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Changes of endocannabinoid plasma levels following type I trauma: A prospective pilot study

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1. Background

1.1 History of endocannabinoid research

Cannabis sativa, being botanically a member of the hemp family, has been cultivated for more than 5000 years both to obtain fibers for manufacturing of textiles and to provide a variety of extracts for medicinal and recreational use. Its stimluating and healing effects for conditions with fever, malaria, obstipation and rheumatic complaints are reported in ancient Chinese and ancient Arabic scripts (Hauer 2008). Since 300 A.D. it was observed that Cannabis can stimulate hunger and increase appetite. The psychomimetic activity of overdoses was described by the Chinese as “seeing the devil”, while the psychic activity was interpreted as a gift of god and the plant was considered holy in the Indo-European culture (Hiley et al. 2004). Cannabinoid research was largely neglected at the beginning of the 20th century, partly because of the political antimarijuana attitude, which officially started in the United States with the Harrison Act in 1914, leading to full prohibition 20 years later. From the past to the present, marijuana and other psychoactive derivatives of Cannabis sativa represent the most widely illegal drug consumed in the Western world. However, despite the social problems related to the abuse of these substances, scientific and social communities have recently started to get again aware of the therapeutic potentials of cannabinoids and of new synthetic compounds interfering with the endogenous cannabinoid system (Cota et al. 2003). In 1964 Tetrahydrocannabinol (THC), the active ingredient of the cannabis plant and of marijuana, could be identified, but only at the beginning of the 1990s the receptor system was found, via which cannabis acts. Thus it also became possible to identify the endogenous ligands of this receptor system as well as to explore the endogenous system. These cannabinoids, which are built by the body itself, were named “endocannabinoids”. The first identified endocannabinoid arachidonylethanolamide was isolated from porcine brain in 1992 (Devane et al. 1992) and labelled “anandamide”. This name was derived from the Sanskrit term “ananda”, which means internal bliss (Cota et al. 2003). Up to the present besides several synthetic cannabinoids and natural derivatives of the hemp plant also some other endogenous ligands were described, from which 2-arachidonoylglycerol is the most known and so far most explored endocannabinoid. The importance of this system is also underlined by the finding of a high degree of evolutionary conservation across species, emphasizing the fundamental physiological role played by cannabinoids in brain function (De Petrocellis et al. 1999).

1.2 Current state of knowledge of the endocannabinoid system

1.2.1 Roles of the endocannabinoid system

The endogenous cannabinoid system was mainly investigated in animals up to date. A myriad of physiological processes is being influenced by this system. The system, comprising specific receptors, endogenous ligands and degradation enzymes for the ligands, seems to act as a neuromodulatory system, influencing the activity of other neurotransmitter systems (Di Marzo et al. 1998).

To get a first glimpse, cannabinoids are involved among others in antinociception, in locomotion, in inhibition of short-term memory (Lutz 2002), emotionality (Hill et al. 2009), cognition, feeding, regulation of neurotransmitter release, energy homeostasis and control of immune cell function (Graham et al. 2009).

The endocannabinoid system has emerged as one of the most important facilitators of stress adaptation in the body (Finn 2010). Cannabinoid 1 (CB1) receptor knockout mice display an increased susceptibility to the anhedonic effects of chronic stress (Hill et al. 2009). Under conditions of acute stress, the endocannabinoid system tonically constrains activation of the HPA axis. During repeated exposure to aversive stimuli, the endocannabinoid system up-regulates in limbic structures, resulting in neural activity in stress circuits, which could contribute to stress habituation (Gorzalka et al. 2008). The endocannabinoid-induced modulation of stress-related behaviors appears to be mediated, at least partly, through the regulation of the serotoninergic system (Haj-Dahmane et al. 2011).

The cannabinoid system also plays an important role in the regulation of hormone secretion (Murphy et al. 1998). For instance, expression of the cannabinoid receptors and the synthesis of endocannabinoids in human pituitary cells, the ability of cannabinoids to inhibit prolactin and growth hormone secretion and to increase adrenocorticotropin (ACTH) secretion were described (Pagotto et al. 2001). The ability to modulate the hypothalamus–pituitary–adrenal (HPA) axis and the involvement in the stress-response are supported by a number of studies in which cannabinoid agonists were shown to produce anxiolytic effects in a dosedependent manner (Navarro et al. 1997).

By enhancing serotonergic and noradrenergic transmission, increasing cellular plasticity and neurotrophin expression within the hippocampus, and dampening activity within the neuroendocrine stress axis, endocannabinoids exert antidepressant effects as well (Hill et al. 2009). It seems also to be relevant for post-traumatic stress disorder (Varvel et al. 2007) by

accelerating the extinction of emotionally aversive memories (Ligresti et al. 2009), and for schizophrenia (Suarez et al. 2010).

Cannabinoids seem to exert neuroprotective roles as well. In fact, endocannabinoids have been shown to promote neuronal maintenance and function (Hwang et al. 2010). There is growing evidence that the cannabinoid system may be important in the progression of neurodegenerative conditions including among others multiple sclerosis, epilepsy, Parkinson´s disease and Alzheimer´s disease(Graham et al. 2009).

Additionally, the hippocampus has been reported to be a relevant neural substrate for cannabinoid-nicotine interactions (Viveros et al. 2007) and the endocannabinoid system is assumed to play a role in the general phenomenon of addiction (Lopez-Moreno et al. 2008).

Furthermore, the cannabinoid system is able to modulate central regulation of body weight and adipose tissue function (Tonstad 2006). Immune and inflammatory responses and various further physiological functions, such as cardiovascular (Mendizabal et al. 2003), respiratory (Pfitzer et al. 2004), reproductive (Wenger et al. 2001), ocular function (Stumpff et al. 2005), anti-emesis or sleep (Hauer 2008) are being modified by the endocannabinoid system, too. Concerning conditions such as arthritis and osteoporosis, cannabinoids may help reduce cartilage resorption and joint destruction (Mbvundula et al. 2006) as well as maintaining bone mass and promoting bone growth (Bab et al. 2008). Other interesting properties of cannabinoids are represented by the antitumoral activity, for instance in breast cancer (Qamri et al. 2009) and in colon cancer (Cianchi et al. 2008).

This overview demonstrates the wide range of physiological and pathophysiological processes in which the endocannabinoid system is involved, and hence the numerous medical fields for which it is highly interesting.

1.2.2 Endogenous cannabinoids

The most known and up to date best understood endocannabinoids are N - arachidonoylethanolamine (anandamide) and 2-Arachidonoylglycerol (2-AG). There is evidence of other endocannabinoids: noladin (Njie et al. 2006), virodhamine (Porter et al. 2002), oleamide (Hiley et al. 2007) and n-arachidonoyl-dopamine (Marinelli et al. 2007). The focus in the following will lie on 2-AG and anandamide, as these were the endocannabinoids investigated in our study.

Anandamide and 2-AG are highly expressed in the brain and are also found in a number of peripheral tissues including heart, liver, kidney (Kondo et al. 1998), testis and blood.

Endocannabinoids are fatty acid derivatives (Kamprath et al. 2009) (Figure 1).

