Published in final edited form as:
Neuroscience. 2012 March 1; 204: 207–229. doi:10.1016/j.neuroscience.2011.11.020.

Contributions of endocannabinoid signaling to psychiatric disorders in humans: Genetic and biochemical evidence

Cecilia J. Hillard, **Kenneth M. Weinlander**, and **Kara L. Stuhr**
Department of Pharmacology and Neuroscience Research Center, Medical College of Wisconsin

Abstract

The endocannabinoid signaling system is a widespread, neuromodulatory system in brain and is also widely utilized in the periphery to modulate metabolic functions and the immune system. Preclinical data demonstrate that endocannabinoid signaling is an important stress buffer and modulates emotional and cognitive functions. These data suggest the hypothesis that endocannabinoid signaling could be dysfunctional in a number of mental disorders. Genetic polymorphisms in the human genes for two important proteins of the endocannabinoid signaling system, the CB1 cannabinoid receptor (CB1R) and fatty acid amide hydrolase (FAAH), have been explored in the context of normal and pathological conditions. In the case the gene for FAAH, the mechanistic relationships among the common genetic polymorphism, the expression of the FAAH protein and its likely impact on endocannabinoid signaling are understood. However, multiple polymorphisms in the gene for the CB1R occur and are associated with human phenotypic differences without an understanding of the functional relationships among the gene, mRNA, protein and protein function. The endocannabinoid ligands are found in the circulation and several studies have identified changes in their concentrations under various conditions. These data are reviewed for the purpose of generating hypotheses and to encourage further studies in this very interesting and important area.

1.0. Introduction

Considerable data support the notion that endocannabinoid signaling has three broad and overlapping functions in mammals. The first is a stress recovery role, operating in a feedback loop in which endocannabinoid signaling is activated by stress and functions to return endocrine, nervous and behavioral systems to homeostatic balance (Hill et al., 2010b). The second function is to control energy balance through regulation of the intake, storage and utilization of food (Di Marzo et al., 2009b). The third function involves immune regulation; endocannabinoid signaling is activated by tissue injury (Pacher and Mechoulam, 2011) and modulates immune and inflammatory responses (Klein and Cabral, 2006). In light of these vital functions, it is not surprising that alterations in endocannabinoid signaling have important biological effects.

Address correspondence to: Cecilia J. Hillard, Ph.D., Center for Neuroscience Research, Department of Pharmacology and Toxicology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226, Phone: 414-955-8493, Fax: 414-955-6057, chillard@mcw.edu.

Based upon thousands of years of human experience with *cannabis sativa* as well as compelling preclinical data, it is reasonable to hypothesize that endogenous cannabinoid signaling is: (1) heterogenous in humans; (2) dysfunctional in some diseases or disorders; and (3) a potential target for therapeutic development. The purpose of this review is to summarize the available data supporting the first two hypotheses, and the corollary hypothesis that heterogeneity in endocannabinoid signaling contributes to variability among humans in the susceptibility to disease or disorders. In part one, two well-studied proteins of the endocannabinoid signaling system, the CB1 cannabinoid receptor (CB1R) and fatty acid amide hydrolase (FAAH), are introduced and evidence for their heterogeneity in humans is summarized. In part two, current understanding of mechanisms that regulate endocannabinoid ligand concentrations in the circulation is presented. Part three summarizes the data pertinent to the hypothesis that heterogeneity in the genes for CB1R (*CNR1*) and FAAH (*FAAH*) or differences in circulating endocannabinoids associate with the symptoms and/or diagnoses of substance use disorders, depression, anxiety and eating disorders, schizophrenia and attention deficit disorder.

2.0 Evidence for genetic heterogeneity of the CB1 cannabinoid receptor and fatty acid amide hydrolase in humans

2.1 CB1 cannabinoid receptor

2.1.1. Introduction—In 1964, Gaoni and Mechoulam published the structure of the psychoactive principal of *cannabis sativa*, Δ^9-tetrahydrocannabinol (Gaoni and Mechoulam, 1964), initiating the modern era of the study of the mechanisms by which *cannabis* affects the body. A series of biochemical studies characterized the target of THC as a G protein-coupled receptor (Howlett and Fleming, 1984, Howlett, 1985, Howlett, 1987, Devane et al., 1988). This was confirmed when a GPCR with high affinity for THC and structural analogs was cloned; it was named the CB1 cannabinoid receptor because of its cannabinoid ligand (Matsuda et al., 1990). CB1Rs are ubiquitously but heterogeneously expressed in the CNS; present at extremely high density in the cingulate gyrus, frontal cortex, hippocampus, cerebellum and the basal ganglia (Mackie, 2005). Moderate receptor densities are found in the basal forebrain, amygdala, nucleus accumbens, periaqueductal grey and hypothalamus; and low densities are seen in the midbrain, pons, medulla, primary motor cortex and thalamus. Within the spinal cord, CB1Rs are expressed by interneurons within the dorsal horn, and on axon terminals of descending inputs and peripheral afferents in the dorsal horn.

Most of the neuronal CB1R in the CNS are found in axons or at presynaptic terminals (Mackie, 2005). CB1R couple predominantly to G proteins of the α_i and α_o subtypes; initiating signaling cascades that result in inhibition of adenylyl cyclase (Howlett, 1985), inhibition of the opening of voltage operated calcium channels (Mackie and Hille, 1992), increased potassium channel conductances (Mackie and Hille, 1992, Deadwyler et al., 1995), and activation of MAP kinases (Bouaboula et al., 1995). Within the CNS, endogenous CB1R activation subserves several types of short- and long-term synaptic plasticity through inhibition of neurotransmitter release (Freund et al., 2003, Patel and Hillard, 2009).

CB1R are also expressed by peripheral neurons (Mackie, 2005). Primary sensory afferents express the CB1R at their terminals in the spinal cord and in the innervated tissues (Ahluwalia et al., 2000). Sympathetic nerve terminals express CB1R, where they inhibit norepinephrine release (Ishac et al., 1996). CB1R are distributed throughout the enteric nervous system and their activation inhibits both intestinal motility and secretion (Izzo and Sharkey, 2010).

Non-neuronal cells also express CB1R. Within the CNS, CB1R are expressed by astrocytes (Salio et al., 2002); cerebral endothelial cells (Golech et al., 2004) and cerebral vascular smooth muscle cells (Gebremedhin et al., 1999). CB1R have also been identified in oligodendrocytes (Moldrich and Wenger, 2000) and in subventricular zones (Berrendero et al., 1998) where postnatal cell proliferation occurs. In addition, the CB1R is expressed by circulating immune cells (Bouaboula et al., 1993), adipocytes (Bensaid et al., 2003), hepatocytes (Jeong et al., 2008), pancreatic islet cells (Li et al., 2010), skeletal muscle (Eckardt et al., 2009) and is present in the adrenal gland (Ziegler et al., 2010a).

2.1.2 *CNR1* gene expression—The CB1 cannabinoid receptor is encoded by the *CNR1* gene which maps to human chromosome 6, specifically to 6q14-q15 (Hoehe et al., 1991). Zhang and colleagues described a scaffold for the *CNR1* gene that includes 4 exons and 3 introns (Zhang et al., 2004). The coding region of the CB1R is at the 5′ end of exon 4. In addition to the conventional 5′ promoter region, these investigators discovered an alternative promoter region in intron 2. These investigators identified 5 different mRNA species that all contained exon 4 but differed in the inclusion of exons 1, 2 and 3. Interestingly, the relative abundance of transcripts that contain exons 1 and 3 differs across brain regions, suggesting that the translation of these exons is regulated and could have functional importance.

Two other splice variants of the human CB1R mRNA have been identified that result in altered CB1R protein. The first mRNA encodes a CB1R (CB1a) that is 61 amino acids shorter at the N-terminal than the full length protein (Shire et al., 1995). The second splice variant results in a protein (CB1b) that is lacking the N-terminal 33 amino acids of the full length CB1R (Ryberg et al., 2005). Although the transcripts for both of these variants are far less abundant than the full length mRNA, they are found in brain and other tissues and have non-overlapping distribution patterns (Ryberg et al., 2005). Importantly, there is evidence that the potency and efficacy of the endocannabinoids at the three proteins differs. In particular, the endocannabinoid, *N*-arachidonylethanolamine (AEA) has extremely low affinity for both CB1a (although in (Rinaldi-Carmona et al., 1996) this difference was not observed) and CB1b (Ryberg et al., 2005). The second endocannabinoid, 2-arachidonoylglycerol (2-AG), is a full efficacy agonist at the full length CB1R but is an inverse agonist at CB1a and CB1b expressed in heterologous systems (Ryberg et al., 2005). If these biochemical properties also occur in human cells, the resulting differences in endocannabinoid signaling would be significant.

2.1.3. Trinucleotide repeat in 3′ untranslated region (UTR) of *CNR1*—The first heterogeneity described in *CNR1* was a microsatellite polymorphism in the 3′ UTR in which the trinucleotide, AAT, is repeated multiple times (Dawson, 1995). Subsequent analysis has revealed that this genomic sequence is 18 kb 3′ to the exon 4 translational start site (Zhang et al., 2004). The consequences of the length of the repeat on CB1R expression and function are not known; however, a recent review of triplet nucleotide repeats (TNR) in the genome concluded “polymorphic TNRs, especially those localized in or close to exons or genetic regulatory elements (promoters, enhancers, microRNA genes, etc.) have considerable phenotype-modifying potential and should be considered high priority genetic variants in genotype–phenotype association studies” (Kozlowski et al., 2010). Several interesting studies have been published recently indicating that the length of the AAT repeat in the 3′ UTR of *CNR1* may indeed have functional consequences in healthy humans.

2.1.4. *CNR1* Single Nucleotide Polymorphisms (SNPs)—A frequent mutation in the coding region of exon 4 that is 1359 bp from the translational start site was first reported in 1999 (rs1049353) (Gadzicki et al., 1999); the common nucleotide is G and the rare is A. This is a silent mutation that does not alter the amino acid (threonine) at position 453 in the CB1R protein. Many other SNPs have been identified in and near the *CNR1* gene (Herman

et al., 2006, Zuo et al., 2007, Baye et al., 2008, Chen et al., 2008, Hamdani et al., 2008, Juhasz et al., 2009b, Ho et al., 2011). Several generalities can be made regarding these heterogeneities. First, none of the identified SNPs result in deletion or mutation of the CB1R protein. Second, several investigators have studied the linkage disequilibrium (LD) among various SNPs in the *CNR1* gene and have identified several groups of SNPs that are in LD, meaning that they are inherited together more often than would be predicted if they were independent genes. These groups of SNPs are called “haplotype blocks”, several of the *CNR1* haplotype blocks identified are shown in Table 1. Third, as is discussed more fully in part 3 of this review, the data regarding the association of SNPs and haplotypes with mental disorders can be contradictory but there are hints that diversity in the *CNR1* gene could contribute in important ways to personality characteristics or other forms of vulnerability to disease.

A very important component to further understanding of the reported associations between *CNR1* genotype and any phenotype is knowledge of the consequence of the associated SNP to CB1R function. The discovery of a significant association between a SNP within or in the neighborhood of a gene and a phenotype suggests that a genetic difference contributes to the phenotype being studied. However, it is often the case that the mechanistic basis for the relationship is not obvious. In particular, while the data discussed below demonstrate some very intriguing association between CNR1 SNPs or haplotypes, the mechanistic links are largely unknown. For example, two SNPs in the coding region of exon 4 (rs1049353 (Gadzicki et al., 1999) and rs3505747 (Ho et al., 2011)) of *CNR1* occur, one of which (rs1049353) has been significantly associated with several human phenotypes (described in section 4.0). However, neither of these changes in nucleotide results in a changed amino acid, leading to the conclusion that the CB1R protein is identical in humans with the two genotypes. It is possible that the SNPs themselves are not causative at all, but are in LD with a causative SNP or other genetic change. Alternatively, the impact of the SNP on mRNA species could be significant and contribute to the mechanism

There are data available supporting the idea that SNPs can have functional effects on *CNR1* through changes in the expression of its mRNA as a result of elimination or creation of a transcription factor binding site. For example, the A allele of rs2180619, a SNP in the 5′ promoter region of *CNR1*, forms sequences that are more than 90% similar to known binding sites for the glucocorticoid receptor and transcription factor II D (Lazary et al., 2009). Conversely, the presence of a G at this position results in putative binding sites for four different transcription factors: sex-determining region Y gene product, T-cell factor 4E, lymphoid enhancer binding factor 1, and transcription factor 4.

Another SNP in a region of exon 4 that has been proposed to serve as an alternative putative promoter region, rs12720071, is within a potential transcription factor binding site (Heinemeyer et al., 1998). Ho, Andreasen and colleagues (Ho et al., 2011) have pointed out that the common nucleotide at this position (A), but not the rare nucleotide (G), results in a putative binding site for CCAAT/enhancer-binding protein beta (C/EBPbeta) (Akira et al., 1990). C/EBP transcription factors have been shown to promote or repress gene expression in the context of adult neuroplasticity, and learning and memory (Alberini et al., 1994, Menard et al., 2002), functions that are known to be affected by CB1R activation (Harkany et al., 2008).

Zhang and colleagues described a haplotype block in the intronic region upstream of exon 3 that consists of SNPs rs806379, rs1535255 and rs2023239 that has become increasingly interesting in relationship to several disorders (Zhang et al., 2004). In the original report, minor allele nucleotides were listed as T, A and G for the three SNPs in the order above. However, it is likely that the authors were using the minus strand rather than the plus strand

of chromosome 6. The more standard convention is to use the plus strand, and by that convention the major/minor alleles for the three SNPs are: A/T, T/G and T/C (Lazary et al., 2009). Heterogeneity within this haplotype block is associated with significantly altered *CNR1* mRNA expression; in particular the TGC (TAG) haplotype was associated with significantly lower *CNR1* mRNA expression compared to the other combinations. (Zhang et al., 2004). In silico data analysis indicates that T alleles at both rs2023239 and rs1535255 results in the formation of specific binding sites for YY1 transcription factor that are not present when the minor alleles are expressed at these sites (Lazary et al., 2009). These data have lead to the hypothesis that the haplotype constructed from the three minor alleles (TGC) is associated with decreased *CNR1* gene expression and, thus, decreased CB1R protein concentrations in brain (Lazary et al., 2009). However, post mortem brain samples from individuals with a C at rs2023239 (the third SNP in this haplotype block) exhibit significantly greater CB1R binding site density in prefrontal cortex compared to those homozygous for T (Hutchison et al., 2008), suggesting that the hypothesis needs further investigation.

Another SNP in the *CNR1* regulatory region, rs806378, could also result in a change in transcription factor binding (Tiwari et al., 2010). The rare nucleotide at this SNP (T), but not the common (C), binds to arylhydrocarbon receptor translocator (ARNT). ARNT heterodimerizes with other transcription factors, including single minded 1 (SIM1) (Yang et al., 2004). The variable presence of a binding site for ARNT/SIM1 in the regulatory region of *CNR1* could contribute to differences in *CNR1* expression under some circumstances. Interestingly, there are associations with SIM1 and hyperphagic obesity in humans (Faivre et al., 2002) and the presence of a T allele at rs806378 is associated with obesity in French individuals (Benzinou et al., 2008). In addition, schizophrenic patients with a T allele at rs806378 gain more weight when treated with atypical antipsychotic agents (Tiwari et al., 2010).

Many other SNPs in the region of the *CNR1* gene have been used as markers for genetic variation in *CNR1* without any understanding at all of their functional consequences. This is a limitation of all gene association studies, but the incidence of significant correlations between *CNR1* SNP genotypes and interesting and important phenotypes supports the need for studies that can elucidate their function.

2.2 Fatty acid amide hydrolase (FAAH)

2.2.1. Introduction—Catabolic inactivation of the endogenous CB1R agonist, AEA, within the brain occurs via FAAH-mediated hydrolysis (Ho and Hillard, 2005). FAAH has a high catalytic efficiency, particularly for AEA as a substrate, and is constitutively active. It also hydrolyzes other members of the *N*-acylethanolamine family of lipids. Recent studies indicate that its activity in brain can be altered by environmental factors, including stress, suggesting that regulation of FAAH is an important mechanism for the regulation of AEA-mediated signaling (Hill et al., 2010a).

2.2.2 *FAAH* gene expression—The gene for FAAH (*FAAH*) is on human chromosome 1p35-34. In 2002, Sipe, Cravatt and colleagues reported a single nucleotide polymorphism (SNP; rs324420) at position 385 in the human *FAAH* gene (Sipe et al., 2002). The common nucleotide at this position is C (82% in Caucasians), the rare nucleotide is A (18% in Caucasians). When the genetic data for 4997 individuals (3460 Caucasian; 1419 Asian and 118 African American) reported in (Sipe et al., 2002, Morita et al., 2005, Flanagan et al., 2006, Iwasaki et al., 2007) are combined, the genotype distribution is 3% AA, 29.5% CA and 67.4% CC. There is some evidence that individuals of African descent have increased occurrence of 385A (26.7%) and twice the likelihood of AA genotype (6.8%) compared to

Caucasians and Asians (Flanagan et al., 2006). Other SNPs have been identified in the region of the *FAAH* gene, none are in the coding region (Flanagan et al., 2006, Dlugos et al., 2010). Analysis of the haplotypes formed from 6 other SNPs in the promoter region and introns of *FAAH* suggests that the C385A mutation occurred at a single origin in Africa more than 114,000 years ago (Flanagan et al., 2006).

