e quatro sujeitos com ansiedade social, nunca tratados, receberam placebo ou canabidiol (CBD) 600 mg (n=12) em um estudo randomizado e duplo-cego. O mesmo número de indivíduos saudáveis realizaram o teste de simulação de falar em público sem receber medicação. A administração do CBD reduziu significativamente a ansiedade, sedação física e outros sentimentos e atitudes durante a fase de estresse, e diminui o nível de alerta na fase pré-estresse. O grupo placebo apresentou níveis elevado de ansiedade, sedação física, outros sentimentos e atitudes, e alerta comparado com o grupo controle. A pontuação do SSPS-N evidenciou aumento significativo durante o teste no grupo placebo, enquanto que o CBD reduziu estes níveis. Não houve diferenças significativas entre os grupos CBD e controle na SSPS-N e nos fatores sedação física, outros sentimentos e atitudes e alerta, da Visual Analogue Mood Scale (VAMS). A segunda parte do estudo avaliou a ansiedade em indivíduos saudáveis que receberam alta dose oral de rimonabanto e submetidos ao teste de simulação de falar em público, para melhor entendimento do possível mecanismo farmacológico para tratamento de transtornos de ansiedade. Vinte e quatro sujeitos saudáveis receberam placebo ou rimonabanto 90 mg (n=12) em um randomizado e duplo-cego. Não foi observado efeitos adversos significativo em ambos grupos. O grupo rimonabanto apresentou maiores níveis de ansiedade na fase pré-estresse e durante o estresse. Não houve diferença significativa quanto aos demais fatores avaliados entre os grupos. O aumento na ansiedade após administração do rimonabanto pode-se ao fato de haver diminuição no sistema endocanabinóide nos receptores CB1 e a possível modulação na ansiedade clínica e patológica. A terceira parte objetivou quantificar canabinóides no sangue total em usuários crônicos de cannabis durante abstinência supervisionada. Trinta usuários crônicos de cannabis, do sexo masculino, permaneceram no centro de pesquisa por até 33 dias, com coleta de sangue uma vez ao dia. Δ^9-tetrahidrocanabinol (THC), 11-hidróxi-THC (11-OH-THC) e 11-nor-9-carbóxi-THC (THCCOOH) foram quantificados no sangue por meio da cromatografia gasosa-espectrometria de massa bidimensional. Vinte e sete de 30 usuários foram positivos para THC no ingresso do estudo, com concentração mediana (variação) de 1.4 ng/mL (0.3–6.3). Níveis de THC diminuíram gradativamente com somente 1 de 11 participantes negativo no dia 26; 2 de 5 indivíduos permaneceram positivos para THC (0.3 ng/mL) por 30 dias. 5.0% dos sujeitos tiveram THC ≥1.0 ng/mL por 12 dias. Concentração mediana de 11-OH-THC foi 1.1 ng/mL no ingresso do estudo, sem valores ≥1.0 ng/mL após 24h. A taxa de detecção de THCCOOH foi 96.7% no ingresso, diminuindo gradativamente para 95.7 e 85.7% nos dias 8 e 22, respectivamente; 4 de 5 sujeitos permaneceram positivo para THCCOOH (0.6–2.7 ng/mL) após 30 dias e um permaneceu positivo no 33º dia. Foi detectado THC em alguns indivíduos por 30 dias, porém em baixas concentrações, devido a extensa eliminação do canabinóide em decorrência da exposição crônica.

Palavras-chave: canabidiol; rimonabanto; cannabis; teste de simulação de falar em público; transtorno de ansiedade social, usuários crônicos de cannabis

ABSTRACT

BERGAMASCHI, M. M. **Subjective effects of cannabidiol in anxiety disorder and cannabinoid excretion in chronic daily cannabis smokers during sustained abstinence**. 2012. 114p. Dissertation (Doctoral). School of Pharmaceutical Sciences of Ribeirão Preto – University of São Paulo, Ribeirão Preto, 2012.

This dissertation is divided into three parts. The first part aimed to investigate the cannabidiol anxiolytic effect in treatment-naïve individuals with social anxiety disorder through simulation of public speaking. Twenty-four never-treated social anxiety disorder subjects were allocated to receive 0 or 600 mg cannabidiol (CBD; n=12) in a double-blind randomized design. The same number of controls performed the simulation of a public speaking test without receiving any medication. Pretreatment with CBD significantly reduced anxiety, cognitive impairment, and discomfort in speech performance and significantly decreased alertness in their anticipatory speech. The placebo group displayed higher anxiety, cognitive impairment, discomfort, and alertness when compared with controls as assessed with the Visual Analogue Mood Scale (VAMS). The SSPS-N scores showed significant increases during testing of the placebo group that was almost abolished in the cannabidiol group. No significant differences were observed between the cannabidiol and control groups in SSPS-N scores or in cognitive impairment, discomfort, and alertness factors of the VAMS. The second part evaluated healthy subjects' anxiety during a public speaking test following a high rimonabant oral dose, to understand better the possible pharmacological approaches for anxiety disorder treatment. Twenty four participants were randomly allocated to receive 0 or 90 mg rimonabant (n=12) in a double-blind design. No significant adverse effects were reported in either group. Participants who received rimonabant showed increased anxiety levels compared to placebo during anticipatory speech and performance measurements. Rimonabant treatment did not affect sedation, cognitive impairment, discomfort, blood pressure, heart rate, self-statements during public speaking, or bodily symptoms scales. Increased anxiety may reflect lower endocannabinoid activity in CB1 receptors and CB1 receptor's possible role in modulation of anxiety and anxiety disorders. The third part aimed to monitor cannabinoid blood concentrations during sustained abstinence from chronic daily cannabis smoking. Thirty male chronic daily cannabis smokers resided on a secure clinical research unit for up to 33 days, with blood collected once daily. Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-THC (11-OH-THC), and 11-nor-9-carboxy-THC (THCCOOH) whole blood concentrations were quantified by two-dimensional gas chromatography-mass spectrometry. Twenty-seven of 30 participants were THC-positive on admission, with a median (range) concentration 1.4 ng/mL (0.3–6.3). THC decreased gradually with only 1 of 11 participants negative at 26 days; 2 of 5 participants remained THC-positive (0.3 ng/mL) for 30 days. 5.0% of participants had THC ≥1.0 ng/mL for 12 days. Median 11-OH-THC concentrations were 1.1 ng/mL on admission, with no results ≥1.0 ng/mL 24h later. THCCOOH detection rates were 96.7 on admission, decreasing slowly to 95.7 and 85.7% on days 8 and 22, respectively; four of 5 participants remained THCCOOH positive (0.6–2.7 ng/mL) after 30 days and one remained positive on discharge at 33 days. THC was quantified in some participants for 30 days, albeit in low concentrations, due to the large cannabinoid body burden from extended exposure.

Keywords: cannabidiol; rimonabant; cannabis; simulated public speaking test; social anxiety disorder, chronic cannabis smokers

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

11-OH-THC	11-hydroxy-THC
5-HT	5-hydroxytryptamine receptor
5-HT1A	5-hydroxytryptamine receptor subtype 1A
AA	African-American
ANOVA	analyses of variance
AP	arterial blood pressure
ATP	adenosine-5'-triphosphate
AUDIT	Alcohol Use Disorders Identification Test scale
BAC	blood alcohol concentration
BAI	Beck Anxiety Inventory
BMI	body mass index
BNST	bed nucleus of the stria terminalis
BSS	Bodily Symptoms Scale
bw	body weight
C	Caucasian
cAMP	cyclic adenosine monophosphate
CAPES	Federal Agency of Support and Evaluation of Graduate Education
CB1	cannabinoid receptor subtype 1
CB2	cannabinoid receptor subtype 2
CBD	cannabidiol
CCSEB	Critério de Classificação Sócio-Econômica Brasil
CYP	cytochrome P450
DBP	diastolic blood pressure
dlPAG	dorsolateral periaqueductal gray
DSM-IV	diagnostic and statistical manual of mental disorders, 4th edition
ECT	electroconvulsive therapy
EEG	electroencephalogram
EKG	electrocardiogram
FAST	Fast Alcohol Screening Test
FCFRP-USP	School of Pharmaceutical Sciences of Ribeirão Preto of University of São Paulo
fMRI	functional magnetic resonance imaging

GABA	gamma-aminobutyric acid
GCMS	gas chromatography–mass spectrometry
GPR55	orphan G-protein-coupled receptor
h	hour
HC	healthy control
HIV	human immunodeficiency virus
HR	heart rate
IRB	Institutional Review Board
logn	natural logarithm
LOQ	limit of quantification
min	minute
MINI-SPIN	short version of Social Phobia Inventory
mL	milliliter
mPFC	medial prefrontal cortex
NA	non-available
ng/mL	nanogram per milliliter
NIDA	National Institute on Drug Abuse
PET	positron emission tomography
PHQ-9	Patient Health Questionnaire-9
rCBF	regional cerebral blood flow
SAD	social anxiety disorder
SBP	systolic blood pressure
SCID-CV	Structured Clinical Interview for the DSM-IV, clinical version
SCL	skin conductance level
SD	standard deviation
SF	spontaneous fluctuations of skin conductance
SPECT	single photon emission computed tomography
SPIN	Social Phobia Inventory
SPST	simulation of public speaking test
SRQ-24	Self-Reporting Questionnaire-24
SSNRIs	selective serotonin and norepinephrine reuptake inhibitors
SSPS	Self-Statements during Public Speaking Scale
SSPS-N	Self-Statements during Public Speaking Scale negative self-evaluation subscale
SSRIs	selective serotonin reuptake inhibitors

THC	Δ^9-tetrahydrocannabinol
THCCOOH	11-nor-9-carboxy-THC
TRPV	transient receptor potential vanilloid
TRPV1	transient receptor potential vanilloid subtype 1
TRPV2	transient receptor potential vanilloid subtype 2
US	United States of America
USP-RP	University of São Paulo, Ribeirão Preto *campi*
VAMS	Visual Analogue Mood Scale

TABLE OF CONTENTS

1. Chapter 1 – Introduction

1.1. Cannabis and Anxiety

Since early ages, *Cannabis sativa* (cannabis) is associated with psychotic symptoms, i.e. panic attack, anxiety, and fear (GROTENHERMEN, 2007; JOHNS, 2001; ZUARDI et al., 2006a), but anxiety symptoms receive little attention as to whether they would be related to psychotic (ARSENEAULT et al., 2004) or withdrawal symptoms (BUDNEY et al., 2004). However, chronic cannabis users report reduced anxiety after smoking cannabis, claiming it as the reason for prolonged cannabis use (ASHTON, 2001; LEE et al., 2009). However, one should consider factors that may be associated with increased anxiety after cannabis use such as duration and frequency of use, individual variability, presence of psychiatric symptoms, and environment (CRIPPA et al., 2009).

Results from epidemiological studies showed chronic cannabis users with high levels of anxiety (REILLY et al., 1998) and also found association with anxiety disorders with at least two-fold more probability than non-cannabis users (BUCKNER et al., 2008; SWADI; BOBIER, 2003). Another hypothesis for repeated cannabis use and development of dependence is self-medication of cannabis as an alternative to reduce anxiety (BONN-MILLER; ZVOLENSKY; BERNSTEIN, 2007; BUCKNER et al., 2007; BUCKNER et al., 2008; INSERM COLLECTIVE EXPERTISE CENTRE, 2001; REILLY et al., 1998). Anxiety associated with cannabis withdrawal symptoms usually manifests after 48 h of last cannabis use and persists for weeks (BUDNEY et al., 2004; HANEY, 2005). In psychiatric and drug treatment perspectives, detection of cannabis withdrawal syndrome is not straightforward as it is still not included in the Diagnostic and Statistical Manual of Mental Disorders, 4^{th} edition (DSM-IV) due to 'uncertain' clinical significance (AMERICAN PSYCHIATRIC ASSOCIATION, 1994).

The mechanisms by which cannabis induced anxiety and anxiety disorders were better described through clinical and animal studies. The most abundant psychoactive compound from cannabis extract, Δ^9-tetrahydrocannabinol (THC), can modulate serotonin, noradrenalin, and endocannabinoid systems, whose mechanism is complex and not fully understood (CRIPPA et al., 2009). Chronic cannabis use may also downregulate cannabinoid receptors with prolonged impairment (HIRVONEN et al., 2012). Future studies concerning cannabinoid receptors density in human brain and endocannabinoid activity may provide better knowledge for the association between cannabis use and anxiety / anxiety disorders.

1.1.1. Cannabinoid Receptors

Two types of G-protein-coupled receptor are known, identified as cannabinoid receptor subtype 1 (CB1) and cannabinoid receptor subtype 2 (CB2) receptors (MATSUDA et al., 1990; MUNRO; THOMAS; ABU-SHAAR, 1993). CB1 receptors are located mainly in the central nervous system and highly expressed in the basal ganglia, hippocampus, amygdala, and cerebellum (GROTENHERMEN, 2004; HERKENHAM et al., 1990), while CB2 receptors are located in immune cells (BELTRAMO et al., 2006; GONG et al., 2006; ROSS et al., 2001; SKAPER et al., 1996; VAN SICKLE et al., 2005; WOTHERSPOON et al., 2005). The discovery of these receptors lead to identification of endogenous cannabinoid agonists (endocannabinoids) named N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (DEVANE et al., 1992; MECHOULAM et al., 1995; SUGIURA et al., 1995).

While the neuronal role of CB2 receptors is still unknown, increased interest emerged on the neuropharmacology of CB1 receptors. Activation of the CB1 receptor can modulate neurotransmitter release by inhibition of excitatory and inhibitory transmitters by elevation of intracellular calcium (DE PETROCELLIS; DI MARZO, 2009; HOWLETT et al., 2002) and inhibition of adenylate cyclase, which converts adenosine-5'-triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (GROTENHERMEN, 2004). However, the mechanism *per se* remains more complex, as these receptors can affect the homeostasis of other neurotransmitters, i.e., acetylcholine, noradrenaline, dopamine, 5-hydroxytryptamine (5-HT), gamma-aminobutyric acid (GABA), glutamate, D-aspartate, and cholecystokinin (PERTWEE; ROSS, 2002; SZABO; SCHLICKER, 2005). In addition to pharmacological modulation of cannabinoid receptors, (endo)cannabinoids can exert multiple actions through diverse mechanisms via transient receptor potential vanilloid (TRPV), orphan G-protein-coupled receptors (GPR55), α-receptors, and endocannabinoid modulation by cannabinoids, i.e., cannabidiol (CBD) inhibits anandamide reuptake (IZZO et al., 2009).

As more than 80 known cannabinoids are located in the *Cannabis sativa* plant (ZUARDI; CRIPPA; HALLAK, 2010), the plethora of pharmacological effects remains unknown, with the most abundant and studied compounds, THC and CBD, as potential therapeutic agents for diverse disorders (PERTWEE, 2008; ZUARDI, 2008; ZUARDI; CRIPPA; HALLAK, 2010).

1.2. Cannabidiol

CBD is a component of *Cannabis sativa* and constitutes up to 40% of plant extracts (GRLIC, 1962). However, CBD concentrations are highly variable and depend on growing conditions, different phenotypes of illicit cannabis, and on the plant parts analyzed (MEHMEDIC et al., 2010; POTTER; CLARK; BROWN, 2008). Evidence suggests that CBD potency decreased in recent years, while THC concentrations increased, as varieties such as sensimilla ('skunk'), provided by illegal cannabis growers, currently dominate cannabis supply in many countries (POTTER; CLARK; BROWN, 2008). CBD induces markedly different psychological effects compared to the best known cannabis compound, THC (PEREZ-REYES et al., 1973; ZUARDI et al., 1982). Despite presenting low affinity for CB1 and CB2 receptors, CBD can still interact with these receptors at doses equal to or lower than 1 μM. Therefore, there is no certainty about whether this antagonism is non-competitive. CBD can also act as a CB1 receptor inverse agonist at concentrations below those needed to bind to the CB1 orthosteric site. Moreover, CBD can antagonize THC effects via non-CB1/CB2 receptors such as GPR55, which is activated by THC and blocked by CBD (PERTWEE, 2008). The time between CBD and THC intake, as well as the CBD/THC ratio, seem to play an important role in the interaction between these two cannabinoids. CBD can increase the potency of THC by pharmacokinetic interaction if CBD is administered before THC, or a pharmacodynamic interaction may occur when both cannabinoids are taken together, mainly at a high dose ratio of CBD/THC (ZUARDI; HALLAK; CRIPPA, 2012).

CBD was first isolated by Adams *et al*. in 1940 (ADAMS; HUNT; CLARK, 1940) and its structure was identified 23 years later (MECHOULAM; SHVO, 1963). Since then, a considerable number of published articles describe its chemistry, biochemistry, pharmacology, and clinical effects. By 2000, the primary research topics regarding possible therapeutic effects of CBD were related to its antiepileptic, sedative, anxiolytic, and antipsychotic activities (CUNHA et al., 1980; ZUARDI et al., 2006a). The last decade has shown a notable increase in scientific literature on CBD, owing to the identification of its anti-inflammatory and neuroprotective effects. These studies raised the possibility of CBD's therapeutic effects for diverse conditions including dementias, cerebral ischemia, diabetes, inflammatory diseases, nausea, and psychiatric disorders (ZUARDI, 2008). This wide range of therapeutic effects can be explained by CBD's multiple mechanisms of action. Despite its low affinity for CB1 and CB2 receptors, CBD is capable of antagonizing CB1 / CB2 receptor agonists at reasonably low concentrations. At CB2 receptors, CBD acts as an inverse agonist.

Other mechanisms of action include antagonism of the recently discovered GPR55 receptor; transient receptor potential vanilloid subtype 1 (TRPV1) agonist; transient receptor potential vanilloid subtype 2 (TRPV2) agonist; 5-hydroxytryptamine receptor subtype 1A ($5\text{-}HT_{1A}$) agonist; antagonism of the putative abnormal-CBD receptor; and regulation of intracellular [Ca^{2+}] (IZZO et al., 2009). Inhibition of adenosine uptake leads to increased adenosine signaling, which may explain the ability of CBD to decrease inflammation and provide neuroprotective effects (CARRIER; AUCHAMPACH; HILLARD, 2006; CASTILLO et al., 2010). A similar mechanism also was reported for CBD, suggesting that this cannabinoid could block anandamide uptake and inhibit its enzymatic hydrolysis (LIGRESTI et al., 2006).

Evidence of CBD anxiolytic effects first appeared in the mid 1970s. Fifteen to 60 mg oral CBD significantly attenuated anxiety, heart rate, and panic induced by 30 mg THC in healthy male participants (KARNIOL et al., 1974). Further studies in animals and humans employing anxiogenic models elucidated CBD's anxiolytic effect. Two initial animal studies reported conflicting results. First, Silveira Filho and Tufik (SILVEIRA; TUFIK, 1981) showed that a high 100 mg/kg CBD dose had no effect on conflict behavior, whereas Zuardi and Karniol (ZUARDI; KARNIOL, 1983) showed that low 10 mg/kg CBD doses decreased conditioned emotional responses in rats. These findings were explained by Guimarães and co-workers (GUIMARAES et al., 1990), who demonstrated in the elevated plus-maze test in rats that CBD has an inverted U-shape dose-effect anxiolytic curve with narrow doses range (2.5 – 10mg/kg). Following this finding, others animal studies confirmed CBD's anxiolytic effect in the elevated plus-maze anxiety model (CAMPOS; GUIMARAES, 2009; 2008; GOMES; RESSTEL; GUIMARAES, 2011; GUIMARAES et al., 1994; ONAIVI; GREEN; MARTIN, 1990), Vogel conflict test (CAMPOS; GUIMARAES, 2008; GOMES; RESSTEL; GUIMARAES, 2011; MOREIRA; AGUIAR; GUIMARAES, 2006), contextual conditioned fear paradigm (LEMOS; RESSTEL; GUIMARAES, 2010; RESSTEL et al., 2006), marble burying test for obsessive-compulsive disorder (CASAROTTO et al., 2010) and attenuation of acute stress responses (RESSTEL et al., 2009).

Direct CBD administration into brain investigates the regions where CBD exerts its anxiolytic effect. Microinjection into the dorsolateral periaqueductal gray (dlPAG) (CAMPOS; GUIMARAES, 2009; 2008) and bed nucleus of the stria terminalis (BNST) (GOMES et al., 2012; GOMES; RESSTEL; GUIMARAES, 2011) in rats suggested that this cannabinoid interacted with 5HT1A (RUSSO et al., 2005; ZANELATI et al., 2010) and TRPV1 to produce anxiolytic-like effects. Another finding (LEMOS; RESSTEL; GUIMARAES, 2010) showed that CBD attenuated the conditioned fear response by paw

shock in rats when CBD is injected into medial prefrontal cortex (mPFC) at the prelimbic region, while anxiogenic response was observed when CBD was injected into the infralimbic prefrontal cortex. In addition, CBD also promoted contextual fear memory extinction after intracerebral ventricular administration antagonized by the cannabinoid (CB1)-antagonist SR141716 (rimonabant), suggesting the role of CB1 receptor on fear extinction (BITENCOURT; PAMPLONA; TAKAHASHI, 2008).

CB1 receptor involvement in anxiety modulation was reported previously (CASAROTTO et al., 2010), but interest increased when the first CB1 receptor antagonist rimonabant was released in the market for obesity treatment in 2006 (MOREIRA; CRIPPA, 2009). Rimonabant was withdrawn from the market two years later due to potential serious psychiatric side effects, i.e. anxiety (MOREIRA; CRIPPA, 2009). CB1 receptors are located primarily in the human central nervous system, particularly those regions responsible for emotions, i.e. hippocampus, amygdala, periaqueductal gray, prefrontal cortex, and hypothalamus (HERKENHAM et al., 1990). CB1 receptor location also explains the reason cannabis smokers experience feelings of relaxation and reduced anxiety (HALL; SOLOWIJ, 1998), also attributed to the role of the endocannabinoid system. The mechanism remains unclear, as antagonism of this receptor is related to anxiogenic-like effects in animals (MOREIRA; CRIPPA, 2009), as observed with administration of THC, a CB1 receptor agonist, in humans and animals (HOWLETT, 1995; KARNIOL; CARLINI, 1973; PERTWEE, 2008; 1997). This controversy could be related to the fact that THC is a partial agonist at CB1 receptors (PERTWEE, 2008) and depending upon several factors, may either facilitate or decrease endocannabinoid transmission.

Thus, pre-clinical studies suggest CBD anxiolytic effects related to specific brain areas modulating emotion and anxiety. Indeed, these findings were also confirmed in clinical human trials. Early studies in healthy participants showed that CBD decreased anxiety provoked by THC, suggesting a non-selective antagonism (ZUARDI et al., 1982).

1.2.1 Cannabidiol and Social Anxiety

Fear of public speaking is one of the pivotal symptoms of social anxiety disorder (SAD) (STEIN; STEIN, 2008). Thus, experimental models based on fear were developed to assess effects of substances on anxiety (HALLAK et al., 2010a; MCNAIR et al., 1982). Zuardi and co-workers (ZUARDI et al., 1993) evaluated CBD's effect on reduction of anxiety during the simulation of public speaking test (SPST) in healthy subjects. This test consisted of

participants speaking in front of the camera while viewing his/her own image on a TV screen. They were also told that the speech would be recorded and further analyzed by a psychologist. A single 300 mg CBD dose significantly reduced post-stress anxiety compared to placebo. A subsequent study performed in treatment-naïve social phobic subjects (BERGAMASCHI et al., 2011a) showed that 600 mg CBD administered 80 minutes before SPST-mitigated anxiety provoked by SPST during the speech. Furthermore, CBD also reduced cognitive impairment and negative self-evaluation compared to placebo before and during the speech, indicating that CBD was able to decrease anxiety *per se* and that it also positively affected self-evaluation during public speaking, crucial for SAD patient therapy. Negative self-evaluation is one of the pivotal aspects of this disorder.

A functional neuroimaging study (CRIPPA et al., 2004) employing single photon emission computed tomography (SPECT) in healthy participants evaluated CBD effects in neural activity in those brain areas modulating anxiety. This test consisted of 0 or 400 mg CBD administration with SPECT image acquisition and subjective ratings performed 110 minutes after CBD intake. This test is considered anxiogenic *per se*, as participants often report increased anxiety before scanning. This study showed that 400 mg CBD could modulate brain activity in regions related to emotion and anxiety, i.e. decrease neural activation in the left amygdala-hippocampal complex and left posterior cingulate gyrus, also related to brain activity modulation by benzodiazepines (i.e. diazepam) and selective serotonin reuptake inhibitors (i.e. citalopram).

Another study investigated regional brain function during emotional processing by functional magnetic resonance imaging (fMRI) (FUSAR-POLI et al., 2009). Functional MRI is a modern imaging technology providing sensitive non-invasive imaging of physiological changes (MATTHEWS; HONEY; BULLMORE, 2006). Administration of 600 mg oral CBD reduced brain activity in the amygdala and anterior and posterior cingulate cortex and decreased skin conductance response when participants were presented fearful faces which elicited different levels of anxiety. A subsequent study investigated connectivity during emotional processing by the fearful face stimuli task. As predicted, CBD modulated prefrontal and subcortical region activity, reducing connectivity between anterior cingulate cortex and amygdala (FUSAR-POLI et al., 2010).

Crippa and co-workers (CRIPPA et al., 2011) conducted the first study evaluating CBD neural effects in treatment-naïve social anxiety disorder participants. Subjects with generalized SAD received 400 mg oral CBD according to the same study design as the previous SPECT study in healthy participants (CRIPPA et al., 2004). CBD decreased activity

in the left parahippocampal gyrus, hippocampus, and inferior temporal gyrus. Interestingly, CBD administration in these SAD subjects did not provoke sedation or alteration in hypothalamic activity, as previously reported (BERGAMASCHI et al., 2011a). In total, these results support the anxiolytic effect of CBD as related to its activity in limbic and paralimbic brain areas.

Although animal and human studies demonstrate significant and strong evidence of CBD's anxiolytic effect, the SAD results must be carefully considered. First, healthy participants in clinical studies have no history of psychiatric disorder or drug abuse. Second, to date, these are only two SAD studies on human pathological anxiety. These participants were treatment-naïve and had no comorbidities. It is known that SAD has an early age of onset and high rate of comorbidity (FILHO et al., 2010) that can affect therapy efficacy, as medication and cognitive behavioral therapy are required (DAVIDSON, 2006; STEIN; STEIN, 2008). Third, after an acute therapeutic response is achieved, long-term treatment is needed to prevent return of symptoms after treatment is stopped. To date, no chronic clinical studies have evaluated CBD's anxiolytic effects.

In conclusion, CBD therapy is a good approach for pharmacological treatment of social anxiety disorder, as acute administration achieves rapid therapeutic effects. However, further clinical trials with larger sample sizes and chronic administration with post-treatment follow-up are needed to confirm these findings.

1.2.2. Cannabidiol Safety in Humans

1.2.2.1. Acute Studies

In the 1970s, human studies showed that oral CBD intake from 15 to 160 mg (CARLINI; MASUR; MAGALHÃES, 1979; HOLLISTER, 1973; KARNIOL et al., 1974), inhalation of 0.15mg/kg body weight (bw) (DALTON et al., 1976), or intravenous injection from 5 to 30 mg (HOLLISTER, 1973; PEREZ-REYES et al., 1973), did not produce adverse effects. CBD did not interfere with several psychomotor and psychological functions. CBD did not affect heart rate, blood pressure, or performance in the verbal paired-associate learning test at doses up to 600 mg (BERGAMASCHI et al., 2011a; CONSROE et al., 1979; KARNIOL et al., 1974; ZUARDI et al., 1982). Subsequent studies on CBD's antipsychotic effects did not report adverse side effects (BHATTACHARYYA et al., 2010; HALLAK et al., 2011; HALLAK et al., 2010b).

1.2.2.2. Chronic studies

Chronic daily oral 10 mg CBD administration for 21 days did not induce changes in neurological [including electroencephalogram (EEG)], clinical [including electrocardiogram [EKG)], psychiatric, blood, or urine examinations (MINCIS et al., 1973). Likewise, oral CBD administration in healthy participants (3 mg/kg bw daily for 30 days) and in epileptic patients (200-300 mg daily for 135 days) was well-tolerated and no signs of toxicity or serious side effects were detected on neurological and physical examinations, blood and urine analysis, or EKG and EEG, which were performed at weekly intervals (CUNHA et al., 1980).

CBD was evaluated for symptomatic efficacy and safety in 15 neuroleptic-free patients with Huntington's disease. Effects after oral CBD (10 mg/kg bw /day for 6 weeks) or placebo (sesame oil for six weeks) were evaluated weekly according to a double-blind, randomized crossover design. CBD showed no significant or clinical differences compared to placebo in the cannabis side effect inventory, clinical laboratory tests, or other safety outcome variables. Also, weekly plasma CBD concentrations by gas chromatography–mass spectrometry (GCMS; mean range 5.9 to 11.2 ng/mL), did not differ significantly over six weeks of CBD administration (CONSROE et al., 1991).

A previous case report of a teenager diagnosed with schizophrenia who experienced severe side effects after treatment with conventional antipsychotics demonstrated significant symptom improvement with no adverse effects after hospitalization and four weeks of treatment with increasing CBD doses up to 1,500 mg/day (ZUARDI et al., 1995). More recently, CBD monotherapy was administered to three patients with treatment-resistant schizophrenia (initial oral 40 mg dose, increasing to 1,280 mg/day for up to four weeks) with no side effects reported, even at the highest dose (ZUARDI et al., 2006b). A similar result was observed in two patients with bipolar affective disorder who received CBD (600-1,200 mg/day) for up to 24 days (ZUARDI et al., 2010). A double-blind study with 42 patients diagnosed with schizophrenia or schizophreniform disorder (diagnosed by DSM-IV) in an acute episode showed that an 800 mg CBD dose significantly reduced psychotic symptoms after two to four weeks of treatment and induced fewer side effects such as extrapyramidal symptoms, increased prolactin levels, and weight gain compared to amisulpride (LEWEKE et al., 2007).

CBD efficacy and safety for Parkinson’s disease patients with psychotic symptoms were evaluated in a four week open trial. Flexible oral CBD dosing ranged from 150 to 400 mg/day in the last week. Patients’ usual treatments showed that psychotic symptoms were

significantly reduced; cognitive and motor symptoms were not affected by the cannabinoid and no serious side effects were reported (ZUARDI et al., 2009). A double-blind placebo controlled trial is currently underway by Zuardi's group to evaluate CBD efficacy, safety, and tolerability in patients with Parkinson's disease and psychosis.

Finally, a 19-year old female with a history of cannabis addiction received 300 mg CBD on day 1, 600 mg/day divided into two doses on days 2 through 10, and 300 mg CBD on day 11. During CBD treatment, the patient did not report any cannabinoid withdrawal symptoms and did not experience anxiety or dissociative symptoms (CRIPPA; ZUARDI; HALLAK, 2010) as assessed by standardized rating scales. Some clinical trials in multiple sclerosis showed that 1:1 mix THC and CBD, available as an oromucosal spray (Sativex®), at doses ranging from 2.5 to 120 mg of each cannabinoid, showed no adverse effects on cognition or mood (WADE et al., 2004), other than those observed with psychoactive drugs for pain treatment (NOTCUTT et al., 2004).

1.3. Rimonabant

Rimonabant is a CB1 receptor inverse agonist or antagonist developed for its anti-obesity and anti-tobacco smoking effects at a therapeutic dose of 5 or 20 mg/day (DESPRES; GOLAY; SJOSTROM, 2005; LE FOLL et al., 2008; PI-SUNYER et al., 2006; VAN GAAL et al., 2005; VAN GAAL et al., 2008b). CB1 receptor antagonist development provided a powerful tool for investigating the endocannabinoid system in animals and humans. *In vitro* studies showed rimonabant antagonized cannabinoids effects, further confirmed by rodent studies (RINALDI-CARMONA et al., 1995; RINALDI-CARMONA et al., 1996). In humans, pretreatment with 90 mg rimonabant significantly antagonized THC effects, documenting for the first time that THC's effects were modulated through the CB1 receptor and that the interaction was pharmacodynamic rather than pharmacokinetic in nature (HUESTIS et al., 2007; HUESTIS et al., 2001).

Evidence for rimonabant's anti-obesity effect in animals showed central and peripheral action on fat metabolism by inducing weight loss and decreasing food intake (KUNOS, 2007; PAGOTTO et al., 2006). This CB1 receptor antagonist was recently approved for anti-obesity treatment but withdrawn from the market due to adverse psychiatric effects such as anxiety and depression (CHRISTENSEN et al., 2007; DESPRES; GOLAY; SJOSTROM, 2005; DESPRES et al., 2009; NISSEN et al., 2008; PI-SUNYER et al., 2006; ROSENSTOCK et al., 2008; RUCKER et al., 2007; SCHEEN et al., 2006; VAN GAAL et al., 2008a; VAN GAAL et al., 2005). However, participants' inclusion with previous depression history and other significant psychiatric disease may have contributed to the observed adverse effects (MOREIRA; CRIPPA, 2009). Rimonabant was also investigated for other therapeutic applications, including tobacco (RIGOTTI et al., 2009) and alcohol cessation (GEORGE et al., 2010; SOYKA et al., 2008). Rimonabant did not affect cardiac function (arterial blood pressure, heart rate, renal function and urine albumin / creatinine ratio in humans (ROSENSTOCK et al., 2008).

Several mechanisms may be proposed to increase anxiety and depressive symptoms after rimonabant administration. One hypothesis would be that the endocannabinoid system maintains a neurochemical balance between glutamate and GABA neurotransmission (MOREIRA; LUTZ, 2008). Indeed, blockade of the CB1 receptor could affect neurotransmitter activity by inhibiting GABA and increasing glutamatergic activity (MOREIRA; LUTZ, 2008). Another hypothesis could be the endocannabinoid anandamide activity at CB1 or TRPV1, whereas anandamide activation of CB1 receptors produces

anxiolytic effects while activation of TRPV1 can induce aversive reactions (MOREIRA; CRIPPA, 2009).