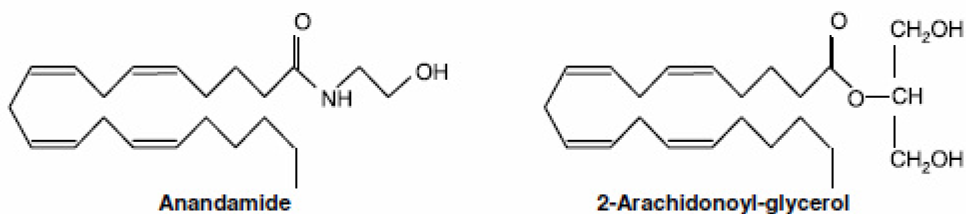


Figure 1: Chemical structures of anandamide and 2-AG (Cota et al. 2003)

In contrast to classical neurotransmitters, endocannabinoids are not stored in the interior of synaptic vesicles, because of the high lipophilicity of these ligands (Di Marzo et al. 1998). Anandamide and 2-AG are synthesized from lipid precursors on demand (McVey et al. 2003) and released from postsynaptic neurons. They serve as retrograde messengers at central synapses (Hashimotodani et al. 2007). The released endocannabinoids travel backward across the synapse (Wilson et al. 2001), activate presynaptic specific G_i-protein-coupled cannabinoid receptors (Di Marzo et al. 2002) on presynaptic terminals and modulate presynaptic functions. Retrograde endocannabinoid signalling is crucial for certain forms of short-term and long-term synaptic plasticity at excitatory or inhibitory synapses in many brain regions (Hashimotodani et al. 2007). Endocannabinoids act like neurotransmitters – their release from neurons and nucleus containing blood cells is depolarization-mediated and they are rapidly removed from the extracellular space by means of an active membrane transport system (Hauer 2008).

Anandamide binds both to CB1 receptor and CB2 receptor (Felder et al. 1995), with a higher affinity to CB1 receptor and is present at highest concentration in hippocampus, cortex, thalamus and cerebellum of different species including humans (Felder et al. 1996).

The synthesis of anandamide takes place as follows: A phosphodiesterase, named phospholipase D, which is being activated by calcium ions, synthezises anandamide by the hydrolysis of a membrane phospholipid (N-arachidonoyl phosphatidyl ethanolamine=NArPE) (Di Marzo et al. 1994; Okamoto et al. 2004). This takes place intracellularly. The transport of the eminently lipophile anandamide to the extracellular space happens via a specific, largely unknown, membrane transport system on the principle of facilitated diffusion, from where it binds to the cannabinoid receptors and unfolds its effects (Figure 2) (Freund et al. 2003). Degradation of anandamide occurs in a two step process that includes transport into the cell by a specific transporter, followed by enzymatic hydrolysis into arachidonic acid and

ethanolamine by cytoplasmatic fatty acid amide hydrolase (FAAH) (Cravatt et al. 1996) in neurons and astrocytes. Therefore, anandamide levels can be eliminated rapidly (Wegener et al. 2009). The degradation products, arachidonic acid (AA) and ethanolamine, are re-esterified to membrane phospholipids (Cota et al. 2003).

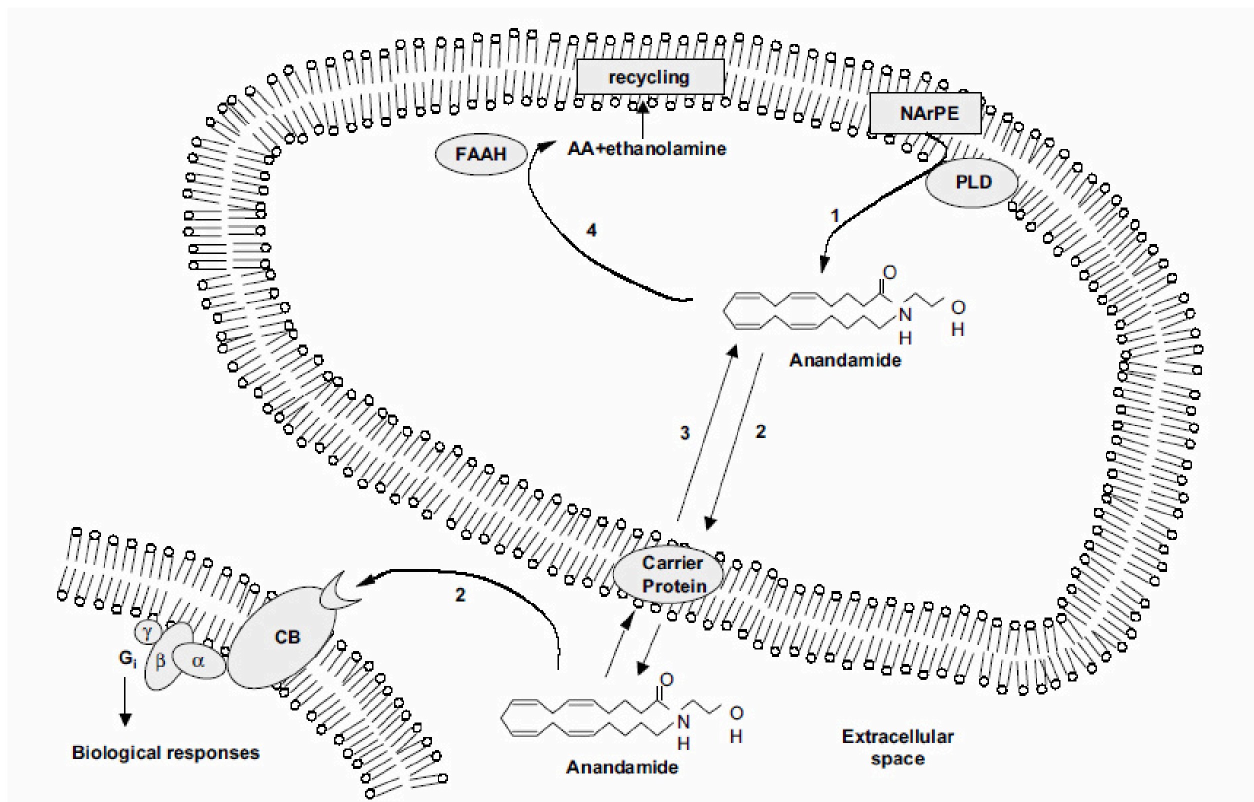


Figure 2: Biosynthesis, release and inactivation of anandamide (Cota et al. 2003)
Anandamide is synthesized from the phospholipase D - catalyzed hydrolysis of the membrane phospholipid precursor N-arachidonoyl phosphatidyl ethanolamine (NArPE) in neurons **(1)**. After synthesis, anandamide is released from neurons through facilitated transport and binds to cannabinoid receptors (CB), leading to biological responses **(2)**. The same carrier involved in the release, probably also mediates anandamide reuptake by neurons **(3)**. After reuptake, the endocannabinoid is degraded through the action of the membrane-bound enzyme fatty acid amide hydrolase (FAAH) **(4)**. Arachidonic acid (AA) and ethanolamine, produced from anandamide hydrolysis, are rapidly re-esterified to membrane phospholipids.

2-AG represents the most abundant endocannabinoid in the brain (Stella et al. 1997). Identified in canine gut in a search for endogenous ligands selective for CB2 receptors, 2-AG displays a lower affinity for CB1 receptor than for CB2 receptor.

2-AG is generated from diacylglycerol (DAG) by DAG lipase. DAG seems to be generated by sequential hydrolyses of inositol phospholipids (Ueda et al. 2011). As well as anandamide, 2-AG can be metabolised by FAAH, nevertheless the hydrolysis of 2-AG via the enzyme monoacylglycerol lipase (MAGL) is more crucial in the brain (Dinh et al. 2002). It was found that approximately 85% of brain 2-AG hydrolase activity can be attributed to MAGL, and the

remaining 15% is mainly catalyzed by two previously unknown enzymes, ABHD6 and ABHD12 (Blankman et al. 2007).