The replacement of A for C at position 385 in the cDNA for FAAH results in a change in amino acid at position 129 in the FAAH protein from proline to threonine (Sipe et al., 2002). Proline at 129 is conserved in FAAH from multiple mammalian species. Although FAAH with threonine at position 129 (P129T) does not differ from wild type in any measure of catalysis, P129T is proteolyzed more quickly by trypsin than the wild type (Sipe et al., 2002). T-lymphocytes from humans with an AA genotype at *FAAH* 385 have approximately 50% of the FAAH enzymatic activity and protein compared to cells from individuals with CC genotype (Chiang et al., 2004). Carriers of FAAH 385A have higher concentrations of plasma AEA and other *N*-acylethanolamine-substrates for FAAH than CC homozygotes (Sipe et al., 2010), which supports the biochemical data. These findings suggest the hypothesis that individuals carrying 385A have greater AEA-activated CB1R signaling. Individuals with an A allele at FAAH 385 also have other SNPs in linkage with rs324420 (Dlugos et al., 2010) that could contribute to the biochemical difference.

2.3. Summary

In summary, heterogeneity in the human genes for both CB1R and FAAH have been identified and studied. While considerable progress has been made and there is clear evidence that they are both genetically polymorphic, critical basic questions about the functionality of the SNPs, particularly those in the *CNR1* remain.

3.0. Endocannabinoid concentrations in blood: useful biomarkers?

3.1. Endocannabinoids are present in plasma and serum

The endocannabinoids can be measured in both plasma and serum. A side-by-side study found that AEA concentrations were 30% higher in serum than in plasma (Lam et al., 2010). In our experience, 2-AG can be measured more reliably and at higher concentrations in serum than plasma (Stuhr and Hillard, unpublished observations). Table 2 contains a list of studies in which plasma or serum endocannabinoid concentrations were determined in blood taken from healthy humans. AEA concentrations reported range from 0.4 to 13 nM; the majority of the studies reported mean concentrations between 1 and 5 nM. Approximately half of the investigations also measured the concentration of 2-AG; the range in this measure was 1-400 nM; the majority of studies reported mean 2-AG concentrations between 5 and 40 nM. When both AEA and 2-AG were measured in the same sample, 2-AG was usually, but not always, higher than AEA. Methodological and demographic differences among the studies likely explain the majority of the variance in endocannabinoid content. Within single studies, the standard deviations are typically less than 50% of the mean values for AEA; the variability in 2-AG is greater with standard deviations typically larger than 100% of the mean values.

Like other circulating lipids, AEA can bind to albumin in the circulation (Giuffrida et al., 2000). Bovine serum albumin has one high-affinity binding site for AEA, with a Kd of 55 nM at 37°C (Bojesen and Hansen, 2003). Considering the data above that the concentrations of AEA in the circulation are in the range of 1-5 nM, it is likely that the majority of the AEA in the circulation is not bound. To our knowledge, the issue of 2-AG binding to albumin has not been examined.

3.2. Sources of circulating endocannabinoids

Since the endocannabinoids are synthesized by tissues and are lipophilic, movement in and out of tissues should be easily accomplished. Several studies support the notion that circulating concentrations of endocannabinoids are in equilibrium with concentrations in tissues. For example, the concentrations of AEA and 2-AG in brain microdialysates, which approximate their concentrations in the extracellular space of the brain, are approximately 2 and 5 nM, respectively (Caille et al., 2007). These concentrations are in the range of the concentrations of AEA and 2-AG in human serum and plasma. In a recent study, a nearly significant ($p=0.0545$) positive relationship was found between the concentrations of AEA in rat plasma and maternal tissues between days 8 and 19 of pregnancy (Fonseca et al., 2010). AEA is produced in the ovarian follicle during ovulation and peaks in the circulation at ovulation (El-Talatini et al., 2010). In addition to the brain and reproductive organs, significant amounts of endocannabinoids could enter the circulation from adipose tissues (Matias et al., 2006, Spoto et al., 2006). It is likely that liver clears the blood of endocannabinoids since both AEA (Caraceni et al., 2010b) and 2-AG (Westerbacka et al., 2010) are present at lower concentrations in the hepatic vein than in the rest of the circulation.

Another significant source of endocannabinoids in the serum and plasma are blood cells. Endocannabinoids are generated in blood, but not plasma, suggesting that blood cells synthesize and release endocannabinoids (Schmidt et al., 2006, Vogeser et al., 2006). In particular, macrophages (Varga et al., 1998, Di Marzo et al., 1999, Chouker et al., 2010) and platelets (Varga et al., 1998) release endocannabinoids when activated *ex vivo* by endotoxin and cytokines. Blood cells also have the capacity to inactivate AEA (Schmidt et al., 2006). This situation has practical as well as mechanistic implications because blood releases AEA in a temperature-dependent manner after harvest; as a result, AEA content can be dependent upon the time between the blood draw and time of plasma or serum separation (Vogeser et al., 2006). After separation of serum or plasma, AEA appears to be stable when stored frozen (Jian et al., 2010); the stability of 2-AG has not been thoroughly tested to our knowledge.

Human pathological conditions with a significant degree of inflammation or tissue injury are associated with significantly elevated plasma or serum endocannabinoids. Both AEA and 2-AG are both several times higher in serum from patients with endotoxic shock (Wang et al., 2001); AEA concentrations are higher in patients with cirrhosis (Fernandez-Rodriguez et al., 2004); and AEA is increased 3.5-fold and 2-AG 7-fold in patients with severe congestive heart failure (Weis et al., 2010b), a condition associated with systemic inflammation (Yndestad et al., 2006). Both AEA and 2-AG are detected in the synovial fluid of patients with arthritis, but not in healthy subjects (Richardson et al., 2008). Plasma 2-AG concentrations increase by several fold in patients undergoing cardiopulmonary bypass (CPB) compared to the same patients immediately after termination of the CPB; an effect that was suggested by the authors to be due to the inflammatory response evoked by this procedure (Weis et al., 2010a). Circulating 2-AG concentrations are increased in men with intra-abdominal adiposity (Bluher et al., 2006, Cote et al., 2007, Di Marzo et al., 2009a), a condition associated with a high degree of systemic inflammation (Marsland et al., 2010). Taken together, these data suggest the hypothesis that the synthesis of 2-AG, and perhaps also AEA, increases in blood cells and tissues in response to triggers of inflammation, which results in an increase of these lipids in the circulation.

Multiple studies have found that circulating endocannabinoids correlate with body mass index and are significantly increased in obese individuals. As mentioned above, 2-AG concentrations are increased in men with intra-abdominal adiposity (Bluher et al., 2006, Cote et al., 2007, Di Marzo et al., 2009a). Other studies have demonstrated significantly

higher AEA concentrations in obese individuals (Sipe et al., 2010, Quercioli et al., 2011). Readers are referred to excellent reviews regarding endocannabinoids and metabolic disorders for further consideration of this topic (Di Marzo et al., 2011, Tam et al., 2011).

One interesting study has examined the correlation of circulating endocannabinoid concentrations with a series of descriptors that comprise the drug reaction scale (DRS) (McPartland et al., 2005). The DRS includes 67 descriptors categorized as indicators of perception, emotion, cognition, and sociability that was designed to discriminate between cannabinoid and non-cannabinoid drugs (Karniol et al., 1975). Serum endocannabinoid concentrations were determined in blood drawn from healthy subjects before and after osteopathic manipulations. Although the manipulation tended to increase serum AEA concentrations, the change did not reach statistical significance. However, significant correlations were observed between the changes in AEA and several DRS categories. In particular, the change in serum AEA concentrations was positively correlated with ratings of *rational* and *cold* and inversely correlated with ratings of *paranoid* and *bad*. These data suggest that AEA concentrations in the circulation might reflect mental state and mood in addition to physiological parameters.

3.3. Relationships between circulating endocannabinoids and stress

Exposure to psychological or physical threat evokes a repertoire of behavioral, endocrine and neuronal changes that heighten awareness, allow for a “fight or flight response”, and increase chances for survival should injury occur. Exposure to stress results in activation of the sympathetic nervous system, which is measured as an increase in heart rate and blood pressure. Stress also induces activation of the hypothalamic-pituitary-adrenal (HPA) axis, the ultimate result of this response is an increase in circulating glucocorticoid concentrations. Less well appreciated are the consistent and reproducible effects of stress to induce inflammatory responses in both the peripheral circulation and in the brain (Dantzer et al., 2008). In particular, acute psychological stress causes a transient elevation in the number of immune cells in the circulation as a result of activation of the sympathetic nervous system (Benschop et al., 1996). Specifically, monocytes are increased in the circulation following acute psychological stress (Bosch et al., 2003, Yamakawa et al., 2009) and exercise (Hong and Mills, 2008), both of which are accompanied by increased sympathetic activation. A meta-analysis of human studies examining the effects of acute psychological stress on pro-inflammatory cytokines found that an increased concentration of IL-6 at 90 min following acute psychological stress was the most consistently observed result (Steptoe et al., 2007). A recent, comprehensive study of the effects of the Trier Social Stress Test (TSST) on a variety of cytokines demonstrated an immediate effect of stress exposure to increase natural killer T cells and the concentrations of interleukin 1beta (IL-1β) in male subjects (Yamakawa et al., 2009). The authors of this paper present the hypothesis that sympathetic activation results in a rapid migration of monocytes and macrophages into the circulation; these cells are also triggered by the sympathetic nervous system to release some cytokines, including IL-1β.

There are several places where endocannabinoid signaling and the three stress responses described intersect. First, endocannabinoid/CB1R signaling regulates the HPA axis response at multiple sites, including the hypothalamus (Di et al., 2003); pituitary (Yasuo et al., 2010) and adrenal gland (Ziegler et al., 2010b). CB1R activation at these sites inhibits HPA axis activation. Within the CNS, endocannabinoid signaling in the amygdala gates the activation of the HPA axis by stress (Hill et al., 2009b) and activation of endocannabinoid/CB1R signaling in prefrontal cortex is required for appropriate recovery of the HPA axis to baseline following stress offset (Hill et al., 2011).

Second, CB1R are present on sympathetic nerve terminals and their activation results in decreased norepinephrine release (Ishac et al., 1996). Therefore, increased endocannabinoid tone at these receptors would result in decreased sympathetic responses.

Third, CB1R signaling is required for appropriate brain stem regulation of sympathetic responses via the baroreflex. The baroreflex is a feedback mechanism triggered by activation of mechanoreceptors, called “baroreceptors” in arteries. Activation of the baroreceptors results in inhibition of sympathetic outflow and, thus, reduces pressure in the vasculature. Sympathoinhibitory responses to activation of the baroreceptor reflex are enhanced by endocannabinoid signaling in the nucleus tractus solitarius (NTS) (Seagard et al., 2004, Seagard et al., 2005, Chen et al., 2010). This mechanism is also consistent with endocannabinoids opposing or buffering stress responses.

Fourth, as was discussed in 3.2., endocannabinoids are released from circulating monocytes and macrophages. Both CB1R and a second subtype of cannabinoid receptors, CB2R, are expressed by immune cells and their activation is generally found to inhibit cytokine secretion and activation (Carrier et al., 2005). In particular, the ability of LPS to increase plasma IL-1β concentrations is inhibited by CB1R agonists (Roche et al., 2006). A study carried out in humans demonstrated that plasma concentrations of 2-AG and the cytokine, tissue necrosis factor alpha, are significantly negatively correlated (Koppel et al., 2009). Taken together, the available data supports the hypothesis that activated immune cells secrete endocannabinoids, which in turn, inhibit the secretion of some cytokines. Thus, the effects of the endocannabinoid system on immune system can also be characterized as stress buffering or enhancing stress recovery.

The effects of various physiological and psychological stressors on circulating endocannabinoids have been determined in humans and most studies find that endocannabinoids are increased by stress. Although our understanding of the sources and targets of the endocannabinoids in the circulation is still very incomplete, it is our working hypothesis that stress-responsive endocannabinoids enter the circulation from either the brain or circulating immune cells. Furthermore, we hypothesize that they function to dampen the initial stress response or serve a recovery role.

Several studies support the hypothesis that endocannabinoids, particularly 2-AG are increased by stress in parallel with the occurrence of an overall pro-inflammatory state. In a study of endocannabinoid responses to surgical stress, 2-AG concentrations were found to increase significantly after the start of cardiac surgery and were increased 3 fold during cardiopulmonary bypass procedure (Weis et al., 2010a). Furthermore, 2-AG concentrations were significantly, positively correlated with IL-6 concentrations in this study. Hemodynamic stress elicited by being held for 30 min (or until syncopal symptoms occurred) at 70° in a head up tilt resulted in significantly increased 2-AG concentrations compared to those obtained in the supine position (Schroeder et al., 2009). Although this study did not examine any parameters of inflammation or immune activation, previous studies have found that head up tilt procedures result in a transient increase in NK cell number that is driven by sympathetic activation (Klokker et al., 1997). However, no correlations between 2-AG and any marker of sympathetic activation were observed in the orthostatic stress study (Schroeder et al., 2009) which is in opposition with our hypothesis in its current form.

In another study, parabolic flight maneuvers were used to stress human volunteers and blood endocannabinoid contents, cortisol and motion sickness were the measured outcomes (Chouker et al., 2010). The results of this study support the hypotheses that circulating endocannabinoids can be affected by stress; interestingly AEA concentrations increased

earlier, during the stress itself while 2-AG increased later and were more closely related to the recovery phase. A study by the same group using the same parabolic stress protocol found that polymorphonuclear cell concentrations were increased in blood immediately after the parabolic flights (Kaufmann et al., 2009b).

Another theme that emerges when these three studies are considered together is that endocannabinoid concentrations are inversely related to the severity of stress-induced sequelae. In the parabolic flight stress study (Chouker et al., 2010), AEA concentrations in the circulation decreased in subjects who developed motion sickness and increased in those who did not. Similarly, 2-AG concentrations did not change significantly in those with motion sickness but increased in those who did not experience motion sickness. Cortisol concentrations were significantly, inversely correlated to AEA concentrations across all subjects. Interestingly, the circulating concentrations of both AEA and 2-AG in flight but before the parabolas occurred were higher in the group that would not experience motion sickness; this could be evidence of differences in stress responsivity in general between high and low endocannabinoid response groups.

Although the concentration of AEA in the circulation was unchanged by the orthostatic stress in the head up tilt study (Schroeder et al., 2009), circulating concentrations of AEA were significantly, positively correlated with orthostatic tolerance. Furthermore, individuals with AEA concentrations above the median exhibited a significant, rightward shift in the relationship between tilt-angle and increased heart rate. AEA concentrations did not correlate with changes in blood pressure. Therefore, although stress did not affect AEA, the basal concentration of AEA affected the reflex responses to the orthostatic stress. One explanation for these data is that individuals with high AEA could have a blunted sympathostimulatory response to unloading of the baroreceptors. Interestingly, in the cardiac surgery study, AEA concentrations were significantly positively correlated with the amount of norepinephrine required to maintain vasomotor tone (Weis et al., 2010a), which is also consistent with this explanation. As was discussed in section 3.3 above, high AEA tone in the NTS is associated with sympathoinhibition (Seagard et al., 2004), so perhaps the circulating AEA reflects AEA signaling in the NTS. Alternatively, high circulating AEA could inhibit sympathetic tone at the level of the sympathetic terminals through CB1R-mediated inhibition of norepinephrine release (Ishac et al., 1996).

Our laboratory has carried out a study of the effects of psychological stress on serum endocannabinoid concentrations in women (Hill et al., 2009c). A seventeen-minute version of the TSST resulted in a significant increase in 2-AG concentrations that returned to basal concentrations 30 min after the end of the stress. Serum AEA concentrations were not significantly different from baseline at either time point. Similarly, a study in which imagery of a previous stressful experience was administered, AEA concentrations were not changed from baseline but slowly dropped until they were significantly lower than baseline 75 min after the end of the stress (Mangieri et al., 2009). As was mentioned above, the TSST has also been shown to result in increased blood monocyte and cytokine concentrations (Yamakawa et al., 2009). A hypothesis that should be tested is whether the changes in 2-AG during the TSST are correlated with cytokines, particularly IL-1β and IL-6.

3.4. Summary

The concentrations of endocannabinoids in the circulation have informational content. There is evidence that endocannabinoid concentrations in the circulation and brain are in equilibrium and, in women, also reflect concentrations in reproductive tissues. There is also evidence from multiple diseases that AEA and, to a greater degree, 2-AG concentrations are increased in the circulation when the immune system is activated during inflammation, infection or injury. The hypothesis that circulating endocannabinoids are related to their

concentrations in brain makes them attractive biomarkers of CNS endocannabinoid tone. However, this notion is complicated by the fact that other tissues also donate endocannabinoids to the circulation, so that a brain biomarker function could be masked by changes in endocannabinoid homeostasis in other tissues or within the immune system. In spite of these caveats, currently available data indicate that circulating endocannabinoid measures, particularly during stressful experiences, could serve as a biomarker for several aspects of the vulnerability to stress-induced sequelae.

4.0. Associations between heterogeneity in endocannabinoid components and human mental disorders

4.1. Associations of heterogeneity in *CNR1* and *FAAH* with CNS function in healthy controls

Mental diseases are very complex in the constellation of symptoms that occur, are heterogeneous in their etiology and exhibit large individual differences in treatment outcomes. The purpose of this section is to examine the evidence that heterogeneity in endocannabinoid signaling could contribute the symptoms, etiology or treatment success of several important mental diseases. There is also evidence that genetic heterogeneity of *CNR1* and *FAAH* could add further variability to these diseases, and to normal variability in the population, affecting basic processes such as personality, reward sensitivity or fear processing. These data will be discussed first.

4.1.1. Reactivity to threat—An important imaging genetic study identified two phenotypic differences between healthy carriers of *FAAH* 385A and CC homozygotes (Hariri et al., 2009). Amygdalar reactivity, measured using BOLD fMRI, in response to threat-related, fearful and angry facial expressions was studied in healthy Caucasians. Subjects with CA or AA genotypes exhibited significantly reduced activation in the amygdala compared to those with CC genotype. In addition, those with CA or AA genotype showed a diminished relationship between amygdalar reactivity and trait anxiety compared to CC. These data suggest that individuals with an A allele at *FAAH* 385 have reduced sensitivity to potential threats or the possibility of harm. It has been hypothesized that blunted amygdalar reactivity to threat contributes to increased risk-taking behavior and to the propensity for addictive disorders in humans (Glahn et al., 2007).