In light of this CB1 receptor antagonist action, healthy humans received up to 90 mg rimonabant to evaluate whether blockade of this cannabinoid receptor could mitigate effects of smoked cannabis. Lower rimonabant doses did not significantly affect subjective measurements, while 90 mg rimonabant attenuated subjective effects with no significant adverse effects and no pharmacokinetics interaction with THC (GORELICK et al., 2006; HUESTIS et al., 2001). Conversely, increased anxiety was observed in animals (COMPTON et al., 1996; NAVARRO et al., 1997; RICHARDSON; AANONSEN; HARGREAVES, 1997; SANTUCCI et al., 1996; TERRANOVA et al., 1996). Further studies confirmed the safety of 40 mg rimonabant administration to humans for up to 15 days without significant adverse effects (GORELICK et al., 2006; HUESTIS et al., 2007; HUESTIS et al., 2001).

1.4. Cannabinoid Blood Pharmacokinetics

THC, the main psychoactive constituent of cannabis, is lipophilic (GARRETT; HUNT, 1974; THOMAS; COMPTON; MARTIN, 1990), thermolabile (JOHNSON et al., 1984), and sensitive to oxidation (AGURELL; LEANDER, 1971; FAIRBAIRN; LIEBMANN; ROWAN, 1976). The most common route of cannabis intake is through smoking, although oral dronabinol, CBD capsules, and other routes of administration are also applied (CRIPPA; ZUARDI; HALLAK, 2010; GROTENHERMEN, 2003; HUESTIS, 2007).

THC and metabolites' peak plasma concentration occur approximately 15 min after smoking initiation (HUESTIS; HENNINGFIELD; CONE, 1992; SCHWOPE et al., 2011a). Due to uncertain smoking topography and consequent drug delivery, cannabinoids have low bioavailability around 10-50% (AGURELL et al., 1986; LINDGREN et al., 1981; OHLSSON et al., 1982). Peak THC plasma concentration after oral intake is 1 - 6 h, (OHLSSON et al., 1980) (TIMPONE et al., 1997; WALL et al., 1983) and demonstrate lower bioavailability than smoked route due to first-pass liver metabolism and acid pH in the stomach (GARRETT; HUNT, 1974). Steady-state volume of distribution is 3.4 L/Kg (GROTENHERMEN, 2003; STICHT; KÄFERSTEIN, 1998), with 95-99% plasma THC bound to proteins in plasma (GROTENHERMEN, 2003; HUESTIS, 2005). Extended cannabinoid excretion due to extensive body burden (HUESTIS, 2005) (HARVEY; LEUSCHNER; PATON, 1982; HO et al., 1970; LEUSCHNER et al., 1986) can be observed in plasma and blood for at least seven days of sustained abstinence (KARSCHNER et al., 2009a; KARSCHNER et al., 2009b) and is present in brain when no longer present in blood (MURA et al., 2005).

Hydroxylation and oxidation of THC by cytochrome P450 (CYP) is the main metabolism pathway (MATSUNAGA et al., 1995; NARIMATSU et al., 1992) with about 100 identified metabolites (HARVEY; SAMARA; MECHOULAM, 1991), mainly by CYP2C9, 2C19, and 3A4 (HUESTIS, 2005) and other tissues such as heart and lung (NAKAZAWA; COSTA, 1971; WIDMAN et al., 1975). Phase I hydroxylation forms the psychoactive metabolite 11-hydroxy-THC (11-OH-THC) and subsequent oxidation to non-psychoactive 11-nor-9-carboxy-THC (THCCOOH) metabolite (HUESTIS, 2005). Phase II conjugation of glucuronic acid (and lesser extend of sulfate, glutathione, amino acids, and fatty acids) to THCCOOH increases water solubility, facilitating urinary excretion (BLACKARD; TENNES, 1984). After smoking a 1.75% or 3.55% cannabis cigarette, THC concentration increased rapidly with mean peak concentrations at 8.4 min at 84.3 and 162.2 ng/mL for the low and high doses, respectively. Time of last THC detection was longer for the high doses

than the low doses, detected for maximum of 27 h (HUESTIS; HENNINGFIELD; CONE, 1992).

CBD has a similar pharmacokinetics pattern as THC, with bioavailability ranging from 11-45% (AGURELL et al., 1981; SAMARA; BIALER; MECHOULAM, 1988) and greater volume of distribution (30 L/Kg) than THC (OHLSSON et al., 1984). CBD metabolism mainly occurs through C-9 and side-chain oxidation and also as cyclized THC and cannabinol (AGURELL et al., 1986; HARVEY; MARTIN; PATON, 1978; HARVEY; MECHOULAM, 1990). Besides significant metabolism by liver enzymes (HUESTIS, 2005), a high percentage of free-CBD is excreted in feces (PATON; PERTWEE, 1972; WALL; BRINE; PEREZ-REYES, 1976). CBD can also alter the pharmacokinetics of other drugs by inactivation of CYP 2C 2D6, 3A4 (BORNHEIM; CORREIA, 1991; 1990; JAEGER; BENET; BORNHEIM, 1996; KLEIN et al., 2011; YAMAORI et al., 2011), decreasing 11-OH-THC and THCCOOH concentrations as a result of covalent binding of CBD metabolite to CYP (BORNHEIM et al., 1994). Chronic CBD treatment showed increased CYP 2B activity, inducing drug metabolism. Besides the ability of CBD to affect THC pharmacokinetics (AGURELL et al., 1985; MCARDLE et al., 2001), CBD extracts or concomitant CBD/THC administration at equivalent dose ratio showed no pharmacokinetics interaction (KARSCHNER et al., 2011) or effect on CYP activity (STOTT et al., 2005).

Cannabinoids half-lives are difficult to measure due to large body burden and sustained drug elimination over days (GROTENHERMEN, 2003). In a recent pharmacokinetics study (SCHWOPE et al., 2011a), participants smoked a 6.8% THC cannabis cigarette. Peak THC and CBD whole blood concentrations were 50 and 1.3 ng/mL, respectively, while plasma concentrations were 76 (THC) and 2 ng/mL (CBD) 15 min after starting smoking. Plasma CBD concentrations was detected up to 1 h after smoking, while THC was still detected in plasma specimens 22 h after smoking. Little is known concerning CBD pharmacokinetics after oral administration and its elimination in urine. Peak CBD concentration was achieved 76.3 min. after oral 2.5 mg CBD intake at mean plasma concentration 2.5 ng/mL (GUY; ROBSON, 2003). In another study with CBD oral administration, participants received 300 mg CBD and mean blood concentrations at 1 and 2 h after administration were 4.7±7.0 and 17±29 ng/mL, respectively (FUSAR-POLI et al., 2009).

1.5. Summary

Cannabidiol anxiolytic effects have not been studied in treatment-naïve social anxiety disorder participants during the public speaking simulation task. Furthermore, there are currently no studies regarding the effects on anxiety after administration of the CB1 receptor antagonist (rimonabant) in healthy humans during the public speaking simulation task. This protocol was approved by Clinics Hospital of Ribeirão Preto of University of São Paulo Institutional Review Board and participants provided written informed consent. The results from these studies would improve knowledge of subjective effects during controlled conditions of experimental anxiety and the therapeutic effect of cannabidiol, a *Cannabis sativa* constituent, which would assist further studies focusing on anxiety treatment. In light of the difficulty in social anxiety disorder treatment with low rates of success, discovery of new potential drugs with rapid onset, minimal side effects, and high efficacy are strongly needed.

Whole blood cannabinoid pharmacokinetics has not been determined in chronic heavy cannabis users during sustained abstinence. The protocol was approved by the National Institute on Drug Abuse (NIDA, Baltimore, MD, USA) Institutional Review Board (IRB) and participants provided written informed consent. This research was done through a doctorate exchange between the School of Pharmaceutical Sciences of Ribeirão Preto and NIDA during 2010 and 2011, generously granted by CAPES (Federal Agency of Support and Evaluation of Graduate Education). Previous studies showed cannabinoids were detected in plasma and whole blood for at least a week in chronic cannabis users during sustained abstinence, leading to questions regarding the duration of cannabinoids excretion. This study would provide important information concerning public safety and assist in the development of evidence-based drug policy and legislation.

2. Chapter 2 – CBD, Clonazepam and Rimonabant Effects in Experimentally-Induced Anxiety in Humans

Data presented in the next two chapters (Chapters 3 and 4) were generated through an IRB – approved clinical research protocol (Appendix A) at Clinics Hospital of Ribeirão Preto of University of São Paulo (Ribeirão Preto, SP, Brazil) from April 2010 through October 2010. Clonazepam administration in SAD participants as detailed during IRB submission and consent form was not performed due to the following reasons: a) benzodiazepines are sedative medications and there is a concern about participants' safety when they leave the research unit by themselves; b) benzodiazepines were previously studied in the simulation of public speaking test (GUIMARAES et al., 1989; ZUARDI et al., 1993), showing decreased anxiety levels during all time points and repeating clonazepam group would not be considered original; c) there are few treatment-naïve social anxiety disorder participants available with no comorbidity, once SAD subjects usually have high comorbidity rates. Therefore, a healthy control (HC) group replaced clonazepam group.

2.1. Study Objectives

- Evaluate CBD anxiolytic effect in treatment-naïve social phobia participants submitted to SPST in a double-blind, placebo-controlled and randomized study;
- Evaluate fear of public speaking and physiological effects after CBD administration in treatment-naïve social phobia participants during SPST;
- Evaluate psychological and physiological effects after CB1 receptor antagonist intake in healthy participants subjected to SPST;

2.2. Participants

2.2.1. Social Anxiety Disorder Participants

2.2.1.1. Inclusion Criteria

- Undergraduate or graduate students at least 18 years old;
- Treatment-naïve generalized social anxiety disorder (for pharmacotherapy or psychotherapy);
- Provide written informed consent.

2.2.1.2. Exclusion Criteria

- Other concomitant psychiatric disorders other than social anxiety disorder;
- History of head trauma, neurological illness, electroconvulsive therapy (ECT), substance abuse, or major medical illness;
- Tobacco smokers who received any medications in the three months prior to the study;
- Cannabis consumption more than five times in their lives or in the last year;
- Use of any other illegal drug;
- History of allergy to cannabis or any component presented in the capsule.
- If female, pregnant or nursing;
- Positive urine pregnancy test prior to admission.

2.2.2. Healthy Participants

2.2.2.1. Inclusion Criteria

- Undergraduate or graduate students at least 18 years old;
- Provide written informed consent.

2.2.2.2. Exclusion Criteria

- History of any psychiatric disorder;
- History of head trauma, neurological illness, ECT, substance abuse, or major medical illness;
- Tobacco smokers that received any medications in the three months prior to the study;
- Cannabis consumption more than five times in their lives or in the last year;
- Use of any other illegal drug;
- History of allergy to rimonabant or any component presented in the capsule;
- If female, pregnant or nursing;
- Positive urine pregnancy test prior to admission.

2.3. Methods

2.3.1. Recruitment

Generalized SAD and healthy participants were graduate or undergraduate students from the School of Pharmaceutical Sciences of Ribeirão Preto of University of São Paulo (FCFRP-USP) and other schools from University of São Paulo, Ribeirão Preto *campi* (USP-RP) during 2010. The database from the Psychiatry Department of the Clinical Hospital of Ribeirão Preto was also utilized for recruitment purposes.

Demographic characteristics of SAD and healthy participants were evaluated by the Critério de Classificação Sócio-Econômica Brasil (CCSEB; Appendix B) that includes 10 Likert scale items and was scored from 0 to 5. The education level was added to the final score, with total score classified from classes A through E. Minor modifications were performed to update the following questions: mp3 player was grouped with radio and DVD/Blue-ray player was grouped with VHS player.

Formal authorization from the director of FCFRP-USP was obtained prior to screening (Appendix C). Students received a package of self-assessment diagnostic instruments, as detailed in the next section, after classes or during the class break.

2.3.2. Screening Procedure

2.3.2.1. Social Anxiety Disorder Participants

Undergraduate and graduate students were screened by self-assessment diagnostic instruments. Social Phobia Inventory (SPIN; Appendix D) is composed of 17 items with Likert scales from 0 to 4, with a total score from 0 to 68 (CONNOR et al., 2000; OSORIO; CRIPPA; LOUREIRO, 2009). MINI-SPIN is a short version of the SPIN scale (CONNOR et al., 2001; OSORIO; CRIPPA; LOUREIRO, 2010) including 3 SPINs items (6, 9, 15) with high sensitivity and specificity for detection of SAD (CONNOR et al., 2001; DE LIMA OSORIO; CRIPPA; LOUREIRO, 2007). Participants who scored a minimum of six points in the three items in the MINI-SPIN were further screened for SAD confirmation.

The Fast Alcohol Screening Test (FAST; Appendix E), a short version of the Alcohol Use Disorders Identification Test scale (AUDIT) including 4 Likert scale items, was used to exclude participants with a total score greater than 3, due to possible alcohol addiction or abuse (HODGSON et al., 2002; MENESES-GAYA et al., 2010).

The Patient Health Questionnaire-9 (PHQ-9; Appendix F) including 9 Likert scale items with scores from 0 to 3, screens the frequency of depressive symptoms over the past 2 weeks. Participants with a total score greater than 3 were excluded from screening, as there is a possibility to have depressive disorder (DE LIMA OSORIO et al., 2009; LOWE et al., 2004).

Participants who fulfilled screening SAD criteria were contacted by telephone in order to respond to the general revision and the social anxiety module of the Structured Clinical Interview for the DSM-IV, clinical version [SCID-CV (FIRST et al., 1997), translated into Portuguese (DEL-BEN et al., 2001)]. Following the telephone interview, subjects were randomly invited to attend an interview for diagnosis confirmation through the full SCID-CV, applied by two examiners familiar with the instrument [the Kappa coefficient between the two interviewers was 0.84 (CRIPPA et al., 2008b)].

2.3.2.2. Healthy Participants

Undergraduate and graduate students were screened by the self-assessment diagnostic instrument. The Beck Anxiety Inventory (BAI; Appendix G) is a self-assessment diagnostic instrument that measures anxiety levels with a Likert scale from 0 to 3 (BECK et al., 1988;

CUNHA, 2001). It is composed of 21 items based on common physiological and psychological symptoms related to anxiety. Participants with minimum anxiety levels (maximum score of 10) were allocated to the next step of the screening process.

The FAST and PHQ-9 were utilized to exclude participants with possible alcohol addiction (or abuse) and depressive symptoms, respectively, with same criteria as previously described for SAD participants.

The Self-Reporting Questionnaire (SRQ-24; Appendix H) includes 30 questions, with 24 questions for screening of psychotic and non-psychotic mental disorders, 1 for tonic-clonic seizure screening and 5 for alcohol abuse screening. The six questions regarding tonic-clonic seizure and alcohol abuse were not included in this questionnaire due to low sensitivity. Each 'yes' response is scored 1 and a 'no' response is 0. Subjects with total scores equal to or greater than 6 were excluded due to the probability of a psychiatric disorder (GONCALVES; STEIN; KAPCZINSKI, 2008; WHO, 1993).

Participants who fulfilled screening criteria were contacted by telephone to respond to the general revision and social anxiety modules of the SCID-CV (FIRST et al., 1997), translated into Portuguese (DEL-BEN et al., 2001). Following the telephone interview, subjects were randomly invited to attend an interview for diagnosis confirmation through the full SCID-CV, applied by two examiners familiar with the instrument [the Kappa coefficient between the two interviewers was 0.84 (CRIPPA et al., 2008b)].

2.3.3. Study Design and Consent Process

The study design was split into 2 sections. The first section (Study 1) consisted of CBD administration to SAD participants and HC (no medications). The second section (Study 2) involved CBD and rimonabant administration to healthy participants randomly allocated into 4 groups (placebo/placebo; rimonabant/placebo; placebo/CBD; rimonabant/CBD). Both sections had similar procedures. Participants from the 1st section provided informed consent I (Appendix H) and the 2nd section provided informed consent II (Appendix I).

The SPST procedure consisted of 6 time points with psychological and physiological measurements performed in each phase as detailed in the following section. Upon arrival, participants rested for 15 min and then completed the consent form (Part 1) after discussion and resolution of questions. Subjects received training and instructions to complete self-assessment scales, followed by drug intake. The second part of the consent form was read to

and discussed with the participant and any questions were resolved prior to SPST instruction and anticipatory speech measurements (A).

After completion of the procedures, participants received remuneration and were allowed to leave the research unit after evaluation and no signs of adverse effects. Participants were advised not to drive vehicles within 24 h of drug administration. The researcher called participants within 48 h after procedures for further evaluation and to assure there were no adverse effects associated with study medications.

2.3.3.1. Study 1

SAD patients were randomly assigned to the two groups to receive 600 mg CBD or placebo in a double-blind study design. An equal number of HC performed the test without receiving medication. Groups were matched according to gender, age, years of education and socioeconomic status and the two SAD groups were matched according to the SPIN (CONNOR et al., 2000).

2.3.3.2. Study 2

Healthy participants were randomly allocated to receive either placebo or 90 mg rimonabant in a double blind study design. Groups were matched according to gender, age, years of education and socioeconomic status, body mass index (BMI), SPIN (CONNOR et al., 2000) and BAI (BECK et al., 1988).

2.3.4. Psychological Measurements

The state-anxiety level and other subjective states were evaluated during the test through the Visual Analogue Mood Scale [VAMS; Appendix J (NORRIS, 1971)], translated into Portuguese (ZUARDI; KARNIOL, 1981). In this scale, the subject is told to mark a point that identifies his/her present subjective state on a 100-mm straight line placed between two words that describe opposite mood states. VAMS contains 16 items that Norris (NORRIS, 1971) grouped into four factors. A factorial analysis performed with the Portuguese version of the VAMS also yielded four factors with similar item composition (ZUARDI et al., 1993). The original name of the anxiety factor was preserved, but the names of the remaining factors were changed to fit the meaning of the items with the highest loads in that particular factor.

Thus, the present factors are: (1) anxiety, comprising the items calm–excited, relaxed–tense, and tranquil–troubled; (2) sedation (formerly mental sedation), including the items alert–drowsy, and attentive–dreamy; (3) cognitive impairment (former physical sedation), including quick-witted–mentally slow, proficient–incompetent, energetic–lethargic, clear-headed–fuzzy, gregarious–withdrawn, well-coordinated–clumsy, and strong–feeble; and (4) discomfort (formerly other feelings and attitudes), containing the items interested–bored, happy–sad, contented–discontented, and amicable–antagonistic (PARENTE et al., 2005).

The Self-Statements during Public Speaking Scale (HOFMANN; DIBARTOLO, 2000) (SSPS; Appendix K), translated into Portuguese (OSÓRIO; CRIPPA, 2008), is a self-report instrument that aims to measure the self-perception of performance in the specific situation of public speaking. It is based upon cognitive theories that propose social anxiety is the result of a negative perception of oneself and of others towards oneself. The scale is comprised of 10 items, rated on a Likert scale from 0 (strongly disagree) to 5 (strongly agree) that are organized into two subscales of five items each, for positive or negative self-evaluation. In this study, we applied the negative self-evaluation subscale (SSPS-N).

The Bodily Symptoms Scale (BSS; Appendix L) was designed to detect physical symptoms that can indirectly influence anxiety measures (ZUARDI et al., 1993). It is organized into 21 items and the intensity of each symptom is rated from 0 (no symptom) to 5 (highest).

2.3.5. Physiological Measurements

The physiological measurements were performed within 5 min on each time point across the procedure. Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) were measured by a multiparametric monitor (Monitor DX 2022, Dixtal, Brazil). Skin conductance level (SCL) and spontaneous fluctuations of skin conductance (SF) were recorded by a computer-controlled module (Contact Precision Instruments, UK).

2.3.6. CBD Preparation

CBD (600 mg) in powder, ~99.9% pure (kindly supplied by STI-Pharm, Brentwood, UK and THC-Pharm, Frankfurt, Germany), was dissolved in corn oil (CRIPPA et al., 2004; ZUARDI et al., 1993). The same amount of corn oil was included as placebo. The drug and placebo were packed inside identical gelatin capsules. The 600 mg CBD dose was based on

the fact that acute anxiolytic effects were observed in HC with doses ranging from 300 (ZUARDI et al., 1993) to 600 mg (FUSAR-POLI et al., 2010; FUSAR-POLI et al., 2009). Time of assessment after the procedure was based on previous studies showing peak plasma CBD concentrations 1–2 h after ingestion (AGURELL et al., 1981; BORGWARDT et al., 2008; CRIPPA et al., 2011; CRIPPA et al., 2004; CRIPPA; ZUARDI; HALLAK, 2010; FUSAR-POLI et al., 2010; FUSAR-POLI et al., 2009).

2.3.7. Rimonabant Preparation

Rimonabant (90 mg; Acomplia®, Sanofi-Aventis, Brazil) was administered inside gelatin capsules. The same amount of wheat flour was used as placebo and packed into identical gelatin capsules. The 90 mg dose was selected based on previous studies demonstrating that this was the minimum required dose to block acutely the CB1 receptor (Huestis, Gorelick et al. 2001; Gorelick, Heishman et al. 2006). Peak rimonabant concentrations occurred 2 h after ingestion.

2.3.8. Study Procedures

Procedures were in accordance with the ethical principles of the Declaration of Helsinki and good clinical practice according to the Ministry of Health (C.N.S. Resolução n° 196 de 10/10/96). The SPST was the same as used by McNair *et al* (MCNAIR et al., 1982) with some modifications (GUIMARAES; ZUARDI; GRAEFF, 1987; HALLAK et al., 2010a). Experiments were performed under the supervision of a pharmacist, nurse, and psychiatrist.

2.3.8.1. Study 1

Subjects were told to have breakfast 2 h prior to the session and to avoid alcoholic drinks 24 h prior to procedures. The experimental session was conducted in a sound-attenuated and temperature-controlled room beginning at 0800. After a 15 min adaptation period, baseline measurements (B) were taken followed by a single oral 600 mg or placebo CBD dose in a double-blind procedure. Pre-stress measurements (P) were made 80 min after drug ingestion. Immediately thereafter, the subject received instructions and had 2 min to prepare a 4-min speech about ‘the public transportation system of your city’. He/she also was

told that the speech would be recorded on videotape and later analyzed by a psychologist. Anticipatory speech measurements (A) were taken before the subject started speaking. Thus, the subject started speaking in front of the camera while viewing his/her own image on the TV screen. The speech was interrupted in the middle and speech performance measurements (S) were taken. The speech was recorded for an additional 2 min. Post-stress measurements (F1 and F2) were made 15 and 35 min after the end of the speech, respectively (Appendix N).

2.3.8.2. Study 2

Subjects were told to have breakfast 2 h prior to the session and to avoid alcoholic drinks 24 h prior to procedures. The experimental session was conducted in a sound-attenuated and temperature-controlled room beginning at 0800. After a 15 min adaptation period and intravenous catheter placement, baseline measurements (B) were taken followed by a single 90 mg rimonabant or placebo dose in a double-blind randomized procedure. Subjects received another 600 mg CBD or placebo dose 40 min later. Pre-stress measurements (P) were made 2 h after the first drug ingestion. Immediately thereafter, the subject received instructions and had 2 min to prepare a 4-min speech about "the public transportation system of your city". He/she also was told that the speech would be recorded on videotape and later analyzed by a psychologist. Anticipatory speech measurements (A) were taken before the subject started speaking. The subject started speaking in front of the camera while viewing his/her own image on the TV screen. The speech was interrupted in the middle and speech performance measurements (S) were taken. The speech was recorded for an additional 2 min. Post-stress measurements (F1 and F2) were made 15 and 35 min after the end of the speech, respectively. Peripheral venous whole blood was collected along with physiological and psychological measurements (-0.25h, 2h, 2.23h, 2.58h, 3.h, 3.5h). Blood was collected in 4 mL green-top (sodium heparin) Vacutainer® tubes through an intravenous catheter, stored on ice, and centrifuged within 5 h to separate plasma (Appendix O).

2.4. Risks and Discomforts

2.4.1. Adverse Events Associated with Cannabidiol

Cannabidiol is one of more than 400 components present in *Cannabis sativa* (SEAMON et al., 2007), but it lacks the typical psychoactive effects of THC observed in

humans (ZUARDI et al., 2006a). CBD was previously administered in high doses up to 1,500 mg/day to humans without significant reported adverse events (BERGAMASCHI et al., 2011b).

2.4.2. Adverse Events Associated with Rimonabant

Rimonabant was previously evaluated for obesity and weight loss treatment and was withdrawn from the market due to psychiatric side effects such as anxiety and depression at a 20mg/day dose (MOREIRA; CRIPPA, 2009). However, no significant psychological or physiological side effects were observed in healthy cannabis smokers (GORELICK et al., 2006; HUESTIS et al., 2001). The following side effects may occur: sleepiness, sedation, fatigue, dizziness, nausea, vomiting, decreased attention, depression, irritability, and/or anxiety (VAN GAAL et al., 2008a). These symptoms, when reported, were mild and disappeared within a few minutes.

2.4.3. Adverse Events Associated with Study Measures

There is no health risk associated with preparing or giving a speech. Participants could talk and discuss with researchers at any time about discomfort during the experimental session. If a participant felt uncomfortable, he/she could interrupt the study with no prejudice for withdrawing.

2.5. Statistical Analyses

VAMS, SSPS-N, BSS, arterial/diastolic/and systolic pressures, heart rate, skin conductance, and total number of spontaneous skin conductance fluctuations were transformed by calculating the difference between the score in each phase and the pre-stress score in the same volunteer. For statistical analysis, skin conductance values were converted into the natural logarithm (logn). These psychological and physiological effects were submitted to repeated-measures analyses of variance (ANOVA) to assess the effect of time on each outcome, followed by *post-hoc* tests. The significance level adopted was two-tailed $p < 0.05$.

2.6. Study Benefits

Study enrollment yielded generalizable knowledge regarding the psychophysiology of anxiety *per se* and anxiety disorders, with a prospective benefit for social anxiety disorder treatment.

2.7. Participant Remuneration

Participants were compensated R$ 40 for time, food, and travel expenses to the research unit.

3. Chapter 3 – Cannabidiol Reduces Anxiety Induced by Simulated Public Speaking in Treatment-Naïve Social Phobia Patients

3.1. Abstract

Generalized SAD is one of the most common anxiety conditions impairing an individual's social life. CBD, a major non-psychotomimetic compound in *Cannabis sativa*, showed anxiolytic effects in humans and animals. This preliminary study aimed to compare the effects of a SPST on HC and treatment-naïve SAD subjects who received a single CBD or placebo dose. Twenty-four never-treated SAD subjects were allocated to receive either 600 mg CBD (n=12) or placebo (n=12) in a double blind randomized design 1.5 h before the test. The same number of HC (n=12) performed the SPST without receiving any medication. Each volunteer participated in only one experimental session in a double-blind procedure. Subjective ratings on the VAMS and SSPS-N and physiological measures (blood pressure, heart rate, and skin conductance) were measured at six different time points during the SPST. The results were submitted to a repeated-measures analysis of variance. Pretreatment with CBD significantly reduced anxiety, cognitive impairment, and discomfort in speech performance and significantly decreased alertness in their anticipatory speech. The placebo group displayed higher anxiety, cognitive impairment, discomfort, and alertness levels when compared with the control group as assessed by the VAMS. The SSPS-N scores showed significant increases during testing of the placebo group that were almost abolished in the CBD group. No significant differences were observed between the CBD and HC groups in SSPS-N scores or in cognitive impairment, discomfort, and alertness factors of the VAMS. The increase in anxiety induced by the SPST in subjects with SAD was reduced by CBD, yielding similar responses as for HC.

3.2. Introduction

SAD is one of the most common anxiety conditions and is associated with impairment in social adjustment to the usual aspects of daily life, increased disability, dysfunction, and a loss of productivity (FILHO et al., 2010; KESSLER, 2007). SAD tends to follow a long-term and unremitting course and is rarely resolved without treatment (CHAGAS et al., 2010; CRIPPA et al., 2007). The pharmacological management of SAD remains problematic, despite several guidelines or consensus statements issued over the past few years (CANADIAN PSYCHIATRIC ASSOCIATION, 2006; MONTGOMERY et al., 2004). As this anxiety disorder is often poorly controlled by currently available drugs [only about 30% of subjects achieve true recovery or remission without residual symptomatology (BLANCO; ANTIA; LIEBOWITZ, 2002)], there is a clear need to search for novel therapeutic agents.

Subjects with SAD appear more likely to smoke cannabis than those without other anxiety disorders to 'self-medicate' anxiety reactions (BUCKNER et al., 2008). However, the relationship between cannabis and anxiety is paradoxical. Cannabis smokers reported reduction in anxiety as one of the motivations for its use; on the other hand, episodes of intense anxiety or panic are among the most common undesirable effects of the drug (CRIPPA et al., 2009). These apparently conflicting statements may partly reflect the fact that low doses of the best-known constituent of the plant, THC, engender anxiolytic-like effects whereas higher doses produce anxiogenic reactions (CRIPPA et al., 2009).

Moreover, other plant components can influence THC's pharmacological activity; in particular, CBD, the major non-psychotomimetic compound in the plant, has psychological effects substantially different from those of THC (ZUARDI, 2008). Oral administration of CBD to healthy volunteers was shown to attenuate the anxiogenic effect of THC and did not involve pharmacokinetic interactions (ZUARDI et al., 1982). In animal studies, CBD has similar effects to anxiolytic drugs in different paradigms, including conditioned emotional response, the Vogel conflict test, and the elevated plus-maze test (ZUARDI, 2008). Previous human studies showed CBD's anxiolytic effects in subjects subjected to the SPST (ZUARDI et al., 1993). Functional neuroimaging in healthy volunteers demonstrated that CBD has anxiolytic properties and that these effects are associated with limbic and paralimbic brain areas (CRIPPA et al., 2004; FUSAR-POLI et al., 2010).

Recently, Crippa *et al* (CRIPPA et al., 2011; CRIPPA; ZUARDI; HALLAK, 2010) investigated CBD's central effects on regional cerebral blood flow (rCBF), using SPECT in patients with SAD. Relative to placebo, CBD was associated with significant decreases in

subjective anxiety induced by the SPECT procedure and modulated the same brain areas as in healthy volunteers.

The data reviewed above led to the hypothesis that CBD may be an effective compound in the treatment of SAD symptoms. As a first step to investigate this hypothesis, we used the SPST, an experimental model for the induction of anxiety. SPST has apparent and predictive validity for SAD because the fear of public speaking is a cardinal SAD manifestation and there is pharmacological evidence that the response pattern to some substances in the SPST is similar to the clinical response presented by SAD patients (BRUNELLO et al., 2000; GRAEFF et al., 2003). In this preliminary study, we aimed to measure the subjective and physiological effects of SPST in HC and in treatment-naïve SAD patients who received a single CBD or placebo dose according to a double-blind design. Due to ethical and economic constraints, we decided on a single CBD dose as a first step in the investigation of a possible anxiolytic action of this cannabinoid in subjects with pathological anxiety. For instance, it is important to confirm whether CBD has the advantage of a rapid onset of action, making it particularly suitable for individuals who have episodic performance-related social phobia and who are able to predict the need for treatment in advance. Considering previous results from a single CBD dose, it is expected that this cannabinoid will reduce the level of fear provoked by the SPST.

3.3. Methods

3.3.1. Subjects

A total of 24 subjects with generalized SAD and 12 HC subjects were selected by the screening procedure described below. SAD subjects were randomly assigned to the two groups with 12 subjects each to receive CBD (600 mg—SAD-CBD) or placebo (SAD-PLAC), in a double-blind study design. To ensure adequacy of the matching procedure, the first participant had his/her treatment chosen blindly; the next participant (whose characteristics were matched to those of the first participant) received the other treatment. An equal number of HC (n=12) performed the test without medication. Groups were matched according to gender, age, years of education, and socioeconomic status. Moreover, the two SAD groups were balanced according to the SPIN (CONNOR et al., 2000). All participants were treatment-naïve for previous pharmacotherapy or psychotherapy and did not present any other concomitant psychiatric disorder. No subject had a history of head trauma, neurological illness, ECT, substance abuse, or major medical illness, based on a semi-standardized medical questionnaire and physical examination. They were all non-tobacco smokers and had no medications for at least 3 months prior to the study. No subjects smoked cannabis more than five times in their lives and had no use in the last year, and none had ever used any other illegal drug. All subjects gave written informed consent after being fully informed about the research procedure to enroll in this ethics committee-approved protocol (HCRP No. 12407/2009).

3.3.2. Screening Procedure and Clinical Assessment

As an initial step, 2319 undergraduate students were screened by a self-assessment diagnostic instrument, the MINI-SPIN (CONNOR et al., 2001; OSORIO; CRIPPA; LOUREIRO, 2010). This led to the identification of subjects with probable SAD, who scored a minimum of six points in the three items that compose the MINI-SPIN. Using this cut-off score, the MINI-SPIN was previously shown to provide high sensitivity and specificity for SAD detection (CONNOR et al., 2001; DE LIMA OSORIO; CRIPPA; LOUREIRO, 2007). A total of 237 subjects with a positive MINI-SPIN and an equal number of subjects with zero points in the three items that compose this instrument were contacted by telephone in order to respond to the general revision and the social anxiety module of the SCID-CV [(FIRST et al.,

1997), translated into Portuguese (DEL-BEN et al., 2001)]. The volunteers who fulfilled SAD criteria and scored ‘very much’ or ‘extremely’ in the 11th item of SPIN (avoids speeches) and those who fulfilled the HC criteria were randomly invited to attend an interview for diagnosis confirmation through the full SCID-CV, applied by two examiners familiar with the instrument [the Kappa coefficient between the two interviewers was 0.84 (CRIPPA et al., 2008b)].