1.2.3 Cannabinoid receptors

The endocannabinoid actions in the human body occur mainly through activation of an own receptor system, the cannabinoid receptors CB1 and CB2, but also through activation of other receptors, for instance the transient receptor potential vanilloid 1 (TRPV1) receptors (Howlett et al. 2000; Begg et al. 2005) and the recently de-“orphanized” receptor GPR55, which was suggested to be a novel cannabinoid receptor (Ryberg et al. 2007) a few years ago, and recently proven to modulate CB2 receptor mediated responses in human neutrophiles (Balenga et al. 2011). Additionally, the endocannabinoid system also interacts with NMDA-receptors (Hampson et al. 2011) and dopamine-receptors (Pandolfo et al. 2011). The focus in this chapter will be on the CB1 and CB2 receptor system.
In 1990, the first cannabinoid receptor (CB1) was cloned (Matsuda et al. 1990), followed 3 years later by the characterization of a second cannabinoid receptor (CB2) (Munro et al. 1993).

CB1 receptors are mainly expressed in the brain, whereas CB2 receptors are found predominantly in the periphery primarily on cells of the immune system (Klein et al. 2003). More precisely, high densities of CB1 receptors are found within frontal regions of the cerebral cortex, basal ganglia, cerebellum, hypothalamus, hippocampus and the amygdala (Campolongo et al. 2009). They were also detected in reward circuits like nucleus accumbens and ventral tegmental area (Wegener et al. 2009). But CB1 receptors also exist in peripheral tissues: reproductive organs, heart, lung, gastrointestinum, ganglion of nervus sympathicus, bladder, adrenal (Pertwee 1997; Pertwee et al. 2002) and nociceptors in the skin (Agarwal et al. 2007).
In contrast, CB2 receptors are situated predominantly on the B-cells and the natural killer cells of the immune system (Iversen 2003), where they modulate apoptosis and immunologic processes (Kaufmann et al. 2008).
CB1 and CB2 receptors belong to the family of G-protein coupled receptors (GPCRs) (Devane et al. 1994) and are located at presynaptic terminals of neurons. CB1 receptors are abundant on GABAergic, glutamatergic and dopaminergic synapses (Wegener et al. 2009).

After activation of the CB1 receptor by endogenous (anandamide, 2-AG) or exogenous (THC) ligands, signalling events characteristic for GPCRs are caused, which finally result in reduction of neurotransmitter release from presynaptic terminals via several independent mechanisms. One of these mechanisms consists in inhibition of adenylate cyclase activity (Howlett et al. 1986), which entails in reduced cAMP formation, inhibition of protein kinase A (PKA) and finally in enhanced potassium ion - current into the synpatic cleft (Wegener et al. 2009). Further mechanisms are opening of inwardly rectifying potassium channels and closure of voltage-activated calcium channels (Felder et al. 1995). Over the G-proteins the CB1 receptors are coupled to ion channels, amongst others calcium channels. The modulation of these calcium channels leads to CB1 - mediated inhibition of presynaptic transmitter release (Figure 3).

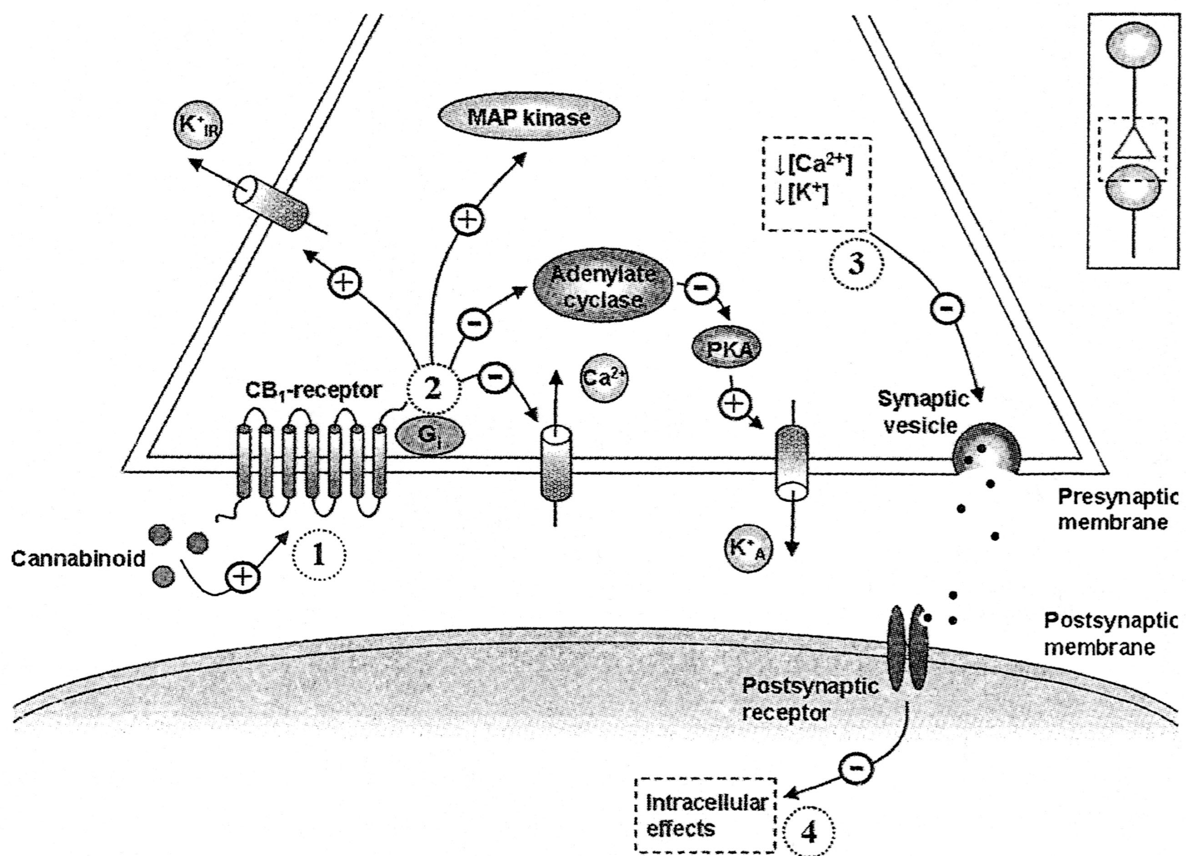


Figure 3: Signal transduction mechanisms affected by the CB 1 receptor in a presynaptic nerve terminal (e.g. on glutamatergic or GABAergic synapses) (Wegener et al. 2009)
(1) Activation of the CB1 receptor by cannabinoids stimulates the coupling to a G-protein thereby initiating a series of intracellular events. **(2)** These events include activation of MAP kinase as well as inhibition of voltage-dependent calcium channels and stimulation of inwardly rectifying potassium channels (K_{IR}). The activation of A-type potassium channels is modulated via inhibition of adenylate cyclase activity, which leads to inhibition of protein kinase A (PKA) and finally to an enhanced outward potassium current. The consequence of CB 1 receptor mediated modulation of cellular ion concentration is the inhibition of neurotransmitter release from synaptic vesicles **(3)**, characterizing this receptor as important modulator of synaptic transmitter release and postsynaptic activity **(4)**.

Diverse effects are mediated through several other systems, as for instance through the mitogen-activated protein (MAP) kinase pathway (Bouaboula et al. 1997), which regulates cellular functions such as cell growth, differentiation and apoptosis, as well as the expression of immediate early genes (Wegener et al. 2009). Dependent of the brain region, CB1 receptors can modify the release of noradrenaline, dopamine, g-aminobutyric acid (GABA), glutamate and serotonin (Schlicker et al. 2001). Besides, there are references indicating that the production of NO and arachidonic acid are being influenced as well (Pertwee et al. 2002).

In contrast to CB1 receptor markedly less detail is known about CB2 receptor signalling. Unlike CB1 receptor, the CB2 receptor is inable to modulate calcium channels or potassium channels, but except for this difference the CB1 and CB2 receptors seem to display similar pharmacological and biochemical properties (Felder et al. 1995).