4.1.2. Reactivity to reward and impulsivity—Subjects with CA and AA genotypes in *FAAH* exhibited significantly greater activation of the ventral striatum in response to reward compared to CC individuals (Hariri et al., 2009). In addition, those with at least one 385A allele demonstrated a significant relationship between ventral striatal reactivity and delay discounting, an index of impulsivity. Therefore, individuals with the 385A allele exhibit heightened reward sensitivity and increased impulsivity, which are both significant risk factors for the development of addiction (De Bank et al., 2005). Thus, individuals with even a single 385A allele exhibit two risk independent factors for addiction, decreased sensitivity to threat and increased sensitivity to reward.

In an interesting study that could have implications for the association of *CNR1* polymorphisms and substance abuse, Ehlers and colleagues have reported that impulsivity, assessed using a scale derived from the Maudsley personality inventory, was significantly associated with several heterogeneities in *CNR1* in Southwest California native Americans (Ehlers et al., 2007). In particular, impulsivity was significantly associated with the length of the AAT repeat, and SNPs at rs1534255, rs2023239, rs1049353 and rs806368. Since impulsivity is an important phenotype for substance use disorders (Hommer et al., 2011), it

is possible that the influence of heterogeneities in *CNR1* on impulsivity underlie their associations with risk for substance use disorders.

CNR1 genotype has been examined in relationship to neuronal responses to emotional faces in ventral striatum using fMRI of 19 healthy Caucasians (Chakrabarti et al., 2006). Four SNPs in *CNR1* were significantly associated with the striatal responses to happy faces (in parentheses are the genotypes with the highest and lowest responses): rs806377 (CC > TT); rs10495353 (GA > GG); rs806380 (GG > AA) and rs6454674 (GG > TT). These SNPs fall into at least 2 haplotype blocks (Table 1), suggesting that they could have independent effects. Interestingly, there were no correlations of these SNPs with the responses to disgust faces. Since happy, and not disgust faces, are innately socially rewarding, these results suggest that genetic variation in *CNR1* in differences among individuals in social reward responsivity. This observation could have implications for *CNR1* genetic contributions to several disorders, including substance abuse, depression and autism.

4.1.3. Learning and memory—The length of the AAT repeat has been shown to associate with THC-induced alterations in the auditory event-related P300 potential, a marker of attentional resource allocation and active working memory (Picton, 1992). Acute treatment of humans with THC reduces the amplitude of the P300 event-related potential (Roser et al., 2008). A greater effect of THC to reduce the amplitude and prolong the latency of the P300 evoked potential was found in individuals with more than 10 AAT repeats compared to those with 10 or fewer (Stadelmann et al., 2011). One hypothesis suggested by these data is that increased AAT repeats in the *CNR1* gene are associated with a CB1R that is more sensitive to THC. Interestingly, subjects with more than 10 AAT repeats also exhibited significantly longer latencies when treated with placebo as well, suggesting that the CB1R encoded by a *CNR1* with a long AAT repeat is also more sensitive to endocannabinoids.

In another study, young healthy subjects were divided into high and low performance groups based upon their efficiency in learning a mirror-tracing task (Ruiz-Contreras et al., 2011). Eight different AAT repeat lengths were observed in this subject group: 7, and 9-15 repeats. The most frequent in the population were 12 and 14 repeats. A significant enrichment in AAT12/14 carriers was found in the high performing group while the AAT12/13 genotype was more frequent among the low performers of the procedural learning task. The effect of the AAT genotype was restricted to procedural learning, neither recognition memory nor motor function were associated with the polymorphism.

4.1.4. Fatigue—A recent study by de Wit and colleagues suggests that a broader consideration of SNPs in the entire FAAH gene could provide a more complete understanding of the contribution of genetic differences in FAAH to drug effects (Dlugos et al., 2010). These investigators identified a haplotype block that includes *FAAH* 385 and two other intronic *FAAH* SNPs, rs3766246 (C/T) and rs2295633 (C/T) with D′ values between 88 and 97. The three most common haplotypes seen in this population were (in order of rs3766246/rs324420/rs2295633) TAT (10.2%); TCT (8.1%) and CCC (48.5%). They examined the association of genotypes at each of these SNPs and the three haplotypes with subjective effects of amphetamine in healthy, young Caucasian adults. Orally administered amphetamine produced increases in measures of Anxiety and Vigor and decreases in Fatigue and Confusion on the Profile of Mood States questionnaire. Fatigue was very significantly associated with genotypes at *FAAH* 385, rs2295633 and rs3766246, such that subjects with C at each of these positions exhibited a significantly greater decrease in Fatigue after amphetamine administration than individuals with the other genotypes. Individuals with the CCC haplotype also exhibited a significant association with Fatigue. These investigators suggested that individuals with the CCC haplotype could be more likely to use amphetamine

repeatedly because of their greater sensitivity to its stimulatory effects. The finding that the C allele at position 385 of *FAAH* has the characteristics of a "risk allele" for amphetamine use in this study is at odds with the findings discussed below (section 4.2.2) that the A allele is highly significantly correlated with problem drug use in Caucasian Americans.

4.2. Substance Use Disorders

CB1Rs are found in human brain regions associated with reward processing, including the ventral tegmental area, the nucleus accumbens, and prefrontal cortex (Glass et al., 1997). Activation of CB1Rs is rewarding in animals (Gardner, 2005, Solinas et al., 2008) and CB1R antagonists reduce the rewarding effects of drugs of abuse such as opiates, nicotine, alcohol, and cocaine, indicating that the endocannabinoid system is involved in the neurobiological mechanism underlying substance abuse (De Vries et al., 2005, Maldonado et al., 2006). Administration of the CB1R antagonist, rimonabant, to healthy subjects reduced the neural response to the sight and taste of chocolate in key reward areas such as the ventral striatum and the orbitofrontal cortex, and increased lateral orbitofrontal activation in response to the sight and taste of molding strawberries, an aversive stimulus (Horder et al., 2010).

The development of a substance use disorder is a step-wise progression from initial use to regular use to dependence. There is evidence that environmental and genetic factors are involved in the progression. In a recent review of the genetics of substance abuse, Bierut suggested that dependence should be considered a pharmacogenetic disorder to which many diverse genes contribute (Bierut, 2011). In light of the role of endocannabinoid signaling in the regulation of reward, many studies have been carried out to examine the hypothesis that genetic heterogeneity in endocannabinoid signaling contributes to the likelihood an individual will develop a substance use disorder.

4.2.1. *CNR1* polymorphisms—Twin studies have demonstrated a role for genetic differences in the liability to develop cannabis dependence (Lynskey et al., 2002); a logical candidate gene that could influence the liability to develop cannabis dependence is *CNR1*. Evidence from independent studies suggests that polymorphisms in *CNR1* are associated with the likelihood of developing cannabis dependence; however, the studies do not converge with respect to specific polymorphism. A meta-analysis published in 2010 combined data published before that time regarding the associations of rs1049353 (coding region), rs806379 (alternative promoter region in intron 2) and the AAT repeat (3′ UTR) (Benyamina et al., 2009). Of the three polymorphisms examined, only the AAT polymorphism showed a significant association with substance use disorders and this only occurred in Caucasians. Risk was associated with having at least 16 repeats and the effect size was small (odds ratio 1.55). In a study not included in the meta-analysis, AAT repeats of 14 or greater were significantly associated with heroin abuse in Caucasians as was the SNP at rs1049353 (Proudnikov et al., 2010).

rs2023239: Haughey and colleagues found a significant association of rs2023239, a SNP that has been hypothesized to regulate *CNR1* transcript in brain (see 2.1.4.; (Zhang et al., 2004)), with self reports of craving and withdrawal in abstinent daily marijuana smokers (Haughey et al., 2008). In particular, carriers of the minor C allele at rs2023239 exhibited significantly greater withdrawal scores and negative affect during abstinence then those homozygous for T. In a follow up study, the same group demonstrated that carriers of a C allele exhibited significantly greater activity in the orbitofrontal cortex, inferior frontal gyrus and anterior cingulate gyrus during exposure to marijuana cues than those with T/T genotype (Filbey et al., 2010). Similarly, individuals with the C allele at rs2023239 also exhibit greater activation of the prefrontal cortex to alcohol-cues and greater subjective

reward when consuming alcohol in moderate to heavy drinkers (Hutchison et al., 2008). Finally, post mortem biochemical analyses demonstrate that the C allele is associated with greater CB1R agonist binding in the prefrontal cortex (Hutchison et al., 2008). Taken together, these findings suggest that rs2023239 is associated with hyperactive CB1R function in the brain, which results in increased sensitivity to reward-related cues and increased unpleasant effects during withdrawal.

The SNP at rs2023239 was also identified as significantly associated with risk for poly-substance use disorders individually and as a haplotype block (rs806379-rs1535255-rs2023239) in African American populations (Zhang et al., 2004). The same study reported that the minor allelic frequency at SNPs rs806379 and rs2023239 is greater in a sample of European Americans with poly-substance abuse. rs2023239 is also significantly associated with nicotine dependence in women but not men (Chen et al., 2008). On the other hand, rs2023239 genotype was not associated with substance abuse disorders in a large study that included samples from nearly 900 individuals with substance use disorders (Herman et al., 2006).

rs806380: A SNP in intron 2 (rs806380) is individually, significantly associated with developing one or more cannabis dependence symptoms; and a haplotype block that includes this SNP (rs6454674-rs806380-rs806377-rs1049353) is associated with the development of cannabis dependence problems in adolescents (Hopfer et al., 2006). In a family-based test of association in which 9 CNR1 SNPs were investigated, rs806380 and a second SNP in the untranslated region of exon 4 (rs806368) were found to be significantly associated with cannabis dependence (Agrawal et al., 2009). As these two SNPs are in low LD, these results suggest independent effects. Both of these studies found that the A at is the risk allele in rs806380 for cannabis dependence. However, another study found that rs806380 was not significantly associated with cannabis dependence in either case-control or family based studies (Hartman et al., 2009).

rs806368 and rs6454674: In a study of 10 polymorphisms in CNR1, risk for substance use disorders in European Americans was associated with two SNPs: the number of G alleles at rs6454674 and a T/T genotype at rs806368 (Zuo et al., 2007). These two SNPs appeared to exert independent, genetic effects because their combination had a significantly greater effect on risk for substance use disorders than either alone. A moderate association of rs806368 was also found with cannabis dependence (Agrawal et al., 2009). In follow-up family-based and population-based studies, significant associations with each of these SNPs and cocaine dependence was confirmed in European Americans and a T/T genotype at rs806368 was significantly associated in a family based study of African Americans (Zuo et al., 2009). This study also confirmed that the two SNPs are independent and calculated the odds ratio for cocaine dependence in European Americans with both at-risk alleles to be 1.4.

4.2.2. *FAAH* polymorphisms—There is evidence that *FAAH* genotype contributes to the risk for alcoholism and problem drug use. Caucasians with the AA genotype have odds ratios of greater than 4 compared to Caucasians with AC or CC to be problem drug and/or alcohol users (Sipe et al., 2002). This finding was confirmed in a second study that also included African-American and Asian drug users and controls (Flanagan et al., 2006). When the data are pooled from the two studies, a highly significant enrichment (p=0.00003) in AA is found in individuals with documented drug addictions over the distribution of AA in control populations. In another study, individuals with AA genotype were found to be at greater risk for regular use of sedatives (Tyndale et al., 2007). However, these findings have not been confirmed in other studies. In particular, no association between *FAAH* 385 genotype and alcoholism (Iwasaki et al., 2007) or methamphetamine dependence (Morita et al., 2005) were found in a study of Japanese. No association of FAAH 385 genotype with

heroin addiction was found in an ethnically mixed sample (Proudnikov et al., 2010). Therefore, drug-specific studies have not replicated the observations made in polydrug abusers.

The influence of *FAAH* 385 on marijuana use and withdrawal also leads to the hypothesis that the C allele at *FAAH* 385 is the "risk allele" for progression to cannabis dependence. Among Caucasians, those with AA genotype were at significantly reduced risk for being THC dependent compared to either CA or CC genotypes (Tyndale et al., 2007). Daily marijuana users with the CC genotype exhibited significantly greater craving during a five day abstinence than individuals with at least one 385A allele (Haughey et al., 2008). Similarly, CC individuals exhibited increased severity of withdrawal after 24 hour abstinence and increased happiness after smoking marijuana than carriers of 385A (Schacht et al., 2009). Interestingly, AC and AA individuals were happier at baseline (before marijuana smoking) than those with CC genotype and displayed decreased happiness with smoking. In accord with the self-report data, BOLD fMRI imaging demonstrated greater activation in widespread regions of the reward circuit in response to marijuana cues in daily users with CC than AC and AA genotypes (Filbey et al., 2010). These data indicate that CC individuals experience more negative effects when they are abstinent and more positive effects when they use marijuana than individuals with 385A. These differences in intermediate phenotypes could underlie the increased liability to progress to cannabis dependence. Thus, while genotype at FAAH 385 is functionally important, the directionality of risk with regard to substance abuse is not easily ascribed to A or C at this stage of our understanding.

4.2.3. Circulating endocannabinoids—Only two studies have explored the interactions of substance use disorders with circulating endocannabinoids. Mangieri and colleagues compared circulating endocannabinoids in healthy social drinkers and recently abstinent alcoholics to stress, alcohol cues and neutral relaxed situations and found that alcohol cues increased circulating AEA in social drinkers but not abstinent alcoholics (Mangieri et al., 2009). In social drinkers but not alcoholics, the maximal alcohol craving was negatively correlated with AEA concentrations at time zero but the change in AEA was positively correlated with peak craving. Interestingly, baseline AEA concentrations were significantly lower in abstinent alcoholics than healthy subjects; perhaps the result of anxiety during early abstinence. As the increase in AEA was correlated with increased heart rate, the authors of this study speculate that the change in AEA concentrations induced by craving in social drinkers involves activation of the autonomic system. However, alcoholics exhibit a disconnected AEA response that could be the result of autonomic dysregulation in alcoholism (Grogan and Kochar, 1994). In the second study, AEA and other NAEs in the circulation were not affected by consumption of a beer with a meal (Joosten et al., 2010).

4.2.4. Summary—It is not surprising, given the well-described role of endocannabinoid signaling in the maintenance of rewarded behaviors in preclinical models, that genetic polymorphisms in the *CNR1* and *FAAH* genes are associated with substance use disorders. A recent study examined both the rs2023239 in CNR1 and the FAAH 385 SNP in abstinent marijuana users and found that carriers of both the G allele at the *CNR1* SNP and a CC genotype at *FAAH* 385 had significantly greater brain activation in reward areas than carriers of only one of these polymorphisms (Filbey et al., 2010). This finding is logical since changes in both the ligand and receptor would be expected to produce a greater phenotypic change than either one alone. Taken together, these studies are consistent with a role for the endocannabinoid system in reward sensitivity and in the emotional responses to cues that have been paired with drug exposure.

4.3 Depression

Humans have used cannabis sativa for thousands of years for a variety of reasons, including to elevate mood. In a study of young, poly-substance users, 69% of the respondents reported that they used cannabis to “make themselves feel better when down or depressed” (Boys et al., 2001). These results suggest that some users of cannabis are self-medicating a depressed mood with the drug. However, evidence indicates depressed subjects actually experienced more depression, aggression and sadness when intoxicated with cannabis than when they are not intoxicated (Arendt et al., 2007). Heavy cannabis use and major depression are co-morbid in clinical and community populations (Degenhardt et al., 2003, Lynskey et al., 2004) and several prospective studies have found that cannabis use precedes the diagnosis of depression (Bovasso, 2001, Patton et al., 2002, Rey and Tennant, 2002). However, a large (greater than 12,000 participants) longitudinal study did not find that past cannabis use was a significant predictor of depression in adults when baseline differences between users and non-users were carefully controlled (Harder et al., 2006). Therefore, neither the evidence that cannabis treats depression nor the evidence that cannabis use causes depression is convincing.

However, Harder and colleagues suggested, based upon the their longitudinal results, that a shared genetic predisposition could underlie the association of depression and cannabis use (Harder et al., 2006). In support of this hypothesis, several recent studies have demonstrated that the genetic factors for cannabis dependence and depression/suicidality are moderately correlated (Fu et al., 2002, Lynskey et al., 2004). Twin studies suggest that shared environmental factors also contribute significantly to the co-morbidity of cannabis dependence and depression (Lynskey et al., 2004).

While THC from cannabis and the endocannabinoid system have in common the CBR1, the effects of cannabis use do not necessarily match the role of the endocannabinoid system. This could be due, in part, to the fact that THC is a partial agonist of the CB1R (Kearn et al., 1999); therefore, THC can produce CB1R agonist effects when endocannabinoid tone is low and antagonist-like effects when endocannabinoid tone is high (Roloff and Thayer, 2009). With regard to a role in depression, data from the clinical experience with the CB1R antagonist, rimonabant, suggest that endocannabinoid/CB1R signaling contributes to maintaining a non-depressed mood. A significant occurrence of depressed mood was found in patients given the CB1R antagonist, rimonabant, for weight loss and obesity-related metabolic disorders (Christensen et al., 2007, de Mattos Viana et al., 2009). As was mentioned in section 4.2, CB1R inhibition reduces the hedonic aspects of sight and smell, which is consistent with a role of endocannabinoid signaling in hedonic processing (Horder et al., 2010).