3.3.3. CBD Preparation

CBD (600 mg) in powder, ~99.9% pure (kindly supplied by STI-Pharm, Brentwood, UK and THC-Pharm, Frankfurt, Germany), was dissolved in corn oil (CRIPPA et al., 2004; ZUARDI et al., 1993). The same amount of corn oil was included as placebo. The drug and placebo were inside identical gelatin capsules. The 600 mg CBD dose was based on the fact that acute anxiolytic effects were observed in HC with doses ranging from 300 (ZUARDI et al., 1993) to 600 mg (FUSAR-POLI et al., 2010; FUSAR-POLI et al., 2009). Although we recently observed that 400 mg CBD significantly decreased subjective anxiety induced by the SPECT procedure in SAD subjects, the SPST has face validity for SAD and the fear of speaking in public is considered to be the most stressful situation in this condition, in contrast with the neuroimaging procedure. Therefore, we decided to administer the highest CBD dose previously found to have anxiolytic effects. The time of assessment after the procedure was chosen based on previous studies demonstrating that the peak oral plasma CBD dose usually occurs 1–2 h after ingestion (AGURELL et al., 1981; BORGWARDT et al., 2008; CRIPPA et al., 2011; CRIPPA et al., 2004; CRIPPA; ZUARDI; HALLAK, 2010; FUSAR-POLI et al., 2010; FUSAR-POLI et al., 2009).

3.3.4. Psychological Measurements

The state-anxiety level and other subjective states were evaluated with the VAMS (NORRIS, 1971), translated into Portuguese (ZUARDI; KARNIOL, 1981). In this scale, the subject is told to mark a point that identifies his/her present subjective state on a 100-mm straight line placed between two words that describe opposite mood states. VAMS contains 16 items that Norris grouped into four factors. A factorial analysis performed with the Portuguese version of the VAMS also yielded four factors with similar item composition (ZUARDI et al., 1993). The original name of the anxiety factor was preserved, but the names

of the remaining factors were changed to fit the meaning of the items with the highest loads in that particular factor. Thus, the present factors are: (1) anxiety, comprising the items calm–excited, relaxed–tense, and tranquil–troubled; (2) sedation (formerly mental sedation), including the items alert–drowsy, and attentive–dreamy; (3) cognitive impairment (formerly physical sedation), including quick-witted–mentally slow, proficient–incompetent, energetic–lethargic, clear-headed–fuzzy, gregarious–withdrawn, well-coordinated–clumsy, and strong–feeble; and (4) discomfort (formerly other feelings and attitudes), made of the items interested–bored, happy–sad, contented–discontented, and amicable–antagonistic (PARENTE et al., 2005).

The SSPS (HOFMANN; DIBARTOLO, 2000), translated into Portuguese (OSÓRIO; CRIPPA, 2008), is a self-report instrument that measures the self-perception of performance in the specific situation of public speaking. It is based upon cognitive theories that propose social anxiety is the result of a negative perception of oneself and of others towards oneself. The scale is comprised of 10 items, rated on a Likert scale from 0 (strongly disagree) to 5 (strongly agree) that are organized into two subscales of five items each for positive or negative self-evaluation. In this study, we applied the negative self-evaluation subscale (SSPS-N).

The BSS was designed to detect physical symptoms that can, indirectly, influence anxiety measures (ZUARDI et al., 1993). It is organized into 21 items and the intensity of each symptom is rated from 0 (no symptom) to 5 (highest).

3.3.5. Physiological Measurements

3.3.5.1. Skin Conductance

A computer-controlled, voltage-constant (0.6 V) module with automatic back off (Contact Precision Instruments, UK) measured skin conductance. Two electrodes (Beckman, UK) were fixed with adhesive tape. Contact with the skin was made through high conductance gel (KY gel, Johnson and Johnson, Brazil). The SCL and SF were recorded.

3.3.5.2. Arterial Blood Pressure

The SBP and DBP were measured by a mercury sphygmomanometer (Becton Dickinson, Brazil).

3.3.5.3. Heart Rate

The HR was estimated by manually counting the pulse.

3.3.6. Procedure

The SPST was the same as described by McNair *et al* (MCNAIR *et al.*, 1982) with some modifications (HALLAK et al., 2010a).

The procedure is summarized in Table 1. After a 15-min adaptation period, baseline measurements (B) were taken and followed by a single oral CBD or placebo dose in a double-blind procedure. Pre-stress measurements (P) were made 80 min after drug ingestion. Immediately thereafter, subjects received the instructions and had 2 min to prepare a 4-min speech about 'the public transportation system of your city'. He/she also was told that the speech would be recorded on videotape and later analyzed by a psychologist. Anticipatory speech measurements (A) were taken before the subject started speaking. Subject started speaking in front of the camera while viewing his/her own image on the TV screen. The speech was interrupted in the middle and speech performance measurements (S) were taken. The speech was recorded for a further 2 min. Post-stress measurements (F1 and F2) were made 15 and 35 min after the end of the speech, respectively.

Table 1 – Timetable of the experimental session.

SESSION (H:MM)	PHASE	PROCEDURE
- 0:30		Adaptation to the laboratory; Instructions about the interview and measurements
- 0:15	Baseline (B)	SCL, SF, HR, AP, VAMS, SSPS, BSS
0		Drug intake: CBD or placebo capsules
+ 1:20	Pre-stress (P)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 1:30		Instructions about the SPST
+ 1:32		Speech preparation
+ 1:34	Anticipatory speech (A)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 1:45		Start of speech
+ 1:47	Speech performance (S)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 1:53		Continuation of speech
+ 1:55		End of speech
+ 2:10	Post-stress 1 (F1)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 2:30	Post-stress 2 (F2)	SCL, SF, HR, AP, VAMS, SSPS, BSS

Abbreviations: SCL, skin conductance level; SF, spontaneous fluctuations of the skin conductance; HR, heart rate; AP, arterial blood pressure; VAMS, visual analogue mood scale; SSPS, self-statements during public speaking; BSS, bodily symptoms scale.

3.3.7. Statistical Analysis

Clinical and demographical characteristics were analyzed with non-parametric tests (gender and socioeconomic level) and with ANOVA, followed by *post-hoc* Bonferroni's test for multiple comparisons (age, age of SAD onset and SPIN).

Scores of VAMS's factors, SSPS-N, BSS, arterial/diastolic/systolic pressure, and heart rate, as well as the SCL and total number of SF, were transformed by calculating the difference between the score in each phase and the pre-stress scores in the same volunteer. For the analysis, SCL values were converted into logn. These delta scores were submitted to a repeated-measures ANOVA, analyzing the factors of phase, group, and phase by group-' interaction. In the case where sphericity conditions were not reached, the degrees of freedom of the repeated factor were corrected with the Huynh–Feldt epsilon. Whenever a significant phase by group interaction occurred, comparisons among the groups were made at each phase

using a one-factor ANOVA followed by multiple comparisons with the Bonferroni's test. Data analysis was performed using the SPSS-17 program, and the significance level adopted was $p<0.05$.

3.4. Results

3.4.1. Subjects

The clinical and demographical characteristics of the subjects are shown in Table 2. The only significant differences among groups were found in the mean scores of SPIN ($F_{2, 35}$=34.3; p<0.001). The SPIN scores were significantly lower in healthy volunteers than in subjects with SAD who received CBD or placebo. No significant difference was observed between the two groups with SAD.

Table 2 – Clinical and demographic characteristic of the groups.

	SAD-placebo	SAD-CBD	Healthy	*p*
Male/female	6/6	6/6	6/6	1.0
Age [mean (SD)]	22.9 (2.4)	24.6 (3.6)	23.3 (1.7)	0.36
Socioeconomic level [a] [Median]	2	2.5	2	0.66
Age of SAD onset [mean (SD)]	12.2 (5.8)	9.6 (6.9)	–	0.36
SPIN [mean (SD)]	36.3 (11.2)	30.9 (12.0)	5.75 (3.3)	<0.001

Abbreviations: SAD, social anxiety disorder; SD, standard deviation; SPIN, social phobia inventory

[a] Socioeconomic level was assessed by the Brazil socioeconomic classification criteria

3.4.2. Psychological Measures

No differences were observed among the initial measures of the three groups on anxiety ($F_{2,35}$=1.4; p=0.27), sedation ($F_{2,35}$=0.4; p=0.70), cognitive impairment ($F_{2,35}$=1.9; p=0.16), and discomfort ($F_{2,35}$=0.6; p=0.55) VAMS factors. Changes in relation to the pre-stress phase of VAMS factors in the three groups are shown in Figure 1.

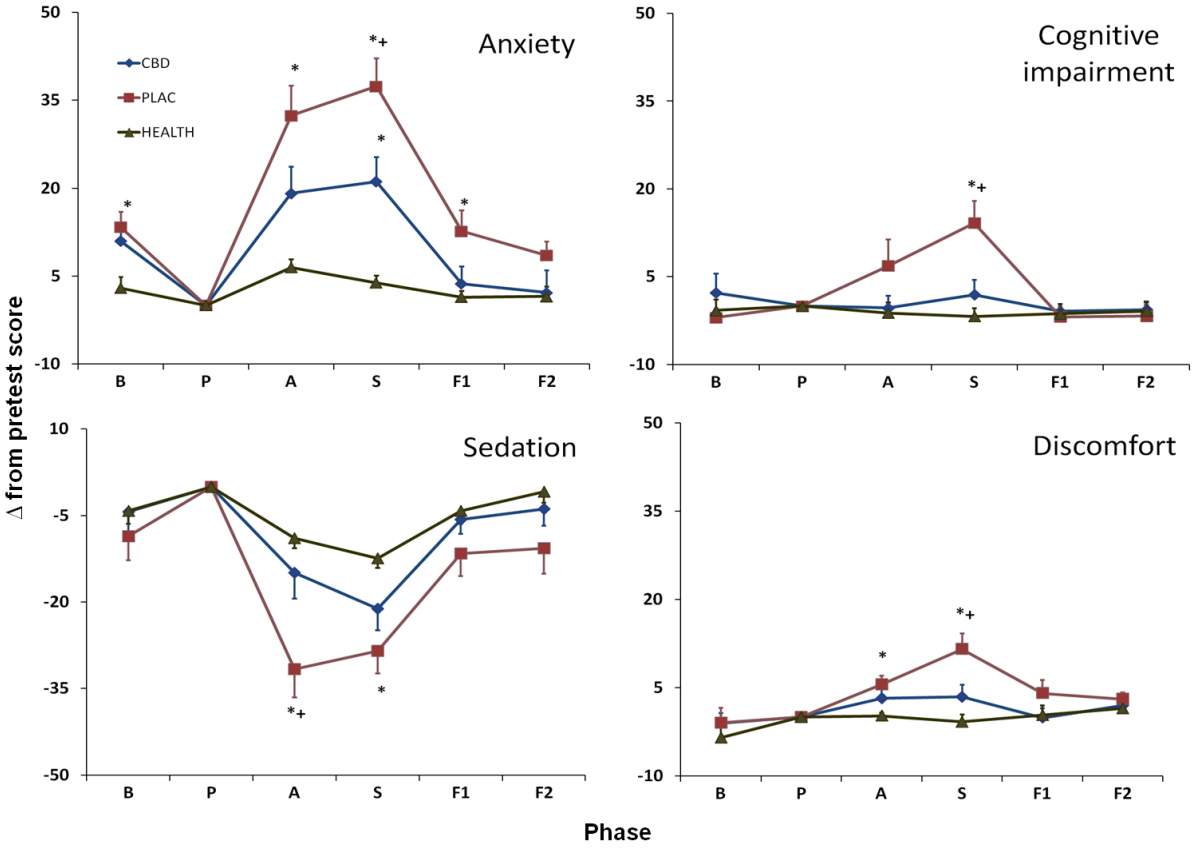

Figure 1 – Changes in VAMS factors induced by simulated public speaking test (SPST). The phases of the experimental session are: B, baseline; P, pre-stress; A, anticipatory speech; S, speech performance; F1, post-stress 1; F2, post-stress 2. Points indicate mean and vertical bars standard error of the mean. * indicates significant differences from healthy control and + from social anxiety subjects who received cannabidiol ($p<0.05$).

Regarding the VAMS anxiety factor, the repeated-measures ANOVA showed a significant effect of phase ($F_{3.6,118.5}=32.7$; $p<0.001$), group ($F_{2,33}=13.5$; $p<0.001$) and phase by group interaction ($F_{7.2,118.5}=6.4$; $p<0.001$). Comparisons among groups evidenced significant differences between SAD-PLAC and HC at the initial ($p=0.018$), anticipatory ($p<0.001$), speech ($p<0.001$) and post-stress ($p=0.018$) phases. The SAD-CBD differs from the SAD-PLAC ($p=0.012$) and HC ($p=0.007$) during the speech phase. Regarding cognitive impairment, repeated-measures ANOVA showed a significant effect of phase ($F_{3.2,105.8}=5.6$; $p=0.001$) and phase by group interaction ($F_{6.4,105.8}=5.1$; $p<0.001$). Comparisons among groups evidenced that SAD-PLAC differed significantly from SAD-CBD ($p=0.009$) and HC ($p=0.001$) at the speech phase. Regarding discomfort, there are significant effects by phase ($F_{4,132}=7.1$; $p<0.001$), group ($F_{2,33}=4.7$; $p=0.016$) and phase by group interaction ($F_{4,132}=2.2$; $p=0.036$). Comparisons among groups evidenced that SAD-PLAC differed significantly from HC at the anticipatory phase ($p=0.047$) and from SAD-CBD ($p=0.029$) and HC ($p=0.001$) at speech phase. On the sedation factor, there are significant effects of phase ($F_{3.1,102.1}=27.1$;

p<0.001), group ($F_{2,33}$=5.3; p=0.010) and phase by group interaction ($F_{6.2,102.1}$=2.4; p=0.032). Comparisons among groups evidenced that SAD-PLAC differed significantly from SAD-CBD (p=0.016) and HC (p=0.001) at the anticipatory phase and from HC at speech phase (p=0.005).

SSPS-N scores at the initial phase differed significantly among groups ($F_{2,35}$=14.8; p<0.001), with the SAS-PLAC and SAD-CBD groups higher than HC (p<0.001). Changes in relation to the pre-stress phase of SSPS-N in the three groups are shown in Figure 2. The repeated-measures ANOVA showed a significant effect of phase ($F_{3.1,101.6}$=9.7; p<0.001), group ($F_{2,33}$=6.6; p=0.004) and phase by group interaction ($F_{6.2,101.6}$=3.2; p=0.006). Comparisons among groups evidenced significant differences between SAD-PLAC and SAD-CBD at the anticipatory (p=0.043) and speech (p=0.001) phases and between SAD-PLAC and HC at the speech (p<0.001) phase. No significant differences were observed between SAD-CBD and HC.

No differences were observed among the initial measures of the three groups on BSS ($F_{2,35}$=1.4; p=0.25), as shown in Figure 2. Changes in relation to the pre-stress phase of BSS in the three groups showed a significant phases effect ($F_{3.3,110.2}$=8.1; p<0.001) and phase by group interaction ($F_{6.7,110.2}$=2.3; p=0.035). Comparisons among groups evidenced significant differences between SAD-PLAC and HC at the speech phase (p=0.05). In this phase, changes in relation to the pre-stress phase were 8.2 for SAD-PLAC and 0.3 for HC. The SAD-CBD group had an intermediate score, which did not differ from SAD-PLAC or HC.

The observed power for the tests used in the statistical analysis of the anxiety VAMS factor and in the negative SSPS, was 0.996 and 0.881, respectively.

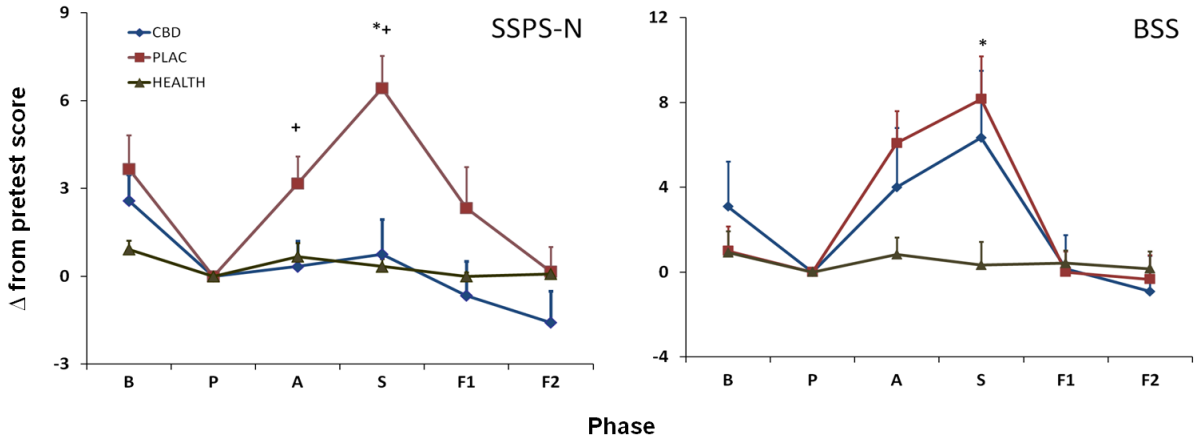

Figure 2 – Changes in Negative Self-Statement during Public Speaking scale (SSPS-N) and Bodily Symptoms Scale (BSS) induced by simulated public speaking test. The phases of the experimental session are: B, baseline; P, pre-stress; A, anticipatory speech; S, speech

performance; F1, post-stress 1; F2, post-stress 2. Points indicate mean and vertical bars standard error of the mean. * indicates significant differences from healthy controls and + from social anxiety subjects who received cannabidiol ($p<0.05$).

3.4.3. Physiological Measures

Systolic pressure ($F_{2,35}=1.2$; $p=0.33$), diastolic pressure ($F_{2,35}=1.7$; $p=0.20$), heart rate ($F_{2,35}=0.4$; $p=0.67$), SCL ($F_{2,5}=1.6$; $p=0.22$), and SF ($F_{2,35}=0.1$; $p=0.90$) did not show significant differences among the three groups in the initial measures. Changes in relation to the pre-stress showed significant repeated-measures ANOVA effect only in phase for the following physiological measures: systolic pressure ($F_{3.7,122.5}=5.9$; $p<0.001$), diastolic pressure ($F_{4,132}=5.1$; $p<0.001$), SCL ($F_{3.2,84.9}=2.8$; $p=0.045$), and SF ($F_{3.6,92.4}=3.8$; $p=0.009$). In these measures, values were significantly elevated during SPS without differences among the groups. For heart rate, repeated-measures ANOVA showed a significant effect of phase ($F_{3.9,127.1}=6.9$; $p<0.001$) and phase by group interaction ($F_{7.7,127.1}=4.6$; $p<0.001$). Comparisons among groups showed a reduction in heart rate from the initial to the pre-stress measures, significantly greater ($p<0.001$) for the SAD-PLAC (delta mean ± standard error; 9.17±1.77) than for the HC (0.50±0.56) group. The SAD-CBD group (4.33±1.56) did not differ significantly from the other two groups.

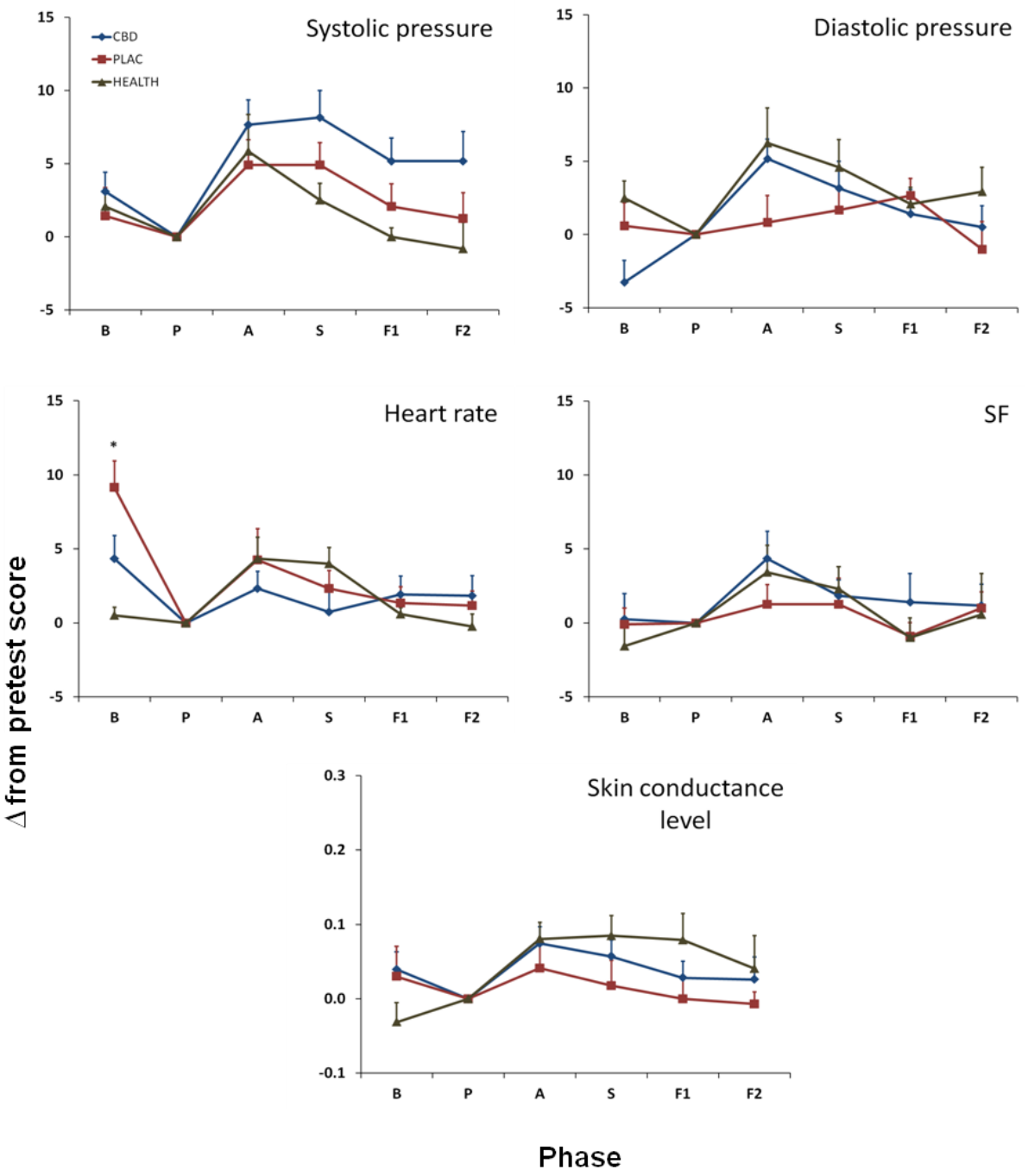

Figure 3 – Changes in systolic and diastolic pressure, heart rate, skin conductance level, and spontaneous fluctuations of skin conductance (SF) induced by simulated public speaking test. The phases of the experimental session are: B, baseline; P, pre-stress; A, anticipatory speech; S, speech performance; F1, post-stress 1; F2, post-stress 2. Points indicate mean and vertical bars standard error of the mean ($p<0.05$).

3.5. Discussion

As observed in another study of SAD subjects' performance on SPST (CRIPPA et al., 2008a), the present results of the VAMS scale showed that the SAD-PLAC group presented a significantly higher anxiety level and greater cognitive impairment, discomfort, and alertness compared with the control group during the test. This was expected as the fear of speaking in public is a cardinal manifestation of SAD (BRUNELLO et al., 2000).

Pretreatment of SAD subjects with CBD significantly reduced anxiety, cognitive impairment, and discomfort in speech performance (S) and significantly decreased alertness in their anticipatory speech (A). The cognitive impairment, discomfort, and alertness of SAD subjects that received CBD had similar results to HC during the SPST. These preliminary results indicate that a single CBD dose can reduce the anxiety-enhancing effect provoked by SPST in SAD subjects, indicating that this cannabinoid inhibits the fear of speaking in public, one of the main symptoms of the disorder.

CBD's anxiolytic effects were extensively demonstrated in animal studies and in healthy volunteers exposed to induced anxiety by several procedures including the simulation of public speaking (CRIPPA et al., 2011; CRIPPA; ZUARDI; HALLAK, 2010). However, there is only one published report of the anxiolytic effect of CBD in an anxiety disorder (CRIPPA et al., 2011; CRIPPA; ZUARDI; HALLAK, 2010). This study was performed with SAD subjects and CBD's anxiolytic effects were detected before provoking anxiety by the tracer injection and scanning procedure of SPECT, suggesting that CBD facilitates habituation of anticipatory anxiety. The SPECT analysis of this study and of a previous one with healthy volunteers (CRIPPA et al., 2004) showed that CBD's effects were associated with parahippocampal gyrus and hippocampus activity. The fMRI detected attenuated responses in the amygdala and in the cingulate cortex induced by 600 mg CBD during the viewing of fearful facial stimuli (FUSAR-POLI et al., 2010). Moreover, CBD disrupted forward intrinsic connectivity between the amygdala and the anterior cingulate during the neural response to fearful faces (FUSAR-POLI et al., 2009). Taken together, these studies demonstrate the CBD activity in limbic and paralimbic brain areas known to be associated with anxiety.

CBD's anxiolytic effect may be mediated by 5-HT_{1A} receptors, as it displaces the agonist [3H]8-OHDPAT from the cloned human 5-HT_{1A} receptor in a concentration-dependent manner and exerts an effect as an agonist at the human 5-HT_{1A} receptor in signal-transduction studies (RUSSO et al., 2005). Additionally, CBD injected into rats' dorsolateral

periaqueductal gray produced anxiolytic-like effects in the elevated plus-maze and elevated T-maze, and these effects were prevented by a 5-HT_{1A} receptor antagonist (CAMPOS; GUIMARAES, 2008; SOARES VDE et al., 2010).

Another important observation of this study was that the increase of negative self-evaluation during public speaking was almost abolished by CBD. In a previous study, we suggested that the negative self-evaluation during the phobic situation of public speaking would be important for the avoidance and impairment in social functioning that support the SAD diagnosis. In that way, the observed CBD's effect for improving self-evaluation during public speaking, one of the pivotal aspects of SAD, will influence the SAD subjects' therapy.

Although physiological measures did not show significant differences among groups, the BSS increased significantly only for the SAD subjects who received placebo during the test. Following the same rationale as above, it is well known that more pronounced bodily symptoms may contribute to the clinical SAD diagnosis, and this result suggests that CBD also protects subjects from subjective physiological abnormalities induced by SPST.

Findings reported herein need to be interpreted with caution given study limitations. First, it would be desirable to measure plasma CBD concentrations and to relate these to changes in VAMS scores; however, it should be pointed out that previous investigations did not confirm whether there is a direct relationship between plasma cannabinoid concentrations, in particular CBD, and clinical effects (AGURELL et al., 1986). Another limitation refers to the sample size; however, the statistical power of the data from the VAMS and SSPS was shown to be relatively robust even with small subject numbers.

An extensive list of medications for the pharmacological treatment of SAD was made available in recent years, including selective serotonin reuptake inhibitors (SSRIs), selective serotonin and norepinephrine reuptake inhibitors (SSNRIs), antidepressants, and benzodiazepines (SCHNEIER, 2001). However, both SSRIs and SSNRIs have an initial activation and a long latency response period and benzodiazepines are limited by their potential to produce motor impairment and sedation and to induce dependence and withdrawal symptoms following discontinuation (BLANCO; ANTIA; LIEBOWITZ, 2002). Conversely, CBD has important advantages in comparison with currently available pharmacological agents for SAD treatment, such as an early onset of action and lack of important side effects both with acute and chronic administration to healthy subjects (CRIPPA et al., 2011; CRIPPA; ZUARDI; HALLAK, 2010). Moreover, repeated treatment with CBD (but not THC) did not result in tolerance or dependence (HAYAKAWA et al., 2007) and possibly reduces drug-seeking behaviors (MORGAN et al., 2010; PARKER et al.,

2004; REN et al., 2009). Thus, because of the absence of psychoactive or cognitive effects, its safety and tolerability profiles, and its broad pharmacological spectrum, CBD is possibly the cannabinoid most likely to have initial correlations in anxiety translated into clinical practice.

Therefore, the effects of a single CBD dose observed in this study in the face of one of the main SAD's phobic stimuli is a promising indication of a rapid onset of therapeutic effect in subjects with SAD. However, randomized, double-blind, placebo-controlled clinical trials with larger samples and chronic use are still needed to confirm these findings. Likewise, because CBD effects are biphasic, determination of adequate treatment ranges for each disorder remains a challenge. Further research to determine the precise mechanisms of CBD action in different anxiety disorders is desirable and opportune.

4. Chapter 4 – Effects of Antagonist CB1 Receptor on Anxiety Induced by Simulated Public Speaking in Healthy Humans

4.1. Abstract

Rimonabant, a cannabinoid receptor type 1 (CB1) antagonist, was developed for obesity and weight loss treatment and later withdrawn due to severe psychiatric side effect, i.e. anxiety and depression. We aimed to assess the SPST effects in healthy subjects after high rimonabant dose administration to understand better the pharmacological approach for anxiety disorder treatment. Twenty four participants were randomly allocated to receive either placebo (n=12) or 90 mg rimonabant (n=12) in a double-blind design. Subjective effects were measured with Visual Analogue Mood Scale, Self-Statements during Public Speaking Scale, and Bodily Symptoms Scale; and physiological measurements with arterial blood pressure and heart rate; made at 6 different time points during SPST. Results were submitted to repeated measures analysis of variance (ANOVA) and groups compared by contrast. No significant adverse effects were reported in both groups. Participants who received rimonabant showed increased anxiety levels compared to placebo during anticipatory speech and performance measurements. Rimonabant treatment did not affect sedation, cognitive impairment, discomfort, blood pressure, heart rate, self-statement during public speaking, and bodily symptoms scales. Increased anxiety may reflect lower endocannabinoid activity in CB1 receptors and its possible role in modulation of anxiety and anxiety disorders.

4.2. Introduction

After CB1 and CB2 discovery in the early 1990s, there was increasing interest to elucidate their pharmacodynamic effects in humans and animals (ZUARDI; CRIPPA; HALLAK, 2010). CB1 receptors are mainly located in the central nervous system, with high expression at hippocampus, amygdala, prefrontal cortex, hypothalamus, and basal ganglia (MACKIE, 2005). THC is the most well known CB1 agonist, which may explain cannabis' psychoactive effects after consumption i.e., motor impairment, amnesia, mood changes, short-term memory, and anxiety (D'SOUZA et al., 2004; PERTWEE, 1997). The discovery of these cannabinoid receptors lead to endocannabinoids' identification (FRIDE; MECHOULAM, 1993), which are integrated to the central and peripheral nervous systems (KUNOS; TAM, 2011; QUARTA et al., 2011), implicated in mood behavior (ASHTON; MOORE, 2011; SAITO; WOTJAK; MOREIRA, 2010) and food intake (DI MARZO; MATIAS, 2005).

As CB1 receptor antagonists were investigated to understand better the physiology of endocannabinoids (MOREIRA; GRIEB; LUTZ, 2009), SR141716 (rimonabant) was developed and later introduced into the market (RINALDI-CARMONA et al., 1994) for obesity and weight loss treatment (DESPRES; GOLAY; SJOSTROM, 2005; PI-SUNYER et al., 2006; SCHEEN et al., 2006; VAN GAAL et al., 2005).

Animal studies first evidenced rimonabant's anxiogenic effect after administration. Acute treatment in rodents with CB1 antagonist showed increased anxiety by elevated-plus maze (NAVARRO et al., 1997; PATEL; HILLARD, 2006), defensive withdrawal (NAVARRO et al., 1997), and increased aversive behavior by paw-shock test (MARSICANO et al., 2002), further supported with clinical trials followed by serious psychiatric side effects reports such as anxiety and depression (CHRISTENSEN et al., 2007; DESPRES; GOLAY; SJOSTROM, 2005; PI-SUNYER et al., 2006; SCHEEN et al., 2006; VAN GAAL et al., 2005). The severity of these side effects lead to drug discontinuation (MOREIRA; CRIPPA, 2009).

Previous studies in humans showed the necessary dose to blockade CB1 receptor is 4.5 times higher than the therapeutic 20 mg dose (GORELICK et al., 2006; HUESTIS et al., 2001). Nonetheless, little is known about the psychological changes implicated with CB1 receptor blockade in healthy humans submitted to controlled conditions of experimental anxiety. Based on previous clinical trial and animals studies, we hypothesized that rimonabant would increase anxiety with no other subjective and physiological side effects. This research focuses to characterize the physiological and psychological effects of 90 mg rimonabant dose

during the simulated public speaking task in healthy humans and to provide better pharmacological approaches for anxiety disorders treatment.

4.3. Methods

4.3.1. Subjects

Healthy participants were selected by the screening procedure described in the next section. Subjects were allocated to receive either placebo (PLAC) or rimonabant (90 mg; RIMO) in a double blind study design. Groups were matched according to gender, age, years of education and socioeconomic status, BMI, SPIN (CONNOR et al., 2000; OSORIO FDE; CRIPPA; LOUREIRO, 2009) and BAI (BECK et al., 1988; CUNHA, 2001). No subject had a history of head trauma, neurological or psychiatric illness, ECT, substance abuse, or major medical illnesses based on a semi-standardized medical questionnaire and physical examination. They were all non-smokers (of tobacco), and they had not taken any medications for at least three months before the study. No subject had used marijuana more than five times in their lives (no use in the last year), and none had ever used any other illegal drug. Female participants who were pregnant or nursing were excluded. Female participants were required to have a negative pregnancy test prior admission. Subjects provided written informed consent after being fully informed about the research procedure, approved by local institutional review board (HCRP No. 12407/2009).