1.2.4 Cannabinoid receptor ligands

Besides the reviewed endocannabinoids anandamide and 2-AG several synthetic and plant-derived cannabinoids exist which activate cannabinoid receptors, as well as pharmacological receptor ligands and receptor antagonists. These will be briefly outlined below for the sake of completeness.

According to their chemical structures the cannabinoid receptor ligands are divided into four major classes. The first group includes the 'classical' cannabinoids, which have a like-3-ring structure (Cota et al. 2003). The cannabinoids from Cannabis plants (more than 60 different compounds, including D9-THC, D8-THC, cannabinol and cannabidiol) and several synthetic compounds, named HU (Horvath et al. 2011), are members of this group. The second group includes the so-called 'nonclassical' cannabinoids and comprises bi- and tricyclic analogs of D9-THC (Howlett et al. 2002). The third group consists of compounds named aminoalkylindols. The most representative member of this class is WIN-55,212, a CB1 agonist widely used in experimental models (Howlett et al. 2002). The fourth group is represented by the endocannabinoids anandamide and 2-AG that are able to bind to cannabinoid receptors and to mimic the effects of plant-derived and synthetic cannabinoids. Remarkably, they are structurally not related to D9-THC.

In the field of drugs the receptor antagonist rimonabant had been used as an anti-obesity agent for 2 years in Europe, but exhibited severe psychiatric side effects, such as increasing likelihood of depression and anxiety, leading to its withdrawal in 2008. Currently,

cannabinoid receptor antagonists, acting primarily in the periphery without crossing the blood-brain barrier are being developed (Wu et al. 2011).

The drug dronabinol, which acts as a receptor agonist, was found to be potentially useful in a variety of medical conditions, while it notably has a very low abuse potential (Calhoun et al. 1998). It can be used for the mitigation of anorexia (Morley 2002) for instance in cancer patients (similarly effective for the treatment of chemotherapy-induced nausea and vomiting as ondansetron) (Meiri et al. 2007), in aging long term care residents (Wilson et al. 2007) as well as in patients with HIV / AIDS (improves appetite and reverses weight loss) (Dejesus et al. 2007). In treatment of multiple sclerosis it displays an analgesic effect on central pain (Svendsen et al. 2004). For chronic pain patients who have significant pain despite constant doses of opioids, dronabinol may provide an additive effect on pain relief (Narang et al. 2008). In a case report, dronabinol was described to be beneficial in a patient with cluster headache who was refractory to multiple acute and preventive medications (Robbins et al. 2009). In Alzheimer´s disease there are results indicating a potential usefulness to improve disturbed behaviour (Volicer et al. 1997) (although it has to be noticed that this observation was made from only 3 patients). There are indications of dronabinol being an alternative in the treatment of intractable cholestatic pruritus (Neff et al. 2002). An interesting case report of a longlasting history of intracranial hypertension exists, in which clinical signs and symptoms disappeared after daily intake of dronabinol (Raby et al. 2006). In the field of ophtalmology, the cannabinoid dronabinol is known to reduce intraocular pressure, which may be relevant in the treatment of ocular circulatory disorders, includig glaucoma (Plange et al. 2007). A singular case report gives account of a pregnant woman with mitochondrial movement disorder, who benefited from dronabinol, especially by supported healthy maternal weight gain (Farooq et al. 2009), although the question of safety considering side effects on the newborn needs to be investigated more precisely. Grant et al. investigated dronabinol recently in the context of trichotillomania (Grant et al. 2011), as the substance has been supposed to reduce compulsive behaviour. In their study a significant reduction in trichotillomania symptoms without negative cognitive effects was observable. Lately, it has been found that dronabinol can be helpful as a substitution strategy for treatment of cannabis dependence, as it showed effectiveness concerning treatment retention and withdrawal symptoms in a randomized, double-blind, placebo-controlled, 12-week trial with 156 cannabis-dependent adults (Levin et al. 2011).

1.2.5 Neuroanatomy of the endocannabinoid system

As described above the endocannabinoid system is an ubiqitous lipid signalling system which is involved in numerous regulatory functions, not only in the central nervous system but also in the autonomic nervous system, the endocrine system, the immune network, the gastrointestinal tract, the reproductive system and in microcirculation (Di Marzo et al. 1998). The distribution of elements of the endocannabinoid system in the nervous system is remarkable: in some regions, for example the hippocampus, there is a complementary distribution of cannabinoid receptors, endocannabinoid transporters and degradation enzymes. However, in other brain areas, for instance the thalamus, there are discrepancies in its distribution (i.e. transport activity and degradation enzymes in the absence of a relevant presence of the CB 1 receptors), which mirrors the present gaps in our knowledge of the composition of the endocannabinoid system (Rodriguez de Fonseca et al. 2005).

High densities of CB1 receptors are found within frontal regions of the cerebral cortex, basal ganglia, cerebellum, hypothalamus, hippocampus and the amygdala (Campolongo et al. 2009). They were also detected in reward circuits like nucleus accumbens and ventral tegmental area (Wegener et al. 2009). The density of CB 1 receptors in the anterior cingulate cortex (ACC) is moderately high (Glass et al. 1997). A significant up-regulation of CB 1 receptor density and CB 2 receptor-stimulated G-protein activation was observed in prefrontal cortex of postmortem brains of depressed suicide victims (Hungund et al. 2004).

The main endocannabinoids are derivatives of arachidonic acid (anandamide and 2-AG), which bind to G-protein-coupled receptors named CB 1 receptor and CB 2 receptor. While CB 1 is found densely in the brain related to motor control, cognition, emotional responses, motivated behaviour and homeostasis, CB 2 receptors are situated on B-cells and the natural killer cells of the immune system. Endocannabinoids are synthesized in a calcium-dependent manner and released upon demand from lipid precursors after cellular depolarization or receptor stimulation and serve as retrograde signalling messengers in GABAergic and glutamatergic synpases, and modulate postsynaptic transmission. They are transported into cells by a specific uptake system and are degraded by two specific enzymatic systems, the fatty acid amide hydrolase and the monoacylglycerol lipase. (Rodriguez de Fonseca et al. 2005) Associated with the inhibitory action of endocannabinoids on presynaptic calcium channels is the inhibition of neurotransmitter release. This presynaptic inhibition of transmitter release by endocannabinoids may adopt two different forms of short-term synaptic plasticity. In the case of GABA transmission it is depolarization-induced suppression of inhibition (DSI) and in case of glutamate transmission it is depolarization-induced

suppression of excitation (DSE) (Wilson et al. 2002) (Diana et al. 2004). The contribution of endocannabinoids to these forms of short-term synaptic plasticity has been described in the hippocampus (Wilson et al. 2001) and the cerebellum (Diana et al. 2002). The role of the endocannabinoid-induced DSI or DSE appears to reflect the coordination of neural networks within the hippocampus and the cerebellum that are involved in physiological processes, such as memory (Rodriguez de Fonseca et al. 2005). Further forms of endocannabinoid modulation of synaptic transmission involve the induction of long-term potention (LTP) and long-term depression (LTD). Activation of the cannabinoid receptors prevents the induction of LTP in the hippocampal synapses (Stella et al. 1997) and a facilitation of LTD in the striatum (Gerdeman et al. 2002).

Of special interest for our work is the role that endocannabinoids play in emotional processing. A key point for the endocannabinoid regulation of emotions is the amygdalar complex. There, endocannabinoids depress the release of glutamate and corticotropin-releasing factor, reducing the amygdalar output and the activity of basolateral inhibitory GABA projections to the central nucleus of the amygdala, thereby activating the amygdalofugal pathway (Navarro et al. 1997). The result will lead to anxiety or anxiolysis, dependent on the rate of activation of descending projections of the ventral nucleaus of the amygdala to the hypothalamus and brain stem. Notwithstanding a a number of studies have showed that the response to enhanced cannabinoid transmission in the limbic system is anxiolysis (Cravatt et al. 2003) (Kathuria et al. 2003).