In further support for a role for the endocannabinoid system in depression, Hungund and colleagues examined CB1R binding site density and ability to signal in post mortem samples from subjects with major depression who died by suicide (Hungund et al., 2004). They found that depressed suicides had 24% greater number of CB1R agonist binding sites and significantly more CB1R protein on western blot in prefrontal cortex than matched controls. Depressed suicides also have increased cannabinoid agonist-evoked GDP/GTP exchange, evidence that the increased receptors are functional. These data are supported by preclinical findings that chronic unpredictable stress in rats (a paradigm that results in symptoms of anhedonia, behavioral inflexibility and hyperactive HPA axis responsivity) also increases CB1R binding site density (Hill et al., 2008a) and CB1R mRNA expression (Bortolato et al., 2007) in prefrontal cortex. On the other hand, chronic unpredictable stress models in rodents decrease CB1R density in subcortical regions, including the hippocampus (Hill et al., 2005, Hill et al., 2008a), suggesting that a shift in the balance of endocannabinoid signaling between cortical and subcortical regions could contribute to depression.

Repeated exposure to stress can have negative consequences on humans and is a contributing factor for several mental disorders. However, most people are resilient to the effects of stress and the biological processes that underlie stress resilience are beginning to be understood (Feder et al., 2009). Among the resilience factors are several psychobiological factors that are associated with a high level of CB1R activation in preclinical studies, including: a low amount of fear-generalization; dampened hypothalamic-pituitary-adrenal axis activation by acute stress; use of active coping strategies; and high reward responsivity (Hill et al., 2010b). As was discussed in section 4.1, carriers of *FAAH* 385A have decreased threat-related amygdalar reactivity and increased reward-related activation of the ventral striatum (Hariri et al., 2009).

4.3.1. *CNR1* polymorphisms—A role for *CNR1* polymorphisms in depression was examined in the context of patients with Parkinson's Disease (Barrero et al., 2005). In a case control study of 48 Parkinson's patients and 41 controls, 14 of the patients and 5 of the controls were diagnosed with depression. Having fewer than 5 repeats of the AAT trinucleotide was significantly associated with depression in the patients with Parkinson's Disease; in fact, the probability of depression was 10 times greater in individuals with at least one short allele than those with two longer alleles. The same trend was seen in the depressed controls, but the sample size was too small for a conclusion. In contrast, an earlier study carried out in Taiwan did not find significant association between the length of the AAT triplet repeat and the diagnoses of either major depression or bipolar disorder compared to controls (Tsai et al., 2001).

Another study examined the association of major depression with genotype at rs1049353 in Caucasians with major depression compared to healthy controls (Monteleone et al., 2010a). A significant shift in the allelic frequency at this SNP was seen such that healthy controls exhibited 86.5% G and 13.4% A alleles at this position, with genotypes GG:GA:AA distributed 21:5.8:1. The group with major depression was significantly enriched in A alleles (27.7% A and 72.3% G) compared to the healthy controls, with genotypes GG:GA:AA distributed 5:3.2:1. These investigators calculated an odds ratio of 2.46 for the contribution of the A allele to the probability of having major depression. This study has not been replicated, and the results seem at odds with the finding that an A allele at rs1049353 is associated with increased striatal reactivity to happy faces (Chakrabarti et al., 2006) since higher responsiveness to positive social cues has been found to be associated with a favorable outcome in major depression (Canli et al., 2005).

Domschke and colleagues tested the hypothesis that *CNR1* genotype could play a role in the responsivity of individuals with major depression to treatment (Domschke et al., 2008). These investigators found that individuals with the G allele at rs1049353 had increased risk for antidepressant treatment resistance, particularly in females with co-morbid anxiety. In agreement with (Chakrabarti et al., 2006), individuals with major depression who had a G allele at rs1049353 exhibited weaker responses to happy faces in several subcortical regions, including bilateral amygdala, pallidum and putamen and in the left caudate and thalamus. The authors of this study suggest that these results support a role for *CNR1* gene variability in depression as a result of its role in subcortical responsiveness to social stimuli.

Preclinical data suggest that endocannabinoid signaling could specifically be involved in the contribution of stress to the risk of developing depression (Hill et al., 2009a). This hypothesis was explored in a population study in which *CNR1* genotype was examined together with personality characteristics and the number of adverse life events experienced by each individual (Juhasz et al., 2009a). Ten SNPs in *CNR1* were examined in haplotypes and while current depression scores significantly associated with some *CNR1* haplotypes, the association diminished when covariation for recent negative life events was added to the

analysis. This suggests a gene × environment interaction such that the *CNR1* gene could affect the experience of negative life events through personality-dependent life choices or by affecting the interpretation or response to a negative life event (Bouchard and McGue, 2003). The same study also demonstrated significant contribution of *CNR1* genetic variation to a high neuroticism, low agreeableness phenotype. One particular SNP, rs7766029, which is not in high LD with either of the primary haplotype blocks of *CNR1*, showed a highly significant interaction with recent negative life events on depression. Neuroticism, which is the ease with which emotions are aroused, magnifies the significance of negative life events and is a well-known predictor of depression (Kendler et al., 2004). Less is known about the contribution of the personality trait of agreeableness to depression, but indirect evidence, pointed out in (Juhasz et al., 2009a) indicates that agreeableness could affect depression through affecting the likelihood of the individual to seek social support or interaction.

4.2.2. *FAAH* polymorphisms—The possible association of *FAAH* genotype with susceptibility to mood disorders has been investigated in two studies, each with a small sample size. In the original paper demonstrating the C385A SNP, no significant associations of genotype with depressive symptoms, suicidal ideation, schizophrenia, bipolar disorder or autism were found (Sipe et al., 2002). In a comparison of genotypes of 108 controls with 120 patients with bipolar disorder and 72 patients with major depression, small but statistically insignificant increases in odds ratio for both disorders were associated with having an A allele at *FAAH* 385 (Monteleone et al., 2010a). No significant contribution of *FAAH* 385 genotype to either paranoid or hebephrenic schizophrenia was seen in study of Japanese patients (n=260; (Morita et al., 2005).

4.2.3. Circulating endocannabinoids—Two studies have explored baseline endocannabinoid concentrations in the serum in women with depressive disorders (Hill et al., 2008b, Hill et al., 2009c). Serum 2-AG concentrations were significantly decreased in patients with major depression compared to demographically matched controls (Hill et al., 2008b, Hill et al., 2009c). The decrease in 2-AG was correlated significantly and negatively with the duration of the depressive episode (Hill et al., 2008b). In one of the studies (Hill et al., 2009c), serum AEA concentrations were also significantly decreased in depressed patients. Both control and depressed women exhibited similar 2-AG responses to administration of the TSST (Hill et al., 2009c).

We presented a hypothesis above that one function of 2-AG in the circulation is to inhibit the release of pro-inflammatory cytokines from monocytes and macrophages that are mobilized by sympathetic activation (section 3.3). It is well known that peripheral inflammatory responses can produce “sickness behavior”, involving depressed mood, social isolation, increased pain sensitivity, fatigue, and anhedonia – all of which are commonly present in major depression (Dantzer et al., 2008). In addition, a meta-analysis of cytokines in major depression demonstrated an increase in circulating TNFalpha and IL-6 in depressed patients (Dowlati et al., 2010). Taken together, these data suggest the hypothesis that low 2-AG in the circulation results in loss of its inhibitory influence on cytokine release, resulting in exacerbation of the symptoms of depression.

An interesting related study examined AEA concentrations in the plasma of patients suffering from fibromyalgia (FM) (Kaufmann et al., 2008). FM is a relatively common syndrome that is characterized by widespread chronic pain that is usually co-morbid with other functional, vegetative and psychiatric symptoms (Arnold et al., 2006). There is a high incidence of extremely stressful experiences in childhood, including childhood sexual abuse, in patients with FM (Weissbecker et al., 2006) and FM is commonly considered a stress-associated disorder (Van Houdenhove and Luyten, 2006). Plasma AEA concentrations were significantly higher in patients with long-standing FM compared to matched controls

(Kaufmann et al., 2008). Interestingly, adhesion and phagocytosis of neutrophils isolated from patients with FM are both significantly, positively correlated with concentrations of AEA. In fact, multiple, backwards regression analysis identified AEA as the main factor determining adhesion and phagocytosis. Although the significance of these data is not apparent, these findings are consistent with a role for circulating AEA as an immune regulatory molecule.

4.4. Anxiety and eating disorders

The most commonly cited reasons for continued recreational cannabis use are relaxation and reduction in tension (Thomas, 1993, Reilly et al., 1998, Schofield et al., 2006). Paradoxically, the most commonly cited reasons for discontinuation of cannabis use are increased anxiety and panic reactions (Szuster et al., 1988, Reilly et al., 1998). Modulation of anxiety reactions by cannabis appears to be complex in that both dose and environmental context can modulate these effects. Subjects under “experimenter harassment” were more likely to experience anxiety reactions under the influence of cannabis than those in neutral environments (Gregg et al., 1976).

Psychiatric adverse effects, including anxiety, were cited as reasons for discontinuation by patients taking rimonabant significantly more than those taking placebo (Van Gaal et al., 2008), although objective measures of anxiety were not significantly increased in patients taking rimonabant (Scheen et al., 2006). A recent meta-analysis pooling data from four large clinical trials indicated that subjects taking rimonabant had a significantly greater increase in anxiety symptoms while taking the drug than patients taking placebo (Christensen et al., 2007). Therefore, human experience with a CB1R agonist (THC) and antagonist (rimonabant) support the hypothesis that endocannabinoid signaling regulates anxiety in humans and suggest that activation of the CB1R by endocannabinoids could produce anxiolytic effects.

Posttraumatic stress disorder (PTSD) occurs in about 30% of individuals following exposure to an extreme traumatic stressor. At the time of the stress exposure, the person's response is intense fear, helplessness or horror. The disorder includes a set of characteristic symptoms including persistent, intrusive recollections or re-experience of the original event, numbing and avoidance and increased arousal (2000). Anecdotal reports of cannabis use causing relief of PTSD symptoms by returning veterans are abundant. A recent, systematic study demonstrated that PTSD diagnoses were associated with increased odds of lifetime history of cannabis use as well as past year daily cannabis use (Cougle et al., 2011). As for other disorders that are associated with heavy cannabis use, the mechanistic relationships between PTSD risk and cannabis use are not known. A small study of the therapeutic benefit of nabilone, an CB1R agonist that is given orally, a majority of patients treated (72% of 47 patients total) experienced a significant reduction in intensity or complete elimination of nightmares (Fraser, 2009). However, this study was not placebo controlled and needs to be confirmed with larger, better-controlled studies.

4.4.1. *CNR1* polymorphisms—The arguments presented in section 4.4 that cannabis use can affect state and trait anxiety in humans beg the question of whether genetic polymorphisms in proteins of the endocannabinoid system contribute to the probability of developing an anxiety disorder. In a study of co-morbidities associated with attention deficit/hyperactivity disorder, a significant association of an A allele at rs1049353 with the diagnosis of PTSD was identified (Lu et al., 2008). However, haplotype analysis indicated that the genotype at rs806368 could be a more important risk factor. In either case, these associations were not replicated in other populations within the study. In a study examining

the role of multiple SNPs, no significant association with haplotype or individual CNR1 SNPs was found (Juhasz et al., 2009a).

Although the association of *CNR1* in isolation does not provide strong evidence of a role in anxiety, an important gene × gene study suggests that anxious phenotype is associated with the co-occurrence of four SNPs in CNR1 together with a previously described low risk polymorphism in a marker (*5-HTTLPR*) the gene for the serotonin reuptake carrier (*SLC64A)* (Lazary et al., 2009). These investigators found that the risk of high anxiety scores in three different inventories of state and trait anxiety was associated with a combination of the minor allele at rs2180619 (G; in the conventional promoter for *CNR1*); minor alleles at the three SNPs in the alternative promoter in intron 2 of CNR1 (rs806379/ rs1535255/rs2023239; TGC haplotype) and an SS homozygous genotype at *5-HTTLPR.* Interestingly, carriers of the major alleles at each of the CNR1 SNPs (A and ATT) are protected from the anxiety-producing effects of SS in *5-HTTLPR.* The authors present an interesting model, based on preclinical data that CB1R activation inhibits serotonin release, hypothesizing that the combination of low CB1R expression and low serotonin reuptake contribute through different mechanisms to excessive 5-HT signaling and, therefore, anxiety.

Genetic factors play a substantial role in the incidence of anorexia nervosa (AN), an eating disorder that occurs primarily in women and is associated with an intense fear of weight gain (Klump et al., 2001). Whole blood CB1R mRNA expression is greater in women with AN and bulimia nervosa (BN) than healthy controls and is negatively associated with measures of perfectionism, impulse regulation and drive for thinness (Frieling et al., 2009), suggesting that CB1R hypoactivity contributes to the disorder. A recent study used the CB1R PET ligand, [^{18}F]MK-9470 to demonstrate that women with AN had 25% higher CB1R binding site density in cortical and subcortical regions than age-matched healthy subjects (Gerard et al., 2011). This study further demonstrated a significant, positive relationship between CB1R binding in the right superior temporal cortex of AN patients and drive for thinness. These authors concluded that the increased CB1R density was a compensatory change driven by hypoactive ECS; future studies will be needed to clarify whether these changes in CB1R are causative or compensatory.

Several genetic association studies have been carried out investigating the relationship between *CNR1* and AN. The contribution of the AAT repeat near CNR1 to AN was examined in a family study (Siegfried et al., 2004). There were no differences found when all patients with AN were combined; however, the 14 repeat allele of AAT was preferentially transmitted in the binging/purging AN group but not in the restricting AN group. A trend for preferential transmission of the 13 AAT repeat in the food-restricting AN group was also found. Another study examined the SNP in the coding region of CNR1 (rs1049353) in a case control study of individuals with either AN or bulimia nervosa (BN) (Monteleone et al., 2009). They found that, compared to healthy controls, both sets of patients had increased expression of the A allele at this SNP. However, this association was not detected in another study of patients with AN (Muller et al., 2008).

4.4.2. FAAH polymorphisms—The *FAAH* 385C to A polymorphism was examined in the same group of AN and BN patients as discussed immediately above (Monteleone et al., 2009). The presence of the rare A allele at this SNP was also found to associate with both AN and BN. Interestingly, individuals with both risk alleles (i.e. an A at rs1049535 and an AC genotype at *FAAH* 385) had an odds ratio of 6.3 for AN and 6.6 for BN. Although a significant fraction of the AN and BN patients had co-morbid depressive disorders, genotypes at the *FAAH* and *CNR1* SNPs examined did not differ between patients with and without co-morbidities. A caution with this study, however, is that the control and AN/BN

groups exhibited many demographic differences and the gene expression profiles deviated from Hardy Weinberg equilibrium.

4.4.3. Circulating endocannabinoids—Support for an relationship between endocannabinoid signaling and anxiety in humans comes from a study of serum endocannabinoids in women with depression (Hill et al., 2008b). In this study, the severity of anxiety experienced by women with major depression was inversely correlated with baseline serum contents of AEA. Although very little is known about the source or potential target of circulating endocannabinoids, these data suggest that some of the somatic manifestations of anxiety could be related to reduced endocannabinoid signaling.

Plasma concentrations of AEA and 2-AG have also been measured in women with AN, BN and binge-eating disorder (Monteleone et al., 2005). Plasma concentrations of AEA were significantly greater in women with AN and binge-eating disorder than controls. AEA concentrations were significantly inversely correlated with leptin concentrations in controls and AN individuals, suggesting that the metabolic disturbances in BN and binge-eating obscure the normal effect of AEA to inhibit leptin. 2-AG concentrations were not different among the groups. Since AEA is an orexigenic factor and is dysregulated in obesity, it is possible that the dysregulated eating patterns of those with AN and binge-eating contribute to the difference in AEA.

4.5. Schizophrenia

Schizophrenia is the second most common mental illness after depression. It usually begins in late adolescence or early adulthood. Symptoms include delusions or hallucinations; loose association; blunted or inappropriate affect and distortion of perceptions. A number of case reports indicate that consumption of large amounts of cannabis with high THC content can result in “cannabinoid psychosis”, with many similarities to schizophrenia (Ujike and Morita, 2004). Cannabis use in adolescence imposes a two-fold increased risk for the development of schizophrenia (Arseneault et al., 2002, Zammit et al., 2002). Meta-analyses estimate that adolescent cannabis use may account for 8-14% of schizophrenia cases (Henquet et al., 2005, Jiang et al., 2007, Moore et al., 2007). Taken together, these studies suggest that excessive activation of CB1R signaling could contribute to the etiology of schizophrenia.

Several studies have examined the density of CB1R in post mortem human brain. Autoradiographic studies have demonstrated that CB1R binding site density in the dorsolateral prefrontal cortex (Dean et al., 2001) and cingulate cortex (Zavitsanou et al., 2004, Newell et al., 2006) of schizophrenics compared to controls. This finding was confirmed in living humans (Wong et al., 2010) using a PET radioligand, [^{11}C]OMAR, which has high selectivity for the CB1R (Horti et al., 2006). In particular, this study demonstrated increased CB1R density throughout the brain in schizophrenics compared to controls, reaching significance only in the pons. On the other hand, no difference in the density of neurons expressing CB1R in the anterior cingulate cortex was observed in an immunohistochemical study (Koethe et al., 2007). Furthermore, a comprehensive and well executed study by Eggan and colleagues demonstrated that CB1R mRNA and CB1R immunoreactivity were significantly lower in GABAergic neurons in the dorsolateral prefrontal cortex of schizophrenics compared to controls (Eggan et al., 2008). Based on the roles of CB1R and GABAergic transmission in the dorsolateral prefrontal cortex, these authors hypothesized that reduced CB1R inhibition of GABA release could serve as a compensatory mechanism to increase GABAergic transmission, which is impaired in schizophrenia (Lewis et al., 2005).