4.3.2. Screening Procedure and Clinical Assessment

Undergraduate and graduate students were recruited by telephone and advertisement and screened by self-assessment diagnostic instruments. BAI is a self-assessment diagnostic instrument that measures anxiety levels with a Likert scale from 0 to 3 (BECK et al., 1988; CUNHA, 2001). It is composed of 21 items based on common physiological and psychological symptoms related to anxiety. Participants with minimum anxiety levels (maximum score of 10), were allocated to the next step of screen process.

The FAST is a short version of the AUDIT constituted by 4 Likert scale items and was used to exclude participants with a total score greater than 3, due to possible alcohol addiction or abuse (HODGSON et al., 2002; MENESES-GAYA et al., 2010).

PHQ-9 is constituted by 9 Likert scale items with score from 0 to 3 to screen the frequency of depressive symptoms over the past 2 weeks. Participants with a total score greater than 3 were excluded from screening steps, as there is a possibility to have depressive disorder (DE LIMA OSORIO et al., 2009; LOWE et al., 2004). Participants who fulfilled

screening criteria were contacted by telephone in order to respond to the general revision and the SCID-CV social anxiety module (FIRST et al., 1997), translated into Portuguese (DEL-BEN et al., 2001), by one examiner familiar with the instrument.

4.3.3. Drug Preparation

Rimonabant (90 mg; Acomplia®, Sanofi-Aventis, Brazil) was administered inside gelatin capsules. The same amount of wheat flour was used as placebo and packed into identical gelatin capsules as the rimonabant's capsules. The 90 mg rimonabant dose was based on previous studies that showed the minimum required dose to blockade acutely CB1 receptor, with peak plasma concentration 2 h after ingestion (GORELICK et al., 2006; HUESTIS et al., 2001).

4.3.4. Psychological Measurements

Subjective states were evaluated with the VAMS Portuguese version (NORRIS, 1971; ZUARDI; KARNIOL, 1981). Participants were told to identify his/her currently subjective state on a 100-mm straight line placed between two words that describe opposite mood states. VAMS contains 16 items that Norris grouped into four factors: (1) anxiety, comprising the items calm–excited, relaxed–tense, and tranquil–troubled; (2) sedation (formerly mental sedation), including the items alert–drowsy, and attentive–dreamy; (3) cognitive impairment (formerly physical sedation), including quick-witted–mentally slow, proficient–incompetent, energetic–lethargic, clear-headed–fuzzy, gregarious–withdrawn, well-coordinated–clumsy, and strong–feeble; and (4) discomfort (formerly other feelings and attitudes), made of the items interested–bored, happy–sad, contented–discontented, and amicable–antagonistic (PARENTE et al., 2005).

The SPSS (HOFMANN; DIBARTOLO, 2000; OSÓRIO; CRIPPA, 2008) is a self-report instrument that measures the self-perception of performance in the specific situation of public speaking. The scale is comprised of 10 items rated on a Likert scale from 0 (strongly disagree) to 5 (strongly agree) that are organized into two subscales of five items each, for positive or negative self-evaluation.

The BSS was designed to detect physical symptoms that can indirectly influence anxiety measures (ZUARDI et al., 1993). It is organized into 21 items and the intensity of each symptom is rated from 0 (no symptom) to 5 (highest).

4.3.5. Physiological Measurements

4.3.5.1. Arterial Blood Pressure and Heart Rate

The SBP, DBP, and HR were measured by a multiparametric monitor (Monitor DX 2022, Dixtal, Brazil).

4.3.6. Procedure

The SPST was the same as described by McNair *et al* (MCNAIR et al., 1982) with some modifications from previous studies (BERGAMASCHI et al., 2011a; HALLAK et al., 2010a). Female participants were required to have a negative urine pregnancy test prior to admission and subjects were told to have breakfast 2 h prior to the session. The experimental session was conducted in a sound attenuated and temperature controlled room and started at 0800. After a 15 min adaptation period, baseline measurements (B) were taken and followed by a single oral rimonabant or placebo dose in a double-blind randomized procedure. Pre-stress measurements (P) were made 2 h after drug ingestion. Immediately thereafter, participants received the instructions and had 2 min to prepare a 4-min speech about “the public transportation system of your city”. He/she also was told that the speech would be recorded on videotape and further analyzed by a psychologist. Anticipatory speech measurements (A) were taken before the subject started speaking. Subjects started speaking in front of the camera while viewing his/her own image on the TV screen. The speech was interrupted in the middle and speech performance measurements (S) were taken. The speech was recorded for an additional 2 min. Post-stress measurements (F1 and F2) were made 15 and 35 min after the end of the speech, respectively (Table 3).

Table 3 – Timetable of the experimental session.

SESSION (H:MM)	PHASE	PROCEDURE
- 0:30		Adaptation to the laboratory; Instructions about the interview and measurements
- 0:15	Baseline (B)	SCL, SF, HR, AP, VAMS, SSPS, BSS
0		Drug intake: rimonabant or placebo capsules
+ 2:00	Pre-stress (P)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 2:10		Instructions about the SPST
+ 2:12		Speech preparation
+ 2:14	Anticipatory speech (A)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 2:25		Start of speech
+ 2:27	Speech performance (S)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 2:33		Continuation of speech
+ 2:35		End of speech
+ 2:50	Post-stress 1 (F1)	SCL, SF, HR, AP, VAMS, SSPS, BSS
+ 3:10	Post-stress 2 (F2)	SCL, SF, HR, AP, VAMS, SSPS, BSS

Abbreviations: SCL, skin conductance level; SF, spontaneous fluctuations of the skin conductance; HR, heart rate; AP, arterial blood pressure; VAMS, visual analogue mood scale; SSPS, self-statements during public speaking; BSS, bodily symptoms scale.

4.3.7. Statistical Analysis

Clinical and demographical characteristics were analyzed with non-parametric tests (gender and socioeconomic level) and with ANOVA (age, BMI, SPIN and BAI). Scores of VAMS's factors, SSPS-P, SSPS-N, BSS, arterial/diastolic/ systolic pressure, and heart rate were transformed by calculating the difference between the score in each phase and the pre-stress score in the same volunteer. These delta scores were submitted to repeated-measures ANOVA, analyzing the factors of phases, groups, and phases by groups contrast. In the case where sphericity conditions were not reached, the degrees of freedom of the repeated factor were corrected with the Huynh–Feldt epsilon. Statistical tests were conducted with SPSS version 19.0 and considered significant if two-tailed $p<0.05$.

4.4. Results

Twenty-four participants provided informed consent to participate in the study with 12 subjects in each group (placebo or rimonabant). Two participants who received placebo reported short temper, while one participant each reported discomfort, diaphoresis and discomfort, and difficult thought in the rimonabant group. No other additional adverse effects were reported and all adverse effects were not serious and expected. Participants were monitored for 48 h after procedure with no adverse events reported.

Clinical and demographic characteristics of the participants are shown in Table 4. No significant differences were observed between the two groups.

Table 4 – Clinical and demographic characteristic of the groups.

	PLACEBO	RIMO	*p*
Male/female	6/6	6/6	1.00
Age [mean (SD)]	24.5 (4.9)	24.9 (3.7)	0.82
Socioeconomic level[1] [Median (range)]	2.5 (1.0 - 3.0)	2.0 (1.0 - 4.0)	0.93
BMI [mean (SD)]	23.8 (4.6)	23.4 (3.3)	0.85
SPIN [mean (SD)]	4.3 (3.1)	6.8 (5.9)	0.19
BAI [mean (SD)]	2.0 (1.7)	3.9 (3.4)	0.10

4.4.1. Psychological Measures

Repeated-measures ANOVA analysis in the VAMS anxiety factor showed a significant effect of phase ($F_{3.17, 69.68} = 9.81$; $p<0.0001$) and phase by group contrast interaction between baseline and anticipatory speech ($F_{1, 22} = 4.53$; $p=0.045$) and baseline and performance measurements ($F_{1, 22} = 4.36$; $p=0.049$). VAMS sedation factor showed only significant effect of phase ($F_{3.80, 83.59} = 11.62$; $p<0.0001$), and no significant effect of phase and phase by group interaction were observed in the VAMS cognitive impairment and discomfort factors (Figure 1).

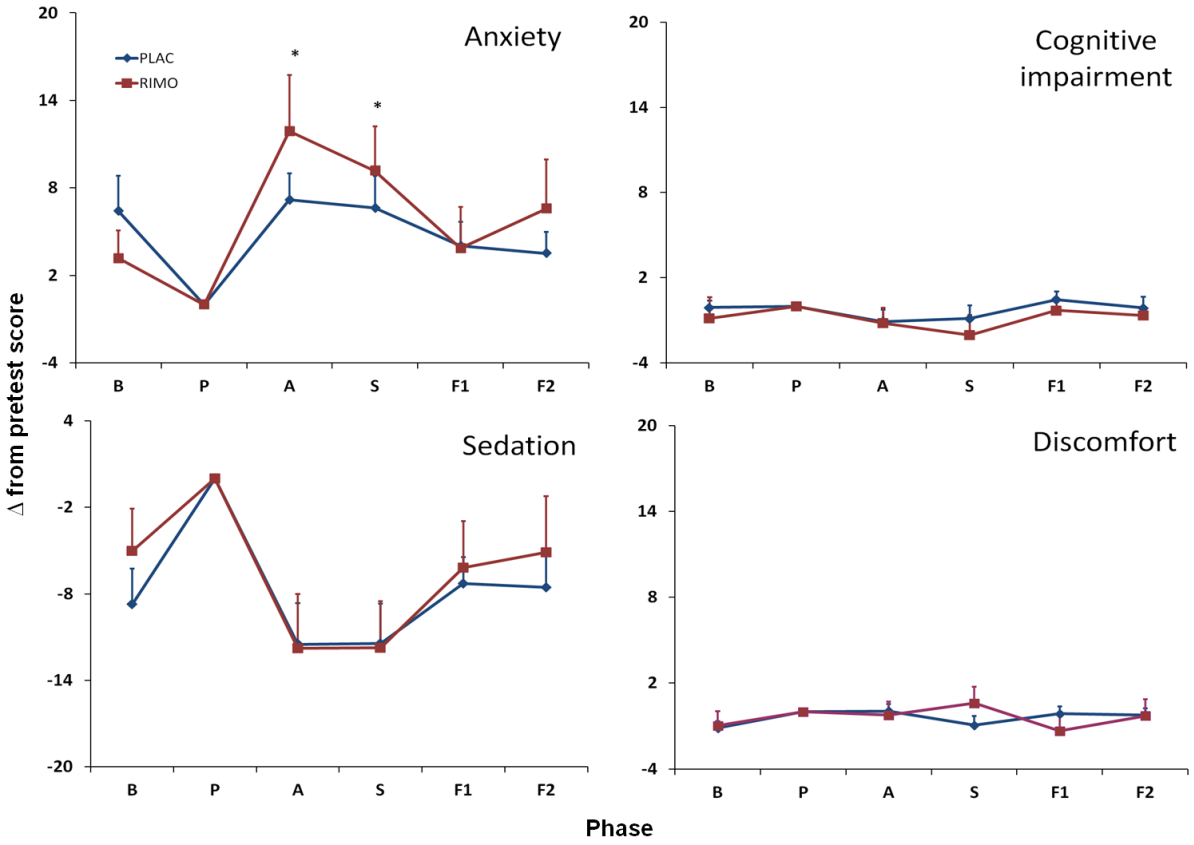

Figure 4 – Changes in Visual Analogue Mood Scale (VAMS) factors induced by simulated public speaking test. The phases of the experimental session are: B, baseline; P, pre-stress; A, anticipatory speech; S, speech performance; F1, post-stress 1; F2, post-stress 2. Points indicate mean and vertical bars standard error of the mean. *Indicates significant differences from placebo group ($p<0.05$).

Significant effect of phases was observed in SSPS-P ($F_{3.43,\ 75.54} = 4.74$; $p=0.003$), SSPS-N ($F_{3.75,\ 82.45} = 3.43$; $p=0.014$) and BSS ($F_{3.14,\ 69.07} = 4.55$; $p=0.005$) with no phase by group contrast interaction (Figure 2).

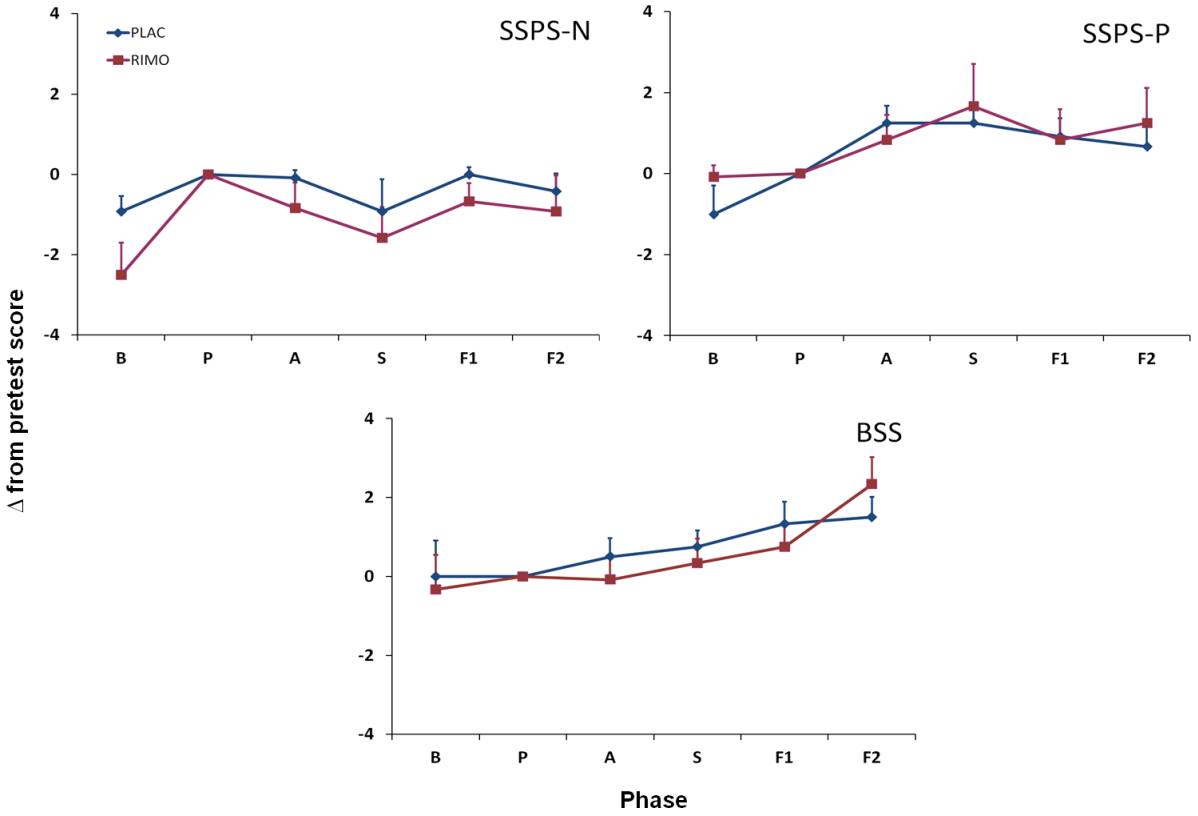

Figure 5 – Changes in Negative (SSPS-N) and Positive (SSPS-P) Self-Statement during Public Speaking scales and Bodily Symptoms Scale (BSS) induced by simulated public speaking test. The phases of the experimental session are: B, baseline; P, pre-stress; A, anticipatory speech; S, speech performance; F1, post-stress 1; F2, post-stress 2. Points indicate mean and vertical bars standard error of the mean ($p<0.05$).

4.4.2. Physiological Measures

Changes in physiological measurements are shown in Figure 3. Systolic pressure and diastolic pressure did not show significant repeated-measures ANOVA effect in phases and phase by group contrast interaction. Heart rate showed significant effect of phases ($F_{3.97, 87.31} = 6.46$; $p<0.0001$).

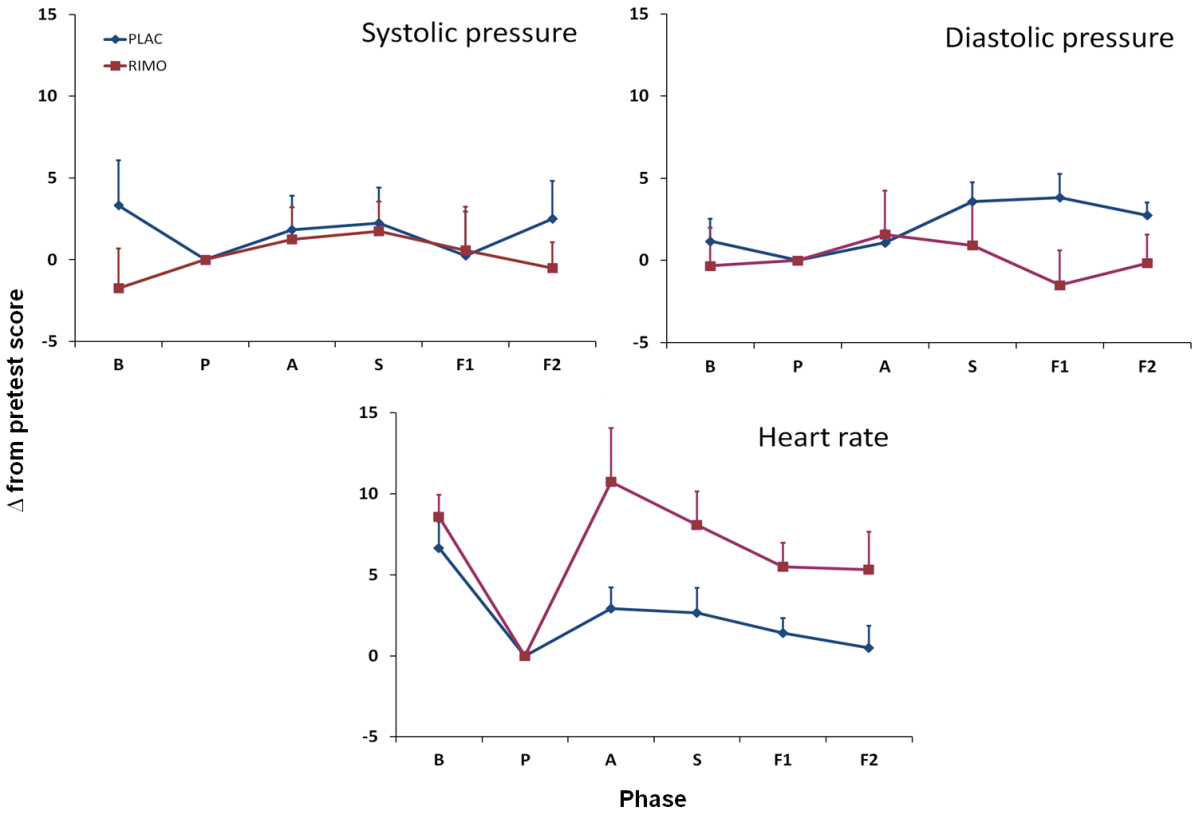

Figure 6 – Changes in systolic and diastolic pressure and heart rate induced by simulated public speaking test. The phases of the experimental session are: B, baseline; P, pre-stress; A, anticipatory speech; S, speech performance; F1, post-stress 1; F2, post-stress 2. Points indicate mean and vertical bars standard error of the mean ($p<0.05$).

4.5. Discussion

These results show for the first time we are aware of, the role of blockade of the CB1 receptor in healthy humans submitted to controlled conditions of experimental anxiety. These data are critical for better understanding the psychophysiology of human anxiety and anxiety disorders. Participants from the rimonabant group showed significantly higher anxiety than the placebo group as reported in previous clinical trials (CHRISTENSEN et al., 2007; VAN GAAL et al., 2008a; VAN GAAL et al., 2008b). Previous studies (GORELICK et al., 2006; HUESTIS et al., 2001) showed that single 90 mg doses of rimonabant significantly attenuated subjective effects of smoked cannabis in humans, with no serious adverse events. Rimonabant alone did not show significant effects on heart rate or arterial blood pressure, consistent with prior studies at the same dose (GORELICK et al., 2006).

Endocannabinoid metabolism was investigated in CB1-knockout mice that showed increased anxiety compared to controls (MARTIN et al., 2002), suggesting mood changes following blockade CB1 receptors. Enhanced levels of endocannabinoids may explain this complex behavior during anxiogenic stimuli in brain areas related to fear and anxiety (MARSICANO et al., 2002). Increased CB1 receptor activation by anandamide showed reduced anxiety in fatty acid amide hydrolase (FAAH) knockout mice (MOREIRA et al., 2008) or after FAAH inhibitor administration (KATHURIA et al., 2003). Alternatively, anandamide can also interact with TRPV1 receptors in brain areas related to anxiety (ROSS, 2003).

Another animal study elucidated the involvement of endocannabinoids in anxiety by administration of anandamide uptake inhibitor in rats, with extinction of contextual fear memory and anxiolytic effect in elevated plus-maze test, antagonized by the CB1 antagonist rimonabant (BITENCOURT; PAMPLONA; TAKAHASHI, 2008). Interestingly, the involvement of CB1 receptor in anxiety is also supported by experiments with THC and/or cannabis, where high dose THC administration is associated with anxiogenic behavior while low dose THC has anxiolytic effects (CRIPPA et al., 2009). In fact, subjects with high levels of anxiety or with anxiety disorders tend to use cannabis in order to alleviate anxiety symptoms (CRIPPA; ZUARDI; HALLAK, 2010).

The endocannabinoid system can play a role in several psychiatric disorders due to multiple action in diverse brain areas, i.e., prefrontal cortex, hippocampus, amygdala and midbrain periaqueductal gray (SAITO; WOTJAK; MOREIRA, 2010). Indeed, modulation of behavioral effects after cannabinoids exposure may be postulated by CB1 location in

forebrain areas related to anxiety, i.e. amygdala (GLASS; DRAGUNOW; FAULL, 1997). The anxiogenic effect can be explained by CB1 receptors located in axon terminals of a distinct subpopulation of GABAergic interneurons that express the peptide cholecystokinin (CCK) and inhibit GABA release (BEINFELD; CONNOLLY, 2001; KATONA et al., 2001). Thus, anxiolytic-like effects followed by CB1 activation by cannabinoids/endocannabinoids may be explained by decreased CCK release (BEINFELD; CONNOLLY, 2001) and decreased calcium influx into the axon terminal with subsequent downregulation of transmitter release (SAITO; WOTJAK; MOREIRA, 2010).

Nonetheless, some limitations arose from the study. First, it would be interesting to evaluate different doses of rimonabant in participants undergoing the SPST. This would provide useful pharmacological implications of CB1 receptor antagonism in experimental anxiety. Second, the availability of the drug was limited due to rimonabant restrictions in the market, and comparison and co-administration with other anxiolytic compounds could not be performed with more participants and sampling size.

Finally, we presented for the first time the anxiogenic-like behavior after blockade of CB1 receptors in humans subjected to controlled conditions of experimental anxiety. 90 mg rimonabant did not produce significant side effects. This suggests the role of endocannabinoid activity during controlled experimental anxiety and increased endocannabinoid activity as an alternate strategy for anxiety disorders treatment. Further randomized placebo-controlled, double-blind clinical trials are needed to determine further the mechanisms of endocannabinoids' action in anxiety and anxiety disorders.

5. Chapter 5 – What is the Impact of Prolonged Cannabinoid Excretion in Chronic Daily Cannabis Smokers' Blood on *Per Se* Drugged Driving Laws?

5.1. Abstract

This study aimed to determine windows of cannabinoid detection in chronic daily cannabis smokers' blood during sustained abstinence. Thirty male chronic daily cannabis smokers resided on a secure research unit for up to 33 days and who underwent daily blood collection; women were excluded because female hormone status may influence brain cannabinoid receptor density, another study measure. THC, 11-OH-THC, and THCCOOH blood concentrations were determined by gas chromatography-mass spectrometry. Twenty-seven of 30 participants were THC-positive on admission, with median (range) concentrations of 1.4 ng/mL (0.3–6.3). THC decreased gradually with only 1 of 11 negative at 26 days; 2 of 5 remained THC-positive (0.3 ng/mL) for 30 days. 5.0% of participants had THC ≥1.0 ng/mL for 12 days. Median 11-OH-THC concentrations were 1.1 ng/mL on admission, with no results ≥1.0 ng/mL 24h later. THCCOOH detection rates were 96.7 on admission, decreasing slowly to 95.7 and 85.7% on days 8 and 22, respectively; 4 of 5 participants remained THCCOOH positive (0.6–2.7 ng/mL) after 30 days and one remained positive on discharge at 33 days. THC was quantifiable for up to 30 days, albeit in low concentrations, due to the large cannabinoid body burden from extended exposure. Although cannabinoid windows of detection in blood are wide for chronic daily cannabis smokers, our recent data showed significant downregulation of CB1-cannabinoid receptors in specific brain areas of these chronic cannabis smokers, and we, and others, reported neurocognitive impairment from one to more than four weeks in chronic cannabis smokers.

5.2. Introduction

Cannabis is the most widely used illicit drug worldwide (UNITED NATIONS OFFICE ON DRUGS AND CRIME (UNODC), 2011). An estimated 17.4 million Americans aged 12 or older smoked cannabis in 2010, with about 6,600 new initiates daily (SUBSTANCE ABUSE AND MENTAL HEALTH SERVICES ADMINISTRATION (SAMHSA), 2011). Acutely intoxicated cannabis smokers show impairment on cognitive, perceptual, and psychomotor tasks, including those assessing short-term memory, sustained or divided attention, complex decision-making, and reaction time (ELKASHEF et al., 2008; HART et al., 2001; LANE et al., 2005; MEYER et al., 1971; RAMAEKERS et al., 2009), as well as experiencing euphoria, relaxation, altered sensory perception, slowing of time, anxiety/paranoia, increased appetite, increased heart rate, and sometimes hallucinations or psychosis (HUESTIS et al., 2001; VANDREY; HANEY, 2009). Acute impairment is well documented for hours after cannabis intake, while the persistence of chronic impairment is less clear. Some studies show neurocognitive impairment 7-28 days or longer after last cannabis intake. Eldreth *et al.* (ELDRETH et al., 2004) showed no impairment in heavy cannabis smokers in executive cognitive functioning 25 days after initiation of abstinence compared with controls. Pope *et al.* (POPE et al., 2001) found neurocognitive impairment for at least 7 days after initiation of abstinence but no significant differences 28 days later in chronic daily cannabis smokers versus less than daily smokers. Bolla *et al*. (BOLLA et al., 2002) found significant impairment after 28 days of monitored abstinence when compared to light users.

Cannabis is second only to alcohol for causing impaired driving and motor vehicle accidents. 12.8% of young adults (aged 18 to 25) reported driving under the influence of illicit drugs in 2009 (SUBSTANCE ABUSE AND MENTAL HEALTH SERVICES ADMINISTRATION, 2010). In the 2007 National Roadside Survey, more drivers tested positive for drugs (16.6%) than for alcohol (12.4%). 16.3% of weekend nighttime drivers who provided oral fluid and/or blood in a random traffic stop were drug-positive, with 8.6% positive for cannabinoids (LACEY et al., 2009), while only 2.2% of drivers had a blood alcohol concentration (BAC) of 0.08% or greater (COMPTON; BERNING, 2009). In 2003, 14% of fatally injured and 19% of non-fatally injured US drivers were positive for THC, the primary psychoactive component of cannabis (JONES; SHINAR; WALSH, 2003).

Cannabis smokers had a 10-fold increase in car crash injury when compared to infrequent or non-users after adjustment for blood alcohol level (BLOWS et al., 2005). THC

blood concentrations >1 ng/mL were associated with a 2.7-fold increase in driver responsibility for their road accidents as compared to drug-free drivers; culpability increased 6.6-fold when THC concentrations were ≥5 ng/mL in driving fatalities (DRUMMER et al., 2004; LAUMON et al., 2005). In laboratory tests, THC serum concentrations of 2–5 ng/mL were associated with perceptual-motor control impairment in 71% of drivers (RAMAEKERS et al., 2006).

In light of this strong association between cannabis use and road accidents, legal *per se* limits were established for blood THC concentrations while driving, analogous to those established for alcohol. Fifteen US states (Arizona, Delaware, Georgia, Illinois, Indiana, Iowa, Michigan, Nevada, North Carolina, Ohio, Pennsylvania, Rhode Island, South Dakota, Utah, and Wisconsin) and 12 European countries (Denmark, Finland, France, Great Britain, Greece, Hungary, Ireland, Italy, Poland, Portugal, Sweden, and Switzerland) established THC concentration limits in blood, while Belgium, Germany, Luxembourg, and Slovenia established limits in plasma/serum (LACEY; BRAINARD; SNITOW, 2010; VERSTRAETE et al., 2011), i.e., blood concentrations above the *per se* limit are considered evidence of driving impairment.

The relationship between THC concentrations and pharmacodynamic effects is complex and non-linear, in contrast to the comparable relationship for alcohol, for which *per se* driving laws are widespread. THC bioavailability is approximately 25% via the smoked route, with a plasma half-life of approximately four days (JOHANSSON et al., 1988). Concentrations initially decrease rapidly due to distribution into tissues, first-pass hepatic metabolism, and excretion into urine and feces (HUESTIS, 2007). The liver enzyme P450 2C9 hydroxylates THC at the C11 position, producing the equipotent metabolite, 11-OH-THC (BORNHEIM; LASKER; RAUCY, 1992). THC was present in brain of motor vehicle fatalities when no longer detectable in blood (MURA et al., 2005). Thus, blood concentrations may be low or not detected, while brain concentrations might be sufficient to cause impairment. These pharmacokinetic characteristics make it difficult to identify a minimum blood THC concentration consistently associated with impairment (JONES; HOLMGREN; KUGELBERG, 2008).

We characterized cannabinoid elimination in daily cannabis smokers' blood during monitored sustained abstinence for up to 33 days. These data inform interpretation of cannabinoid blood concentrations in clinical and forensic cases, including impaired driving.

5.3. Methods

5.3.1. Participants

Participants with a history of chronic, daily cannabis smoking were recruited by print, radio, internet, and television advertisements. Subjects were required to be male, 18-65 years old, and physically and psychologically healthy based on comprehensive medical and psychological evaluation. Women were excluded because this was part of a larger study of positron emission tomography (PET) imaging to evaluate brain cannabinoid CB1 receptors (HIRVONEN et al., 2012); female hormonal cycle may affect CB1 receptor density (FATTORE; FRATTA, 2010). Self-reported cannabis smoking of more than one year, typical smoking pattern of more than five days per week for the six months prior to admission, and a positive urine cannabinoid screen were required for inclusion.

Exclusion criteria were clinically significant illness, schizophrenia, bipolar or other psychotic disorder diagnosis, participation in drug or alcohol abuse treatment within 90 days, or dependence on any substance other than cannabis, nicotine, or caffeine. Additional exclusion criteria due to the PET scanning component (HIRVONEN et al., 2012) included positive HIV test, metallic foreign body in the head, history of head trauma or seizures, fetal alcohol syndrome or other neurodevelopment disorder, and radiation exposure in the prior year.

All subjects provided written informed consent to participate in this National Institute on Drug Abuse Institutional Review Board-approved study. Participants resided on a secure clinical research unit for up to 33 days with constant 24 h surveillance, preventing access to unauthorized illicit substances. There were no dietary or physical activity restrictions.

5.3.2. Specimen Collection

Three mL blood was collected each morning by indwelling peripheral intravenous catheters into sodium heparin BD Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ, USA). Specimens were stored at −20°C in 4 mL polypropylene cryotubes until analysis.

5.3.3. Blood Cannabinoid Analysis

Blood cannabinoid extraction was performed with modifications to a previously published validated plasma cannabinoid method (LOWE et al., 2007). Calibration curves were linear from 0.25 – 25 ng/mL for THC; 0.50 – 50 ng/mL for 11-OH-THC and 0.25 – 50 ng/mL for THCCOOH. Three quality controls concentrations were analyzed in each batch across the linear range of the assay. Intra- and inter-assay imprecision were less than 6.4%, and analytical bias was within 91.6 – 111.5%.

5.3.4. Data Analysis

Body mass index, calculated as BMI = weight (Kg) / height^2 (m^2), classified participants as underweight (<18.5), normal weight (18.5 – 24.9), overweight (≥25.0), or obese (≥30.0). Cannabinoid blood detection rates were calculated at the limits of quantification (LOQ) of the method 0.25 (THC, THCCOOH) or 0.50 ng/mL (11-OH-THC), 1 ng/mL (cutoff concentration in many forensic toxicology laboratories), and 5 ng/mL, a concentration shown to have a 6.6 odds ratio for fatal accident culpability and observed impairment in cognitive performance and motor tasks related to driving skills (DRUMMER et al., 2004).

Associations between time of last detectable cannabinoid concentration and participant demographics were evaluated with Spearman's rank correlation. Differences in cannabinoid concentrations between days were evaluated by Wilcoxon rank test. A Kaplan-Meier survival analysis was performed to evaluate the duration of cannabinoid detection in blood after admission, based on last detection times in each participant. Participant data were censored if they left the study before achieving negative THC or negative 11-OH-THC results on four or two consecutive days, respectively, i.e. if participants failed to fulfill the THC or 11-OH-THC criteria, they were not considered negative on discharge. The THC criterion required more consecutive negative days than the 11-OH-THC criterion because there frequently were negative THC specimens interspersed between positive ones. Requiring a fewer number of negative days for THC would have biased the study towards classifying participants as falsely negative on discharge. THCCOOH was not included in the survival analysis because only one participant was negative for at least 2 consecutive days prior to discharge.