As an interesting novel perspective concerning the perception of the endocannabinoid system is the thesis of a functional division between neuronal and glial elements of the endocannabinoid system. It was found that the functional neuroanatomy of the endocannabinoid system in the healthy brain appears to be quite different from that described unter pathological conditions. While CB 1 receptors and fatty acid amide hydrolase, together with the endocannabinoids and their putative uptake mechanisms, play essential roles in the normal state, profound changes occur under inflammatory insults. CB 2 expression seems to be induced in vivo in microglial cells, while fatty acid amide hydrolase is profusely expressed in astroglia. For instance, several groups have found significant increases in endocannabinoid levels in different animal models of human disease, such as amongst others multiple sclerosis (Baker et al. 2001), in which glia is assumed to play an important role. So it seems that a shift from mainly neuronal function of the endocannabinoid system to a major glial participation takes place under pathological conditions (Pazos et al. 2005).

1.2.6 Trauma and neurobiological sequelae

1.2.6.1 Overview of neural circuits (amygdala, hippocampus and ACC) after trauma

Hippocampal volumes of trauma survivors with post-traumatic stress disorder are reduced compared to those of nontraumatized persons (Yehuda 1999). Most studies noted that volumes of the right hippocampus are smaller than volumes of the left hippocampus in post-traumatic stress disorder patients compared to healthy subjects (Pavic et al. 2007), although some studies report of bilaterally equal volume reductions. These abnormalities may represent a pretraumatic vulnerability to develop post-traumatic stress disorder or they may be a consequence of traumatic exposure. Apfel and colleagues measured hippocampal volumes in subjects with chronic post-traumatic stress disorder and subjects with remitted post-traumatic stress disorder: subjects with chronic post-traumatic stress disorder had, on average, a smaller hippocampus compared with those with remitted post-traumatic stress disorder (Apfel 2011). This again raises two possible conclusions: either a smaller hippocampus is a risk factor for lack of recovery from post-traumatic stress disorder, or traumatic effects on hippocampal volume are reversible once post-traumatic stress disorder symptoms remit and the patient recovers (Apfel 2011). Supporting the notion of reduced hippocampal volumes being a genetic vulnerability is a study with monozygotic twins discordant for trauma exposure (Gilbertson et al. 2002). On the other side, long-term treatment with paroxetine is associated with increased hippocampal volumes in post-traumatic stress disorder (Vermetten et al. 2003), making it unlikely that smaller hippocampal volumes represent exclusively a risk factor for the development of post-traumatic stress disorder. In Vietnam veterans hippocampal volume was directly correlated with combat exposure (Gurvits et al. 1996). Elevated sensitivity of the glucocorticoid receptor, associated with post-traumatic stress disorder, has been shown to lead to hippocampal volume loss, and this may explain the volume loss in PTSD (Yehuda 2001). Woon found in a meta-analysis of 39 hippocampal volumetric studies that hippocampal volume reduction is associated with trauma exposure independent of post-traumatic stress disorder diagnosis, although additional hippocampal reduction was found in post-traumatic stress disorder compared to the trauma-exposed group without post-traumatic stress disorder (Woon et al. 2010). A corresponding result has been reported by Winter (Winter et al. 2004).

Besides hippocampal volume reduction also hippocampal function appears impaired among post-traumatic stress disorder patients (Nemeroff et al. 2006). The posterior hippocampus is a nodal point in storage, processing, and retrieval of spatiotemporal memories, central to the

protective function of fear conditioning. So smaller posterior hippocampi may indicate malfunction in this faculty, leading to the exaggerated conditioned fear response (Bonne et al. 2008). Negative correlations were found between re-experiencing symptoms and left hippocampal volume (Lindauer et al. 2006). The stress-induced dysfunction of the hippocampus might mediate symptoms which are related to memory dysregulation, including explicit memory deficits as well as fragmentation of memory in abuse survivors (Bremner 2006).

Functional neuroimaging symptom provocation and cognitive activation paradigms using PET measurement of regional cerebral blood flow have revealed greater activation of the amygdala and anterior paralimbic structures (which are known to be involved in processing negative emotions such as fear), and failure of activation of the cingulate cortex (which is assumed to play an inhibitory role) in response to trauma-related stimuli in individuals with post-traumatic stress disorder. Functional MRI research has shown the amygdala to be hyperresponsive to fear-related stimuli in this disorder (Pitman et al. 2001) which may reflect the amygdala´s role in emotional memory and conditioned fear. Concerning amygdala volume, after the World Trade Center trauma.of 09/11/01 interestingly even healthy adults who were situated to the disaster with closer proximity had lower gray matter volume in amygdala after the incident (Ganzel et al. 2008).
The amygdala is essential in memory for the emotional valence of events. Several neurological and psychological studies suggest that hyper-reactivity in the amygdala corresponds to a constellation of symptoms of anxiety in post-traumatic stress disorder, while the lowered activation of the prefrontal and the anterior cingular cortex could reflect slower extinction of a conditioned emotional response (Damsa et al. 2005).
Regarding the influence of the endocannabinoid system on brain structures, a neuroprotective effect was described for the case of traumatic brain injury in the rat and the mouse (Mechoulam et al. 2002), as well as an essential role in acute fear adaptation for CB1 proteins in the amygdala of mice (Kamprath et al. 2011).

Lower gray matter volume in the anterior cingulate after trauma (Ganzel et al. 2008) has been described, as well as decreased medial prefrontal / anterior cingulate function in post-traumatic stress disorder (Bremner 2006). These brain regions comprise a neural circuit that has been demonstrated as important for emotional processing and emotional regulation, conditioned fear and attention on the one side, and in resilience to trauma on the other side. A

deficiency of medial prefrontal / anterior cingulate activation with re-experiencing of traumatic situations is hypothesized to represent a neural correlate of the failure of extinction known in post-traumatic stress disorder and subsequent re-experiencing and heightened arousal / vegetative reactions. The mentioned regulation of emotional responsiveness of the anterior cingulate gyrus is achieved through inhibition of amygdala function. Shin et al. found a correlation between increased amygdala function and decreased medial prefrontal function with traumatic reminders, suggesting a failure of inhibition of the amygdala by the medial prefrontal cortex which could account for increased post-traumatic stress symptoms with traumatic reminders (Shin et al. 2004).

As far as the dorsal anterior cingulate cortex is concerned, hyperresponsivity during trauma script has been described (Nardo et al. 2011) and appears to be a familial risk factor for the development of post-traumatic stress disorder (Shin et al. 2011). In a script-driven trauma imagery study the symptom of avoidance was correlated negatively with anterior cingulate activity (Hopper et al. 2007). The anterior cingulate is part of the neural fear network and is an interface between emotion, cognition and behaviour. Accordingly, a reduced function of the anterior cingulate is associated with the inability to monitor and regulate emotional processes, such as fear, and furthermore inability to explain these rationally (Hull 2002). It has been postulated that resting state connectivity of the posterior cingulate with the perigenual anterior cingulate and the right amygdala is associated with current post-traumatic stress disorder symptoms and that correlation with the right amygdala predicts future post-traumatic stress disorder symptoms (Lanius et al. 2010).

Summarized, the three factors that are responsible for the symptoms in post-traumatic stress disorder are: stress sensitization, fear conditioning and failure of extinction.