4.4.1. *CNR1* polymorphisms—There is a strong genetic component for the development of schizophrenia; a susceptibility locus has been mapped to chromosome 6q (Levi et al., 2005), near to the location of CNR1. Several schizophrenia-*CNR1* genetic association studies have been carried out with mixed results. All of the studies of the *CNR1* coding SNP rs1049353 have shown no significant association with schizophrenia (Ujike et al., 2002, Seifert et al., 2007, Zammit et al., 2007). Inconsistent results have been reported for the AAT repeat as several studies have shown significant associations (Ujike et al., 2002, Martinez-Gras et al., 2006, Chavarria-Siles et al., 2008) and while others have shown non-significant associations (Dawson, 1995, Tsai et al., 2000, Seifert et al., 2007). One study confirmed that rs1049353 genotype did not associate with susceptibility for schizophrenia but that the G allele frequency was significantly higher in patients non-responsive to antipsychotic treatment compared to responsive patients (Hamdani et al., 2008), suggesting that *CNR1* genotype is not a susceptibility gene but instead might be a pharmacogenetic factor. In sum, the available data do not support a strong association between the genotype of *CNR1* and susceptibility to schizophrenia.

A recent study examined a slightly different hypothesis, that *CNR1* polymorphisms contribute to the development of schizophrenia in the context of heavy cannabis use (Ho et al., 2011). In other words, the tested hypothesis was that *CNR1* genetic status modulates the contribution cannabis use to the susceptibility to schizophrenia. The approach taken by this group was to compare brain volume and neurocognition in schizophrenics who were and were not heavy cannabis users and to examine the contribution of *CNR1* genotype to the differences (Ho et al., 2011). Significant main effects of three SNPs, rs7766029, rs12720071 and rs9450898 were associated with white matter volume in schizophrenics. In addition, a significant gene x cannabis use interaction was found with rs12720071 with regard to white matter volume and neurocognitive impairment. These results suggest that *CNR1* genotypes can alter the contribution of heavy cannabis use to worsen white matter volume decreases and cognitive impairment in schizophrenics.

A very common adverse effect of the treatment of schizophrenia with antipsychotics is weight gain. In light of the relationships between excessive endocannabinoid signaling and body weight, several studies have explored the relationship between weight gain upon antipsychotic treatment and several *CNR1* SNPs. A nominal association between the genotype at rs806378 and weight gain in response to clozapine or olanzapine; with the T allele carriers gaining on average 2.2 kg more weight than CC carriers (Tiwari et al., 2010). The CNR1 SNP at rs1049353 was not found to be associated with weight gain in another study (Monteleone et al., 2010b).

4.4.2. FAAH polymorphisms—One study has investigated the possible association of the *FAAH* 385C/A SNP in schizophrenia, and did not find significant association (Morita et al., 2005). However, this SNP was found to be associated with antipsychotic-induced weight gain (Monteleone et al., 2010b). In particular, the A allele at this site (which results in lower FAAH expression) was more frequent in those that gained more than 7% of their baseline body weight.

4.4.3. Circulating endocannabinoids—A series of reports have examined the concentration of AEA in the cerebrospinal fluid (CSF) of patients with schizophrenia. AEA concentrations are substantially higher in schizophrenics than controls (Leweke et al., 1999, Giuffrida et al., 2004, Leweke et al., 2007, Koethe et al., 2009). In fact, AEA concentrations are 8-fold higher in drug-naïve, paranoid schizophrenics and are significantly inversely correlated with psychotic symptoms (Giuffrida et al., 2004). The increase in CSF AEA occurs very early in the course of the disease (the prodromal phase), and those with the lowest AEA concentrations are at highest risk for converting to a psychotic state (Koethe et

al., 2009). Interestingly, heavy cannabis users who are schizophrenics were found to have significantly lower CSF AEA concentrations than schizophrenics who are low frequency cannabis users (Leweke et al., 2007). These data lead to the hypothesis that elevated AEA concentrations are protective against the symptoms of schizophrenia. In one study, AEA concentrations in the plasma were also found to be significantly higher in untreated schizophrenic patients compared to controls and decline with clinical remission (De Marchi et al., 2003). In another study, plasma AEA was elevated in schizophrenics with substance use disorders and did not change with quetiapine treatment although substance use was reduced. In yet another study, no difference in plasma AEA occurred between controls and schizophrenics (Leweke et al., 2007).

Taken together, elevated CSF AEA concentrations are consistently observed in schizophrenic patients, even early in the disease. The negative correlation with symptoms suggests that AEA is increased, perhaps as a result of increased D2 receptor activation (Giuffrida et al., 1999), and that it acts to normalize dysregulated signaling.

4.8 Attention deficit disorder

The data discussed in section 4.1.2. suggest a role for the endocannabinoids in attention deficit/hyperactivity disorder (ADHD). Specifically, several SNPs and the length of the AAT repeat in one ethnic group exhibit significant relationships with measures of impulsivity (Ehlers et al., 2007) and individuals with an A allele at *FAAH* 385 exhibit more impulsivity in the delay discounting task (Hariri et al., 2009). Two studies have examined this issue directly. In the first, FAAH activity was found to be lower in peripheral lymphocytes of boys diagnosed with ADHD than matched controls (Centonze et al., 2009). Although genotype was not determined in this study, these data are in accord with the finding that subjects expressing *FAAH* 385A, which results in reduced FAAH protein expression (Chiang et al., 2004). The second study examined the contribution of *CNR1* polymorphisms of two, two-SNP haplotype blocks (rs806368/rs1049353 and rs806377/rs6454574) that fall into haplotype blocks 2 and 1, respectively, in Table 1 (Lu et al., 2008). A significantly increased risk for ADHD was associated with a C/G genotype at rs806377/rs6454574; none of the other combinations were significantly associated. The association was gender-dependent, contributing more strongly to risk in males than females. Interestingly, there were no significant associations of ADHD with the other haplotype block examined (rs806368/rs1049353), in contrast to the finding that both of these SNPs were associated with impulsivity in another study (Ehlers et al., 2007).

ADHD, in addition to impulsivity, also involves emotional dysregulation and there is evidence to support increased amygdalar reactivity (Plessen et al., 2006); differences in prefrontal-limbic circuitry (Durston et al., 2006) and poor HPA axis responsivity (King et al., 1998) in individuals with ADHD compared to controls. Taken together with the data above, it is possible that the role of endocannabinoid signaling in the regulation of stress responses and emotional regulation underlies its contribution to ADHD risk.

5.0. Conclusion

Two things are evident from the data presented in this review. First, there is good reason to believe that human differences in endocannabinoid signaling contribute to the probability of the occurrence of several important mental diseases. Because endocannabinoid signaling is vital to the regulation of brain function, metabolism and the immune system, it is not surprising that the genetic differences in *CNR1* and *FAAH* preserve basic protein function. It is likely that mutations leading to complete loss of function could not be sustained in a population. On the other hand, we argue that the data gathered thus far strongly suggest that polymorphisms in *CNR1* and *FAAH* contribute to individual differences in personality that

can, in combination with environment and social circumstances, significantly impact the probability of the individual developing a mental disorder.

Many very important questions need to be answered. In particular, future studies in which the *CNR1* SNPs are studied at a function level are essential for mechanistic conclusions to be made. This includes deep sequencing of the *CNR1* gene as well as in vitro studies of mRNA stability and transcription factor binding to *CNR1* gene variants. Second, fundamental questions remain regarding the sources and targets of AEA and 2-AG in the circulation. Third, other proteins of the endocannabinoid signaling system, including diacylglycerol lipase and monoacylglycerol lipase, critical for the synthesis and degradation of 2-AG, could also contribute to human variability in endocannabinoid signaling.

The endocannabinoid system is altered in response to experience and environment; exhibits significant genetic diversity and modulates mood, reward, cognition and many other functions. We hypothesize that better understanding of individual differences in endocannabinoid signaling will impact diagnosis, treatment and prevention of mental disorders.

Bibliography

Diagnostic and statistical manual of mental disorders, DSM-IV-TR. Washington, DC: American Psychiatric Association; 2000.

Agrawal A, Wetherill L, Dick DM, Xuei X, Hinrichs A, Hesselbrock V, Kramer J, Nurnberger JI Jr, Schuckit M, Bierut LJ, Edenberg HJ, Foroud T. Evidence for association between polymorphisms in the cannabinoid receptor 1 (CNR1) gene and cannabis dependence. Am J Med Genet B Neuropsychiatr Genet. 2009; 150B:736–740. [PubMed: 19016476]

Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience. 2000; 100:685–688. [PubMed: 11036202]

Akira S, Isshiki H, Sugita T, Tanabe O, Kinoshita S, Nishio Y, Nakajima T, Hirano T, Kishimoto T. A nuclear factor for IL-6 expression (NF-IL6) is a member of a C/EBP family. EMBO J. 1990; 9:1897–1906. [PubMed: 2112087]

Alberini CM, Ghirardi M, Metz R, Kandel ER. C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. Cell. 1994; 76:1099–1114. [PubMed: 8137425]

Arendt M, Rosenberg R, Fjordback L, Brandholdt J, Foldager L, Sher L, Munk-Jorgensen P. Testing the self-medication hypothesis of depression and aggression in cannabis-dependent subjects. Psychol Med. 2007; 37:935–945. [PubMed: 17202003]

Arnold LM, Hudson JI, Keck PE, Auchenbach MB, Javaras KN, Hess EV. Comorbidity of fibromyalgia and psychiatric disorders. The Journal of clinical psychiatry. 2006; 67:1219–1225. [PubMed: 16965199]

Arseneault L, Cannon M, Poulton R, Murray R, Caspi A, Moffitt TE. Cannabis use in adolescence and risk for adult psychosis: longitudinal prospective study. Bmj. 2002; 325:1212–1213. [PubMed: 12446537]

Balvers MG, Verhoeckx KC, Witkamp RF. Development and validation of a quantitative method for the determination of 12 endocannabinoids and related compounds in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877:1583–1590.

Barrero FJ, Ampuero I, Morales B, Vives F, de Dios Luna Del Castillo J, Hoenicka J, Garcia Yebenes J. Depression in Parkinson's disease is related to a genetic polymorphism of the cannabinoid receptor gene (CNR1). Pharmacogenomics J. 2005; 5:135–141. [PubMed: 15668727]

Baye TM, Zhang Y, Smith E, Hillard CJ, Gunnell J, Myklebust J, James R, Kissebah AH, Olivier M, Wilke RA. Genetic variation in cannabinoid receptor 1 (CNR1) is associated with derangements in lipid homeostasis, independent of body mass index. Pharmacogenomics. 2008; 9:1647–1656. [PubMed: 19018721]

Bensaid M, Gary-Bobo M, Esclangon A, Maffrand JP, Le Fur G, Oury-Donat F, Soubrie P. The cannabinoid CB1 receptor antagonist SR141716 increases Acrp30 mRNA expression in adipose tissue of obese fa/fa rats and in cultured adipocyte cells. Mol Pharmacol. 2003; 63:908–914. [PubMed: 12644592]

Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M. Catecholamine-induced leukocytosis: early observations, current research, and future directions. Brain Behav Immun. 1996; 10:77–91. [PubMed: 8811932]

Benyamina A, Bonhomme-Faivre L, Picard V, Sabbagh A, Richard D, Blecha L, Rahioui H, Karila L, Lukasiewicz M, Farinotti R, Marill C, Reynaud M. Association between ABCB1 C3435T polymorphism and increased risk of cannabis dependence. Prog Neuropsychopharmacol Biol Psychiatry. 2009; 33:1270–1274. [PubMed: 19625010]

Benzinou M, Chevre JC, Ward KJ, Lecoeur C, Dina C, Lobbens S, Durand E, Delplanque J, Horber FF, Heude B, Balkau B, Borch-Johnsen K, Jorgensen T, Hansen T, Pedersen O, Meyre D, Froguel P. Endocannabinoid receptor 1 gene variations increase risk for obesity and modulate body mass index in European populations. Hum Mol Genet. 2008; 17:1916–1921. [PubMed: 18375449]

Berrendero F, Garcia-Gil L, Hernandez ML, Romero J, Cebeira M, de Miguel R, Ramos JA, Fernandez-Ruiz JJ. Localization of mRNA expression and activation of signal transduction mechanisms for cannabinoid receptor in rat brain during fetal development. Development. 1998; 125:3179–3188. [PubMed: 9671590]

Bierut LJ. Genetic vulnerability and susceptibility to substance dependence. Neuron. 2011; 69:618–627. [PubMed: 21338875]

Bluher M, Engeli S, Kloting N, Berndt J, Fasshauer M, Batkai S, Pacher P, Schon MR, Jordan J, Stumvoll M. Dysregulation of the peripheral and adipose tissue endocannabinoid system in human abdominal obesity. Diabetes. 2006; 55:3053–3060. [PubMed: 17065342]

Bojesen IN, Hansen HS. Binding of anandamide to bovine serum albumin. J Lipid Res. 2003; 44:1790–1794. [PubMed: 12837852]

Bortolato M, Mangieri RA, Fu J, Kim JH, Arguello O, Duranti A, Tontini A, Mor M, Tarzia G, Piomelli D. Antidepressant-like activity of the fatty acid amide hydrolase inhibitor URB597 in a rat model of chronic mild stress. Biol Psychiatry. 2007; 62:1103–1110. [PubMed: 17511970]

Bosch JA, Berntson GG, Cacioppo JT, Dhabhar FS, Marucha PT. Acute stress evokes selective mobilization of T cells that differ in chemokine receptor expression: a potential pathway linking immunologic reactivity to cardiovascular disease. Brain Behav Immun. 2003; 17:251–259. [PubMed: 12831827]

Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G, Casellas P. Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. Biochem J. 1995; 312:637–641. [PubMed: 8526880]

Bouaboula M, Rinaldi M, Carayon P, Carillon C, Delpech B, Shire D, Le Fur G, Casellas P. Cannabinoid-receptor expression in human leukocytes. Eur J Biochem. 1993; 214:173–180. [PubMed: 8508790]

Bouchard TJ Jr, McGue M. Genetic and environmental influences on human psychological differences. J Neurobiol. 2003; 54:4–45. [PubMed: 12486697]

Bovasso GB. Cannabis abuse as a risk factor for depressive symptoms. Am J Psychiatry. 2001; 158:2033–2037. [PubMed: 11729021]

Boys A, Marsden J, Strang J. Understanding reasons for drug use amongst young people: a functional perspective. Health Educ Res. 2001; 16:457–469. [PubMed: 11525392]

Caille S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH. Specific alterations of extracellular endocannabinoid levels in the nucleus accumbens by ethanol, heroin, and cocaine self-administration. J Neurosci. 2007; 27:3695–3702. [PubMed: 17409233]

Canli T, Cooney RE, Goldin P, Shah M, Sivers H, Thomason ME, Whitfield-Gabrieli S, Gabrieli JD, Gotlib IH. Amygdala reactivity to emotional faces predicts improvement in major depression. Neuroreport. 2005; 16:1267–1270. [PubMed: 16056122]

Caraceni P, Viola A, Piscitelli F, Giannone F, Berzigotti A, Cescon M, Domenicali M, Petrosino S, Giampalma E, Riili A, Grazi G, Golfieri R, Zoli M, Bernardi M, Di Marzo V. Circulating and

hepatic endocannabinoids and endocannabinoid-related molecules in patients with cirrhosis. Liver Int. 2010a; 30:816–825. [PubMed: 19840245]

Caraceni P, Viola A, Piscitelli F, Giannone F, Berzigotti A, Cescon M, Domenicali M, Petrosino S, Giampalma E, Riili A, Grazi G, Golfieri R, Zoli M, Bernardi M, Di Marzo V. Circulating and hepatic endocannabinoids and endocannabinoid-related molecules in patients with cirrhosis. Liver Int. 2010b; 30:816–825. [PubMed: 19840245]

Carrier EJ, Patel S, Hillard CJ. Endocannabinoids in neuroimmunology and stress. Curr Drug Targets CNS Neurol Disord. 2005; 4:657–665. [PubMed: 16375683]

Centonze D, Bari M, Di Michele B, Rossi S, Gasperi V, Pasini A, Battista N, Bernardi G, Curatolo P, Maccarrone M. Altered anandamide degradation in attention-deficit/hyperactivity disorder. Neurology. 2009; 72:1526–1527. [PubMed: 19398708]

Chakrabarti B, Kent L, Suckling J, Bullmore E, Baron-Cohen S. Variations in the human cannabinoid receptor (CNR1) gene modulate striatal responses to happy faces. Eur J Neurosci. 2006; 23:1944–1948. [PubMed: 16623851]

Chavarria-Siles I, Contreras-Rojas J, Hare E, Walss-Bass C, Quezada P, Dassori A, Contreras S, Medina R, Ramirez M, Salazar R, Raventos H, Escamilla MA. Cannabinoid receptor 1 gene (CNR1) and susceptibility to a quantitative phenotype for hebephrenic schizophrenia. Am J Med Genet B Neuropsychiatr Genet. 2008

Chen CY, Bonham AC, Dean C, Hopp FA, Hillard CJ, Seagard JL. Retrograde release of endocannabinoids inhibits presynaptic GABA release to second-order baroreceptive neurons in NTS. Auton Neurosci. 2010; 158:44–50. [PubMed: 20580326]

Chen X, Williamson VS, An SS, Hettema JM, Aggen SH, Neale MC, Kendler KS. Cannabinoid receptor 1 gene association with nicotine dependence. Arch Gen Psychiatry. 2008; 65:816–824. [PubMed: 18606954]

Chiang KP, Gerber AL, Sipe JC, Cravatt BF. Reduced cellular expression and activity of the P129T mutant of human fatty acid amide hydrolase: evidence for a link between defects in the endocannabinoid system and problem drug use. Hum Mol Genet. 2004; 13:2113–2119. [PubMed: 15254019]

Chouker A, Kaufmann I, Kreth S, Hauer D, Feuerecker M, Thieme D, Vogeser M, Thiel M, Schelling G. Motion sickness, stress and the endocannabinoid system. PLoS One. 2010; 5:e10752. [PubMed: 20505775]

Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet. 2007; 370:1706–1713. [PubMed: 18022033]

Cote M, Matias I, Lemieux I, Petrosino S, Almeras N, Despres JP, Di Marzo V. Circulating endocannabinoid levels, abdominal adiposity and related cardiometabolic risk factors in obese men. Int J Obes (Lond). 2007; 31:692–699. [PubMed: 17224929]

Cougle JR, Bonn-Miller MO, Vujanovic AA, Zvolensky MJ, Hawkins KA. Posttraumatic stress disorder and Cannabis use in a nationally representative sample. Psychol Addict Behav. 2011; 25:554–558. [PubMed: 21480682]

Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nature reviews Neuroscience. 2008; 9:46–56.