Investigation via boxplot and Kolmogorov-Smirnov normality test showed that data were not normally distributed, therefore, non-parametric tests were conducted with SPSS

Statistics for Windows version 19.0. Values below limit of quantification (LOQ) were replaced with 0.5*LOQ for statistical comparisons. Statistical tests were considered significant if two-tailed $p<0.05$.

5.4. Results

Thirty male chronic daily cannabis smokers (26 African Americans, 3 whites, 1 mixed race; mean (±SD) age 28.3±7.9 years) participated (Table 4). Subjects' BMI indicated 2 were underweight, 16 normal weight, 9 overweight, and 3 obese. Subjects smoked a median 9 cannabis joints per day on 14 days in the 14 days prior to study screening. Subjects began smoking cannabis at a median age of 14 years, with 10 years median duration of use. All participants reported alcohol consumption, only two reported illicit opioid ingestion, one used amphetamine, one used minor tranquilizers, and 80% smoked tobacco. One participant reported cocaine consumption in the two weeks prior to admission and one was administratively withdrawn because of cocaine use during transfer for a PET scan.

Table 5 – Demographic characteristics and self-reported cannabis use history for 30 male participants.

Subject	Age	Race	BMI	Self-reported Cannabis joints smoked per day*	Days smoked in prior 14*	Age at 1st use (y)	Lifetime duration of cannabis smoking (y)
A	33	AA	20.4	6	14	6	13
B	25	AA	29.5	6	13	16	7
C	38	AA	25.6	18	14	21	15
D	19	AA	24.4	12	10	14	4
E	43	AA	25.1	4	12	13	28
F	29	AA	20.6	18	14	14	14
G	29	AA	21.0	12	13	14	10
H	27	AA	24.4	15	14	16	10
I	26	C	20.2	5	14	16	10
J	24	AA	19.7	18	14	18	5
K	22	AA	25.2	6	14	12	6
L	29	AA	23.7	9	14	14	15
M	36	C	16.4	1	13	22	10
N	30	AA	30.2	18	14	14	17
O	29	AA	29.3	9	14	11	17
P	25	AA	32.8	12	14	17	7
Q	24	AA	26.4	18	14	13	10
R	25	AA	27.7	6	14	15	10
S	21	AA	17.6	30	13	11	9
T	40	AA	25.4	6	12	18	22
U	25	AA	31.7	6	13	13	4
V	25	AA	20.5	12	13	17	5
W	52	C	24.9	3	14	14	38
X	38	AA	22.4	3	14	17	17
Y	20	AA	19.0	9	13	13	6
Z	31	AA	20.8	6	14	16	15
2A	21	AA	20.9	6	12	14	5
2B	21	AA	20.5	15	14	13	8
2C	21	AA	21.1	9	14	12	6
2D	21	AA+C	26.3	8	11	14	7
Mean	28.3		23.8	10.2	13.3	14.6	11.7
SD	7.9		4.2	6.3	1.0	3.1	7.5

*prior to study screening

Abbreviations: AA, African-American; C, Caucasian; SD, standard deviation; BMI, body mass index.

Participants (n=30) provided a total of 570 blood specimens: 326 were THC positive (57.2%), with concentrations ranging from 0.25 to 6.3 ng/mL. 531 specimens (93.2%) were THCCOOH positive and 33 were 11-OH-THC positive (5.8%). 11-OH-THC concentrations were ≤4.1 ng/mL. Participants resided on the secure unit for at least 1 (n=24), 2 (n=20), 3 (n=14), or 4 (n=11) weeks, with the longest residence 33 days.

Table 5 and Figure 7 present cannabinoid concentrations and detection rates (cannabinoids ≥LOQ) in blood during sustained abstinence. Twenty-seven of 30 participants (90% at 0.25 ng/mL) were THC-positive on admission (Day 0). 77.8% were ≥1.0 ng/mL, and only 3 (11.1%) ≥5.0 ng/mL. Two were THC-negative at admission, but later positive. One participant (M) was THC negative from admission through discharge on Day 29.

Less than 24 h after admission (Day 1), 59.1% of subjects had THC concentrations ≥1.0, but none ≥5.0 ng/mL. The highest THC concentration was 2.9 ng/mL. All subjects' THC concentrations were ≤1 ng/mL within 7 days. THC median and maximum concentrations and % of subjects THC-positive did not always decrease in a consistent manner. Less than 50% of chronic daily cannabis smokers' blood was THC positive after 16 days. Two participants' last THC-positive blood samples occurred on Day 30 (0.3 ng/mL for both), with interspersed negative and positive specimens prior to this time. These subjects ("C" and "O") smoked cannabis for 15-17 years and during screening, reported smoking 9-18 cannabis joints/blunts per day every day, similar to other participants (Table 1). One subject's THC concentrations were above 1.0 ng/mL for 12 consecutive days after admission, the longest consecutive period at this threshold.

Twenty-two of 30 participants (73.3%) were 11-OH-THC-positive on admission, 40.0% ≥1 ng/mL. Less than 24 h later, median 11-OH-THC concentrations significantly decreased (p=0.028) to 0.7 ng/mL (maximum 0.8 ng/mL). Only 1 of 28 participants' blood was still 11-OH-THC positive less than 72 h after admission, at a concentration of 0.5 ng/mL. Specimens from seven participants were never 11-OH-THC positive.

All but one participant (96.7%) was THCCOOH-positive at admission and all were positive 24 h later. THCCOOH concentrations were <10 ng/mL by Day 3, and <5 ng/mL by 6 days, even in these chronic daily cannabis smokers. All samples were THCCOOH positive through Day 7 and 85% through Day 22. One participant was still THCCOOH-positive (0.7 ng/mL) at discharge after 33 days of abstinence. Negative specimens were interspersed with positive specimens for THC, 11-OH-THC, and THCCOOH in 12, 1, and 2 participants, respectively.

Table 6 – Whole blood cannabinoid concentrations (ng/mL) in chronic daily male cannabis smokers during sustained monitored abstinence.

		THC			11-OH-THC			THCCOOH		
Day	n	% ≥ LOQ	Median	Maximum Conc.	% ≥ LOQ	Median	Maximum Conc.	% ≥ LOQ	Median	Maximum Conc.
Adm	30	90.0	1.4	6.3	73.3	1.1	4.1	96.7	26.5	93.4
1	22	68.2	1.8	2.9	31.8	0.7	0.8	100.0	11.3	35.2
2	30	80.0	1.2	2.2	10.0	0.5	0.6	100.0	10.6	26.3
3	28	78.6	1.3	2.6	3.6	0.5	0.5	100.0	8.0	26.1
4	28	78.6	1.1	2.3	NA	NA	<LOQ	100.0	6.2	20.3
5	26	76.9	1.0	1.9	NA	NA	<LOQ	100.0	5.2	19.4
6	25	72.0	1.0	2.2	NA	NA	<LOQ	100.0	4.1	17.8
7	24	79.2	0.9	2.0	NA	NA	<LOQ	100.0	3.1	14.4
8	23	65.2	0.8	2.4	NA	NA	<LOQ	95.7	3.0	12.7
9	21	61.9	0.7	2.0	NA	NA	<LOQ	95.2	2.3	7.6
10	21	61.9	0.5	1.8	NA	NA	<LOQ	95.2	2.0	6.6
11	21	71.4	0.5	1.2	NA	NA	<LOQ	95.2	2.0	6.9
12	20	65.0	0.5	1.0	NA	NA	<LOQ	95.0	1.7	5.4
13	20	55.0	0.4	0.8	NA	NA	<LOQ	90.0	1.8	4.3
14	20	60.0	0.4	1.0	NA	NA	<LOQ	85.0	1.8	5.1
15	18	72.2	0.4	0.9	NA	NA	<LOQ	94.4	1.5	5.0
16	18	44.4	0.3	0.8	NA	NA	<LOQ	94.4	1.6	4.8
17	17	47.1	0.5	0.8	NA	NA	<LOQ	94.1	1.3	5.2
18	16	43.8	0.4	0.7	NA	NA	<LOQ	87.5	1.3	5.7
19	14	42.9	0.4	0.5	NA	NA	<LOQ	85.7	1.1	4.7
20	15	40.0	0.3	0.7	NA	NA	<LOQ	86.7	1.2	4.0
21	14	42.9	0.4	0.5	NA	NA	<LOQ	85.7	1.2	3.4
22	14	21.4	0.4	0.5	NA	NA	<LOQ	85.7	0.9	3.2
23	13	23.1	0.4	0.5	NA	NA	<LOQ	84.6	0.8	3.3
24	12	25.0	0.4	0.7	NA	NA	<LOQ	83.3	1.1	3.1
25	12	8.3	0.3	0.3	NA	NA	<LOQ	91.7	0.7	2.5
26	11	9.1	0.4	0.4	NA	NA	<LOQ	81.8	0.6	2.8
27	10	0.0	NA	<LOQ	NA	NA	<LOQ	70.0	0.7	2.5
28	11	0.0	NA	<LOQ	NA	NA	<LOQ	90.9	0.7	2.6
29	8	0.0	NA	<LOQ	NA	NA	<LOQ	75.0	0.6	1.8
30	5	40.0	0.3	0.3	NA	NA	<LOQ	80.0	1.1	2.7
31	1	0.0	NA	<LOQ	NA	NA	<LOQ	0.0	NA	<LOQ
32	1	0.0	NA	<LOQ	NA	NA	<LOQ	100.0	0.9	0.9
33	1	0.0	NA	<LOQ	NA	NA	<LOQ	100.0	0.7	0.7

Abbreviations: NA, non-available; THC, Δ^9-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; LOQ, limit of quantification; 0.25 ng/mL (THC and THCCOOH) and 0.50 ng/mL (11-OH-THC).

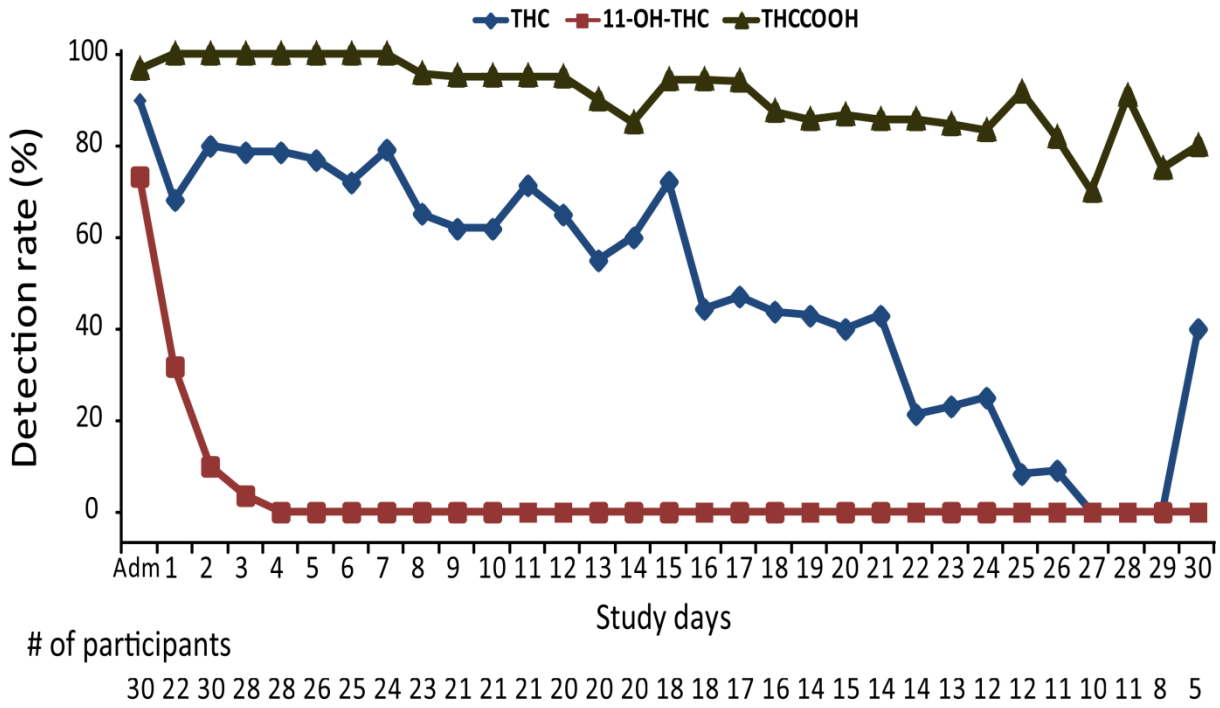

Figure 7 – Cannabinoid detection rates in chronic daily cannabis smokers based on the method's limit of quantifications 0.25 ng/mL for Δ^9-tetrahydrocannabinol (THC**)** and 11-nor-9-carboxy-THC (THCCOOH**)** and 0.5 ng/mL for 11-hydroxy-THC (11-OH-THC).

Eleven participants had exponential THC decreases. In four subjects, THC concentrations increased from admission to Day 1; 3 participants' blood was positive only on admission. In contrast, 25 participants showed exponential THCCOOH declines, and THCCOOH increases from admission to Day 1 were observed in three individuals' blood.

Last THC detection times were significantly but weakly correlated with self-reported quantity of cannabis smoking in the two weeks prior to screening (r=0.372; p=0.047). The median last detection time in blood after admission was 22 days (95% confidence interval 17.8–26.2 days) for THC and 1 day (95% confidence interval 0 day) for 11-OH-THC (Figure 8).

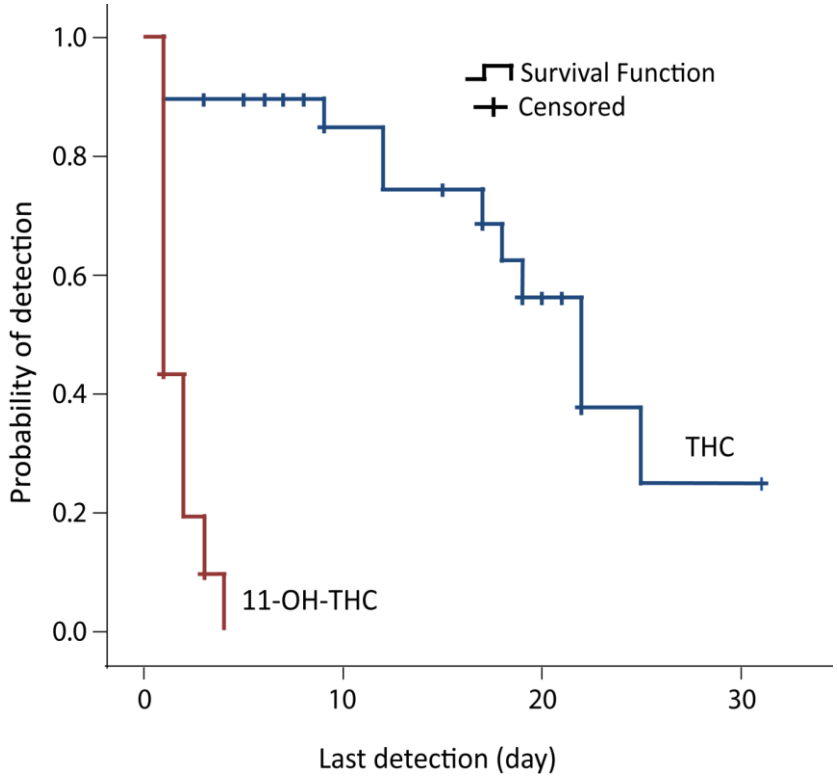

Figure 8 – Kaplan-Meier survival curves for Δ^9-tetrahydrocannabinol (THC) and 11-hydroxy-THC (11-OH-THC**)** detection in whole blood during 33 days of sustained abstinence in chronic daily cannabis smokers. THCCOOH was not included because it was positive throughout the study.

5.5. Discussion

To our knowledge, these are the first blood cannabinoid concentrations in chronic daily cannabis smokers during extended (up to 33 days) continuously monitored abstinence. These data are critical for understanding cannabinoid pharmacokinetics in this population and for interpreting blood cannabinoid tests. Both THC and its inactive metabolite THCCOOH were detected in blood up to one month after last smoking, four times longer than previously described (longest prior study assessed for seven days after last smoking) (KARSCHNER et al., 2009a). In contrast, the active THC metabolite, 11-OH-THC, had a maximum detection window of 72 h after admission, shorter than the seven days reported in a previous study of cannabis smokers monitoring abstinence for one week (KARSCHNER et al., 2009a). This difference may be due in part to gender difference because in the prior study, females had longer THC and 11-OH-THC detection windows than males.

Participants showed highly variable THC and THCCOOH concentrations over time, with positive specimens occurring days to weeks after initiation of abstinence. The variable THC detection rate throughout the study with positive specimens interspersed with negatives ones (e.g. two participants THC positive on day 30), reflects large THC body burden (HUESTIS, 2007). Although THC is mainly stored in adipose tissue (BRUNET et al., 2006; JOHANSSON et al., 1989), we did not find significant correlation between BMI and time of last detectable THC concentration. Persistence of THC impairment was shown for at least several weeks after initiation of abstinence in multiple studies (BOLLA et al., 2002; ELDRETH et al., 2004; POPE et al., 2001; SOLOWIJ et al., 2002). Thus, our findings suggest an association between residual cannabinoid concentrations and impairment over the initial few weeks of abstinence, consistent with the approach of *per se* drugged driving laws. However, additional research is warranted on development of and dissipation of pharmacodynamic tolerance (GORELICK et al., 2011; HANEY et al., 1997; JONES, 1978; RAMAEKERS et al., 2011), acute cannabis withdrawal (which may also impair performance) (GONZALEZ, 2007; POPE; YURGELUN-TODD, 1996), and the relationship between concentrations in blood and brain (the site of action of impairment) (MURA et al., 2005).

THC serum concentrations of 2 – 5 ng/mL were shown to impair driving (RAMAEKERS et al., 2006), and concentrations of 7 – 10 ng/mL produced impairment equivalent to a blood alcohol concentration of 0.05%. (GROTENHERMEN et al., 2007) Sweden and Australia have zero tolerance for illegal drugs in drivers. If a 5 ng/mL THC blood cut-off were adopted in Sweden, 90% of convicted impaired drivers would not have been

prosecuted; 61% of prosecuted drivers would have been missed with a >1 ng/mL cut-off (JONES; HOLMGREN; KUGELBERG, 2008).

Two states, Nevada and Ohio, set blood *per se* limits of ≥2 ng/mL for THC or ≥5 ng/mL for THCCOOH (LACEY; BRAINARD; SNITOW, 2010). In our study sample, 1 of 21 participants (4.8%) met this THC *per se* limit after 9 days of abstinence, and 1 of 16 participants (6.3%) met this THCCOOH *per se* limit after 18 days of abstinence, and thus would be prosecuted. Recommended blood cut-offs for forensic toxicology laboratories of 2, 2, and 5 ng/mL for THC, 11-OH-THC, and THCCOOH, respectively (FARRELL; KERRIGAN; LOGAN, 2007), would result in 1 of 21 subjects (4.8%) prosecuted after 9 days of abstinence, no subjects 24 hours after abstinence and 1 of 16 subjects (6.3%) after 18 days of abstinence for THC, 11-OH-THC, and THCCOOH, respectively. Under the highest *per se* limits in Europe (VERSTRAETE et al., 2011), 3 ng/mL for THC in Portugal or 50 ng/mL for THCCOOH in Poland, no participants would be prosecuted after 24 h of abstinence. Colorado (USA) is currently considering a *per se* limit of 5.0 ng/mL THC in blood. If applied to our study results, only 3 of 30 subjects (10%) would be prosecuted at admission, when subjects frequently self-reported recent smoking, and no participants after 24 h abstinence. While existing laws focus on THC and THCCOOH *per se* levels, an appropriate cutoff might also be selected for 11-OH-THC due its shorter detection window. THC-glucuronide, CBN, and CBD concentrations in blood may also indicate recent cannabis smoking (SCHWOPE et al., 2011b).

This study has several limitations. Time of last cannabis smoking was based on participant self-report. However, our data report objective data- time from admission and document drug detection over days to weeks. If participants actually abstained from cannabis smoking immediately prior to admission, length of detection could only be longer than times reported. Also, the majority (87%) of the study population was African-American; race/ethnicity may affect drug metabolism due to cytochrome P450 polymorphism and possibly excretion; additional research in other populations should be performed.

In conclusion, our results demonstrate for the first time of which we are aware, that cannabinoids can be detected in blood of chronic daily cannabis smokers during a month of sustained abstinence. This is consistent with the time course of persisting neurocognitive impairment reported in recent studies (BOLLA et al., 2002; HIRVONEN et al., 2012; POPE et al., 2001; SOLOWIJ et al., 2002). There is a strong public safety need to reduce morbidity and mortality from cannabis-impaired driving. Extended residual THC excretion in chronic daily cannabis smokers complicates prosecution. Establishment of *per se* THC legislation

might achieve such a reduction in motor vehicle injuries and deaths. *Per se* alcohol legislation improved prosecution of drunk drivers and dramatically reduced alcohol-related deaths. By analogy, one way to protect the public from drugged drivers is to establish legislation making it illegal to smoke cannabis and drive.

6. Chapter 6 – Conclusions

Cannabidiol has extensive pharmacological effects by multiple mechanisms (IZZO et al., 2009). Previous studies demonstrated anticonvulsant, antipsychotic, antidepressant and anxiolytic effect of CBD in psychiatry (CRIPPA; ZUARDI; HALLAK, 2010). Chapter 3 extended the knowledge CBD anxiolytic effect in SAD participants. A single dose of CBD significantly reduced anxiety, cognitive impairment, and discomfort at speech performance and significantly decreased alertness at anticipatory speech. Acute CBD administration mitigated the increase of negative self-evaluation during public speaking and the self-report of somatic symptoms, with no effects on physiological measures.

Chapter 4 provided addition information about anxiogenic-like behavior after acute blockade of CB1 receptor in humans submitted to controlled conditions of experimental anxiety. Rimonabant administration had no effect on VAMS factors besides ‘anxiety’ and no effect on other measurements. This finding can extended our knowledge about neurobiological mechanisms of anxiety and the possible role of endocannabinoids in anxiety / anxiety disorders. However, additional studies are necessary to investigate modulation of endocannabinoids as alternative for SAD treatment.

Cannabinoids quantification in whole blood presented in Chapter 5 provided novel insight into THC, 11-OH-THC and THCCOOH excretion over days to weeks. These novel data provide important information regarding extended cannabinoid excretion in chronic daily cannabis smokers and impact development of *per se* laws to reduce morbidity and mortality from cannabis-impaired driving.

7. References

ADAMS, R.; HUNT, M.; CLARK, J. H. Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp I. **J Am Chem Soc**, v. 62, n. 1, p. 196-200, 1940.

AGURELL, S.; CARLSSON, S.; LINDGREN, J. E.; OHLSSON, A.; GILLESPIE, H.; HOLLISTER, L. Interactions of delta 1-tetrahydrocannabinol with cannabinol and cannabidiol following oral administration in man. Assay of cannabinol and cannabidiol by mass fragmentography. **Experientia**, v. 37, n. 10, p. 1090-2, 1981.

AGURELL, S.; GILLESPIE, H.; HALLDIN, M.; HOLLISTER, L. E.; JOHANSSON, E.; LINDGREN, J. E.; OHLSSON, A.; SZIRMAI, M.; WIDMAN, M. A review of recent studies on the pharmacokinetics and metabolism of delta-1-tetrahydrocannabinol, cannabidiol and cannabinol in man. In: PATON, S. W.; NAHAS, G. G.**Marijuana '84**. Oxford: IRL Press Limited, 1985, p 49-62.

AGURELL, S.; HALLDIN, M.; LINDGREN, J. E.; OHLSSON, A.; WIDMAN, M.; GILLESPIE, H.; HOLLISTER, L. Pharmacokinetics and metabolism of delta 1-tetrahydrocannabinol and other cannabinoids with emphasis on man. **Pharmacol Rev**, v. 38, n. 1, p. 21-43, 1986.

AGURELL, S.; LEANDER, K. Stability, transfer and absorption of cannabinoid constituents of cannabis (hashish) during smoking. **Acta Pharmaceutica Suecica**, v. 8, p. 391-402, 1971.

AMERICAN PSYCHIATRIC ASSOCIATION, A. **Diagnostic and Statistical Manual of Mental Disorders, DSM-IV**. (4). Washington, DC: American Psychiatric Association, 1994.

ARSENEAULT, L.; CANNON, M.; WITTON, J.; MURRAY, R. M. Causal association between cannabis and psychosis: examination of the evidence. **Br J Psychiatry**, v. 184, p. 110-7, 2004.

ASHTON, C. H. Pharmacology and effects of cannabis: a brief review. **Br J Psychiatry**, v. 178, p. 101-106, 2001.

ASHTON, C. H.; MOORE, P. B. Endocannabinoid system dysfunction in mood and related disorders. **Acta Psychiatr Scand**, v. 124, n. 4, p. 250-61, 2011.

BECK, A. T.; EPSTEIN, N.; BROWN, G.; STEER, R. A. An inventory for measuring clinical anxiety: psychometric properties. **J Consult Clin Psychol**, v. 56, n. 6, p. 893-7, 1988.

BEINFELD, M. C.; CONNOLLY, K. Activation of CB1 cannabinoid receptors in rat hippocampal slices inhibits potassium-evoked cholecystokinin release, a possible mechanism contributing to the spatial memory defects produced by cannabinoids. **Neurosci Lett**, v. 301, n. 1, p. 69-71, 2001.

BELTRAMO, M.; BERNARDINI, N.; BERTORELLI, R.; CAMPANELLA, M.; NICOLUSSI, E.; FREDDUZZI, S.; REGGIANI, A. CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. **Eur J Neurosci**, v. 23, n. 6, p. 1530-8, 2006.

BERGAMASCHI, M. M.; QUEIROZ, R. H.; CHAGAS, M. H.; DE OLIVEIRA, D. C.; DE MARTINIS, B. S.; KAPCZINSKI, F.; QUEVEDO, J.; ROESLER, R.; SCHRODER, N.; NARDI, A. E.; MARTIN-SANTOS, R.; HALLAK, J. E.; ZUARDI, A. W.; CRIPPA, J. A. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. **Neuropsychopharmacology**, v. 36, n. 6, p. 1219-26, 2011a.

BERGAMASCHI, M. M.; QUEIROZ, R. H.; ZUARDI, A. W.; CRIPPA, J. A. Safety and side effects of cannabidiol, a Cannabis sativa constituent. **Curr Drug Saf**, v. 6, n. 4, p. 237-49, 2011b.

BHATTACHARYYA, S.; MORRISON, P. D.; FUSAR-POLI, P.; MARTIN-SANTOS, R.; BORGWARDT, S.; WINTON-BROWN, T.; NOSARTI, C.; CM, O. C.; SEAL, M.; ALLEN, P.; MEHTA, M. A.; STONE, J. M.; TUNSTALL, N.; GIAMPIETRO, V.; KAPUR, S.; MURRAY, R. M.; ZUARDI, A. W.; CRIPPA, J. A.; ATAKAN, Z.; MCGUIRE, P. K. Opposite effects of delta-9-tetrahydrocannabinol and cannabidiol on human brain function and psychopathology. **Neuropsychopharmacology**, v. 35, n. 3, p. 764-74, 2010.

BITENCOURT, R. M.; PAMPLONA, F. A.; TAKAHASHI, R. N. Facilitation of contextual fear memory extinction and anti-anxiogenic effects of AM404 and cannabidiol in conditioned rats. **Eur Neuropsychopharmacol**, v. 18, n. 12, p. 849-59, 2008.

BLACKARD, C.; TENNES, K. Human placental transfer of cannabinoids. **N Engl J Med**, v. 311, p. 797, 1984.

BLANCO, C.; ANTIA, S. X.; LIEBOWITZ, M. R. Pharmacotherapy of social anxiety disorder. **Biol Psychiatry**, v. 51, n. 1, p. 109-20, 2002.

BLOWS, S.; IVERS, R. Q.; CONNOR, J.; AMERATUNGA, S.; WOODWARD, M.; NORTON, R. Marijuana use and car crash injury. **Addiction**, v. 100, n. 5, p. 605-11, 2005.

BOLLA, K. I.; BROWN, K.; ELDRETH, D.; TATE, K.; CADET, J. L. Dose-related neurocognitive effects of marijuana use. **Neurology**, v. 59, n. 9, p. 1337-43, 2002.

BONN-MILLER, M. O.; ZVOLENSKY, M. J.; BERNSTEIN, A. Marijuana use motives: concurrent relations to frequency of past 30-day use and anxiety sensitivity among young adult marijuana smokers. **Addict Behav**, v. 32, n. 1, p. 49-62, 2007.

BORGWARDT, S. J.; ALLEN, P.; BHATTACHARYYA, S.; FUSAR-POLI, P.; CRIPPA, J. A.; SEAL, M. L.; FRACCARO, V.; ATAKAN, Z.; MARTIN-SANTOS, R.; O'CARROLL, C.; RUBIA, K.; MCGUIRE, P. K. Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. **Biol Psychiatry**, v. 64, n. 11, p. 966-73, 2008.

BORNHEIM, L. M.; CORREIA, M. A. Purification and characterization of the major hepatic cannabinoid hydroxylase in the mouse: a possible member of the cytochrome P-450IIC subfamily. **Mol Pharmacol**, v. 40, n. 2, p. 228-34, 1991.

BORNHEIM, L. M.; CORREIA, M. A. Selective inactivation of mouse liver cytochrome P-450IIIA by cannabidiol. **Mol Pharmacol**, v. 38, n. 3, p. 319-26, 1990.

BORNHEIM, L. M.; EVERHART, E. T.; LI, J.; CORREIA, M. A. Induction and genetic regulation of mouse hepatic cytochrome P450 by cannabidiol. **Biochem Pharmacol**, v. 48, n. 1, p. 161-71, 1994.

BORNHEIM, L. M.; LASKER, J. M.; RAUCY, J. L. Human hepatic microsomal metabolism of delta-1-tetrahydrocannabinol. **Drug Metab Dispos**, v. 20, n. 2, p. 241-246, 1992.

BRUNELLO, N.; DEN BOER, J. A.; JUDD, L. L.; KASPER, S.; KELSEY, J. E.; LADER, M.; LECRUBIER, Y.; LEPINE, J. P.; LYDIARD, R. B.; MENDLEWICZ, J.; MONTGOMERY, S. A.; RACAGNI, G.; STEIN, M. B.; WITTCHEN, H. U. Social phobia: diagnosis and epidemiology, neurobiology and pharmacology, comorbidity and treatment. **J Affect Disord**, v. 60, n. 1, p. 61-74, 2000.

BRUNET, B.; DOUCET, C.; VENISSE, N.; HAUET, T.; HEBRARD, W.; PAPET, Y.; MAUCO, G.; MURA, P. Validation of Large White Pig as an animal model for the study of cannabinoids metabolism: application to the study of THC distribution in tissues. **Forensic Science International**, v. 161, n. 2-3, p. 169-74, 2006.

BUCKNER, J. D.; BONN-MILLER, M. O.; ZVOLENSKY, M. J.; SCHMIDT, N. B. Marijuana use motives and social anxiety among marijuana-using young adults. **Addictive Behaviors**, v. 32, n. 10, p. 2238-2252, 2007.

BUCKNER, J. D.; SCHMIDT, N. B.; LANG, A. R.; SMALL, J. W.; SCHLAUCH, R. C.; LEWINSOHN, P. M. Specificity of social anxiety disorder as a risk factor for alcohol and cannabis dependence. **J Psychiatr Res**, v. 42, n. 3, p. 230-9, 2008.

BUDNEY, A. J.; HUGHES, J. R.; MOORE, B. A.; VANDREY, R. Review of the validity and significance of cannabis withdrawal syndrome. **American Journal of Psychiatry**, v. 161, n. 11, p. 1967-77, 2004.

CAMPOS, A. C.; GUIMARAES, F. S. Evidence for a potential role for TRPV1 receptors in the dorsolateral periaqueductal gray in the attenuation of the anxiolytic effects of cannabinoids. **Prog Neuropsychopharmacol Biol Psychiatry**, v. 33, n. 8, p. 1517-21, 2009.

CAMPOS, A. C.; GUIMARAES, F. S. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. **Psychopharmacology (Berl)**, v. 199, n. 2, p. 223-30, 2008.

CANADIAN PSYCHIATRIC ASSOCIATION, C. Clinical practice guidelines. Management of anxiety disorders. **Can J Psychiatry**, v. 51, n. 8 Suppl 2, p. 9S-91S, 2006.

CARLINI, E. A.; MASUR, J.; MAGALHÃES, C. C. P. B. Possível efeito hipnótico do cannabidiol no ser humano. Estudo preliminar. **Ciência e Cultura**, v. 31, p. 315-322, 1979.

CARRIER, E. J.; AUCHAMPACH, J. A.; HILLARD, C. J. Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. **Proc Natl Acad Sci U S A**, v. 103, n. 20, p. 7895-900, 2006.

CASAROTTO, P. C.; GOMES, F. V.; RESSTEL, L. B.; GUIMARAES, F. S. Cannabidiol inhibitory effect on marble-burying behaviour: involvement of CB1 receptors. **Behav Pharmacol**, v. 21, n. 4, p. 353-8, 2010.

CASTILLO, A.; TOLON, M. R.; FERNANDEZ-RUIZ, J.; ROMERO, J.; MARTINEZ-ORGADO, J. The neuroprotective effect of cannabidiol in an in vitro model of newborn hypoxic-ischemic brain damage in mice is mediated by CB(2) and adenosine receptors. **Neurobiol Dis**, v. 37, n. 2, p. 434-40, 2010.

CHAGAS, M. H.; NARDI, A. E.; MANFRO, G. G.; HETEM, L. A.; ANDRADA, N. C.; LEVITAN, M. N.; SALUM, G. A.; ISOLAN, L.; FERRARI, M. C.; CRIPPA, J. A. Guidelines of the Brazilian Medical Association for the diagnosis and differential diagnosis of social anxiety disorder. **Rev Bras Psiquiatr**, v. 32, n. 4, p. 444-52, 2010.