1.2.6.2 Endocannabinoids in the context of trauma

One of the little clinical studies investigating endocannabinoid receptor mediated effects in humans after a trauma revealed the following result: out of 46 patients diagnosed with PTSD and having ongoing nightmares in spite of conventional antidepressants and hypnotics, 72% of patients experienced a cessation of nightmares or a significant reduction in nightmare intensity, as well as some patients reported reduced daytime flashbacks, after receiving the synthetic cannabinoid nabilone (Fraser 2009). Concerning aversive memories, it was found that deletion of CB1 receptors appears to selectively promote the consolidation and diminish the extinction of these (Hill et al. 2009). More precisely, findings suggest that endocannabinoids enhance memory consolidation in the basolateral amygdala and CB1

activity within this brain region is needed for enabling glucocorticoid effects on memory consolidation enhancement (Campolongo et al. 2009). According to the proposed role of the CB1 receptor as a ‘stress-recovery signal’ mediating receptor, CB1-agonists and inhibitors of endocannabinoid inactivation were found to facilitate the extinction of aversive memories in laboratory animals (Ligresti et al. 2009). The finding that CB1 antagonism in obese humans increases the odds of developing anxiety and depression (Despres 2009) indicates similar effects of the endocannabinoid system in humans.

Another study states that the consolidation of aversive memories is modulated by the dorsal hippocampus endocannabinoid system, and that its recruitment seems to be mediated by glucocorticoids (de Oliveira Alvares et al. 2010). In this context, it is also interesting that among patients with PTSD, higher levels of cannabis consumption are observable, which might represent a form of self medication (Calhoun et al. 2000). On a genetical level, a number of variations in cannabinoid receptor genes in humans have been associated with PTSD (Onaivi 2009).

Regarding the endocannabinoid system and *psychic* trauma, no further clinical studies have been published to date. Nonetheless the data outlined above demonstrate a crucial role of the endocannabinoid system in the context of trauma.

Furthermore, a neuroprotective effect of endocannabinoids after traumatic injury has been hypothesized. Neurodegenerative stimuli result in increased endocannabinoid levels and animals with genetic deletions of CB1 receptors are more susceptible to the deleterious effects of such stimuli (Fowler et al. 2010). For instance, significantly elevated 2-AG levels were measured after closed head injury in mice and administration of synthetic 2-AG lead to significant reduction of brain oedema, reduced infarct volume and reduced hippocampal cell death compared with controls (Panikashvili et al. 2001). Not only 2-AG levels are significantly elevated after traumatic brain injury, but also CB2 receptors were shown to reduce infarct size (Shohami et al. 2011). On the other hand, also neurotoxic effects have been described (Sarne et al. 2011), of which future implications are being discussed and not clear yet.

The endocannabinoid system has also been examined regarding its connection to the field of anxiety and fear. A growing body of evidence distinctively shows that deficits in endocannabinoid signalling may result in anxiogenic and depressive responses, while pharmacological augmentation of endocannabinoid signalling can produce anxiolytic as well as antidepressant responses (Hill et al. 2009). For instance, a study with mice demonstrated augmentation of the extinction of conditioned fear by facilitating endocannabinoid

neurotransmission (Chhatwal et al. 2005). In humans, the CB 1 receptor antagonist rimonabant exhibited severe psychiatric side effects, such as increasing the likelihood of depression and anxiety (Wu et al. 2011). When it comes to the issue of dose, interestingly low doses of a metabolically stable anandamide analog (methanandamide), microinjected into the prefrontal cortex, produced an anxiolytic-like response in rats, while higher doses induced anxiety-like behavior (Rubino et al. 2008).

1.2.6.3 Endocannabinoids in the context of stress

Coming to the relationship of the endocannabinoid system with stress, stress is defined as follows:
“The term “stress” generally indicates an internal (i.e. infection, psychological condition) or external (i.e. physical danger or damage) circumstance that threatens the homeostasis of the organism. Thus, stress results in a discrepancy, either real or perceived, between the demands of a situation and the organism´s resources.” (Cota 2008)

Results of previous studies on endocannabinoids and stress were mainly based on laboratory animal studies.
One of the few studies in humans investigated the influence of somatic stress on the endocannabinoid system: in case of human chronic heart failure, a shift of the CB1 / CB2 receptor ratio towards expression of CB2 receptors combined with significantly elevated peripheral blood levels of endocannabinoids indicating an activation of the endocannabinoid system was described (Weis et al. 2010). Another trial examined not the connection with stress itself, but the association with the sequelae of a stress reduction by loss of consciousness caused by general anaesthesia with isoflurane during cardiac surgery. This reduction in stress lead to significantly reduced plasma anandamide concentrations (Weis et al. 2010).
Roughly summarized, the majority of studies with laboratory animals suggested that the endocannabinoid system possibly counteracts the harmful consequences of stressful stimuli (Resstel et al. 2009) and elicits anxiolytic-like effects (Kinsey et al. 2011).

In order to scrutinize this issue more detailed, a distinction of acute stress and chronic / repeated stress makes sense.

Most of the research in animals suggests that *acute* stress produces a mobilization of 2-AG while concurrently reducing the tissue content of the other endocannabinoid ligand anandamide (Hill et al. 2010). Augmentation of endocannabinoid signalling seems to suppress stress-responsive systems (Hill et al. 2006). In a study with CB1 knockout mice, the lack of CB1 receptor resulted in an enhanced response to stress (Aso et al. 2008). Genetic and pharmacological studies demonstrate that a reduction in anandamide signalling may be involved in the initiation of HPA axis activation and reactions in emotional behaviour, while the increase in 2-AG signalling may be involved in terminating the stress response, limiting neuronal activation and contributing to changes in motivated behaviours (Hill et al. 2010). On the other side there is also contrary data in literature: emotionally stressful conditions in rats cause a reduction of 2-AG levels in the hypothalamus, without changes of the tissue levels of anandamide (Patel et al. 2004). Further work of Rademacher et al. in mice yielded reduced anandamide levels without affecting 2-AG levels after acute stress in the amygdala, without any changes of endocannabinoids in the medial prefrontal cortex and the ventral striatum (Rademacher et al. 2008).
One possible explanation for diverging results was described by Hill and Bruce: a species difference appears to exist between the consequences of stress on the endocannabinoid system in mice and rats. In rats, stress was described to mobilize 2-AG signalling and to suppress anandamide signalling, in a variety of limbic structures. In mice, there does not appear to be an effect of stress on 2-AG (at least through the examination of tissue levels of endocannabinoid content), whereas the reduction in anandamide still seems to be present.
The only investigation regarding the endocannabinoid system in humans after stress was conducted by Hill et al. by exposing 15 medication-free women diagnosed with major depression, and 15 healthy matched controls to the Trier social stress test (17 minutes mock job interview). Basal endocannabinoid levels were significantly reduced in women with major depression compared to matched controls, indicating a deficit in peripheral endocannabinoid activity. A rapid increase in circulating levels of 2-AG while not affecting circulating levels of anandamide was found after completion of the Trier social stress test in patients and controls, suggesting that stress-induced mobilization of 2-AG is similar in rats and humans (Hill et al. 2009).

Concerning *chronic* stress, existing data is controversial as well. Animal studies conducted by Taber and Hurley support the theory that a progressive increase of 2-AG and decrease of anandamide within limbic-related brain areas may, in part, mediate behavioral habituation to

repeated predictable stress (Taber et al. 2009). In accord with this hypothesis, Hill et al. could demonstrate one year later that the anandamide level is persistently decreased throughout the corticolimbic stress circuit in repeatedly stressed rats, whereas 2-AG is exclusively elevated within the amygdala in a stress-dependent manner (Hill et al. 2010). Similarly, there is further data suggesting that 2-AG may act at CB1 receptors in limbic brain regions to facilitate behavioural adaptation or habituation to repeated restraint stress (Finn 2010). Results of a mice study of Patel et al. indicate that exposure to repeated, but not acute, stress produces neuroadaptations that confer basolateral amygdala neurons with an enhanced capacity to elevate 2-AG content and engage in 2-AG-mediated short-term retrograde synaptic signalling (Patel et al. 2009).