Dawson E. Identification of a polymorphic triplet repeat marker for the brain cannabinoid receptor gene: use in linkage and association studies. Psychiat Genetics. 1995; 5:S50.

De Bank PA, Kendall DA, Alexander SP. A spectrophotometric assay for fatty acid amide hydrolase suitable for high-throughput screening. Biochem Pharmacol. 2005; 69:1187–1193. [PubMed: 15794939]

De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V. Endocannabinoid signalling in the blood of patients with schizophrenia. Lipids Health Dis. 2003; 2:5. [PubMed: 12969514]

de Mattos Viana B, Prais HA, Daker MV. Melancholic features related to rimonabant. Gen Hosp Psychiatry. 2009; 31:583–585. [PubMed: 19892218]

De Vries TJ, de Vries W, Janssen MC, Schoffelmeer AN. Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. Behav Brain Res. 2005; 161:164–168. [PubMed: 15904723]

Deadwyler SA, Hampson RE, Mu J, Whyte A, Childers S. Cannabinoids modulate voltage sensitive potassium A-current in hippocampal neurons via a cAMP-dependent process. J Pharmacol Exp Ther. 1995; 273:734–743. [PubMed: 7538581]

Dean B, Sundram S, Bradbury R, Scarr E, Copolov D. Studies on [3H]CP-55940 binding in the human central nervous system: regional specific changes in density of cannabinoid-1 receptors associated with schizophrenia and cannabis use. Neuroscience. 2001; 103:9–15. [PubMed: 11311783]

Degenhardt L, Hall W, Lynskey M. Exploring the association between cannabis use and depression. Addiction. 2003; 98:1493–1504. [PubMed: 14616175]

Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol. 1988; 34:605–613. [PubMed: 2848184]

Di Marzo V, Bisogno T, De Petrocellis L, Melck D, Orlando P, Wagner JA, Kunos G. Biosynthesis and inactivation of the endocannabinoid 2-arachidonoylglycerol in circulating and tumoral macrophages. Eur J Biochem. 1999; 264:258–267. [PubMed: 10447696]

Di Marzo V, Cote M, Matias I, Lemieux I, Arsenault BJ, Cartier A, Piscitelli F, Petrosino S, Almeras N, Despres JP. Changes in plasma endocannabinoid levels in viscerally obese men following a 1 year lifestyle modification programme and waist circumference reduction: associations with changes in metabolic risk factors. Diabetologia. 2009a; 52:213–217. [PubMed: 18972095]

Di Marzo V, Ligresti A, Cristino L. The endocannabinoid system as a link between homoeostatic and hedonic pathways involved in energy balance regulation. Int J Obes (Lond). 2009b; 33 2:S18–24. [PubMed: 19528974]

Di Marzo V, Piscitelli F, Mechoulam R. Cannabinoids and endocannabinoids in metabolic disorders with focus on diabetes. Handb Exp Pharmacol. 2011:75–104. [PubMed: 21484568]

Di S, Malcher-Lopes R, Halmos KC, Tasker JG. Nongenomic glucocorticoid inhibition via endocannabinoid release in the hypothalamus: a fast feedback mechanism. J Neurosci. 2003; 23:4850–4857. [PubMed: 12832507]

Dlugos AM, Hamidovic A, Hodgkinson CA, Goldman D, Palmer AA, de Wit H. More aroused, less fatigued: fatty acid amide hydrolase gene polymorphisms influence acute response to amphetamine. Neuropsychopharmacology. 2010; 35:613–622. [PubMed: 19890266]

Domschke K, Dannlowski U, Ohrmann P, Lawford B, Bauer J, Kugel H, Heindel W, Young R, Morris P, Arolt V, Deckert J, Suslow T, Baune BT. Cannabinoid receptor 1 (CNR1) gene: impact on antidepressant treatment response and emotion processing in major depression. Eur Neuropsychopharmacol. 2008; 18:751–759. [PubMed: 18579347]

Dowlati Y, Herrmann N, Swardfager W, Liu H, Sham L, Reim EK, Lanctot KL. A meta-analysis of cytokines in major depression. Biological psychiatry. 2010; 67:446–457. [PubMed: 20015486]

Durston S, Mulder M, Casey BJ, Ziermans T, van Engeland H. Activation in ventral prefrontal cortex is sensitive to genetic vulnerability for attention-deficit hyperactivity disorder. Biological psychiatry. 2006; 60:1062–1070. [PubMed: 16712804]

Eckardt K, Sell H, Taube A, Koenen M, Platzbecker B, Cramer A, Horrighs A, Lehtonen M, Tennagels N, Eckel J. Cannabinoid type 1 receptors in human skeletal muscle cells participate in the negative crosstalk between fat and muscle. Diabetologia. 2009; 52:664–674. [PubMed: 19089403]

Eggan SM, Hashimoto T, Lewis DA. Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. Arch Gen Psychiatry. 2008; 65:772–784. [PubMed: 18606950]

Ehlers CL, Slutske WS, Lind PA, Wilhelmsen KC. Association between single nucleotide polymorphisms in the cannabinoid receptor gene (CNR1) and impulsivity in southwest California Indians. Twin Res Hum Genet. 2007; 10:805–811. [PubMed: 18179391]

El-Talatini MR, Taylor AH, Konje JC. The relationship between plasma levels of the endocannabinoid, anandamide, sex steroids, and gonadotrophins during the menstrual cycle. Fertil Steril. 2010; 93:1989–1996. [PubMed: 19200965]

Faivre L, Cormier-Daire V, Lapierre JM, Colleaux L, Jacquemont S, Genevieve D, Saunier P, Munnich A, Turleau C, Romana S, Prieur M, De Blois MC, Vekemans M. Deletion of the SIM1 gene (6q16.2) in a patient with a Prader-Willi-like phenotype. J Med Genet. 2002; 39:594–596. [PubMed: 12161602]

Feder A, Nestler EJ, Charney DS. Psychobiology and molecular genetics of resilience. Nat Rev Neurosci. 2009; 10:446–457. [PubMed: 19455174]

Fernandez-Rodriguez CM, Romero J, Petros TJ, Bradshaw H, Gasalla JM, Gutierrez ML, Lledo JL, Santander C, Fernandez TP, Tomas E, Cacho G, Walker JM. Circulating endogenous cannabinoid anandamide and portal, systemic and renal hemodynamics in cirrhosis. Liver Int. 2004; 24:477–483. [PubMed: 15482346]

Filbey FM, Schacht JP, Myers US, Chavez RS, Hutchison KE. Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. Neuropsychopharmacology. 2010; 35:967–975. [PubMed: 20010552]

Flanagan JM, Gerber AL, Cadet JL, Beutler E, Sipe JC. The fatty acid amide hydrolase 385 A/A (P129T) variant: haplotype analysis of an ancient missense mutation and validation of risk for drug addiction. Hum Genet. 2006; 120:581–588. [PubMed: 16972078]

Fonseca BM, Correia-da-Silva G, Taylor AH, Lam PM, Marczylo TH, Konje JC, Bell SC, Teixeira NA. N-acylethanolamine levels and expression of their metabolizing enzymes during pregnancy. Endocrinology. 2010; 151:3965–3974. [PubMed: 20534733]

Fraser GA. The use of a synthetic cannabinoid in the management of treatment-resistant nightmares in posttraumatic stress disorder (PTSD). CNS Neurosci Ther. 2009; 15:84–88. [PubMed: 19228182]

Freund TF, Katona I, Piomelli D. Role of endogenous cannabinoids in synaptic signaling. Physiol Rev. 2003; 83:1017–1066. [PubMed: 12843414]

Frieling H, Albrecht H, Jedtberg S, Gozner A, Lenz B, Wilhelm J, Hillemacher T, de Zwaan M, Kornhuber J, Bleich S. Elevated cannabinoid 1 receptor mRNA is linked to eating disorder related behavior and attitudes in females with eating disorders. Psychoneuroendocrinology. 2009; 34:620–624. [PubMed: 19046818]

Fu Q, Heath AC, Bucholz KK, Nelson E, Goldberg J, Lyons MJ, True WR, Jacob T, Tsuang MT, Eisen SA. Shared genetic risk of major depression, alcohol dependence, and marijuana dependence: contribution of antisocial personality disorder in men. Arch Gen Psychiatry. 2002; 59:1125–1132. [PubMed: 12470129]

Gadzicki D, Muller-Vahl K, Stuhrmann M. A frequent polymorphism in the coding exon of the human cannabinoid receptor (CNR1) gene. Mol Cell Probes. 1999; 13:321–323. [PubMed: 10441206]

Gaoni Y, Mechoulam R. Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc. 1964; 86:1646–1647.

Gardner EL. Endocannabinoid signaling system and brain reward: emphasis on dopamine. Pharmacol Biochem Behav. 2005; 81:263–284. [PubMed: 15936806]

Gebremedhin D, Lange AR, Campbell WB, Hillard CJ, Harder DR. Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. Am J Physiol. 1999; 276:H2085–H2093. [PubMed: 10362691]

Gerard N, Pieters G, Goffin K, Bormans G, Van Laere K. Brain type 1 cannabinoid receptor availability in patients with anorexia and bulimia nervosa. Biol Psychiatry. 2011; 70:777–784. [PubMed: 21718968]

Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J, Klosterkotter J, Piomelli D. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. Neuropsychopharmacology. 2004; 29:2108–2114. [PubMed: 15354183]

Giuffrida A, Parsons LH, Kerr TM, Rodriguez de Fonseca F, Navarro M, Piomelli D. Dopamine activation of endogenous cannabinoid signaling in dorsal striatum. Nat Neurosci. 1999; 2:358–363. [PubMed: 10204543]

Giuffrida A, Rodriguez de Fonseca F, Nava F, Loubet-Lescoulie P, Piomelli D. Elevated circulating levels of anandamide after administration of the transport inhibitor, AM404. Eur J Pharmacol. 2000; 408:161–168. [PubMed: 11080522]

Glahn DC, Lovallo WR, Fox PT. Reduced amygdala activation in young adults at high risk of alcoholism: studies from the Oklahoma family health patterns project. Biol Psychiatry. 2007; 61:1306–1309. [PubMed: 17306772]

Glass M, Dragunow M, Faull RL. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. Neuroscience. 1997; 77:299–318. [PubMed: 9472392]

Golech SA, McCarron RM, Chen Y, Bembry J, Lenz F, Mechoulam R, Shohami E, Spatz M. Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors. Brain Res Mol Brain Res. 2004; 132:87–92. [PubMed: 15548432]

Gregg JM, Small EW, Moore R, Raft D, Toomey TC. Emotional response to intravenous delta9tetrahydrocannabinol during oral surgery. J Oral Surg. 1976; 34:301–313. [PubMed: 1062533]

Grogan JR, Kochar MS. Alcohol and hypertension. Arch Fam Med. 1994; 3:150–154. [PubMed: 7994437]

Hamdani N, Tabeze JP, Ramoz N, Ades J, Hamon M, Sarfati Y, Boni C, Gorwood P. The CNR1 gene as a pharmacogenetic factor for antipsychotics rather than a susceptibility gene for schizophrenia. Eur Neuropsychopharmacol. 2008; 18:34–40. [PubMed: 17669634]

Harder VS, Morral AR, Arkes J. Marijuana use and depression among adults: Testing for causal associations. Addiction. 2006; 101:1463–1472. [PubMed: 16968348]

Hariri AR, Gorka A, Hyde LW, Kimak M, Halder I, Ducci F, Ferrell RE, Goldman D, Manuck SB. Divergent effects of genetic variation in endocannabinoid signaling on human threat- and reward-related brain function. Biol Psychiatry. 2009; 66:9–16. [PubMed: 19103437]

Harkany T, Mackie K, Doherty P. Wiring and firing neuronal networks: endocannabinoids take center stage. Curr Opin Neurobiol. 2008; 18:338–345. [PubMed: 18801434]

Hartman CA, Hopfer CJ, Haberstick B, Rhee SH, Crowley TJ, Corley RP, Hewitt JK, Ehringer MA. The association between cannabinoid receptor 1 gene (CNR1) and cannabis dependence symptoms in adolescents and young adults. Drug Alcohol Depend. 2009; 104:11–16. [PubMed: 19443135]

Haughey HM, Marshall E, Schacht JP, Louis A, Hutchison KE. Marijuana withdrawal and craving: influence of the cannabinoid receptor 1 (CNR1) and fatty acid amide hydrolase (FAAH) genes. Addiction. 2008; 103:1678–1686. [PubMed: 18705688]

Heinemeyer T, Wingender E, Reuter I, Hermjakob H, Kel AE, Kel OV, Ignatieva EV, Ananko EA, Podkolodnaya OA, Kolpakov FA, Podkolodny NL, Kolchanov NA. Databases on transcriptional regulation: TRANSFAC, TRRD and COMPEL. Nucleic Acids Res. 1998; 26:362–367. [PubMed: 9399875]

Henquet C, Murray R, Linszen D, van Os J. The environment and schizophrenia: the role of cannabis use. Schizophr Bull. 2005; 31:608–612. [PubMed: 15976013]

Herman AI, Kranzler HR, Cubells JF, Gelernter J, Covault J. Association study of the CNR1 gene exon 3 alternative promoter region polymorphisms and substance dependence. Am J Med Genet B Neuropsychiatr Genet. 2006; 141B:499–503. [PubMed: 16741937]

Hill MN, Carrier EJ, McLaughlin RJ, Morrish AC, Meier SE, Hillard CJ, Gorzalka BB. Regional alterations in the endocannabinoid system in an animal model of depression: effects of concurrent antidepressant treatment. J Neurochem. 2008a; 106:2322–2336. [PubMed: 18643796]

Hill MN, Hillard CJ, Bambico FR, Patel S, Gorzalka BB, Gobbi G. The therapeutic potential of the endocannabinoid system for the development of a novel class of antidepressants. Trends Pharmacol Sci. 2009a; 30:484–493. [PubMed: 19732971]

Hill MN, McLaughlin RJ, Bingham B, Shrestha L, Lee TT, Gray JM, Hillard CJ, Gorzalka BB, Viau V. Endogenous cannabinoid signaling is essential for stress adaptation. Proc Natl Acad Sci U S A. 2010a; 107:9406–9411. [PubMed: 20439721]

Hill MN, McLaughlin RJ, Morrish AC, Viau V, Floresco SB, Hillard CJ, Gorzalka BB. Suppression of amygdalar endocannabinoid signaling by stress contributes to activation of the hypothalamic-pituitary-adrenal axis. Neuropsychopharmacology. 2009b; 34:2733–2745. [PubMed: 19710634]

Hill MN, McLaughlin RJ, Pan B, Fitzgerald ML, Roberts CJ, Lee TT, Karatsoreos IN, Mackie K, Viau V, Pickel VM, McEwen BS, Liu QS, Gorzalka BB, Hillard CJ. Recruitment of Prefrontal

Cortical Endocannabinoid Signaling by Glucocorticoids Contributes to Termination of the Stress Response. J Neurosci. 2011; 31:10506–10515. [PubMed: 21775596]

Hill MN, Miller GE, Carrier EJ, Gorzalka BB, Hillard CJ. Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. Psychoneuroendocrinology. 2009c; 34:1257–1262. [PubMed: 19394765]

Hill MN, Miller GE, Ho WS, Gorzalka BB, Hillard CJ. Serum endocannabinoid content is altered in females with depressive disorders: a preliminary report. Pharmacopsychiatry. 2008b; 41:48–53. [PubMed: 18311684]

Hill MN, Patel S, Campolongo P, Tasker JG, Wotjak CT, Bains JS. Functional interactions between stress and the endocannabinoid system: from synaptic signaling to behavioral output. J Neurosci. 2010b; 30:14980–14986. [PubMed: 21068301]

Hill MN, Patel S, Carrier EJ, Rademacher DJ, Ormerod BK, Hillard CJ, Gorzalka BB. Downregulation of endocannabinoid signaling in the hippocampus following chronic unpredictable stress. Neuropsychopharmacology. 2005; 30:508–515. [PubMed: 15525997]

Ho BC, Wassink TH, Ziebell S, Andreasen NC. Cannabinoid receptor 1 gene polymorphisms and marijuana misuse interactions on white matter and cognitive deficits in schizophrenia. Schizophr Res. 2011; 128:66–75. [PubMed: 21420833]

Ho, WSV.; Hillard, CJ. Modulators of endocannabinoid enzymic hydrolysis and membrane transport. In: Pertwee, R., editor. Cannabinoids. Vol. 168. Freiburg; Springer: 2005. p. 187-207.

Hoehe MR, Caenazzo L, Martinez MM, Hsieh WT, Modi WS, Gershon ES, Bonner TI. Genetic and physical mapping of the human cannabinoid receptor gene to chromosome 6q14-q15. New Biol. 1991; 3:880–885. [PubMed: 1931832]

Hommer DW, Bjork JM, Gilman JM. Imaging brain response to reward in addictive disorders. Ann N Y Acad Sci. 2011; 1216:50–61. [PubMed: 21272010]

Hong S, Mills PJ. Effects of an exercise challenge on mobilization and surface marker expression of monocyte subsets in individuals with normal vs. elevated blood pressure. Brain, behavior, and immunity. 2008; 22:590–599.