CHRISTENSEN, R.; KRISTENSEN, P. K.; BARTELS, E. M.; BLIDDAL, H.; ASTRUP, A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. **Lancet**, v. 370, n. 9600, p. 1706-13, 2007.

COMPTON, D. R.; ACETO, M. D.; LOWE, J.; MARTIN, B. R. In vivo characterization of a specific cannabinoid receptor antagonist (SR141716A): Inhibition of delta-9-tetrahydrocannabinol-induced responses and apparent agonist activity. **J Pharmacol Exp Ther**, v. 277, p. 586-594, 1996.

COMPTON, R.; BERNING, A. Results of the 2007 National Roadside Survey of Alcohol and Drug Use by Drivers. **Report**. Washington, DC, 2009.

CONNOR, K. M.; DAVIDSON, J. R.; CHURCHILL, L. E.; SHERWOOD, A.; FOA, E.; WEISLER, R. H. Psychometric properties of the Social Phobia Inventory (SPIN). New self-rating scale. **Br J Psychiatry**, v. 176, p. 379-86, 2000.

CONNOR, K. M.; KOBAK, K. A.; CHURCHILL, L. E.; KATZELNICK, D.; DAVIDSON, J. R. Mini-SPIN: A brief screening assessment for generalized social anxiety disorder. **Depress Anxiety**, v. 14, n. 2, p. 137-40, 2001.

CONSROE, P.; CARLINI, E. A.; ZWICKER, A. P.; LACERDA, L. A. Interaction of cannabidiol and alcohol in humans. **Psychopharmacology (Berl)**, v. 66, n. 1, p. 45-50, 1979.

CONSROE, P.; LAGUNA, J.; ALLENDER, J.; SNIDER, S.; STERN, L.; SANDYK, R.; KENNEDY, K.; SCHRAM, K. Controlled clinical trial of cannabidiol in Huntington's disease. **Pharmacol Biochem Behav**, v. 40, n. 3, p. 701-8, 1991.

CRIPPA, J. A.; DERENUSSON, G. N.; FERRARI, T. B.; WICHERT-ANA, L.; DURAN, F. L.; MARTIN-SANTOS, R.; SIMOES, M. V.; BHATTACHARYYA, S.; FUSAR-POLI, P.; ATAKAN, Z.; SANTOS FILHO, A.; FREITAS-FERRARI, M. C.; MCGUIRE, P. K.; ZUARDI, A. W.; BUSATTO, G. F.; HALLAK, J. E. Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. **J Psychopharmacol**, v. 25, n. 1, p. 121-30, 2011.

CRIPPA, J. A.; LOUREIRO, S. R.; BAPTISTA, C. A.; OSORIO, F. Are there differences between early- and late-onset social anxiety disorder? **Rev Bras Psiquiatr**, v. 29, n. 2, p. 195-6; author reply 196-7, 2007.

CRIPPA, J. A.; ZUARDI, A. W.; BUSATTO, G. F.; SANTOS-FILHO, A.; GRAEFF, F. G.; BORDUQUI, T.; SANTOS, A. C.; ARAUJO, D.; DURAN, F.; DEL-BEN, C. M.; FREITAS, M. C. Grey matter correlates of cognitive measures of the simulated public speaking test in social anxiety spectrum: a voxel-based study. **Eur Psychiatry**, v. 23, p. S212-S212, 2008a.

CRIPPA, J. A.; ZUARDI, A. W.; GARRIDO, G. E.; WICHERT-ANA, L.; GUARNIERI, R.; FERRARI, L.; AZEVEDO-MARQUES, P. M.; HALLAK, J. E.; MCGUIRE, P. K.; FILHO BUSATTO, G. Effects of cannabidiol (CBD) on regional cerebral blood flow. **Neuropsychopharmacology**, v. 29, n. 2, p. 417-26, 2004.

CRIPPA, J. A.; ZUARDI, A. W.; HALLAK, J. E. Therapeutical use of the cannabinoids in psychiatry. **Rev Bras Psiquiatr**, v. 32 Suppl 1, p. S56-66, 2010.

CRIPPA, J. A.; ZUARDI, A. W.; MARTIN-SANTOS, R.; BHATTACHARYYA, S.; ATAKAN, Z.; MCGUIRE, P.; FUSAR-POLI, P. Cannabis and anxiety: a critical review of the evidence. **Hum Psychopharmacol**, v. 24, n. 7, p. 515-23, 2009.

CRIPPA, J. A. S.; OSORIO, F. D.; DEL-BEN, C. M.; SANTOS, A.; FREITAS, M. C. D.; LOUREIRO, S. R. Comparability between telephone and face-to-face Structured Clinical Interview for DSM-IV in assessing social anxiety disorder. **Perspect Psychiatr C**, v. 44, n. 4, p. 241-247, 2008b.

CUNHA, J. A. **Manual da versão em português das escalas Beck**. São Paulo: Casa do Psicólogo, 2001.

CUNHA, J. M.; CARLINI, E. A.; PEREIRA, A. E.; RAMOS, O. L.; PIMENTEL, C.; GAGLIARDI, R.; SANVITO, W. L.; LANDER, N.; MECHOULAM, R. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. **Pharmacology**, v. 21, n. 3, p. 175-85, 1980.

D'SOUZA, D. C.; PERRY, E.; MACDOUGALL, L.; AMMERMAN, Y.; COOPER, T.; WU, Y. T.; BRALEY, G.; GUEORGUIEVA, R.; KRYSTAL, J. H. The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. **Neuropsychopharmacology**, v. 29, n. 8, p. 1558-72, 2004.

DALTON, W. S.; MARTZ, R.; LEMBERGER, L.; RODDA, B. E.; FORNEY, R. B. Influence of cannabidiol on delta-9-tetrahydrocannabinol effects. **Clin Pharmacol Ther**, v. 19, n. 3, p. 300-9, 1976.

DAVIDSON, J. R. T. Pharmacotherapy of social anxiety disorder: What does the evidence tell us? **J Clin Psychiatry**, v. 67, p. 20-26, 2006.

DE LIMA OSORIO, F.; CRIPPA, J. A.; LOUREIRO, S. R. A study of the discriminative validity of a screening tool (MINI-SPIN) for social anxiety disorder applied to Brazilian university students. **Eur Psychiatry**, v. 22, n. 4, p. 239-43, 2007.

DE LIMA OSORIO, F.; VILELA MENDES, A.; CRIPPA, J. A.; LOUREIRO, S. R. Study of the discriminative validity of the PHQ-9 and PHQ-2 in a sample of Brazilian women in the context of primary health care. **Perspect Psychiatr Care**, v. 45, n. 3, p. 216-27, 2009.

DE PETROCELLIS, L.; DI MARZO, V. Role of endocannabinoids and endovanilloids in Ca2+ signalling. **Cell Calcium**, v. 45, n. 6, p. 611-24, 2009.

DEL-BEN, C. M.; VILELA, J. A. A.; CRIPPA, J. A. S.; HALLAK, J. E. C.; LABATE, C. M.; ZUARDI, A. W. Confiabilidade da "Entrevista Clínica Estruturada para o DSM-IV - Versão Clínica" traduzida para o português. **Rev Bras Psiquiatr**, v. 23, p. 156-159, 2001.

DESPRES, J. P.; GOLAY, A.; SJOSTROM, L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. **N Engl J Med**, v. 353, n. 20, p. 2121-34, 2005.

DESPRES, J. P.; ROSS, R.; BOKA, G.; ALMERAS, N.; LEMIEUX, I. Effect of rimonabant on the high-triglyceride/ low-HDL-cholesterol dyslipidemia, intraabdominal adiposity, and liver fat: the ADAGIO-Lipids trial. **Arterioscler Thromb Vasc Biol**, v. 29, n. 3, p. 416-23, 2009.

DEVANE, W. A.; HANUS, L.; BREUER, A.; PERTWEE, R. G.; STEVENSON, L. A.; GRIFFIN, G.; GIBSON, D.; MANDELBAUM, A.; ETINGER, A.; MECHOULAM, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. **Science**, v. 258, p. 1946-1949, 1992.

DI MARZO, V.; MATIAS, I. Endocannabinoid control of food intake and energy balance. **Nat Neurosci**, v. 8, n. 5, p. 585-9, 2005.

DRUMMER, O. H.; GEROSTAMOULOS, J.; BATZIRIS, H.; CHU, M.; CAPLEHORN, J.; ROBERTSON, M. D.; SWANN, P. The involvement of drugs in drivers of motor vehicles killed in Australian road traffic crashes. **Accid Anal Prev**, v. 36, n. 2, p. 239-248, 2004.

ELDRETH, D. A.; MATOCHIK, J. A.; CADET, J. L.; BOLLA, K. I. Abnormal brain activity in prefrontal brain regions in abstinent marijuana users. **NeuroImage**, v. 23, n. 3, p. 914-20, 2004.

ELKASHEF, A.; VOCCI, F.; HUESTIS, M.; HANEY, M.; BUDNEY, A.; GRUBER, A.; EL-GUEBALY, N. Marijuana neurobiology and treatment. **Subst Abus**, v. 29, n. 3, p. 17-29, 2008.

FAIRBAIRN, J. W.; LIEBMANN, J. A.; ROWAN, M. G. The stability of cannabis and its preparations on storage. **J Pharm Pharmacol**, v. 28, n. 1, p. 1-7, 1976.

FARRELL, L. J.; KERRIGAN, S.; LOGAN, B. K. Recommendations for toxicological investigation of drug impaired driving. **J Forensic Sci**, v. 52, n. 5, p. 1214-8, 2007.

FATTORE, L.; FRATTA, W. How important are sex differences in cannabinoid action? **Br J Pharmacol**, v. 160, n. 3, p. 544-8, 2010.

FILHO, A. S.; HETEM, L. A.; FERRARI, M. C.; TRZESNIAK, C.; MARTIN-SANTOS, R.; BORDUQUI, T.; DE LIMA OSORIO, F.; LOUREIRO, S. R.; BUSATTO FILHO, G.; ZUARDI, A. W.; CRIPPA, J. A. Social anxiety disorder: what are we losing with the current diagnostic criteria? **Acta Psychiatr Scand**, v. 121, n. 3, p. 216-26, 2010.

FIRST, M. B.; SPITZER, R. L.; GIBBON, M.; WILLIAMS, J. B. W. **Structured Clinical Interview for DSM–IV Axis I Disorders-Clinician Version (SCID-CV)**. Washington, DC: American Psychiatric Press, 1997.

FRIDE, E.; MECHOULAM, R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. **Eur J Pharmacol**, v. 231, p. 313-314, 1993.

FUSAR-POLI, P.; ALLEN, P.; BHATTACHARYYA, S.; CRIPPA, J. A.; MECHELLI, A.; BORGWARDT, S.; MARTIN-SANTOS, R.; SEAL, M. L.; O'CARROL, C.; ATAKAN, Z.; ZUARDI, A. W.; MCGUIRE, P. Modulation of effective connectivity during emotional processing by Delta 9-tetrahydrocannabinol and cannabidiol. **Int J Neuropsychopharmacol**, v. 13, n. 4, p. 421-32, 2010.

FUSAR-POLI, P.; CRIPPA, J. A.; BHATTACHARYYA, S.; BORGWARDT, S. J.; ALLEN, P.; MARTIN-SANTOS, R.; SEAL, M.; SURGULADZE, S. A.; O'CARROL, C.; ATAKAN, Z.; ZUARDI, A. W.; MCGUIRE, P. K. Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. **Arch Gen Psychiatry**, v. 66, n. 1, p. 95-105, 2009.

GARRETT, E. R.; HUNT, C. A. Physiochemical properties, solubility, and protein binding of delta-9-tetrahydrocannabinol. **J Pharm Sci**, v. 63, p. 1056-1064, 1974.

GEORGE, D. T.; HERION, D. W.; JONES, C. L.; PHILLIPS, M. J.; HERSH, J.; HILL, D.; HEILIG, M.; RAMCHANDANI, V. A.; GEYER, C.; SPERO, D. E.; SINGLEY, E. D.; O'MALLEY, S. S.; BISHAI, R.; RAWLINGS, R. R.; KUNOS, G. Rimonabant (SR141716) has no effect on alcohol self-administration or endocrine measures in nontreatment-seeking heavy alcohol drinkers. **Psychopharmacology (Berl)**, v. 208, n. 1, p. 37-44, 2010.

GLASS, M.; DRAGUNOW, M.; FAULL, R. L. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. **Neuroscience**, v. 77, n. 2, p. 299-318, 1997.

GOMES, F. V.; REIS, D. G.; ALVES, F. H.; CORREA, F. M.; GUIMARAES, F. S.; RESSTEL, L. B. Cannabidiol injected into the bed nucleus of the stria terminalis reduces the expression of contextual fear conditioning via 5-HT1A receptors. **J Psychopharmacol**, v. 26, n. 1, p. 104-13, 2012.

GOMES, F. V.; RESSTEL, L. B.; GUIMARAES, F. S. The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. **Psychopharmacology (Berl)**, v. 213, n. 2-3, p. 465-73, 2011.

GONCALVES, D. M.; STEIN, A. T.; KAPCZINSKI, F. Performance of the Self-Reporting Questionnaire as a psychiatric screening questionnaire: a comparative study with Structured Clinical Interview for DSM-IV-TR. **Cad Saude Publica**, v. 24, n. 2, p. 380-90, 2008.

GONG, J. P.; ONAIVI, E. S.; ISHIGURO, H.; LIU, Q. R.; TAGLIAFERRO, P. A.; BRUSCO, A.; UHL, G. R. Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. **Brain Res**, v. 1071, n. 1, p. 10-23, 2006.

GONZALEZ, R. Acute and non-acute effects of cannabis on brain functioning and neuropsychological performance. **Neuropsychol Rev**, v. 17, n. 3, p. 347-61, 2007.

GORELICK, D. A.; GOODWIN, R. S.; SCHWILKE, E.; SCHWOPE, D. M.; DARWIN, W. D.; KELLY, D. L.; MCMAHON, R. P.; LIU, F.; ORTEMANN-RENON, C.; BONNET, D.; HUESTIS, M. A. Antagonist-elicited cannabis withdrawal in humans. **J Clin Psychopharmacol**, v. 31, n. 5, p. 603-612, 2011.

GORELICK, D. A.; HEISHMAN, S. J.; PRESTON, K. L.; NELSON, R. A.; MOOLCHAN, E. T.; HUESTIS, M. A. The cannabinoid CB1 receptor antagonist rimonabant attenuates the hypotensive effect of smoked marijuana in male smokers. **Am Heart J**, v. 151, n. 3, p. 754 e1-754 e5, 2006.

GRAEFF, F. G.; PARENTE, A.; DEL-BEN, C. M.; GUIMARAES, F. S. Pharmacology of human experimental anxiety. **Braz J Med Biol Res**, v. 36, n. 4, p. 421-32, 2003.

GRLIC, L. A Comparative-Study on Some Chemical and Biological Characteristics of Various Samples of Cannabis Resin. **Bull Narcotics**, v. 14, n. 3, p. 37-46, 1962.

GROTENHERMEN, F. Pharmacokinetics and pharmacodynamics of cannabinoids. **Clin Pharmacokinet**, v. 42, n. 4, p. 327-60, 2003.

GROTENHERMEN, F. Pharmacology of cannabinoids. **Neuro Endocrinol Lett**, v. 25, n. 1-2, p. 14-23, 2004.

GROTENHERMEN, F. The toxicology of cannabis and cannabis prohibition. **Chem Biodivers**, v. 4, n. 8, p. 1744-69, 2007.

GROTENHERMEN, F.; LESON, G.; BERGHAUS, G.; DRUMMER, O. H.; KRUGER, H. P.; LONGO, M.; MOSKOWITZ, H.; PERRINE, B.; RAMAEKERS, J. G.; SMILEY, A.; TUNBRIDGE, R. Developing limits for driving under cannabis. **Addiction**, v. 102, n. 12, p. 1910-7, 2007.

GUIMARAES, F. S.; CHIARETTI, T. M.; GRAEFF, F. G.; ZUARDI, A. W. Antianxiety effect of cannabidiol in the elevated plus-maze. **Psychopharmacology (Berl)**, v. 100, n. 4, p. 558-9, 1990.

GUIMARAES, F. S.; DE AGUIAR, J. C.; MECHOULAM, R.; BREUER, A. Anxiolytic effect of cannabidiol derivatives in the elevated plus-maze. **Gen Pharmacol**, v. 25, n. 1, p. 161-4, 1994.

GUIMARAES, F. S.; KOHEM, C. L.; GUS, G.; FILLMANN, H. S.; DE-VECINO, M. C.; DE-PAOLI, C. L.; RIBEIRO, A. M.; TEIXEIRA, C. C.; WANNMACHER, L. A simple simulated public speaking test for evaluating anxiolytic drugs. **Braz J Med Biol Res**, v. 22, n. 9, p. 1083-9, 1989.

GUIMARAES, F. S.; ZUARDI, A. W.; GRAEFF, F. G. Effect of chlorimipramine and maprotiline on experimental anxiety in humans. **J Psychopharmacol**, v. 1, n. 3, p. 184-92, 1987.

GUY, G. W.; ROBSON, P. J. A Phase I, open label, four-way crossover study to compare the pharmacokinetic profiles of a single dose of 20 mg of a cannabis based medicine extract (CBME) administered on 3 different areas of the buccal mucosa and to investigate the pharmacokinetics of CBME per oral in healthy male and female volunteers (GWPK0112). **Journal of Cannabis Therapeutics**, v. 3, n. 4, p. 79-120, 2003.

HALL, W.; SOLOWIJ, N. Adverse effects of cannabis. **Lancet**, v. 352, n. 9140, p. 1611-6, 1998.

HALLAK, J. E.; CRIPPA, J. A.; QUEVEDO, J.; ROESLER, R.; SCHRODER, N.; NARDI, A. E.; KAPCZINSKI, F. National Science and Technology Institute for Translational Medicine (INCT-TM): advancing the field of translational medicine and mental health. **Rev Bras Psiquiatr**, v. 32, n. 1, p. 83-90, 2010a.

HALLAK, J. E.; DURSUN, S. M.; BOSI, D. C.; DE MACEDO, L. R.; MACHADO-DE-SOUSA, J. P.; ABRAO, J.; CRIPPA, J. A.; MCGUIRE, P.; KRYSTAL, J. H.; BAKER, G. B.; ZUARDI, A. W. The interplay of cannabinoid and NMDA glutamate receptor systems in humans: preliminary evidence of interactive effects of cannabidiol and ketamine in healthy human subjects. **Prog Neuropsychopharmacol Biol Psychiatry**, v. 35, n. 1, p. 198-202, 2011.

HALLAK, J. E.; MACHADO-DE-SOUSA, J. P.; CRIPPA, J. A.; SANCHES, R. F.; TRZESNIAK, C.; CHAVES, C.; BERNARDO, S. A.; REGALO, S. C.; ZUARDI, A. W. Performance of schizophrenic patients in the Stroop Color Word Test and electrodermal responsiveness after acute administration of cannabidiol (CBD). **Rev Bras Psiquiatr**, v. 32, n. 1, p. 56-61, 2010b.

HANEY, M. The marijuana withdrawal syndrome: diagnosis and treatment. **Curr Psychiatry Rep**, v. 7, n. 5, p. 360-6, 2005.

HANEY, M.; COMER, S. D.; WARD, A. S.; FOLTIN, R. W.; FISCHMAN, M. W. Factors influencing marijuana self-administration by humans. **Behav Pharmacol**, v. 8, n. 2-3, p. 101-112, 1997.

HART, C. L.; VAN GORP, W.; HANEY, M.; FOLTIN, R. W.; FISCHMAN, M. W. Effects of acute smoked marijuana on complex cognitive performance. **Neuropsychopharmacology**, v. 25, n. 5, p. 757-765, 2001.

HARVEY, D. J.; LEUSCHNER, J. T. A.; PATON, W. D. M. Gas chromatographic and mass spectrometric studies on the metabolism and pharmacokinetics of delta-tetrahydrocannabinol in the rabbit. **J Chromatogr**, v. 239, p. 243-250, 1982.

HARVEY, D. J.; MARTIN, B. R.; PATON, W. D. Identification and measurement of cannabinoids and their in vivo metabolites in liver by gas chromatography--mass spectrometry. **Adv Biosci**, v. 22-23, p. 45-62, 1978.

HARVEY, D. J.; MECHOULAM, R. Metabolites of cannabidiol identified in human urine. **Xenobiotica**, v. 20, n. 3, p. 303-20, 1990.

HARVEY, D. J.; SAMARA, E.; MECHOULAM, R. Comparative metabolism of cannabidiol in dog, rat and man. **Pharmacol Biochem Behav**, v. 40, n. 3, p. 523-32, 1991.

HAYAKAWA, K.; MISHIMA, K.; NOZAKO, M.; OGATA, A.; HAZEKAWA, M.; LIU, A. X.; FUJIOKA, M.; ABE, K.; HASEBE, N.; EGASHIRA, N.; IWASAKI, K.; FUJIWARA, M. Repeated treatment with cannabidiol but not Delta9-tetrahydrocannabinol has a neuroprotective effect without the development of tolerance. **Neuropharmacology**, v. 52, n. 4, p. 1079-87, 2007.

HERKENHAM, M.; LYNN, A. B.; LITTLE, M. D.; JOHNSON, M. R.; MELVIN, L. S.; DE COSTA, B. R.; RICE, K. C. Cannabinoid receptor localization in brain. **Proc Natl Acad Sci U S A**, v. 87, n. 5, p. 1932-6, 1990.

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. Reversible and regionally selective downregulation of brain cannabinoid CB(1) receptors in chronic daily cannabis smokers. **Mol Psychiatry**, v. 17, n. 6, p. 642-649, 2012.

HO, B. T.; FRITCHIE, G. E.; KRALIK, P. M.; ENGLERT, L. F.; MCISAAC, W. M.; IDANPAAN-HEIKKILA, J. Distribution of tritiated-1 delta 9tetrahydrocannabinol in rat tissues after inhalation. **J Pharm Pharmacol**, v. 22, n. 7, p. 538-9, 1970.

HODGSON, R.; ALWYN, T.; JOHN, B.; THOM, B.; SMITH, A. The FAST Alcohol Screening Test. **Alcohol Alcohol**, v. 37, n. 1, p. 61-6, 2002.

HOFMANN, S. G.; DIBARTOLO, P. M. An instrument to assess self-statements during public speaking: scale development and preliminary psychometric properties. **Behav Ther**, v. 31, n. 3, p. 499-515, 2000.

HOLLISTER, L. E. Cannabidiol and cannabinol in man. **Experientia**, v. 29, n. 7, p. 825-6, 1973.

HOWLETT, A. C. Pharmacology of cannabinoid receptors. **Annu Rev Pharmacol Toxicol**, v. 35, p. 607-34, 1995.

HOWLETT, A. C.; BARTH, F.; BONNER, T. I.; CABRAL, G.; CASELLAS, P.; DEVANE, W. A.; FELDER, C. C.; HERKENHAM, M.; MACKIE, K.; MARTIN, B. R.; MECHOULAM, R.; PERTWEE, R. G. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. **Pharmacol Rev**, v. 54, n. 2, p. 161-202, 2002.

HUESTIS, M. A. Human cannabinoid pharmacokinetics. **Chem Biodivers**, v. 4, n. 8, p. 1770-804, 2007.

HUESTIS, M. A. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. **Handb Exp Pharmacol**, v. 168, p. 657-690, 2005.

HUESTIS, M. A.; BOYD, S. J.; HEISHMAN, S. J.; PRESTON, K. L.; BONNET, D.; LE FUR, G.; GORELICK, D. A. Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users. **Psychopharmacology (Berl)**, v. 194, n. 4, p. 505-515, 2007.

HUESTIS, M. A.; GORELICK, D. A.; HEISHMAN, S. J.; PRESTON, K. L.; NELSON, R. A.; MOOLCHAN, E. T.; FRANK, R. A. Blockade of effects of smoked marijuana by the CB1-selective cannabinoid receptor antagonist SR141716. **Arch Gen Psychiatry**, v. 58, n. 4, p. 322-330, 2001.

HUESTIS, M. A.; HENNINGFIELD, J. E.; CONE, E. J. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. **Anal Toxicol**, v. 16, n. 5, p. 276-282, 1992.

INSERM COLLECTIVE EXPERTISE CENTRE. **Cannabis: Effects of consumption on health**. Paris: Institut national de la santé et de la recherche médicale, 2001.

IZZO, A. A.; BORRELLI, F.; CAPASSO, R.; DI MARZO, V.; MECHOULAM, R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. **Trends Pharmacol Sci**, v. 30, n. 10, p. 515-27, 2009.

JAEGER, W.; BENET, L. Z.; BORNHEIM, L. M. Inhibition of cyclosporine and tetrahydrocannabinol metabolism by cannabidiol in mouse and human microsomes. **Xenobiotica**, v. 26, n. 3, p. 275-84, 1996.

JOHANSSON, E.; AGURELL, S.; HOLLISTER, L. E.; HALLDIN, M. M. Prolonged apparent half-life of delta-1-tetrahydrocannabinol in plasma of chronic marijuana users. **J Pharm Pharmacol**, v. 40, n. 5, p. 374-375, 1988.

JOHANSSON, E.; NOREN, K.; SJOVALL, J.; HALLDIN, M. M. Determination of delta 1-tetrahydrocannabinol in human fat biopsies from marihuana users by gas chromatography-mass spectrometry. **Biomed Chromatogr**, v. 3, n. 1, p. 35-8, 1989.

JOHNS, A. Psychiatric effects of cannabis. **Br J Psychiatry**, v. 1787, p. 116-122, 2001.

JOHNSON, J. R.; JENNISON, T. A.; PEAT, M. A.; FOLTZ, R. L. Stability of delta-9-tetrahydrocannabinol (THC), 11-hydroxy-THC, and 11-nor-9-carboxy-THC in blood and plasma. **J Anal Toxicol**, v. 8, p. 202-204, 1984.

JONES, A. W.; HOLMGREN, A.; KUGELBERG, F. C. Driving under the influence of cannabis: a 10-year study of age and gender differences in the concentrations of tetrahydrocannabinol in blood. **Addiction**, v. 103, n. 3, p. 452-61, 2008.

JONES, R. K.; SHINAR, D.; WALSH, J. M. State of Knowledge of Drug-Impaired Driving. **Report**. Washington, DC, 2003, 1-120).

JONES, R. T. Behavioral Tolerance: Lessons Learned from Cannabis Research. **NIDA Research Monograph**, v. 18, p. 118-126, 1978.

KARNIOL, I. G.; CARLINI, E. A. Comparative studies in man and in laboratory animals on 8 - and 9 -trans-tetrahydrocannabinol. **Pharmacology**, v. 9, n. 2, p. 115-26, 1973.

KARNIOL, I. G.; SHIRAKAWA, I.; KASINSKI, N.; PFEFERMAN, A.; CARLINI, E. A. Cannabidiol interferes with the effects of delta 9 - tetrahydrocannabinol in man. **Eur J Pharmacol**, v. 28, n. 1, p. 172-7, 1974.

KARSCHNER, E.; SCHWILKE, E.; LOWE, R.; DARWIN, W.; POPE, H., JR; HERNING, R.; CADET, J.; HUESTIS, M. Do Delta9-tetrahydrocannabinol concentrations indicate recent use in chronic cannabis users? **Addiction**, v. 104, n. 12, p. 2041-2048, 2009a.

KARSCHNER, E.; SCHWILKE, E.; LOWE, R.; DARWIN, W. D.; HERNING, R.; CADET, J.; HUESTIS, M. Implications of plasma Delta9-tetrahydrocannabinol, 11-hydroxy-THC, and 11-nor-9-carboxy-THC concentrations in chronic cannabis smokers. **J Anal Toxicol**, v. 33, n. 8, p. 469-477, 2009b.

KARSCHNER, E. L.; DARWIN, W. D.; GOODWIN, R. S.; WRIGHT, S.; HUESTIS, M. A. Plasma cannabinoid pharmacokinetics following controlled oral delta9-tetrahydrocannabinol and oromucosal cannabis extract administration. **Clin Chem**, v. 57, n. 1, p. 66-75, 2011.

KATHURIA, S.; GAETANI, S.; FEGLEY, D.; VALINO, F.; DURANTI, A.; TONTINI, A.; MOR, M.; TARZIA, G.; LA RANA, G.; CALIGNANO, A.; GIUSTINO, A.; TATTOLI, M.; PALMERY, M.; CUOMO, V.; PIOMELLI, D. Modulation of anxiety through blockade of anandamide hydrolysis. **Nature Medicine**, v. 9, n. 1, p. 76-81, 2003.

KATONA, I.; RANCZ, E. A.; ACSADY, L.; LEDENT, C.; MACKIE, K.; HAJOS, N.; FREUND, T. F. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. **J Neurosci**, v. 21, n. 23, p. 9506-18, 2001.

KESSLER, R. C. The global burden of anxiety and mood disorders: putting the European Study of the Epidemiology of Mental Disorders (ESEMeD) findings into perspective. **J Clin Psychiatry**, v. 68 Suppl 2, p. 10-9, 2007.

KLEIN, C.; KARANGES, E.; SPIRO, A.; WONG, A.; SPENCER, J.; HUYNH, T.; GUNASEKARAN, N.; KARL, T.; LONG, L. E.; HUANG, X. F.; LIU, K.; ARNOLD, J. C.; MCGREGOR, I. S. Cannabidiol potentiates Delta-tetrahydrocannabinol (THC) behavioural effects and alters THC pharmacokinetics during acute and chronic treatment in adolescent rats. **Psychopharmacology (Berl)**, v. 218, n. 2, p. 443-57, 2011.

KUNOS, G. Understanding metabolic homeostasis and imbalance: what is the role of the endocannabinoid system? **Am J Med**, v. 120, n. 9 Suppl 1, p. S18-24; discussion S24, 2007.

KUNOS, G.; TAM, J. The case for peripheral CB(1) receptor blockade in the treatment of visceral obesity and its cardiometabolic complications. **Br J Pharmacol**, v. 163, n. 7, p. 1423-31, 2011.

LACEY, J.; BRAINARD, K.; SNITOW, S. Drug Per Se Laws: A Review of Their Use in States. **Report**. Washington, DC, 2010.

LACEY, J. H.; KELLEY-BAKER, T.; FURR-HOLDEN, D.; VOAS, R. B.; ROMANO, E.; RAMIREZ, A.; BRAINARD, K.; MOORE, C.; TORRES, P.; BERNING, A. 2007 National Roadside Survey of Alcohol and Drug Use by Drivers: Drug Results. **Report**. Washington, DC, 2009.

LANE, S. D.; CHEREK, D. R.; TCHEREMISSINE, O. V.; LIEVING, L. M.; PIETRAS, C. J. Acute marijuana effects on human risk taking. **Neuropsychopharmacology**, v. 30, n. 4, p. 800-9, 2005.

LAUMON, B.; GADEGBEKU, B.; MARTIN, J. L.; BIECHELER, M. B. Cannabis intoxication and fatal road crashes in France: population based case-control study. **BMJ**, v. 331, n. 7529, p. 1371, 2005.

LE FOLL, B.; FORGET, B.; AUBIN, H. J.; GOLDBERG, S. R. Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies. **Addict Biol**, v. 13, n. 2, p. 239-52, 2008.

LEE, C. M.; NEIGHBORS, C.; HENDERSHOT, C. S.; GROSSBARD, J. R. Development and preliminary validation of a comprehensive marijuana motives questionnaire. **J Stud Alcohol Drugs**, v. 70, n. 2, p. 279-87, 2009.

LEMOS, J. I.; RESSTEL, L. B.; GUIMARAES, F. S. Involvement of the prelimbic prefrontal cortex on cannabidiol-induced attenuation of contextual conditioned fear in rats. **Behav Brain Res**, v. 207, n. 1, p. 105-11, 2010.

LEUSCHNER, J. T. A.; HARVEY, D. J.; BULLINGHAM, R. E. S.; PATON, W. D. M. Pharmacokinetics of delta-9-tetrahydrocannabinol in rabbits following single or multiple intravenous doses. **Drug Metab Dispos**, v. 14, p. 230-238, 1986.

LEWEKE, F. M.; KOETHE, D.; GERTH, C. W.; NOLDEN, B. M.; SCHREIBER, D.; GROSS, S.; SCHULTZE-LUTTER, F.; HELLMICH, M.; KLOSTERKOTTER, J. Cannabidiol as an antipsychotic agent. **Eur Psychiatry**, v. 22, p. S21-S21, 2007.

LIGRESTI, A.; MORIELLO, A. S.; STAROWICZ, K.; MATIAS, I.; PISANTI, S.; DE PETROCELLIS, L.; LAEZZA, C.; PORTELLA, G.; BIFULCO, M.; DI MARZO, V. Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. **J Pharmacol Exp Ther**, v. 318, n. 3, p. 1375-87, 2006.

LINDGREN, J. E.; OHLSSON, A.; AGURELL, S.; HOLLISTER, L.; GILLESPIE, H. Clinical effects and plasma levels of delta 9-tetrahydrocannabinol (delta 9-THC) in heavy and light users of cannabis. **Psychopharmacology (Berl)**, v. 74, n. 3, p. 208-12, 1981.

LOWE, B.; UNUTZER, J.; CALLAHAN, C. M.; PERKINS, A. J.; KROENKE, K. Monitoring depression treatment outcomes with the patient health questionnaire-9. **Med Care**, v. 42, n. 12, p. 1194-201, 2004.

LOWE, R. H.; KARSCHNER, E. L.; SCHWILKE, E. W.; BARNES, A. J.; HUESTIS, M. A. Simultaneous quantification of delta-9-tetrahydrocannabinol (THC), 11-hydroxy-delta-9-tetrahydrocannabinol (11-OH-THC), and 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in human plasma using two-dimensional gas chromatography,

cryofocusing, and electron impact-mass spectrometry. **J Chromatogr A**, v. 1163, n. 1-2, p. 318-327, 2007.