Patel and Hillard provided a thesis of endocannabinoid level changes after repeated stress dependent on the brain region. Two sets of brain regions with general patterns were described: a cortical-like pattern was observed within amygdala, hippocampus, the medial prefrontal cortex, and the hypothalamus. Within these regions, repeated stress induced an anandamide deficiency. In contrast to anandamide, 2-AG increased in these cortical-like structures, and displayed a sensitized response to repeated restraint stress.

A contrary pattern was observed within the ventral striatum, assigned to a subcortical pattern: here data indicated that repeated restraint results in a 2-AG deficit together with enhanced anandamide signalling (Patel et al. 2008).

Derived from a male mice model, it was hypothesized in that increased CB1 receptor activity contributes to the process of habituation to homotypic stress (Patel et al. 2005). In the following years this was corroborated, which is outlined in a paper of Gorzalka: under conditions of repeated stress, a progressive increase in limbic 2-AG / CB receptor signalling contributes to the development and expression of neuroendocrine habituation (Gorzalka et al. 2009). Interestingly, the relationship between glucocorticoids and the endocannabinoid system appears to be functionally bidirectional: not only anandamide signalling influences the HPA axis, but also the CB1 receptor is under negative regulation of glucocortidoids in the hippocampus, which suggests that hippocampal CB1 receptor signalling might be reduced under conditions associated with hypersecretion of glucocorticoids, such as chronic stress (Hill et al. 2008). Correspondingly, downregulation of CB1 receptor expression and 2-AG levels within the hippocampus under chronic stress was described in an animal study (Hill et al. 2005). In an animal study, Hill et al. reported a downregulation of CB1 receptor expression and 2-AG levels within the hippocampus under chronic stress, while interestingly CB1 receptor density and 2-AG content were unaffected in the limbic forebrain (Hill et al. 2005).

Regarding the nature of *repeated* stress, Campos et al. conducted a study with rats suggesting that facilitation of the endocannabinoid system in the ventral hippocampus regulates anxiety-like behaviors and that this effect is dependent of previous stress experience (Campos et al. 2010). Taking early maternal deprivation as a model for neurodevelopmental stress, an investigation in rats provided evidence for a connection of early maternal deprivation with dysregulation of the endocannabinoid system, in terms of significant increase in DAGLalpha immunoreactivity (enzyme involved in 2-AG biosynthesis) and decrease in MAGL immunoreactivity (enzyme involved in 2-AG degradation) (Suarez et al. 2010). Gender might have an influence on the endocannabinoid system in chronic unpredictable stress, as the endocannabinoid system is preferentially organized in male and female animals to respond differentially to chronic stress (Reich et al. 2009).
An interesting theory is the following postulated by Carrier et al.: they report of direct and indirect evidence that stress affects endocannabinoids, whereas chronic stress affects CB1 *receptor density* (Carrier et al. 2005).

Taken together, the picture of the endocannabinoid system is largely one of a system that serves to facilitate habituation to stress, reduce innate anxiety responses, promote extinction of conditioned fear responding to unconditioned and conditioned stress, as well as diverse effects on memory and plasticity (Abush et al. 2010).
In other words, the endocannabinoid system promotes activities and responses which are beneficial for survival in the face of challenges to homeostasis (Finn 2010). Moreover, there is evidence that among others the central amygdala is an important neural substrate mediating the interactions between cannabinoids and environmental stress (Patel et al. 2005).

2. Research objectives

Given that post-traumatic stress disorder is an anxiety disorder characterized by reexperiencing intrusive recollections of the traumatic event in ways that are highly distressing (Yehuda 2004), agents which are beneficial in the extinction of aversive memories, such as facilitators of endocannabinoid signalling, may exhibit a therapeutic benefit in PTSD (Hill et al. 2009).
So far, little to no research took place concerning endocannabinoid response to stress in humans. In this study we investigated the influence of a type I traumatic event on the endocannabinoid system in humans over the course of time. We measured plasma endocannabinoid levels in subjects who experienced a psychic trauma and compared these to

non-traumatized controls. The goal was to contribute to the understanding of the role the endocannabinoid system plays in pathophysiological processes on one hand, and get first indications for the endocannabinoid system as a potential therapeutic target on the other hand. Moreover, based on previous findings of structural alterations of distinct brain regions, e.g. the hippocampus, we have analyzed peripheral endocannabinoid levels in relation to structural changes in key regions of the brain using MRI and voxel based morphometry.

3. Materials and methods

3.1 Participants and setting

Participants were 4 women and 10 men who were admitted to the outpatient clinics of the Munich University Psychiatric Hospital and the Munich University Department of Surgery . All participants had experienced a type I trauma, i.e. a single trauma of short duration, provoked by an accidental cause. Eleven of the participants were underground / streetcar / city train drivers and traumatized by experiencing a suicidal jump of an unknown person in front of their train. Further three cases were as follows: In one case a pedestrian was hit by a streetcar, in another case a pedestrian was struck by a car and one case was an occupational accident (Table 1).

The participants were between 29 and 58 years of age, with a mean age of 44.5 years. The control group consisted of healthy non-traumatized subjects matched for age and gender. There were no significant differences in age, sex, psychiatric history, psychiatric family history or history of addiction between the two groups (Table 2).

Subject	Role / Profession	Accident
1	Streetcar driver	Suicidal jump in front of streetcar driver
2	Underground train driver	Suicidal jump in front of underground train driver
3	Pedestrian	Pedestrian struck by car
4	Workman	Occupational accident (falling down a ventilation shaft)
5	Pedestrian	Pedestrian struck by streetcar
6	Streetcar driver	Suicidal jump in front of streetcar driver
7	Underground train driver	Suicidal jump in front of underground train driver
8	Underground train driver	Suicidal jump in front of underground train driver
9	Streetcar driver	Suicidal jump in front of streetcar driver
10	Streetcar driver	Suicidal jump in front of streetcar driver
11	Underground train driver	Suicidal jump in front of underground train driver
12	Streetcar driver	Suicidal jump in front of streetcar driver
13	City train driver	Suicidal jump in front of city train driver
14	Streetcar driver	Suicidal jump in front of streetcar driver

Table 1: Type of traumatic experience

Demographics	Trauma	Control	Statistics
Age (SD)	44.50 (8.25)	43.36 (8.56)	n.s.
Gender (male/female)	10/4	10/4	n.s.
Years of education	14	15	n.s.
Marital status (single, married, divorced)	2/12/0	4/7/3	n.s.
Psychiatric history	4	0	n.s.
Psychiatric family history	4	3	n.s.
Substance abuse history (> 10 years prior to study)	5	1	n.s.
Regular intake of any medication	8	4	n.s.
Somatic history	9	1	p=0.004
Past traumata (yes/no)	7/7	0/14	p=0.006

Table 2: Demographic and clinical variables

Participants were evaluated within fourty-eight hours after the traumatic event, one month later and six months later. Controls were evaluated analogously (Figure 4). At each point of time a psychiatric interview was conducted by an experienced psychiatrist and the Clinician Administered PTSD Scale (CAPS) was administered to the participants. Depressive symptoms were surveyed with Hamilton Depression Scale (HAMD) and Becks Depression Inventory (BDI). Acute stress disorder and PTSD were diagnosed according to DSM-IV criteria. "Subthreshold PTSD" was diagnosed according to Stein et al.: presence of at least one symptom of the reexperiencing cluster plus either three symptoms or more of the avoidance cluster or at least two symptoms of the hyperarousal cluster (Stein et al. 1997). Endocannabinoid measurements (anandamide and 2-AG) were performed at all three points of time in traumatized and controls. Furthermore, subjects underwent MRI scanning at all three time points using diffusion tensor imaging and voxel-based morphometry of amygdala, hippocampus and cingulum. Detailed results of these investigations are reported in a separate dissertation (Dino Santangelo de Souza).