Hopfer CJ, Young SE, Purcell S, Crowley TJ, Stallings MC, Corley RP, Rhee SH, Smolen A, Krauter K, Hewitt JK, Ehringer MA. Cannabis receptor haplotype associated with fewer cannabis dependence symptoms in adolescents. Am J Med Genet B Neuropsychiatr Genet. 2006

Horder J, Harmer CJ, Cowen PJ, McCabe C. Reduced neural response to reward following 7 days treatment with the cannabinoid CB(1) antagonist rimonabant in healthy volunteers. Int J Neuropsychopharmacol. 2010; 13:1103–1113. [PubMed: 20426883]

Horti AG, Fan H, Kuwabara H, Hilton J, Ravert HT, Holt DP, Alexander M, Kumar A, Rahmim A, Scheffel U, Wong DF, Dannals RF. 11C-JHU75528: a radiotracer for PET imaging of CB1 cannabinoid receptors. J Nucl Med. 2006; 47:1689–1696. [PubMed: 17015906]

Howlett AC. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. Mol Pharmacol. 1985; 27:429–436. [PubMed: 2984538]

Howlett AC. Cannabinoid inhibition of adenylate cyclase: relative activity of constituents and metabolites of marihuana. Neuropharmacology. 1987; 26:507–512. [PubMed: 3601007]

Howlett AC, Fleming RM. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response of neuroblastoma cell membranes. Molec Pharmacol. 1984; 26:532–538. [PubMed: 6092901]

Hungund BL, Vinod KY, Kassir SA, Basavarajappa BS, Yalamanchili R, Cooper TB, Mann JJ, Arango V. Upregulation of CB1 receptors and agonist-stimulated [35S]GTPgammaS binding in the prefrontal cortex of depressed suicide victims. Mol Psychiatry. 2004; 9:184–190. [PubMed: 14966476]

Hutchison KE, Haughey H, Niculescu M, Schacht J, Kaiser A, Stitzel J, Horton WJ, Filbey F. The incentive salience of alcohol: translating the effects of genetic variant in CNR1. Arch Gen Psychiatry. 2008; 65:841–850. [PubMed: 18606956]

Ishac EJ, Jiang L, Lake KD, Varga K, Abood ME, Kunos G. Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. Br J Pharmacol. 1996; 118:2023–2028. [PubMed: 8864538]

Iwasaki S, Ishiguro H, Higuchi S, Onaivi ES, Arinami T. Association study between alcoholism and endocannabinoid metabolic enzyme genes encoding fatty acid amide hydrolase and

monoglyceride lipase in a Japanese population. Psychiatr Genet. 2007; 17:215–220. [PubMed: 17621164]

Izzo AA, Sharkey KA. Cannabinoids and the gut: new developments and emerging concepts. Pharmacol Ther. 2010; 126:21–38. [PubMed: 20117132]

Jean-Gilles L, Feng S, Tench CR, Chapman V, Kendall DA, Barrett DA, Constantinescu CS. Plasma endocannabinoid levels in multiple sclerosis. J Neurol Sci. 2009

Jeong WI, Osei-Hyiaman D, Park O, Liu J, Batkai S, Mukhopadhyay P, Horiguchi N, Harvey-White J, Marsicano G, Lutz B, Gao B, Kunos G. Paracrine activation of hepatic CB1 receptors by stellate cell-derived endocannabinoids mediates alcoholic fatty liver. Cell Metab. 2008; 7:227–235. [PubMed: 18316028]

Jian W, Edom R, Weng N, Zannikos P, Zhang Z, Wang H. Validation and application of an LC-MS/MS method for quantitation of three fatty acid ethanolamides as biomarkers for fatty acid hydrolase inhibition in human plasma. J Chromatogr B Analyt Technol Biomed Life Sci. 2010; 878:1687–1699.

Jiang S, Fu Y, Williams J, Wood J, Pandarinathan L, Avraham S, Makriyannis A, Avraham HK. Expression and function of cannabinoid receptors CB1 and CB2 and their cognate cannabinoid ligands in murine embryonic stem cells. PLoS ONE. 2007; 2:e641. [PubMed: 17653268]

Joosten MM, Balvers MG, Verhoeckx KC, Hendriks HF, Witkamp RF. Plasma anandamide and other N-acylethanolamines are correlated with their corresponding free fatty acid levels under both fasting and non-fasting conditions in women. Nutr Metab (Lond). 2010; 7:49. [PubMed: 20546561]

Juhasz G, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Elliott R, Anderson IM, Deakin JW. CNR1 gene is associated with high neuroticism and low agreeableness and interacts with recent negative life events to predict current depressive symptoms. Neuropsychopharmacology. 2009a; 34:1504–1513.

Juhasz G, Lazary J, Chase D, Pegg E, Downey D, Toth ZG, Stones K, Platt H, Mekli K, Payton A, Anderson IM, Deakin JF, Bagdy G. Variations in the cannabinoid receptor 1 gene predispose to migraine. Neurosci Lett. 2009b; 461:116–120. [PubMed: 19539700]

Jumpertz R, Wiesner T, Bluher M, Engeli S, Batkai S, Wirtz H, Bosse-Henck A, Stumvoll M. Circulating endocannabinoids and N-acyl-ethanolamides in patients with sleep apnea--specific role of oleoylethanolamide. Exp Clin Endocrinol Diabetes. 2010; 118:591–595. [PubMed: 20429051]

Karniol IG, Shirakawa I, Takahashi RN, Knobel E, Musty RE. Effects of delta9-tetrahydrocannabinol and cannabinol in man. Pharmacology. 1975; 13:502–512. [PubMed: 1221432]

Kaufmann I, Hauer D, Huge V, Vogeser M, Campolongo P, Chouker A, Thiel M, Schelling G. Enhanced Anandamide Plasma Levels in Patients with Complex Regional Pain Syndrome following Traumatic Injury: A Preliminary Report. Eur Surg Res. 2009a; 43:325–329. [PubMed: 19729930]

Kaufmann I, Schachtner T, Feuerecker M, Schelling G, Thiel M, Chouker A. Parabolic flight primes cytotoxic capabilities of polymorphonuclear leucocytes in humans. European journal of clinical investigation. 2009b; 39:723–728. [PubMed: 19473213]

Kaufmann I, Schelling G, Eisner C, Richter HP, Krauseneck T, Vogeser M, Hauer D, Campolongo P, Chouker A, Beyer A, Thiel M. Anandamide and neutrophil function in patients with fibromyalgia. Psychoneuroendocrinology. 2008; 33:676–685. [PubMed: 18395993]

Kearn CS, Greenberg MJ, DiCamelli R, Kurzawa K, Hillard CJ. Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. J Neurochem. 1999; 72:2379–2387. [PubMed: 10349847]

Kendler KS, Kuhn J, Prescott CA. The interrelationship of neuroticism, sex, and stressful life events in the prediction of episodes of major depression. Am J Psychiatry. 2004; 161:631–636. [PubMed: 15056508]

King JA, Barkley RA, Barrett S. Attention-deficit hyperactivity disorder and the stress response. Biological psychiatry. 1998; 44:72–74. [PubMed: 9646887]

Klein TW, Cabral GA. Cannabinoid-induced immune suppression and modulation of antigen-presenting cells. J Neuroimmune Pharmacol. 2006; 1:50–64. [PubMed: 18040791]

Klokker M, Secher NH, Madsen P, Pedersen M, Pedersen BK. Adrenergic beta 1-and beta 1 + 2-receptor blockade suppress the natural killer cell response to head-up tilt in humans. J Appl Physiol. 1997; 83:1492–1498. [PubMed: 9375311]

Klump KL, Kaye WH, Strober M. The evolving genetic foundations of eating disorders. Psychiatr Clin North Am. 2001; 24:215–225. [PubMed: 11416922]

Koethe D, Giuffrida A, Schreiber D, Hellmich M, Schultze-Lutter F, Ruhrmann S, Klosterkotter J, Piomelli D, Leweke FM. Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. Br J Psychiatry. 2009; 194:371–372. [PubMed: 19336792]

Koethe D, Llenos IC, Dulay JR, Hoyer C, Torrey EF, Leweke FM, Weis S. Expression of CB(1) cannabinoid receptor in the anterior cingulate cortex in schizophrenia, bipolar disorder, and major depression. J Neural Transm. 2007; 114:1055–1063. [PubMed: 17370106]

Koppel J, Bradshaw H, Goldberg TE, Khalili H, Marambaud P, Walker MJ, Pazos M, Gordon ML, Christen E, Davies P. Endocannabinoids in Alzheimer's disease and their impact on normative cognitive performance: a case-control and cohort study. Lipids Health Dis. 2009; 8:2. [PubMed: 19144193]

Kozlowski P, de Mezer M, Krzyzosiak WJ. Trinucleotide repeats in human genome and exome. Nucleic Acids Res. 2010; 38:4027–4039. [PubMed: 20215431]

Lam PM, Marczylo TH, Konje JC. Simultaneous measurement of three N-acylethanolamides in human bio-matrices using ultra performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2010; 398:2089–2097. [PubMed: 20835819]

Lazary J, Lazary A, Gonda X, Benko A, Molnar E, Hunyady L, Juhasz G, Bagdy G. Promoter variants of the cannabinoid receptor 1 gene (CNR1) in interaction with 5-HTTLPR affect the anxious phenotype. Am J Med Genet B Neuropsychiatr Genet. 2009

Levi A, Kohn Y, Kanyas K, Amann D, Pae CU, Hamdan A, Segman RH, Avidan N, Karni O, Korner M, Jun TY, Beckmann JS, Macciardi F, Lerer B. Fine mapping of a schizophrenia susceptibility locus at chromosome 6q23: increased evidence for linkage and reduced linkage interval. Eur J Hum Genet. 2005; 13:763–771. [PubMed: 15812564]

Leweke FM, Giuffrida A, Koethe D, Schreiber D, Nolden BM, Kranaster L, Neatby MA, Schneider M, Gerth CW, Hellmich M, Klosterkotter J, Piomelli D. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: Impact of cannabis use. Schizophr Res. 2007; 94:29–36. [PubMed: 17566707]

Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D. Elevated endogenous cannabinoids in schizophrenia. Neuroreport. 1999; 10:1665–1669. [PubMed: 10501554]

Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. Nat Rev Neurosci. 2005; 6:312–324. [PubMed: 15803162]

Li C, Bowe JE, Jones PM, Persaud SJ. Expression and function of cannabinoid receptors in mouse islets. Islets. 2010; 2:293–302. [PubMed: 21099327]

Lu AT, Ogdie MN, Jarvelin MR, Moilanen IK, Loo SK, McCracken JT, McGough JJ, Yang MH, Peltonen L, Nelson SF, Cantor RM, Smalley SL. Association of the cannabinoid receptor gene (CNR1) with ADHD and post-traumatic stress disorder. Am J Med Genet B Neuropsychiatr Genet. 2008; 147B:1488–1494. [PubMed: 18213623]

Lynskey MT, Glowinski AL, Todorov AA, Bucholz KK, Madden PA, Nelson EC, Statham DJ, Martin NG, Heath AC. Major depressive disorder, suicidal ideation, and suicide attempt in twins discordant for cannabis dependence and early-onset cannabis use. Arch Gen Psychiatry. 2004; 61:1026–1032. [PubMed: 15466676]

Lynskey MT, Heath AC, Nelson EC, Bucholz KK, Madden PA, Slutske WS, Statham DJ, Martin NG. Genetic and environmental contributions to cannabis dependence in a national young adult twin sample. Psychol Med. 2002; 32:195–207. [PubMed: 11871371]

Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol. 2005:299–325. [PubMed: 16596779]

Mackie K, Hille B. Cannabinoids inhibit N-type calcium channels in neuroblastoma-glioma cells. Proc Natl Acad Sci. 1992; 89:3825–3829. [PubMed: 1315042]

Maldonado R, Valverde O, Berrendero F. Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. 2006; 29:225–232. [PubMed: 16483675]

Mangieri RA, Hong KI, Piomelli D, Sinha R. An endocannabinoid signal associated with desire for alcohol is suppressed in recently abstinent alcoholics. Psychopharmacology (Berl). 2009; 205:63–72. [PubMed: 19343330]

Marsland AL, McCaffery JM, Muldoon MF, Manuck SB. Systemic inflammation and the metabolic syndrome among middle-aged community volunteers. Metabolism. 2010; 59:1801–1808. [PubMed: 20619428]

Martinez-Gras I, Hoenicka J, Ponce G, Rodriguez-Jimenez R, Jimenez-Arriero MA, Perez-Hernandez E, Ampuero I, Ramos-Atance JA, Palomo T, Rubio G. (AAT)n repeat in the cannabinoid receptor gene, CNR1: association with schizophrenia in a Spanish population. Eur Arch Psychiatry Clin Neurosci. 2006

Matias I, Gonthier MP, Orlando P, Martiadis V, De Petrocellis L, Cervino C, Petrosino S, Hoareau L, Festy F, Pasquali R, Roche R, Maj M, Pagotto U, Monteleone P, Di Marzo V. Regulation, function and dysregulation of endocannabinoids in models of adipose and {beta}-pancreatic cells and in obesity and hyperglycemia. J Clin Endocrinol Metab. 2006; 91:3171–3180. [PubMed: 16684820]

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990; 346:561–564. [PubMed: 2165569]

McPartland JM, Giuffrida A, King J, Skinner E, Scotter J, Musty RE. Cannabimimetic effects of osteopathic manipulative treatment. J Am Osteopath Assoc. 2005; 105:283–291. [PubMed: 16118355]

Menard C, Hein P, Paquin A, Savelson A, Yang XM, Lederfein D, Barnabe-Heider F, Mir AA, Sterneck E, Peterson AC, Johnson PF, Vinson C, Miller FD. An essential role for a MEK-C/EBP pathway during growth factor-regulated cortical neurogenesis. Neuron. 2002; 36:597–610. [PubMed: 12441050]

Moldrich G, Wenger T. Localization of the CB(1) cannabinoid receptor in the rat brain. An immunohistochemical study*. Peptides. 2000; 21:1735–1742. [PubMed: 11090929]

Monteleone P, Bifulco M, Di Filippo C, Gazzerro P, Canestrelli B, Monteleone F, Proto MC, Di Genio M, Grimaldi C, Maj M. Association of CNR1 and FAAH endocannabinoid gene polymorphisms with anorexia nervosa and bulimia nervosa: evidence for synergistic effects. Genes Brain Behav. 2009; 8:728–732. [PubMed: 19659925]

Monteleone P, Bifulco M, Maina G, Tortorella A, Gazzerro P, Proto MC, Di Filippo C, Monteleone F, Canestrelli B, Buonerba G, Bogetto F, Maj M. Investigation of CNR1 and FAAH endocannabinoid gene polymorphisms in bipolar disorder and major depression. Pharmacol Res. 2010a; 61:400–404. [PubMed: 20080186]

Monteleone P, Matias I, Martiadis V, De Petrocellis L, Maj M, Di Marzo V. Blood Levels of the Endocannabinoid Anandamide are Increased in Anorexia Nervosa and in Binge-Eating Disorder, but not in Bulimia Nervosa. Neuropsychopharmacology. 2005; 30:1216–1221. [PubMed: 15841111]

Monteleone P, Milano W, Petrella C, Canestrelli B, Maj M. Endocannabinoid Pro129Thr FAAH functional polymorphism but not 1359G/A CNR1 polymorphism is associated with antipsychotic-induced weight gain. J Clin Psychopharmacol. 2010b; 30:441–445. [PubMed: 20631561]

Moore TH, Zammit S, Lingford-Hughes A, Barnes TR, Jones PB, Burke M, Lewis G. Cannabis use and risk of psychotic or affective mental health outcomes: a systematic review. Lancet. 2007; 370:319–328. [PubMed: 17662880]

Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Ohtani K, Kishimoto M, Morio A, Imamura T, Sakai A, Inada T, Harano M, Komiyama T, Yamada M, Sekine Y, Iwata N, Iyo M, Sora I, Ozaki N, Kuroda S. A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia. Neurosci Lett. 2005; 376:182–187. [PubMed: 15721218]

Muller TD, Reichwald K, Bronner G, Kirschner J, Nguyen TT, Scherag A, Herzog W, Herpertz-Dahlmann B, Lichtner P, Meitinger T, Platzer M, Schafer H, Hebebrand J, Hinney A. Lack of association of genetic variants in genes of the endocannabinoid system with anorexia nervosa. Child Adolesc Psychiatry Ment Health. 2008; 2:33. [PubMed: 19014633]

Newell KA, Deng C, Huang XF. Increased cannabinoid receptor density in the posterior cingulate cortex in schizophrenia. Exp Brain Res. 2006; 172:556–560. [PubMed: 16710682]

Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? Prog Lipid Res. 2011; 50:193–211. [PubMed: 21295074]

Patel, S.; Hillard, CJ. Endocannabinoids as modulators of synaptic signaling. In: Reggio, PH., editor. The Cannabinoid Receptors. New York: Humana Press; 2009. p. 281-308.