MACKIE, K. Distribution of cannabinoid receptors in the central and peripheral nervous system. **Handb Exp Pharmacol**, n. 168, p. 299-325, 2005.

MARSICANO, G.; WOTJAK, C. T.; AZAD, S. C.; BISOGNO, T.; RAMMES, G.; CASCIO, M. G.; HERMANN, H.; TANG, J.; HOFMANN, C.; ZIEGLGANSBERGER, W.; DI MARZO, V.; LUTZ, B. The endogenous cannabinoid system controls extinction of aversive memories. **Nature**, v. 418, n. 6897, p. 530-4, 2002.

MARTIN, M.; LEDENT, C.; PARMENTIER, M.; MALDONADO, R.; VALVERDE, O. Involvement of CB1 cannabinoid receptors in emotional behaviour. **Psychopharmacology (Berl)**, v. 159, n. 4, p. 379-87, 2002.

MATSUDA, L. A.; LOLAIT, S. J.; BROWNSTEIN, M. J.; YOUNG, A. C.; BONNER, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. **Nature**, v. 346, p. 561-564, 1990.

MATSUNAGA, T.; IWAWAKI, Y.; WATANABE, K.; YAMAMOTO, I.; KAGEYAMA, T.; YOSHIMURA, H. Metabolism of delta-9-tetrahydrocannabinol by cytochrome P450 isozymes purified from hepatic microsomes of monkeys. **Life Sciences**, v. 56, p. 2089-2095, 1995.

MATTHEWS, P. M.; HONEY, G. D.; BULLMORE, E. T. Applications of fMRI in translational medicine and clinical practice. **Nat Rev Neurosci**, v. 7, n. 9, p. 732-44, 2006.

MCARDLE, K.; MACKIE, P.; PERTWEE, R.; GUY, G.; WHITTLE, B.; HAWKSWORTH, G. Selective inhibition of delta-9-tetrahydrocannabinol metabolite formation by cannabidiol in vitro (abstract) in Proceedings of the BTS Annual Congress. **Toxicology**, v. 168, p. 133-134, 2001.

MCNAIR, D. M.; FRANKENTHALER, L. M.; CZERLINSKY, T.; WHITE, T. W.; SASSON, S.; FISHER, S. Simulated public speaking as a model of clinical anxiety. **Psychopharmacology (Berl)**, v. 77, n. 1, p. 7-10, 1982.

MECHOULAM, R.; BEN-SHABAT, S.; HANUS, L.; LIGUMSKY, M.; KAMINSKI, N. E.; SCHATZ, A. R.; GOPHER, A.; ALMOG, S.; MARTIN, B. R.; COMPTON, D. R.; ET AL. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. **Biochem Pharmacol**, v. 50, n. 1, p. 83-90, 1995.

MECHOULAM, R.; SHVO, Y. Hashish. I. The structure of cannabidiol. **Tetrahedron**, v. 19, n. 12, p. 2073-8, 1963.

MEHMEDIC, Z.; CHANDRA, S.; SLADE, D.; DENHAM, H.; FOSTER, S.; PATEL, A. S.; ROSS, S. A.; KHAN, I. A.; ELSOHLY, M. A. Potency trends of Delta9-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. **J Forensic Sci**, v. 55, n. 5, p. 1209-17, 2010.

MENESES-GAYA, C.; CRIPPA, J. A.; ZUARDI, A. W.; LOUREIRO, S. R.; HALLAK, J. E.; TRZESNIAK, C.; MACHADO DE SOUSA, J. P.; CHAGAS, M. H.; SOUZA, R. M.; MARTIN-SANTOS, R. The fast alcohol screening test (FAST) is as good as the AUDIT to screen alcohol use disorders. **Subst Use Misuse**, v. 45, n. 10, p. 1542-57, 2010.

MEYER, R. E.; PILLARD, R. C.; SHAPIRO, L. M.; MIRIN, S. M. Administration of marijuana to heavy and casual marijuana users. **Am J Psychiatry**, v. 128, n. 2, p. 198-204, 1971.

MINCIS, M.; PFEFERMAN, A.; GUIMARAES, R. X.; RAMOS, O. L.; ZUKERMAN, E.; KARNIOL, I. G.; CARLINI, E. A. Chronic administration of cannabidiol in man. Pilot study. **AMB Rev Assoc Med Bras**, v. 19, n. 5, p. 185-90, 1973.

MONTGOMERY, S. A.; LECRUBIER, Y.; BALDWIN, D. S.; KASPER, S.; LADER, M.; NIL, R.; STEIN, D.; VAN REE, J. M. ECNP Consensus Meeting, March 2003. Guidelines for the investigation of efficacy in social anxiety disorder. **Eur Neuropsychopharmacol**, v. 14, n. 5, p. 425-33, 2004.

MOREIRA, F. A.; AGUIAR, D. C.; GUIMARAES, F. S. Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. **Prog Neuropsychopharmacol Biol Psychiatry**, v. 30, n. 8, p. 1466-71, 2006.

MOREIRA, F. A.; CRIPPA, J. A. The psychiatric side-effects of rimonabant. **Rev Bras Psiquiatr**, v. 31, n. 2, p. 145-53, 2009.

MOREIRA, F. A.; GRIEB, M.; LUTZ, B. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. **Best Pract Res Clin Endocrinol Metab**, v. 23, n. 1, p. 133-44, 2009.

MOREIRA, F. A.; 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 CB1 receptors. **Neuropharmacology**, v. 54, n. 1, p. 141-50, 2008.

MOREIRA, F. A.; LUTZ, B. The endocannabinoid system: emotion, learning and addiction. **Addict Biol**, v. 13, n. 2, p. 196-212, 2008.

MORGAN, C. J.; FREEMAN, T. P.; SCHAFER, G. L.; CURRAN, H. V. Cannabidiol attenuates the appetitive effects of Delta 9-tetrahydrocannabinol in humans smoking their chosen cannabis. **Neuropsychopharmacology**, v. 35, n. 9, p. 1879-85, 2010.

MUNRO, S.; THOMAS, K. L.; ABU-SHAAR, M. Molecular characterization of a peripheral receptor for cannabinoids. **Nature**, v. 365, p. 61-65, 1993.

MURA, P.; KINTZ, P.; DUMESTRE, V.; RAUL, S.; HAUET, T. THC can be detected in brain while absent in blood. **J Anal Toxicol**, v. 29, n. 8, p. 842-843, 2005.

NAKAZAWA, K.; COSTA, E. Metabolism of delta 9 -tetrahydrocannabinol by lung and liver homogenates of rats treated with methylcholanthrene. **Nature**, v. 234, n. 5323, p. 48-9, 1971.

NARIMATSU, S.; WATANABE, K.; MATSUNAGA, T.; YAMAMOTO, I.; IMAOKA, S.; FUNAE, Y.; YOSHIMURA, H. Cytochrome P-450 isozymes involved in the oxidative metabolism of delta 9-tetrahydrocannabinol by liver microsomes of adult female rats. **Drug Metab Dispos**, v. 20, n. 1, p. 79-83, 1992.

NAVARRO, M.; HERNANDEZ, E.; MUNOZ, R. M.; DEL ARCO, I.; VILLANUA, M. A.; CARRERA, M. R. A.; DE FONSECA, F. R. Acute administration of the CB1, cannabinoid receptor antagonist SR141716A induces anxiety-like responses in the rat. **NeuroReport**, v. 8, p. 491-496, 1997.

NISSEN, S. E.; NICHOLLS, S. J.; WOLSKI, K.; RODES-CABAU, J.; CANNON, C. P.; DEANFIELD, J. E.; DESPRES, J. P.; KASTELEIN, J. J.; STEINHUBL, S. R.; KAPADIA, S.; YASIN, M.; RUZYLLO, W.; GAUDIN, C.; JOB, B.; HU, B.; BHATT, D. L.; LINCOFF, A. M.; TUZCU, E. M. Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. **JAMA**, v. 299, n. 13, p. 1547-60, 2008.

NORRIS, H. The action of sedatives on brain stem oculomotor systems in man. **Neuropharmacology**, v. 10, n. 21, p. 181-91, 1971.

NOTCUTT, W.; PRICE, M.; MILLER, R.; NEWPORT, S.; PHILLIPS, C.; SIMMONS, S.; SANSOM, C. Initial experiences with medicinal extracts of cannabis for chronic pain: results from 34 'N of 1' studies. **Anaesthesia**, v. 59, n. 5, p. 440-52, 2004.

OHLSSON, A.; LINDGREN, J. E.; ANDERSSON, S.; AGURELL, S.; GILLESPIE, H.; HOLLISTER, L. E. Single dose kinetics of cannabidiol in man. In: AGURELL, S.; DEWEY, W. L.; WILLETTE, R. E.**The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects**. Orlando: Academic Press, 1984, p 219-225.

OHLSSON, A.; LINDGREN, J. E.; WAHLEN, A.; AGURELL, S.; HOLLISTER, L. E.; GILLESPIE, H. K. Plasma delta-9-tetrahydrocannabinol concentrations and clinical effects after oral and intravenous administration and smoking. **Clin Pharmacol Ther**, v. 28, p. 409-416, 1980.

OHLSSON, A.; LINDGREN, J. E.; WAHLEN, A.; AGURELL, S.; HOLLISTER, L. E.; GILLESPIE, H. K. Single dose kinetics of deuterium labelled delta-1-tetrahydrocannabinol in heavy and light cannabis users. **Biomed Mass Spectrom**, v. 9, p. 6-10, 1982.

ONAIVI, E. S.; GREEN, M. R.; MARTIN, B. R. Pharmacological characterization of cannabinoids in the elevated plus maze. **J Pharmacol Exp Ther**, v. 253, n. 3, p. 1002-9, 1990.

OSORIO, F. D.; CRIPPA, J. A. S.; LOUREIRO, S. R. Further Study of the Psychometric Qualities of a Brief Screening Tool for Social Phobia (MINI-SPIN) Applied to Clinical and Nonclinical Samples. **Perspect Psychiatr C**, v. 46, n. 4, p. 266-278, 2010.

OSORIO FDE, L.; CRIPPA, J. A.; LOUREIRO, S. R. Cross- cultural validation of the Brazilian Portuguese version of the Social Phobia Inventory (SPIN): study of the items and internal consistency. **Rev Bras Psiquiatr**, v. 31, n. 1, p. 25-9, 2009.

OSORIO, F. L.; CRIPPA, J. A.; LOUREIRO, S. R. Cross- cultural validation of the Brazilian Portuguese version of the Social Phobia Inventory (SPIN): study of the items and internal consistency. **Rev Bras Psiquiatr**, v. 31, n. 1, p. 25-9, 2009.

OSÓRIO, F. L.; CRIPPA, J. A. S. L., S. R. Self statements during public speaking scale (SSPS): cross-cultural adaptation for Brazilian Portuguese and internal consistency. **Revista de Psiquiatria Clínica**, v. 35, n. 6, p. 207-211, 2008.

PAGOTTO, U.; MARSICANO, G.; COTA, D.; LUTZ, B.; PASQUALI, R. The emerging role of the endocannabinoid system in endocrine regulation and energy balance. **Endocr Rev**, v. 27, n. 1, p. 73-100, 2006.

PARENTE, A. C.; GARCIA-LEAL, C.; DEL-BEN, C. M.; GUIMARAES, F. S.; GRAEFF, F. G. Subjective and neurovegetative changes in healthy volunteers and panic patients performing simulated public speaking. **Eur Neuropsychopharmacol**, v. 15, n. 6, p. 663-71, 2005.

PARKER, L. A.; BURTON, P.; SORGE, R. E.; YAKIWCHUK, C.; MECHOULAM, R. Effect of low doses of delta9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. **Psychopharmacology (Berl)**, v. 175, n. 3, p. 360-6, 2004.

PATEL, S.; HILLARD, C. J. 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**, v. 318, n. 1, p. 304-11, 2006.

PATON, W. D.; PERTWEE, R. G. Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. **Br J Pharmacol**, v. 44, n. 2, p. 250-61, 1972.

PEREZ-REYES, M.; TIMMONS, M. C.; DAVIS, K. H.; WALL, E. M. A comparison of the pharmacological activity in man of intravenously administered delta9-tetrahydrocannabinol, cannabinol, and cannabidiol. **Experientia**, v. 29, n. 11, p. 1368-9, 1973.

PERTWEE, R. G. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. **Br J Pharmacol**, v. 153, n. 2, p. 199-215, 2008.

PERTWEE, R. G. Pharmacology of cannabinoid CB1 and CB2 receptors. **Pharmacol Ther**, v. 74, p. 129-180, 1997.

PERTWEE, R. G.; ROSS, R. A. Cannabinoid receptors and their ligands. **Prostaglandins Leukot Essent Fatty Acids**, v. 66, n. 2-3, p. 101-21, 2002.

PI-SUNYER, F. X.; ARONNE, L. J.; HESHMATI, H. M.; DEVIN, J.; ROSENSTOCK, J. Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. **JAMA**, v. 295, n. 7, p. 761-75, 2006.

POPE, H.; GRUBER, A.; HUDSON, J.; HUESTIS, M.; YURGELUN-TODD, D. Neuropsychological performance in long-term cannabis users. **Arch Gen Psychiatry**, v. 58, n. 10, p. 909-915, 2001.

POPE, H. G.; YURGELUN-TODD, D. The residual cognitive effects of heavy marijuana use in college students. **JAMA**, v. 275, n. 7, p. 521-527, 1996.

POTTER, D. J.; CLARK, P.; BROWN, M. B. Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. **J Forensic Sci**, v. 53, n. 1, p. 90-4, 2008.

QUARTA, C.; MAZZA, R.; OBICI, S.; PASQUALI, R.; PAGOTTO, U. Energy balance regulation by endocannabinoids at central and peripheral levels. **Trends Mol Med**, v. 17, n. 9, p. 518-26, 2011.

RAMAEKERS, J.; KAUERT, G.; THEUNISSEN, E.; TOENNES, S.; MOELLER, M. Neurocognitive performance during acute THC intoxication in heavy and occasional cannabis users. **J Psychopharmacol**, v. 23, n. 3, p. 266-277, 2009.

RAMAEKERS, J.; THEUNISSEN, E.; DE BROUWER, M.; TOENNES, S.; MOELLER, M.; KAUERT, G. Tolerance and cross-tolerance to neurocognitive effects of THC and alcohol in heavy cannabis users. **Psychopharmacology (Berl)**, v. 214, n. 2, p. 391-401, 2011.

RAMAEKERS, J. G.; MOELLER, M. R.; VAN RUITENBEEK, P.; THEUNISSEN, E. L.; SCHNEIDER, E.; KAUERT, G. Cognition and motor control as a function of Delta9-THC concentration in serum and oral fluid: limits of impairment. **Drug Alcohol Depend**, v. 85, n. 2, p. 114-22, 2006.

REILLY, D.; DIDCOTT, P.; SWIFT, W.; HALL, W. Long-term cannabis use: characteristics of users in an Australian rural area. **Addiction**, v. 93, n. 6, p. 837-846, 1998.

REN, Y.; WHITTARD, J.; HIGUERA-MATAS, A.; MORRIS, C. V.; HURD, Y. L. Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. **J Neurosci**, v. 29, n. 47, p. 14764-9, 2009.

RESSTEL, L. B.; JOCA, S. R.; MOREIRA, F. A.; CORREA, F. M.; GUIMARAES, F. S. Effects of cannabidiol and diazepam on behavioral and cardiovascular responses induced by contextual conditioned fear in rats. **Behav Brain Res**, v. 172, n. 2, p. 294-8, 2006.

RESSTEL, L. B.; TAVARES, R. F.; LISBOA, S. F.; JOCA, S. R.; CORREA, F. M.; GUIMARAES, F. S. 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. **Br J Pharmacol**, v. 156, n. 1, p. 181-8, 2009.

RICHARDSON, J. D.; AANONSEN, L.; HARGREAVES, K. M. SR 141716A, a cannabinoid receptor antagonist, produces hyperalgesia in untreated mice. **Eur J Pharmacol**, v. 319, p. R3-R4, 1997.

RIGOTTI, N. A.; GONZALES, D.; DALE, L. C.; LAWRENCE, D.; CHANG, Y. A randomized controlled trial of adding the nicotine patch to rimonabant for smoking cessation: efficacy, safety and weight gain. **Addiction**, v. 104, n. 2, p. 266-76, 2009.

RINALDI-CARMONA, M.; BARTH, F.; HEAULME, M.; ALONSO, R.; SHIRE, D.; CONGY, C.; SOUBRIE, P.; BRELIERE, J. C.; LE FUR, G. Biochemical and pharmacological characterisation of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. **Life Sci**, v. 56, n. 23-24, p. 1941-7, 1995.

RINALDI-CARMONA, M.; BARTH, F.; HEAULME, M.; SHIRE, D.; CALANDRA, B.; CONGY, C.; MARTINEZ, S.; MARUANI, J.; NELIAT, G.; CAPUT, D.; ET AL. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. **FEBS Lett**, v. 350, n. 2-3, p. 240-4, 1994.

RINALDI-CARMONA, M.; PIALOT, F.; CONGY, C.; REDON, E.; BARTH, F.; BACHY, A.; BRELIERE, J. C.; SOUBRIE, P.; LE FUR, G. Characterization and distribution of binding sites for [3H]-SR 141716A, a selective brain (CB1) cannabinoid receptor antagonist, in rodent brain. **Life Sci**, v. 58, n. 15, p. 1239-47, 1996.

ROSENSTOCK, J.; HOLLANDER, P.; CHEVALIER, S.; IRANMANESH, A. SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naive Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naive type 2 diabetes. **Diabetes Care**, v. 31, n. 11, p. 2169-76, 2008.

ROSS, R. A. Anandamide and vanilloid TRPV1 receptors. **Br J Pharmacol**, v. 140, n. 5, p. 790-801, 2003.

ROSS, R. A.; COUTTS, A. A.; MCFARLANE, S. M.; ANAVI-GOFFER, S.; IRVING, A. J.; PERTWEE, R. G.; MACEWAN, D. J.; SCOTT, R. H. Actions of cannabinoid receptor ligands on rat cultured sensory neurones: implications for antinociception. **Neuropharmacology**, v. 40, n. 2, p. 221-32, 2001.

RUCKER, D.; PADWAL, R.; LI, S. K.; CURIONI, C.; LAU, D. C. Long term pharmacotherapy for obesity and overweight: updated meta-analysis. **British Medical Journal**, v. 335, n. 7631, p. 1194-9, 2007.

RUSSO, E. B.; BURNETT, A.; HALL, B.; PARKER, K. K. Agonistic properties of cannabidiol at 5-HT1a receptors. **Neurochem Res**, v. 30, n. 8, p. 1037-1043, 2005.

SAITO, V. M.; WOTJAK, C. T.; MOREIRA, F. A. Pharmacological exploitation of the endocannabinoid system: new perspectives for the treatment of depression and anxiety disorders? **Rev Bras Psiquiatr**, v. 32 Suppl 1, p. S7-14, 2010.

SAMARA, E.; BIALER, M.; MECHOULAM, R. Pharmacokinetics of cannabidiol in dogs. **Drug Metab Dispos**, v. 16, n. 3, p. 469-72, 1988.

SANTUCCI, V.; STORME, J. J.; SOUBRIE, P.; LE FUR, G. Arousal-enhancing properties of the CB1 cannabinoid receptor antagonist SR141716A in rats as assessed by

electroencephalographic spectral and sleep-waking cycle analysis. **Life Sciences**, v. 58, p. 103-110, 1996.

SCHEEN, A. J.; FINER, N.; HOLLANDER, P.; JENSEN, M. D.; VAN GAAL, L. F. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. **Lancet**, v. 368, n. 9548, p. 1660-72, 2006.

SCHNEIER, F. R. Treatment of social phobia with antidepressants. **J Clin Psychiatry**, v. 62 Suppl 1, p. 43-8; discussion 49, 2001.

SCHWOPE, D. M.; KARSCHNER, E. L.; GORELICK, D. A.; HUESTIS, M. A. Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. **Clin Chem**, v. 57, n. 10, p. 1406-14, 2011a.

SCHWOPE, D. M.; KARSCHNER, E. L.; GORELICK, D. A.; HUESTIS, M. A. Identification of recent cannabis use: whole-blood and plasma free and glucuronidated cannabinoid pharmacokinetics following controlled smoked cannabis administration. **Clin Chem**, v. 57, n. 10, p. 1406-1414, 2011b.

SEAMON, M. J.; FASS, J. A.; MANISCALCO-FEICHTL, M.; ABU-SHRAIE, N. A. Medical marijuana and the developing role of the pharmacist. **Am J Health Syst Pharm**, v. 64, n. 10, p. 1037-44, 2007.

SILVEIRA, N. G.; TUFIK, S. Comparative effects between cannabidiol and diazepam on neophobia, food-intake and conflict behavior. **Res Commun Psych Psy**, v. 6, n. 3, p. 251-266, 1981.

SKAPER, S. D.; BURIANI, A.; DAL TOSO, R.; PETRELLI, L.; ROMANELLO, S.; FACCI, L.; LEON, A. The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. **Proc Natl Acad Sci U S A**, v. 93, n. 9, p. 3984-9, 1996.

SOARES VDE, P.; CAMPOS, A. C.; BORTOLI, V. C.; ZANGROSSI, H., JR.; GUIMARAES, F. S.; ZUARDI, A. W. Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. **Behav Brain Res**, v. 213, n. 2, p. 225-9, 2010.

SOLOWIJ, N.; STEPHENS, R. S.; ROFFMAN, R. A.; BABOR, T.; KADDEN, R.; MILLER, M.; CHRISTIANSEN, K.; MCREE, B.; VENDETTI, J. Cognitive functioning of long-term heavy cannabis users seeking treatment. **JAMA**, v. 287, n. 9, p. 1123-31, 2002.

SOYKA, M.; KOLLER, G.; SCHMIDT, P.; LESCH, O. M.; LEWEKE, M.; FEHR, C.; GANN, H.; MANN, K. F. Cannabinoid receptor 1 blocker rimonabant (SR 141716) for treatment of alcohol dependence: results from a placebo-controlled, double-blind trial. **J Clin Psychopharmacol**, v. 28, n. 3, p. 317-24, 2008.

STEIN, M. B.; STEIN, D. J. Social anxiety disorder. **Lancet**, v. 371, n. 9618, p. 1115-25, 2008.

STICHT, G.; KÄFERSTEIN, H. Grundbegriffe, toxikokinetik und toxikodynamik. In: BERGHAUS, G.; KRUGER, H. P.**Cannabis im Strassenverkehr**. Stuttgart: Gustav Fischer Verlag, 1998. cap. 1, p 1-11.

STOTT, C. G.; GUY, G. W.; WRIGHT, S.; WHITTLE, B. A. The effects of cannabis extracts Tetranabinex and Nabidiolex on human cytochrome P450-mediated metabolism. **Symposium on the Cannabinoids**, p. 163, 2005.

SUBSTANCE ABUSE AND MENTAL HEALTH SERVICES ADMINISTRATION. Results from the 2009 National Survey on Drug Use and Health: National Findings **Report**. Rockville, MD, 2010.

SUBSTANCE ABUSE AND MENTAL HEALTH SERVICES ADMINISTRATION (SAMHSA). Results from the 2010 National Survey on Drug Use and Health: Summary of National Findings. **Report**. Rockville, MD, 2011.

SUGIURA, T.; KONDO, S.; SUKAGAWA, A.; NAKANE, S.; SHINODA, A.; ITOH, K.; YAMASHITA, A.; WAKU, K. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. **Biochem Biophys Res Commun**, v. 215, n. 1, p. 89-97, 1995.

SWADI, H.; BOBIER, C. Substance use disorder comorbidity among inpatient youths with psychiatric disorder. **Australian and New Zealand Journal of Psychiatry**, v. 37, n. 3, p. 294-298, 2003.

SZABO, B.; SCHLICKER, E. Effects of cannabinoids on neurotransmission. **Handb Exp Pharmacol**, n. 168, p. 327-65, 2005.

TERRANOVA, J. P.; STORME, J. J.; LAFON, N.; PERIO, A.; RINALDI-CARMONA, M.; LEFUR, G.; SOUBRIE, P. Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. **Psychopharmacology**, v. 126, p. 165-172, 1996.

THOMAS, B. F.; COMPTON, D. R.; MARTIN, B. R. Characterization of the lipophilicity of natural and synthetic analogs of delta 9-tetrahydrocannabinol and its relationship to pharmacological potency. **J Pharmacol Exp Ther**, v. 255, n. 2, p. 624-30, 1990.

TIMPONE, J. G.; WRIGHT, D. J.; LI, N.; EGORIN, M. J.; ENAMA, M. E.; MAYERS, J.; GALETTO, G. The safety and pharmacokinetics of single-agent and combination therapy with megestrol acetate and dronabinol for the treatment of HIV wasting syndrome. The DATRI 004 Study Group. Division of AIDS Treatment Research Initiative. **AIDS Res Hum Retroviruses**, v. 13, n. 4, p. 305-15, 1997.

UNITED NATIONS OFFICE ON DRUGS AND CRIME (UNODC). World Drug Report 2011. **Report**. New York, 2011, 272).

VAN GAAL, L.; PI-SUNYER, X.; DESPRES, J. P.; MCCARTHY, C.; SCHEEN, A. Efficacy and safety of rimonabant for improvement of multiple cardiometabolic risk factors in overweight/obese patients: pooled 1-year data from the Rimonabant in Obesity (RIO) program. **Diabetes Care**, v. 31 Suppl 2, p. S229-40, 2008a.

VAN GAAL, L. F.; RISSANEN, A. M.; SCHEEN, A. J.; ZIEGLER, O.; ROSSNER, S.; GROUP, R.-E. S. Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. **Lancet**, v. 365, n. 9468, p. 1389-1397, 2005.

VAN GAAL, L. F.; SCHEEN, A. J.; RISSANEN, A. M.; ROSSNER, S.; HANOTIN, C.; ZIEGLER, O. Long-term effect of CB1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe Study. **Eur Heart J**, v. 29, n. 14, p. 1761-71, 2008b.

VAN SICKLE, M. D.; DUNCAN, M.; KINGSLEY, P. J.; MOUIHATE, A.; URBANI, P.; MACKIE, K.; STELLA, N.; MAKRIYANNIS, A.; PIOMELLI, D.; DAVISON, J. S.; MARNETT, L. J.; DI MARZO, V.; PITTMAN, Q. J.; PATEL, K. D.; SHARKEY, K. A. Identification and functional characterization of brainstem cannabinoid CB2 receptors. **Science**, v. 310, n. 5746, p. 329-32, 2005.

VANDREY, R.; HANEY, M. Pharmacotherapy for cannabis dependence: how close are we? **CNS Drugs**, v. 23, n. 7, p. 543-553, 2009.

VERSTRAETE, A.; KNOCHE, A.; JANTOS, R.; SKOPP, G.; GJERDE, H.; VINDENES, V.; MØRLAND, J.; LANGEL, K.; LILLSUNDE, P. Per se limits - Methods of defining cut-off values for zero tolerance. **Report**. 2011. (6th Framework Programme, Deliverable 142).

WADE, D. T.; MAKELA, P.; ROBSON, P.; HOUSE, H.; BATEMAN, C. 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**, v. 10, n. 4, p. 434-41, 2004.

WALL, M. E.; BRINE, D. R.; PEREZ-REYES, M. Metabolism of cannabinoids in man. In: BRAUDE, M. C.; SZARA, S.**The Pharmacology of Marihuana**. New York: Raven Press, 1976, p 93-113.

WALL, M. E.; SADLER, B. M.; BRINE, D.; TAYLOR, H.; PEREZ-REYES, M. Metabolism, disposition, and kinetics of delta-9-tetrahydrocannabinol in men and women. **Clin Pharmacol Ther**, v. 34, p. 352-363, 1983.

WHO. A user's guide to Self-Reporting Questionnaire. **Report**. Geneva, 1993.

WIDMAN, M.; NORDQVIST, M.; DOLLERY, C. T.; BRIANT, R. H. Metabolism of delta-1-tetrahydrocannabinol by the isolated perfused dog lung. Comparison with in vitro liver metabolism. **J Pharm Pharmacol**, v. 27, p. 842-848, 1975.

WOTHERSPOON, G.; FOX, A.; MCINTYRE, P.; COLLEY, S.; BEVAN, S.; WINTER, J. Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons. **Neuroscience**, v. 135, n. 1, p. 235-45, 2005.

YAMAORI, S.; OKAMOTO, Y.; YAMAMOTO, I.; WATANABE, K. Cannabidiol, a major phytocannabinoid, as a potent atypical inhibitor for CYP2D6. **Drug Metab Dispos**, v. 39, n. 11, p. 2049-56, 2011.

ZANELATI, T. V.; BIOJONE, C.; MOREIRA, F. A.; GUIMARAES, F. S.; JOCA, S. R. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. **Br J Pharmacol**, v. 159, n. 1, p. 122-8, 2010.

ZUARDI, A.; CRIPPA, J.; DURSUN, S.; MORAIS, S.; VILELA, J.; SANCHES, R.; HALLAK, J. Cannabidiol was ineffective for manic episode of bipolar affective disorder. **J Psychopharmacol**, v. 24, n. 1, p. 135-7, 2010.

ZUARDI, A. W. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. **Rev Bras Psiquiatr**, v. 30, n. 3, p. 271-80, 2008.

ZUARDI, A. W.; COSME, R. A.; GRAEFF, F. G.; GUIMARAES, F. S. Effects of ipsapirone and cannabidiol on human experimental anxiety. **J Psychopharmacol**, v. 7, n. 1 Suppl, p. 82-8, 1993.

ZUARDI, A. W.; CRIPPA, J. A.; HALLAK, J. E. Cannabis sativa: the plant that can induce unwanted effects and also treat them. **Rev Bras Psiquiatr**, v. 32, n. Suppl 1, p. S1-2, 2010.

ZUARDI, A. W.; CRIPPA, J. A.; HALLAK, J. E.; MOREIRA, F. A.; GUIMARAES, F. S. Cannabidiol, a Cannabis sativa constituent, as an antipsychotic drug. **Braz J Med Biol Res**, v. 39, n. 4, p. 421-9, 2006a.

ZUARDI, A. W.; CRIPPA, J. A.; HALLAK, J. E.; PINTO, J. P.; CHAGAS, M. H.; RODRIGUES, G. G.; DURSUN, S. M.; TUMAS, V. Cannabidiol for the treatment of psychosis in Parkinson's disease. **J Psychopharmacol**, v. 23, n. 8, p. 979-83, 2009.

ZUARDI, A. W.; HALLAK, J. E.; DURSUN, S. M.; MORAIS, S. L.; SANCHES, R. F.; MUSTY, R. E.; CRIPPA, J. A. Cannabidiol monotherapy for treatment-resistant schizophrenia. **J Psychopharmacol**, v. 20, n. 5, p. 683-6, 2006b.

ZUARDI, A. W.; HALLAK, J. E. C.; CRIPPA, J. A. S. Interaction between cannabidiol (CBD) and Delta(9)-tetrahydrocannabinol (THC): influence of administration interval and dose ratio between the cannabinoids. **Psychopharmacology**, v. 219, n. 1, p. 247-249, 2012.

ZUARDI, A. W.; KARNIOL, I. G. Changes in the conditioned emotional response of rats, induced by delta-9-THC, CBD and mixture of the 2 cannabinoids. **Arq Biol Tecnol**, v. 26, n. 3, p. 391-397, 1983.

ZUARDI, A. W.; KARNIOL, I. G. Transcultural evaluation of a self-evaluation scale of subjective states. **Jornal Brasileiro de Psiquiatria**, v. 131, p. 403-406, 1981.

ZUARDI, A. W.; MORAIS, S. L.; GUIMARAES, F. S.; MECHOULAM, R. Antipsychotic effect of cannabidiol. **J Clin Psychiatry**, v. 56, n. 10, p. 485-6, 1995.

ZUARDI, A. W.; SHIRAKAWA, I.; FINKELFARB, E.; KARNIOL, I. G. Action of cannabidiol on the anxiety and other effects produced by delta-9-THC in normal subjects. **Psychopharmacology (Berl)**, v. 76, p. 245-250, 1982.

Appendix A – Institutional Review Board (IRB) Approval

HOSPITAL DAS CLÍNICAS DA FACULDADE DE MEDICINA
DE RIBEIRÃO PRETO DA UNIVERSIDADE DE SÃO PAULO

www.hcrp.usp.br

USP - RIBEIRÃO
assistência - ensino - pesquisa

Ribeirão Preto, 17 de março de 2010

Ofício nº 712/2010
CEP/MGV

Prezados Senhores,

O trabalho intitulado **"EFEITOS DO CANABIDIOL, CLONAZEPAM E RIMONABANTO NA ANSIEDADE EXPERIMENTAL INDUZIDA EM HUMANOS"** foi analisado pelo Comitê de Ética em Pesquisa, em sua 303ª Reunião Ordinária realizada em 15/03/2010 e enquadrado na categoria: **APROVADO, bem como o Termo de Consentimento Livre e Esclarecido,** de acordo com o Processo HCRP nº 12407/2009.

Este Comitê segue integralmente a Conferência Internacional de Harmonização de Boas Práticas Clínicas (IGH-GCP), bem como a Resolução nº 196/96 CNS/MS.

Lembramos que devem ser apresentados a este CEP, o Relatório Parcial e o Relatório Final da pesquisa.

Atenciosamente.