Patton GC, Coffey C, Carlin JB, Degenhardt L, Lynskey M, Hall W. Cannabis use and mental health in young people: cohort study. BMJ. 2002; 325:1195–1198. [PubMed: 12446533]

Picton TW. The P300 wave of the human event-related potential. J Clin Neurophysiol. 1992; 9:456–479. [PubMed: 1464675]

Plessen KJ, Bansal R, Zhu H, Whiteman R, Amat J, Quackenbush GA, Martin L, Durkin K, Blair C, Royal J, Hugdahl K, Peterson BS. Hippocampus and amygdala morphology in attention-deficit/hyperactivity disorder. Archives of general psychiatry. 2006; 63:795–807. [PubMed: 16818869]

Potvin S, Kouassi E, Lipp O, Bouchard RH, Roy MA, Demers MF, Gendron A, Astarita G, Piomelli D, Stip E. Endogenous cannabinoids in patients with schizophrenia and substance use disorder during quetiapine therapy. J Psychopharmacol. 2008; 22:262–269. [PubMed: 18308802]

Proudnikov D, Kroslak T, Sipe JC, Randesi M, Li D, Hamon S, Ho A, Ott J, Kreek MJ. Association of polymorphisms of the cannabinoid receptor (CNR1) and fatty acid amide hydrolase (FAAH) genes with heroin addiction: impact of long repeats of CNR1. Pharmacogenomics J. 2010; 10:232–242. [PubMed: 20010914]

Quercioli A, Pataky Z, Vincenti G, Makoundou V, Di Marzo V, Montecucco F, Carballo S, Thomas A, Staub C, Steffens S, Seimbille Y, Golay A, Ratib O, Harsch E, Mach F, Schindler TH. Elevated endocannabinoid plasma levels are associated with coronary circulatory dysfunction in obesity. Eur Heart J. 2011; 32:1369–1378. [PubMed: 21303779]

Reilly D, Didcott P, Swift W, Hall W. Long-term cannabis use: characteristics of users in an Australian rural area. Addiction. 1998; 93:837–846. [PubMed: 9744119]

Rey JM, Tennant CC. Cannabis and mental health. BMJ. 2002; 325:1183–1184. [PubMed: 12446510]

Richardson D, Pearson RG, Kurian N, Latif ML, Garle MJ, Barrett DA, Kendall DA, Scammell BE, Reeve AJ, Chapman V. Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. Arthritis Res Ther. 2008; 10:R43. [PubMed: 18416822]

Rinaldi-Carmona M, Calandra B, Shire D, Bouaboula M, Oustric D, Barth F, Casellas P, Ferrara P, Le Fur G. Characterization of two cloned human CB1 cannabinoid receptor isoforms. J Pharmacol Exp Ther. 1996; 278:871–878. [PubMed: 8768742]

Roche M, Diamond M, Kelly JP, Finn DP. In vivo modulation of LPS-induced alterations in brain and peripheral cytokines and HPA axis activity by cannabinoids. Journal of neuroimmunology. 2006; 181:57–67. [PubMed: 17011047]

Roloff AM, Thayer SA. Modulation of excitatory synaptic transmission by Delta9-tetrahydrocannabinol switches from agonist to antagonist depending on firing rate. Mol Pharmacol. 2009; 75:892–900. [PubMed: 19118122]

Roser P, Juckel G, Rentzsch J, Nadulski T, Gallinat J, Stadelmann AM. Effects of acute oral Delta(9)-tetrahydrocannabinol and standardized cannabis extract on the auditory P300 event-related potential in healthy volunteers. Eur Neuropsychopharmacol. 2008; 18:569–577. [PubMed: 18544469]

Ruiz-Contreras AE, Delgado-Herrera M, Garcia-Vaca PA, Almeida-Rosas GA, Soria-Rodriguez G, Soriano-Bautista A, Cadena-Valencia J, Bazan-Frias JR, Gomez-Lopez N, Espejel-Nunez A, Vadillo-Ortega F, Carrillo-Sanchez K, Verdin-Reyes JC, March-Mifsut S, Mendez-Diaz M, Prospero-Garcia O. Involvement of the AATn polymorphism of the CNR1 gene in the efficiency of procedural learning in humans. Neurosci Lett. 2011; 494:202–206. [PubMed: 21396980]

Ryberg E, Vu HK, Larsson N, Groblewski T, Hjorth S, Elebring T, Sjogren S, Greasley PJ. Identification and characterisation of a novel splice variant of the human CB1 receptor. FEBS Lett. 2005; 579:259–264. [PubMed: 15620723]

Salio C, Doly S, Fischer J, Franzoni MF, Conrath M. Neuronal and astrocytic localization of the cannabinoid receptor-1 in the dorsal horn of the rat spinal cord. Neurosci Lett. 2002; 329:13–16. [PubMed: 12161251]

Schacht JP, Selling RE, Hutchison KE. Intermediate cannabis dependence phenotypes and the FAAH C385A variant: an exploratory analysis. Psychopharmacology (Berl). 2009; 203:511–517. [PubMed: 19002671]

Scheen AJ, Finer N, Hollander P, Jensen MD, Van Gaal LF. Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. Lancet. 2006; 368:1660–1672. [PubMed: 17098084]

Schmidt A, Brune K, Hinz B. Determination of the endocannabinoid anandamide in human plasma by high-performance liquid chromatography. Biomed Chromatogr. 2006; 20:336–342. [PubMed: 16189813]

Schofield D, Tennant C, Nash L, Degenhardt L, Cornish A, Hobbs C, Brennan G. Reasons for cannabis use in psychosis. Aust N Z J Psychiatry. 2006; 40:570–574. [PubMed: 16756582]

Schroeder C, Batkai S, Engeli S, Tank J, Diedrich A, Luft FC, Jordan J. Circulating endocannabinoid concentrations during orthostatic stress. Clin Auton Res. 2009; 19:343–346. [PubMed: 19756829]

Seagard JL, Dean C, Patel S, Rademacher DJ, Hopp FA, Schmeling WT, Hillard CJ. Anandamide content and interaction of endocannabinoid/GABA modulatory effects in the NTS on baroreflex-evoked sympathoinhibition. Am J Physiol Heart Circ Physiol. 2004; 286:H992–H1000. [PubMed: 14615281]

Seagard JL, Hopp FA, Hillard CJ, Dean C. Effects of endocannabinoids on discharge of baroreceptive NTS neurons. Neurosci Lett. 2005; 381:334–339. [PubMed: 15896495]

Seifert J, Ossege S, Emrich HM, Schneider U, Stuhrmann M. No association of CNR1 gene variations with susceptibility to schizophrenia. Neurosci Lett. 2007; 426:29–33. [PubMed: 17881126]

Shire D, Carillon C, Kaghad M, Calandra B, Rinaldi-Carmona M, Le Fur G, Caput D, Ferrara P. An amino-terminal variant of the central cannabinoid receptor resulting from alternative splicing. J Biol Chem. 1995; 270:3726–3731. published erratum appears in J Biol Chem 1996 Dec 27;271(52):33706. [PubMed: 7876112]

Siegfried Z, Kanyas K, Latzer Y, Karni O, Bloch M, Lerer B, Berry EM. Association study of cannabinoid receptor gene (CNR1) alleles and anorexia nervosa: differences between restricting and binging/purging subtypes. Am J Med Genet. 2004; 125B:126–130. [PubMed: 14755457]

Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci U S A. 2002; 99:8394–8399. [PubMed: 12060782]

Sipe JC, Scott TM, Murray S, Harismendy O, Simon GM, Cravatt BF, Waalen J. Biomarkers of endocannabinoid system activation in severe obesity. PLoS One. 2010; 5:e8792. [PubMed: 20098695]

Solinas M, Goldberg SR, Piomelli D. The endocannabinoid system in brain reward processes. Br J Pharmacol. 2008; 154:369–383. [PubMed: 18414385]

Spoto B, Fezza F, Parlongo G, Battista N, Sgro E, Gasperi V, Zoccali C, Maccarrone M. Human adipose tissue binds and metabolizes the endocannabinoids anandamide and 2-arachidonoylglycerol. Biochimie. 2006

Stadelmann AM, Juckel G, Arning L, Gallinat J, Epplen JT, Roser P. Association between a cannabinoid receptor gene (CNR1) polymorphism and cannabinoid-induced alterations of the auditory event-related P300 potential. Neurosci Lett. 2011; 496:60–64. [PubMed: 21513772]

Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. Brain, behavior, and immunity. 2007; 21:901–912.

Szuster RR, Pontius EB, Campos PE. Marijuana sensitivity and panic anxiety. J Clin Psychiatry. 1988; 49:427–429. [PubMed: 3182732]

Tam J, Liu J, Mukhopadhyay B, Cinar R, Godlewski G, Kunos G. Endocannabinoids in liver disease. Hepatology. 2011; 53:346–355. [PubMed: 21254182]

Thomas H. Psychiatric symptoms in cannabis users. Br J Psychiatry. 1993; 163:141–149. [PubMed: 8075903]

Tiwari AK, Zai CC, Likhodi O, Lisker A, Singh D, Souza RP, Batra P, Zaidi SH, Chen S, Liu F, Puls I, Meltzer HY, Lieberman JA, Kennedy JL, Muller DJ. A common polymorphism in the cannabinoid receptor 1 (CNR1) gene is associated with antipsychotic-induced weight gain in Schizophrenia. Neuropsychopharmacology. 2010; 35:1315–1324. [PubMed: 20107430]

Tsai SJ, Wang YC, Hong CJ. Association study of a cannabinoid receptor gene (CNR1) polymorphism and schizophrenia. Psychiatr Genet. 2000; 10:149–151. [PubMed: 11204352]

Tsai SJ, Wang YC, Hong CJ. Association study between cannabinoid receptor gene (CNR1) and pathogenesis and psychotic symptoms of mood disorders. Am J Med Genet. 2001; 105:219–221. [PubMed: 11353438]

Tyndale RF, Payne JI, Gerber AL, Sipe JC. The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: Studies of drug use and dependence in caucasians. Am J Med Genet B Neuropsychiatr Genet. 2007; 144:660–666. [PubMed: 17290447]

Ujike H, Morita Y. New perspectives in the studies on endocannabinoid and cannabis: cannabinoid receptors and schizophrenia. J Pharmacol Sci. 2004; 96:376–381. [PubMed: 15613777]

Ujike H, Takaki M, Nakata K, Tanaka Y, Takeda T, Kodama M, Fujiwara Y, Sakai A, Kuroda S. CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. Mol Psychiatry. 2002; 7:515–518. [PubMed: 12082570]

Van Gaal L, Pi-Sunyer X, Despres JP, 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. 2008; 31 2:S229–240. [PubMed: 18227491]

Van Houdenhove B, Luyten P. Stress, depression and fibromyalgia. Acta Neurol Belg. 2006; 106:149–156. [PubMed: 17323832]

Varga K, Wagner JA, Bridgen DT, Kunos G. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. Faseb J. 1998; 12:1035–1044. [PubMed: 9707176]

Vogeser M, Hauer D, Christina Azad S, Huber E, Storr M, Schelling G. Release of anandamide from blood cells. Clin Chem Lab Med. 2006; 44:488–491. [PubMed: 16599845]

Wang Y, Liu Y, Ito Y, Hashiguchi T, Kitajima I, Yamakuchi M, Shimizu H, Matsuo S, Imaizumi H, Maruyama I. Simultaneous measurement of anandamide and 2-arachidonoylglycerol by polymyxin b-selective adsorption and subsequent high-performance liquid chromatography analysis: increase in endogenous cannabinoids in the sera of patients with endotoxic shock. Anal Biochem. 2001; 294:73–82. [PubMed: 11412008]

Weis F, Beiras-Fernandez A, Hauer D, Hornuss C, Sodian R, Kreth S, Briegel J, Schelling G. Effect of anaesthesia and cardiopulmonary bypass on blood endocannabinoid concentrations during cardiac surgery. Br J Anaesth. 2010a; 105:139–144. [PubMed: 20525978]

Weis F, Beiras-Fernandez A, Sodian R, Kaczmarek I, Reichart B, Beiras A, Schelling G, Kreth S. Substantially altered expression pattern of cannabinoid receptor 2 and activated endocannabinoid system in patients with severe heart failure. J Mol Cell Cardiol. 2010b; 48:1187–1193. [PubMed: 19931541]

Weissbecker I, Floyd A, Dedert E, Salmon P, Sephton S. Childhood trauma and diurnal cortisol disruption in fibromyalgia syndrome. Psychoneuroendocrinology. 2006; 31:312–324. [PubMed: 16274933]

Westerbacka J, Kotronen A, Fielding BA, Wahren J, Hodson L, Perttila J, Seppanen-Laakso T, Suortti T, Arola J, Hultcrantz R, Castillo S, Olkkonen VM, Frayn KN, Oresic M, Yki-Jarvinen H. Splanchnic balance of free fatty acids, endocannabinoids, and lipids in subjects with nonalcoholic fatty liver disease. Gastroenterology. 2010; 139:1961–1971. e1961. [PubMed: 20600015]

Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, Ye W, Dannals RF, Ravert HT, Nandi A, Rahmim A, Ming JE, Grachev I, Roy C, Cascella N. Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. Neuroimage. 2010; 52:1505–1513. [PubMed: 20406692]

Yamakawa K, Matsunaga M, Isowa T, Kimura K, Kasugai K, Yoneda M, Kaneko H, Ohira H. Transient responses of inflammatory cytokines in acute stress. Biol Psychol. 2009; 82:25–32. [PubMed: 19446599]

Yang C, Boucher F, Tremblay A, Michaud JL. Regulatory interaction between arylhydrocarbon receptor and SIM1, two basic helix-loop-helix PAS proteins involved in the control of food intake. J Biol Chem. 2004; 279:9306–9312. [PubMed: 14660629]

Yasuo S, Unfried C, Kettner M, Geisslinger G, Korf HW. Localization of an endocannabinoid system in the hypophysial pars tuberalis and pars distalis of man. Cell Tissue Res. 2010; 342:273–281. [PubMed: 20957495]

Yndestad A, Damas JK, Oie E, Ueland T, Gullestad L, Aukrust P. Systemic inflammation in heart failure--the whys and wherefores. Heart Fail Rev. 2006; 11:83–92. [PubMed: 16819581]

Zammit S, Allebeck P, Andreasson S, Lundberg I, Lewis G. Self reported cannabis use as a risk factor for schizophrenia in Swedish conscripts of 1969: historical cohort study. Bmj. 2002; 325:1199. [PubMed: 12446534]

Zammit S, Spurlock G, Williams H, Norton N, Williams N, O'Donovan MC, Owen MJ. Genotype effects of CHRNA7, CNR1 and COMT in schizophrenia: interactions with tobacco and cannabis use. Br J Psychiatry. 2007; 191:402–407. [PubMed: 17978319]

Zavitsanou K, Garrick T, Huang XF. Selective antagonist [3H]SR141716A binding to cannabinoid CB1 receptors is increased in the anterior cingulate cortex in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2004; 28:355–360. [PubMed: 14751433]

Zhang PW, Ishiguro H, Ohtsuki T, Hess J, Carillo F, Walther D, Onaivi ES, Arinami T, Uhl GR. Human cannabinoid receptor 1: 5′candidate regulatory regions, polymorphisms, haplotypes and association with polysubstance abuse. Mol Psychiatry. 2004; 9:916–931. [PubMed: 15289816]

Ziegler CG, Mohn C, Lamounier-Zepter V, Rettori V, Bornstein SR, Krug AW, Ehrhart-Bornstein M. Expression and function of endocannabinoid receptors in the human adrenal cortex. Horm Metab Res. 2010a; 42:88–92. [PubMed: 19862666]

Ziegler CG, Mohn C, Lamounier-Zepter V, Rettori V, Bornstein SR, Krug AW, Ehrhart-Bornstein M. Expression and Function of Endocannabinoid Receptors in the Human Adrenal Cortex. Horm Metab Res. 2010b; 42:88–92. [PubMed: 19862666]

Zuo L, Kranzler HR, Luo X, Covault J, Gelernter J. CNR1 variation modulates risk for drug and alcohol dependence. Biol Psychiatry. 2007; 62:616–626. [PubMed: 17509535]

Zuo L, Kranzler HR, Luo X, Yang BZ, Weiss R, Brady K, Poling J, Farrer L, Gelernter J. Interaction between two independent CNR1 variants increases risk for cocaine dependence in European Americans: a replication study in family-based sample and population-based sample. Neuropsychopharmacology. 2009; 34:1504–1513. [PubMed: 19052543]

Highlights

- The polymorphisms in CNR1 and FAAH are described
- Hypotheses regarding the source and targets of circulating endocannabinoids are presented
- Associations between CNR1 and FAAH polymorphisms and psychiatric disorders are reviewed

Table 1

Haplotype blocks of SNPs in *CNR1*

Haplotype block	Citation
rs6454674; rs806380; rs806377;rs1049353	(Hopfer et al., 2006)
rs6454674; rs806379; rs806377	(Zuo et al., 2009)
rs806379; rs1535255; rs2023239	(Zhang et al., 2004, Juhasz et al., 2009a, Lazary et al., 2009)
rs806368; rs1049353	(Zuo et al., 2009)
rs806369; rs1049353; rs4707436; rs12720071; rs806368	(Juhasz et al., 2009a)

Table 2
Plasma and serum concentrations of AEA and 2-AG in healthy, normal subjects

Plasma		
AEA concentration (nM)	**2-AG concentration (nM)**	**Citation**
13	5.6	(Sipe et al., 2010)
9.0	not reported	(Mangieri et al., 2009)
5.9	not reported	(Joosten et al., 2010)
5.1	1.6	(Caraceni et al., 2010a)
4.3	not reported	(Kaufmann et al., 2008)
4.1	not reported	(Kaufmann et al., 2009a)
3.7	not reported	(Vogeser et al., 2006)
2.9	1.0	(Cote et al., 2007)
2.6	not reported	(De Marchi et al., 2003)
2.1	22.5	(Potvin et al., 2008)
2.0	7.9	(Schroeder et al., 2009)
1.9	11.0	(Koppel et al., 2009)
2.1	19.5	(Jumpertz et al., 2010)
1.6	5.3	(Quercioli et al., 2011)
0.8	20.0	(Jean-Gilles et al., 2009)
0.7-1.0	not reported	(El-Talatini et al., 2010)
0.7	18.7	(Balvers et al., 2009)
0.7	not reported	(Lam et al., 2010)
0.36	not reported	(Fernandez-Rodriguez et al., 2004)
Serum		
5	10	(Wang et al., 2001)
3.0	1.0	(McPartland et al., 2005)
2.6	400	(Hill et al., 2009c)
1.0	not reported	(Lam et al., 2010)
0.7	20	(Hill et al., 2008b)
0.4	not reported	(Giuffrida et al., 2004)