DRª MARCIA GUIMARÃES VILLANOVA
Vice-Coordenadora do Comitê de Ética em Pesquisa do HCRP e da FMRP-USP

Ilustríssimos Senhores
MATEUS MACHADO BERGAMASCHI
PROFª DRª REGINA HELENA COSTA QUEIROZ
Faculdade de Ciências Farmacêuticas – Laboratório de Toxicologia

Comitê de Ética em Pesquisa HCRP e FMRP-USP - Campus Universitário
FWA – 0000 2733; IRB – 0000 2186 e Registro SISNEP/CONEP nº 4
Fone (16) 3602-2228 - E-mail : cep@hcrp.usp.br
Monte Alegre 14048-900 Ribeirão Preto SP

Appendix B – Brazil Socioeconomic Classification Criteria

CRITÉRIO DE CLASSIFICAÇÃO SÓCIOECONÔMICA BRASIL (CCSEB)
ABA, ANEP, ABIPEME; 1997

SISTEMA DE PONTOS
Posse de itens

	Não tem	Tem			
		1	2	3	4 ou +
Televisão em cores	0	2	3	4	5
Rádio / Tocador de mp3	0	1	2	3	4
Banheiro	0	2	3	4	4
Automóvel	0	2	4	5	5
Empregada / Mensalista	0	2	4	4	4
Aspirador de pó	0	1	1	1	1
Máquina de lavar	0	1	1	1	1
Videocassete / DVD player / Blue-ray player	0	2	2	2	2
Geladeira	0	2	2	2	2
Freezer (aparelho independende ou parte da geladeira duplex)	0	1	1	1	1

Grau de instrução

Analfabeto / Primário incompleto	**0**
Primário completo / Ginasial incompleto	**1**
Ginasial completo / Colegial incompleto	**2**
Colegial completo / Superior incompleto	**3**
Superior completo	**5**

CORTES DO CRITÉRIO BRASIL
Dados do Levantamento Sócio-econômico / 1996

Classe	**Pontos**
A (1)	**25 – 34**
B (2)	**17 – 24**
C (3)	**11 – 16**
D (4)	**6 – 10**
E (5)	**0 – 5**

CCSE do sujeito _____
CCSE dos pais _____

Appendix C – FCFRP/USP Director's Authorization

Faculdade de Ciências Farmacêuticas de Ribeirão Preto
Universidade de São Paulo – USP

Avenida do Café, s/n – Telefone: (16) 3602–4259
Fax: (16) 3602–4725
14040-903 – Ribeirão Preto – SP – Brasil

Ribeirão Preto, 19 de Fevereiro de 2010.

Prezado Diretor,

Solicitamos a V.Sª. a autorização para aplicar Escalas de triagem, em alunos de Graduação e de Pós-Graduação da Faculdade de Ciências Farmacêuticas de Ribeirão Preto, ao término das aulas, após o aceite dos alunos e consentimento dos docentes. Esta triagem visa selecionar Voluntários Saudáveis e Voluntários portadores de Transtorno de Ansiedade Social, a fim de participarem do estudo intitulado "Efeitos do canabidiol, clonazepam e rimonabanto na ansiedade experimental induzida em humanos". Ressaltamos que o trabalho está de acordo com o Comitê de Ética do Hospital das Clínicas da FMRP-USP, que o processo está em tramitação e esta é uma das exigências do referido "Comitê de Ética", que o Responsável pela Instituição, seja informado e autorize a realização da Triagem. Ainda gostaríamos de esclarecer que todas as informações pessoais serão mantidas em sigilo, encaminhadas a Psiquiatria Clínica da FMRP-USP, que definirá os grupos e após esta definição, os escolhidos serão consultados através do "Consentimento Livre e Esclarecido em Pesquisa" e assim o Projeto se iniciará.

Sendo só o que se apresenta para o momento e esperando contar com a colaboração de V.Sª, antecipadamente agradecemos.

Atenciosamente,

Mateus Machado Bergamaschi — Prof. Dr. Regina Helena Costa Queiroz

AUTORIZADO. DE ACORDO.

Prof. Dr. Sérgio de Albuquerque
Diretor da FCFRP-USP

Appendix D – Social Phobia Inventory (SPIN)

INVENTÁRIO DE FOBIA SOCIAL (SPIN)

Por favor, indique quanto os seguintes problemas incomodaram você durante a última semana.
Marque somente um item para cada problema, e verifique se respondeu todos os itens.

	nada	um pouco	modera-damente	bas-tante	extre-mamente
1. Tenho medo de autoridades	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
2. Incomodo-me por ficar vermelho na frente das pessoas	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
3. Festas e eventos sociais me assustam	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
4. Evito falar com pessoas que não conheço	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
5. Fico muito assustado ao ser criticado	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
6. Evito fazer coisas ou falar com certas pessoas por medo de ficar envergonhado	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
7. Transpirar na frente das pessoas me incomoda	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
8. Evito ir a festas	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
9. Evito atividades nas quais sou o centro das atenções	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
10. Conversar com estranhos me assusta	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
11. Evito falar para uma platéia ou dar discursos (ex. apresentações em sala de aula)	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
12. Faço qualquer coisa para não ser criticado	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
13. Sentir palpitações cardíacas me incomoda quanto estou na meio de outras pessoas	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
14. Tenho receio de fazer coisas quando posso estar sendo observado	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
15. Ficar envergonhado ou parecer bobo são meus maiores temores	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
16. Evito falar com qualquer autoridade	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐
17. Tremer ou estremecer na frente das outras pessoas me angustia	**0** ☐	**1** ☐	**2** ☐	**3** ☐	**4** ☐

COPYRIGHT Jonathan Davidson (1995)
Tradução e adaptação para o Português:
Crippa JAS, Graeff FG, Zuardi AW,
Hetem LA, Busatto GF, Loureiro SR (2003).
Digramação: Marcelo Mazza (2004).

Appendix E – Fast Alcohol Screening Test (FAST)

FAST

UM DRINKE / DOSE EQUIVALE A:

- **Um copo de pinga, vodca ou uísque** (37 mL) ou;
- **Uma taça pequena de vinho** (140 mL) ou;
- **Uma latinha de cerveja** (350 mL) ou;
- **Um cálice de Martini ou vermute** (50 mL)

POR FAVOR, CONSIDERANDO O QUADRO ACIMA, PARA AS PERGUNTAS ABAIXO CIRCULE AS ALTERNATIVAS MAIS APROPRIADAS.

1. HOMENS: *Com que frequência que você consome 8 (OITO) ou mais doses de bebida alcoólica em uma mesma ocasião?*

MULHERES: *Com que frequência que você consome 6 (SEIS) ou mais doses de bebida alcoólica em uma mesma ocasião?*

(0) Nunca (1) Menos que mensalmente (2) Mensalmente
(3) Semanalmente (4) Diariamente ou quase diariamente

---

2. *Com que frequência durante o último ano você não conseguiu se lembrar do que aconteceu na noite anterior porque havia bebido?*

(0) Nunca (1) Menos que mensalmente (2) Mensalmente
(3) Semanalmente (4) Diariamente ou quase diariamente

---

3. *Com que frequência durante o último ano você deixou de fazer o que era esperado devido ao uso de bebidas alcoólicas?*

(0) Nunca (1) Menos que mensalmente (2) Mensalmente
(3) Semanalmente (4) Diariamente ou quase diariamente

---

4. *Durante o último ano algum parente, amigo, médico ou outro profissional da área de saúde mostrou-se preocupado com modo de beber ou sugeriu que você parasse de beber?*

(0) Não (2) Sim, em uma ocasião (4) Sim, em mais do que uma ocasião

---

Appendix F – Patient Health Questionnaire-9 (PHQ-9)

QUESTIONÁRIO SOBRE A SAÚDE DO/A PACIENTE-9 72883
(Portuguese for Brazil version of the PHQ-9)

THIS SECTION FOR USE BY STUDY PERSONNEL ONLY.

Were data collected? **No** ☐ *(provide reason in comments)*

If **Yes**, data collected on visit date ☐ **or** specify date: _____
DD-Mon-YYYY

Comments:

Only the patient (subject) should enter information onto this questionnaire.

Durante as <u>últimas 2 semanas</u>, com que freqüência você foi incomodado/a por qualquer um dos problemas abaixo?	**Nenhuma vez**	**Vários dias**	**Mais da metade dos dias**	**Quase todos os dias**
1. Pouco interesse ou pouco prazer em fazer as coisas	0	1	2	3
2. Se sentir "para baixo", deprimido/a ou sem perspectiva	0	1	2	3
3. Dificuldade para pegar no sono ou permanecer dormindo, ou dormir mais do que de costume	0	1	2	3
4. Se sentir cansado/a ou com pouca energia	0	1	2	3
5. Falta de apetite ou comendo demais	0	1	2	3
6. Se sentir mal consigo mesmo/a — ou achar que você é um fracasso ou que decepcionou sua família ou você mesmo/a	0	1	2	3
7. Dificuldade para se concentrar nas coisas, como ler o jornal ou ver televisão	0	1	2	3
8. Lentidão para se movimentar ou falar, a ponto das outras pessoas perceberem? Ou o oposto – estar tão agitado/a ou irrequieto/a que você fica andando de um lado para o outro muito mais do que de costume	0	1	2	3
9. Pensar em se ferir de alguma maneira ou que seria melhor estar morto/a	0	1	2	3

SCORING FOR USE BY STUDY PERSONNEL ONLY

0 + _____ + _____ + _____

= Total Score: _____

Se você assinalou <u>qualquer</u> um dos problemas, indique o grau de <u>dificuldade</u> que os mesmos lhe causaram para realizar seu trabalho, tomar conta das coisas em casa ou para se relacionar com as pessoas?

Nenhuma dificuldade	**Alguma dificuldade**	**Muita dificuldade**	**Extrema dificuldade**
☐	☐	☐	☐

Copyright © 2005 Pfizer Inc. Todos os direitos reservados. Reproduzido sob permissão. EPI0905.PH

Declaro que as informações contidas neste questionário são verdadeiras.	Iniciais do/a paciente:	Data:

Appendix G – Beck Anxiety Inventory (BAI)

Nome: _____ ***Data:*** ____/____/____

Abaixo, está uma lista de sintomas comuns de ansiedade. Por favor, leia cuidadosamente cada item da lista. Indique **quanto** você foi incomodado, por cada um dos sintomas listados à esquerda, durante a **última semana, inclusive hoje**, marcando um X no grau de incômodo correspondente a uma das caselas das colunas à direita.

	Quanto foi incomodado			
	Nada 0	***Fraco 1***	***Moderadamente 2***	***Muito forte***
Sintomas	***Não incomodou nada***	***Incomodou-me um pouco***	***Foi muito desagradável, mas consegui aguentar***	***Quase não consegui aguentar***
1. Dormência ou formigamento				
2. Calores				
3. Pernas bambas				
4. Incapaz de relaxar				
5. Medo do pior acontecer				
6. Tonteira ou cabeça leve				
7. Coração batendo forte ou acelerado				
8. Inquieto(a)				
9. Aterrorizado(a)				
10. Nervoso(a)				
11. Sensação de sufocamento				
12. Mãos tremendo				
13. Trêmulo(a)				
14. Medo de perder o controle				
15. Dificuldade de respirar				
16. Medo de morrer				
17. Assustado(a)				
18. Indigestão ou desconforto no abdômen				
19. Desmaio				
20. Face ruborizada				
21. Suores (não devido a calor)				
			Total:	

Appendix H – Self-Reporting Questionnaire-24 (SRQ-24)

SRQ-24

O(A) SR(A). PODERIA POR FAVOR RESPONDER ÀS SEGUINTES PERGUNTAS A RESPEITO DA SUA SAÚDE:

01- Tem dores de cabeça freqüentes?.	1- Sim 2- Não	
02- Tem falta de apetite?.	1- Sim 2- Não	
03- Dorme mal?	1- Sim 2- Não	
04- Assusta-se com facilidade?	1- Sim 2- Não	
05- Tem tremores de mão?	1- Sim 2- Não	
06- Sente-se nervoso(a), tenso(a) ou preocupado(a)	1- Sim 2- Não	
07- Tem má digestão?	1- Sim 2- Não	
08- Tem dificuldade de pensar com clareza?	1- Sim 2- Não	
09- Tem se sentido triste ultimamente?	1- Sim 2- Não	
10- Tem chorado mais do que de costume?	1- Sim 2- Não	
11- Encontra dificuldades para realizar com satisfação suas atividades diárias?	1- Sim 2- Não	
12- Tem dificuldades para tomar decisões?	1- Sim 2- Não	
13- Tem dificuldades no serviço (seu trabalho é penoso, causa sofrimento)?	1- Sim 2- Não	
14- É incapaz de desempenhar um papel útil em sua vida?	1- Sim 2- Não	
15- Tem perdido o interesse pelas coisas?	1- Sim 2- Não	
16-Você se sente uma pessoa inútil, sem préstimo?	1- Sim 2- Não	
17-Tem tido idéias de acabar com a vida	1- Sim 2- Não	
18- Sente-se cansado(a) o tempo todo?	1- Sim 2- Não	
19- Tem sensações desagradáveis no estômago?	1- Sim 2- Não	
20- Você se cansa com facilidade?	1- Sim 2- Não	

A - Total de sim |___||___|

21- Sente que tem alguém que de alguma maneira quer lhe fazer mal?	1- Sim 2- Não	
22- Você é alguém muito mais importante do que a maioria das pessoas pensa?	1- Sim 2- Não	
23- Tem notado alguma interferência ou outro problema estranho c/ seu pensamento?	1- Sim 2- Não	
24- Ouve vozes que não sabe de onde vêm, ou que outras pessoas não podem ouvir?	1- Sim 2- Não	

B - Total de sim |___||___|

Appendix I – Informed Consent I

TERMO DE CONSENTIMENTO PÓS INFORMAÇÃO (primeira parte)

NOME DA PESQUISA: Efeitos do Canabidiol, Clonazepam e Rimonabanto na ansiedade experimental induzida em humanos
PESQUISADOR RESPONSÁVEL: Mateus Machado Bergamaschi
Contato: 3602-4259 / 3602-2703 / 9786-6088 e-mail: mateusbergamaschi@yahoo.com.br

1. Justificativa e objetivo da pesquisa

Vários estudos mostram que uma substância extraída da planta *Cannabis sativa*, chamada canabidiol, poderia ter efeitos de diminuir a ansiedade. Dessa forma, estamos desenvolvendo um estudo com a finalidade de verificar o efeito que esta substância teria quando é administrado o rimonabanto e gostaríamos de convidá-lo (a) a participar deste estudo.

2. Procedimentos que serão utilizados e seu propósito.

Este estudo é composto de duas partes. Nesta primeira parte, você deverá preencher escalas para nos dizer como você está se sentindo naquele momento. Estas escalas são bastante simples, mas nós faremos um treinamento antes de iniciarmos os procedimentos. Também mediremos sua pressão arterial e seu pulso. Geralmente, quando as pessoas ficam nervosas, as mãos ficam suadas. Por isso vamos verificar o suor da sua pele, através de um aparelho específico. Ao final da entrevista, você deverá tomar uma cápsula que poderá conter canabidiol na dose de 600 mg, clonazepam na dose de 1 mg ou placebo. Nós precisamos esperar 1 hora e 20 minutos até que a droga atinja os níveis adequados no seu sangue.

3. Desconfortos e riscos esperados.

O canabidiol é um dos cerca de 400 componentes da *cannabis sativa*, mas não é o componente responsável pelos efeitos psicológicos comumente observados com a cannabis em humanos. O canabidiol já foi estudado em outras pessoas e não apresentou efeitos indesejáveis a não ser sonolência em doses muito altas. O clonazepam é uma medicação bastante segura, largamente utilizada na prática clínica para o tratamento de transtornos de ansiedade. Excepcionalmente, alguns efeitos colaterais podem ocorrer, como sonolência, sedação, cansaço, diminuição da atenção e tonturas. Quando ocorrem, esses sintomas costumam ser leves e desaparecer em poucos minutos.

Em vista do possível efeito sedativo das drogas utilizadas, solicitamos que você não dirija após a sessão experimental. Caso você sinta desconforto nas próximas 48 horas, você poderá entrar em contato com um dos psiquiatras de nossa equipe para uma avaliação. Caso você se sinta incomodado, nós poderemos interromper os procedimentos, se assim você desejar. No final desta primeira parte, eu lhe explicarei a segunda metade do estudo e você me dirá se deseja continuar participando do estudo ou não.

4. Benefícios que se pode obter

A sua participação neste estudo contribuirá para que possamos ampliar a nossa compreensão a respeito das alterações que ocorrem nas reações normais de ansiedade e também nos quadros de ansiedade patológica. Com estas informações poderemos ajudar pessoas portadoras de transtornos ansiosos, melhorando a forma de tratamento dessas pessoas.

Os resultados serão tornados públicos independente de serem favoráveis ou não, porém respeitando a sua identidade, que não será revelada.

Ciente: _____

TERMO DE CONSENTIMENTO PÓS INFORMAÇÃO (segunda parte)

NOME DA PESQUISA: Efeitos do Canabidiol, Clonazepam e Rimonabanto na ansiedade experimental induzida em humanos
PESQUISADOR RESPONSÁVEL: Mateus Machado Bergamaschi

1. Justificativa e objetivo da pesquisa

Conforme eu havia lhe dito anteriormente, estamos interessados em verificar o efeito do canabidiol sobre a ansiedade induzida em humanos.

2. Procedimentos que serão utilizados e seu propósito.

A sua participação nesta parte do trabalho consiste na realização de um discurso sobre meios de transporte. Você terá 2 minutos para preparar o discurso, que deverá ter uma duração de 4 minutos. Este discurso será gravado em videoteipe e posteriormente analisado por psicólogo. Durante e após a realização do discurso, você deverá responder alguns questionários e mediremos sua pressão arterial, seu pulso e o suor da sua pele, como fizemos na primeira parte do estudo

3. Desconfortos e riscos esperados.

Preparar e realizar o discurso não apresenta riscos para sua saúde. Caso você sinta desconforto durante a sessão experimental, poderemos conversar sobre isso, e caso você continue a se sentir muito incomodado interromperemos a atividade, se você assim desejar. Caso você recuse a participar do estudo, não haverá prejuízo ao atendimento que lhe for prestado.

4. Benefícios que se pode obter

A sua participação neste estudo contribuirá para que possamos ampliar a nossa compreensão a respeito das alterações que ocorrem nas reações normais de ansiedade e também nos quadros de ansiedade patológica. Com estas informações poderemos ajudar pessoas portadoras de transtornos ansiosos, melhorando a forma de tratamento dessas pessoas.

Os resultados serão tornados públicos independente de serem favoráveis ou não, porém respeitando a sua identidade, que não será revelada.

Ciente: _____

Eu, _____, R.G._____, abaixo assinado, tendo recebido as informações acima, e ciente dos meus direitos abaixo relacionados concordo em participar.

1. A garantia de receber a resposta a qualquer pergunta ou esclarecimento a qualquer dúvida acerca dos procedimentos, riscos, benefícios e outros relacionados com a pesquisa e o tratamento a que serei submetido;
2. A liberdade de retirar meu consentimento a qualquer momento e deixar de participar no estudo sem que isso traga prejuízo à continuidade do meu cuidado e tratamento;
3. A segurança de que não serei identificado e que será mantido o caráter confidencial da informação relacionada com a minha privacidade;
4. O compromisso de me proporcionar informação atualizada durante o estudo, ainda que esta possa afetar minha vontade de continuar participando;
5. O compromisso de que serei devidamente acompanhado e assistido durante todo o período de minha participação no projeto, bem como de que será garantida a continuidade do meu tratamento, após a conclusão dos trabalhos de pesquisa;
6. Eventuais despesas decorrentes da minha participação no projeto, tais como transporte e alimentação ou outras semelhantes, poderão ser ressarcidas pelo pesquisador responsável pela pesquisa.

Declaro, ainda, que concordo inteiramente com as condições que me foram apresentadas e que, livremente, manifesto a minha vontade em participar do referido projeto.

Ribeirão Preto, _____ de _____ de _____

ASSINATURA DO VOLUNTÁRIO

ASSINATURA DO PESQUISADOR RESPONSÁVEL
Mateus Machado Bergamaschi

Appendix J – Informed Consent II

TERMO DE CONSENTIMENTO PÓS INFORMAÇÃO (primeira parte)

NOME DA PESQUISA: Efeitos do Canabidiol, Clonazepam e Rimonabanto na ansiedade experimental induzida em humanos
PESQUISADOR RESPONSÁVEL: Mateus Machado Bergamaschi
Contato: 3602-4259 / 3602-2703 / 9786-6088 e-mail: mateusbergamaschi@yahoo.com.br

1. Justificativa e objetivo da pesquisa

Vários estudos mostram que uma substância extraída da planta *Cannabis sativa*, chamada canabidiol, poderia ter efeitos de diminuir a ansiedade. Dessa forma, estamos desenvolvendo um estudo com a finalidade de verificar o efeito que esta substância teria quando é administrado o rimonabanto e gostaríamos de convidá-lo (a) a participar deste estudo.

2. Procedimentos que serão utilizados e seu propósito.

Este estudo é composto de duas partes. Nesta primeira parte, você deverá preencher escalas para nos dizer como você está se sentindo naquele momento. Estas escalas são bastante simples, mas nós faremos um treinamento antes de iniciarmos os procedimentos. Também mediremos sua pressão arterial e seu pulso. Geralmente, quando as pessoas ficam nervosas, as mãos ficam suadas. Por isso vamos verificar o suor da sua pele, através de um aparelho específico. Precisaremos colher seu sangue (4 mL) em vários momentos do exame e para isto será necessário instalar um soro na sua veia. Ao final da entrevista, você deverá tomar uma cápsula que poderá conter canabidiol na dose de 600 mg e placebo, canabidiol na dose de 600 mg e rimonabanto 90 mg ou placebo e placebo. Nós precisamos esperar 2 horas até que as drogas atinjam os níveis adequados no seu sangue.

3. Desconfortos e riscos esperados.

Como qualquer injeção, o soro que será instalado em sua veia poderá lhe causar dor. O canabidiol é um dos cerca de 400 componentes da *Cannabis sativa*, mas não é o componente responsável pelos efeitos psicológicos comumente observados com a cannabis em humanos. O canabidiol já foi estudado em outras pessoas e não apresentou efeitos indesejáveis a não ser sonolência em doses muito altas. O rimonabanto foi utilizado para o tratamento da obesidade e recentemente retirado do mercado, devido a seus efeitos adversos psiquiátricos, que são depressão e ansiedade em doses de 20 mg/dia. No entanto, em pessoas saudáveis, não há relatos de efeitos fisiológicos e psicológicos. Excepcionalmente, alguns efeitos colaterais podem ocorrer, como sonolência, sedação, cansaço, diminuição da atenção, tonturas, aumento da ansiedade, depressão aguda, náusea, vômitos e agitação. Quando ocorrem, esses sintomas costumam ser leves e desaparecer em poucos minutos.

Em vista do possível efeito sedativo dos medicamentos utilizados, solicitamos que você não dirija após a sessão experimental. Caso você sinta desconforto nas próximas 48 horas, você poderá entrar em contato com um dos psiquiatras de nossa equipe para uma avaliação. Caso você se sinta incomodado, nós poderemos interromper os procedimentos, se assim você desejar. Caso você recuse a participar do estudo, não haverá prejuízo ao atendimento que lhe for prestado. No final desta primeira parte, eu lhe explicarei a segunda metade do estudo e você me dirá se deseja continuar participando do estudo ou não.

4. Benefícios que se pode obter

A sua participação neste estudo contribuirá para que possamos ampliar a nossa compreensão a respeito das alterações que ocorrem nas reações normais de ansiedade e também nos quadros de ansiedade patológica. Com estas informações poderemos ajudar pessoas portadoras de transtornos ansiosos, melhorando a forma de tratamento dessas pessoas.

Os resultados serão tornados públicos independente de serem favoráveis ou não, porém respeitando a sua identidade, que não será revelada.

Ciente: _____

TERMO DE CONSENTIMENTO PÓS INFORMAÇÃO (segunda parte)

NOME DA PESQUISA: Efeitos do Canabidiol, Clonazepam e Rimonabanto na ansiedade experimental induzida em humanos
PESQUISADOR RESPONSÁVEL: Mateus Machado Bergamaschi

1. Justificativa e objetivo da pesquisa

Conforme eu havia lhe dito anteriormente, estamos interessados em verificar o efeito do canabidiol sobre a ansiedade induzida em humanos.

2. Procedimentos que serão utilizados e seu propósito.

A sua participação nesta parte do trabalho consiste na realização de um discurso sobre meios de transporte. Você terá 2 minutos para preparar o discurso, que deverá ter uma duração de 4 minutos. Este discurso será gravado em videoteipe e posteriormente analisado por psicólogo. Durante e após a realização do discurso, você deverá responder alguns questionários e mediremos sua pressão arterial, seu pulso e o suor da sua pele, como fizemos na primeira parte do estudo

3. Desconfortos e riscos esperados.

Preparar e realizar o discurso não apresenta riscos para sua saúde. Caso você sinta desconforto durante a sessão experimental, poderemos conversar sobre isso, e caso você continue a se sentir muito incomodado interromperemos a atividade, se você assim desejar. Caso você recuse a participar do estudo, não haverá prejuízo ao atendimento que lhe for prestado.

4. Benefícios que se pode obter

A sua participação neste estudo contribuirá para que possamos ampliar a nossa compreensão a respeito das alterações que ocorrem nas reações normais de ansiedade e também nos quadros de ansiedade patológica. Com estas informações poderemos ajudar pessoas portadoras de transtornos ansiosos, melhorando a forma de tratamento dessas pessoas.

Os resultados serão tornados públicos independente de serem favoráveis ou não, porém respeitando a sua identidade, que não será revelada.

Ciente: _____

Eu, _____, R.G._____, abaixo assinado, tendo recebido as informações acima, e ciente dos meus direitos abaixo relacionados concordo em participar.

1. A garantia de receber a resposta a qualquer pergunta ou esclarecimento a qualquer dúvida acerca dos procedimentos, riscos, benefícios e outros relacionados com a pesquisa e o tratamento a que serei submetido;
2. A liberdade de retirar meu consentimento a qualquer momento e deixar de participar no estudo sem que isso traga prejuízo à continuidade do meu cuidado e tratamento;
3. A segurança de que não serei identificado e que será mantido o caráter confidencial da informação relacionada com a minha privacidade;
4. O compromisso de me proporcionar informação atualizada durante o estudo, ainda que esta possa afetar minha vontade de continuar participando;
5. O compromisso de que serei devidamente acompanhado e assistido durante todo o período de minha participação no projeto, bem como de que será garantida a continuidade do meu tratamento, após a conclusão dos trabalhos de pesquisa;
6. Eventuais despesas decorrentes da minha participação no projeto, tais como transporte e alimentação ou outras semelhantes, poderão ser ressarcidas pelo pesquisador responsável pela pesquisa.

Declaro, ainda, que concordo inteiramente com as condições que me foram apresentadas e que, livremente, manifesto a minha vontade em participar do referido projeto.

Ribeirão Preto, _____ de _____ de _____

ASSINATURA DO VOLUNTÁRIO

ASSINATURA DO PESQUISADOR RESPONSÁVEL
Mateus Machado Bergamaschi

Appendix K – Visual Analogue Mood Scale (VAMS)

V A M S

SUJEITO: ..... FASE:.....

INSTRUÇÕES: Avalie como você se sente agora em relação aos itens abaixo e marque cada linha com um traço vertical no ponto que melhor descreve seus sentimentos. O centro de cada linha indica como você habitualmente se encontra e as extremidades indicam o máximo de cada condição.

ALERTA	_____	SONOLENTO
CALMO	_____	AGITADO
FORTE	_____	FRACO
CONFUSO	_____	COM IDÉIAS CLARAS
ÁGIL	_____	DESAJEITADO
APÁTICO	_____	DINÂMICO
SATISFEITO	_____	INSATISFEITO
PREOCUPADO	_____	TRANQUILO
RACIOCÍNIO DIFÍCIL	_____	PERSPICAZ
TENSO	_____	RELAXADO
ATENTO	_____	DISTRAÍDO
INCAPAZ	_____	CAPAZ
ALEGRE	_____	TRISTE
HOSTIL	_____	AMISTOSO
INTERESSADO	_____	DESINTERESSADO
RETRAÍDO	_____	SOCIÁVEL

Appendix L – Self-Statements During Public Speaking Scale (SSPS)

AUTO-AVALIAÇÃO AO FALAR EM PÚBLICO – ESTADO

Imagine as coisas que você está pensando sobre mesmo *agora* nesta situação de falar em público diante da câmera. Tendo em mente esta situação, até que ponto você concorda com as afirmações abaixo? Por favor, dê uma nota de 0 (se você discorda totalmente) a 5 (se você concorda totalmente com a afirmação.

	0	1	2	3	4	5
1. O que tenho a perder? Vale a pena tentar.	☐	☐	☐	☐	☐	☐
2. Sou um fracasso.	☐	☐	☐	☐	☐	☐
3. Esta é uma situação difícil, mas posso dar conta dela.	☐	☐	☐	☐	☐	☐
4. Um fracasso nesta situação seria mais uma prova da minha incopetência.	☐	☐	☐	☐	☐	☐
5. Mesmo que não dê certo, não é o fim do mundo.	☐	☐	☐	☐	☐	☐
6. Posso dar conta de tudo.	☐	☐	☐	☐	☐	☐
7. Qualquer coisa que eu disser vai parecer bobagem.	☐	☐	☐	☐	☐	☐
8. Acho que vou me dar mal de qualquer jeito.	☐	☐	☐	☐	☐	☐
9. Ao invés de me preocupar, eu deveria me concentrar no que eu vou dizer.	☐	☐	☐	☐	☐	☐
10. Eu me sinto desajeitado e tolo, certamente eles vão notar.	☐	☐	☐	☐	☐	☐

COPYRIGHT Stefan G. Hoffman
Tradução e adaptação para o Português:
Crippa JAS, Osório F, Graeff FG, Zuardi AW,
de Pinho M, Chaves M, Loureiro SR (2004).

Appendix M – Bodily Symptoms Scale (BSS)

ESCALA DE SINTOMAS SOMÁTICOS

NOME: _____ DATA: _____SCORE: ____

Instruções: Avalie como você se sente agora em relação aos itens abaixo e faça um círculo ao redor do número que melhor expresse este seu estado atual.

	NADA	MUITO POUCO	POUCO	MODERA-DAMENTE	MUITO	EXTREMA-MENTE
	(0)	(1)	(2)	(3)	(4)	(5)
01. Cansado (a)	0	1	2	3	4	5
02. Fraco (a)	0	1	2	3	4	5
03. Letárgico (a)	0	1	2	3	4	5
04. Com dor ou peso na cabeça	0	1	2	3	4	5
05. Com tensão muscular	0	1	2	3	4	5
06. Com temor	0	1	2	3	4	5
07. Com fome	0	1	2	3	4	5
08. Com sede	0	1	2	3	4	5
09. Com dificuldade de coordenação	0	1	2	3	4	5
10. Suando	0	1	2	3	4	5
11. Com palpitação	0	1	2	3	4	5
12. Com dificuldade de respirar	0	1	2	3	4	5
13. Agitado (a)	0	1	2	3	4	5
14. Com vontade de urinar	0	1	2	3	4	5
15. Com náusea ou mal-estar gástrico	0	1	2	3	4	5
16. Com boca seca	0	1	2	3	4	5
17. Com visão turva	0	1	2	3	4	5
18. Com tontura	0	1	2	3	4	5
19. Com vontade de evacuar	0	1	2	3	4	5
20. Com dificuldade de urinar	0	1	2	3	4	5
21. Com formigamento	0	1	2	3	4	5

Appendix N – Study 1 Experimental Plan

Session (min)	Phase	Procedure
- 0:30		Adaptation to the laboratory; instructions about the interview and measurements
- 0:15	Baseline (B)	SCL, SF, HR, AP, VAMS, SSPS and BSS
0		Drug intake: CBD or placebo capsules
+ 1:20	Pre-stress (P)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 1:30		Instructions about the SPST
+ 1:32		Speech preparation
+ 1:34	Anticipatory speech (A)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 1:45		Start of speech
+ 1:47	Speech performance (S)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 1:53		Continuation of speech
+ 1:55		End of speech
+ 2:10	Post-stress 1 (F1)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 2:30	Post-stress 2 (F2)	SCL, SF, HR, AP, VAMS, SSPS and BSS

SCL, skin level conductance; SF, number of spontaneous fluctuations of the skin conductance; HR, heart rate; AP, arterial blood pressure; VAMS, Visual Analogue Mood Scale; SSPS, Self-Statements during Public Speaking Scale; BSS, Bodily Symptoms Scale.

Appendix O – Study 2 Experimental Plan

Session (min)	Phase	Procedure
- 0:30		Adaptation to the laboratory; instructions about the interview and measurements
- 0:15	Baseline (B)	SCL, SF, HR, AP, VAMS, SSPS and BSS 1st blood draw
0		Drug intake: Rimonabant or placebo capsules
+ 0:40		Drug intake: CBD or placebo capsules
+ 2:00	Pre-stress (P)	SCL, SF, HR, AP, VAMS, SSPS and BSS, 2nd blood draw
+ 2:10		Instructions about the SPST
+ 2:12		Speech preparation
+ 2:14	Anticipatory speech (A)	SCL, SF, HR, AP, VAMS, SSPS and BSS 3rd blood draw
+ 2:25		Start of speech
+ 2:27	Speech performance (S)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 2:33		Continuation of speech
+ 2:35		End of speech; 4th blood draw
+ 2:50	Post-stress 1 (F1)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 3:00		5th blood draw
+ 3:10	Post-stress 2 (F2)	SCL, SF, HR, AP, VAMS, SSPS and BSS
+ 3:30		6th blood draw

SCL, skin level conductance; SF, number of spontaneous fluctuations of the skin conductance; HR, heart rate; AP, arterial blood pressure; VAMS, Visual Analogue Mood Scale; SSPS, Self-Statements during Public Speaking Scale; BSS, Bodily Symptoms Scale.