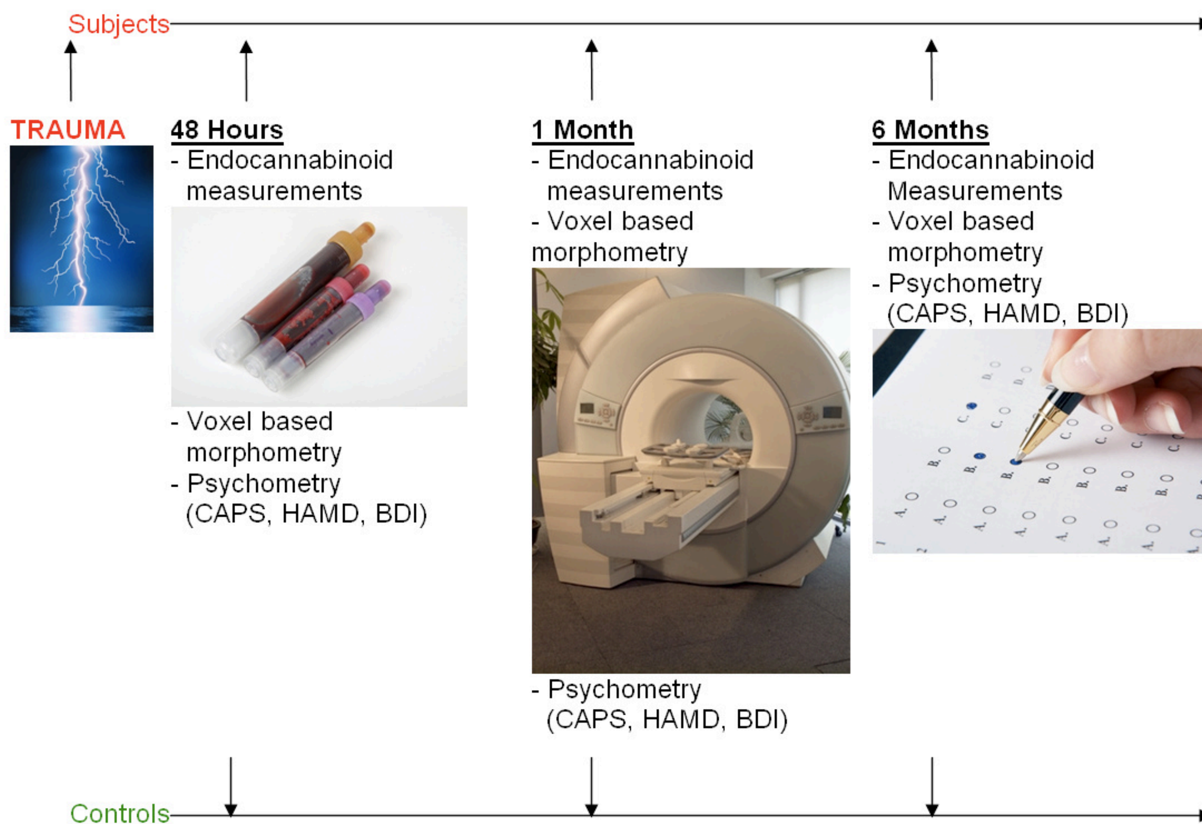


Figure 4: Study design

The study was approved by the local ethics committee of the Faculty of Medicine Ludwig-Maximilians-University Munich, Germany and carried out in accordance with the Declaration of Helsinki. Patient confidentiality was in no way breached and a statement of written informed consent was made before starting examination.

3.2 Endocannabinoid measurement

Blood samples for endocannabinod measurements were drawn into EDTA containing tubes (S-Monovette®, Sarstedt, Numbrecht, Germany) and centrifuged immediately (within five minutes). The time interval between blood sampling and centrifugation was minimized as previous experiments have shown that endocannabinoid generation in blood samples is continued ex-vivo, which could lead to false positive increases in measured plasma endocannabinoid concentrations.

Peripheral endocannabinoid activity was determined by measuring the endocannabinoids anandamide (ANA) and 2-AG in plasma using high performance liquid chromatography-tandem mass spectrometry (HPLC / MS-MS). Measurements followed automated on-line

solid phase extraction using column switching with subsequent direct transfer to HPLC and a tandem mass spectrometry system (Waters Quattro Ultima Pt; Waters Corporation, 34 Maple Street, Milford, MA 01757, USA). Stable isotope labeled anandamide (fourfold deuterated) was synthesized by Roche Diagnostics, Mannheim, Germany and fivefold deuterated 2-AG was obtained from Cayman Europe, Tallinn, Estonia. These compounds were used as the internal standard for HPLC / MS-MS measurements. The purity of these materials amounts >97.2%. Pure solutions of anandamide (0.1 to 2 ng/ml) and 2-AG (0.5 to 10 ng/ml) were applied for calibration. Anandamide and 2-AG were supplied by Sigma, Deisenhofen, Germany. Electrospray ionization in the positive mode was used; the following mass transitions are monitored: native anandamide, 348 > 62; 4d3 anandamide (internal standard), 352>66. The method has a linear response within the calibration ranges and a total coefficient of variation of 34% at a mean concentration of 0.2 ng/l. The lower limit of detection of the method (defined as a signal / noise ration > 4:1) was 0.025 ng/ml for anandamide and 0.33 ng/ml for 2-AG.

3.3 Data processing for volumetric data analysis

The analysis was done using SPM5 and a code written in Matlab R2007a. Initially the DICOM pictures were converted via MRIcro® (Version 1.39 on Linux) to the Analyze format. Anatomical parcellation was semi-automatically performed on the basis of high-resolution MPRAGE data using IBASPM (Individual Brain Atlas using Statistical Parametric Mapping Software), a specialized toolbox for the statistical parametric mapping package (SPM 5, J. Ashburner an K.J. Friston, Wellcome Department of Imaging Neuroscience, UCL, London). The software uses a standardized procedure. Three principal elements for the labeling process are used: gray matter segmentation, normalization transform matrix (that maps voxels from individual space to standardized one) and MaxPro MNI Atlas. All three are combined to yield a good performance in segmenting gross cortical structures. The programs here can be used in general for any standardized Atlas and any MRI image modality. In a first step the MRI volume is normalized to MNI (Montreal Neurological Institute) space using a set of non-linear basis functions to obtain the spatial transformation matrix. After that individual images are segmented in three different brain tissues: cerebral spinal fluid, grey matter and white matter. Thereafter each individual grey matter voxel is labelled based on MNI anatomical atlas and the transformation obtained in the previous step. For further analysis the resulting volumetric data were transferred to SPSS 14.0® (SPSS Inc., Chicago, Illinois, USA). (Each of the regions-of-interest (ROI) volumes is depicted in cm^3.)

The three work steps of MRI acquisition, image preprocessing and analysis of imaging data will be described in detail in the doctor´s thesis of the colleague (Dino Santangelo de Souza) who performed these steps (ditto for the three investigated key regions, which were selected according to literature).

3.4 Data analysis

Analyses were performed in SPSS, Version 17.0. Traumatized subjects and controls were compared for differences in demographic and clinical variables at baseline using exact fisher tests for categorical variables and t-tests for dimensional variables. Endocannabinoid levels and psychometric scales over the three points of time were analyzed for change with a repeated-measures design (ANOVA), testing for main effects of time and group as well as time x group interactions. Hippocampal and amygdala volumes were correlated with endocannabinoid levels by use of Pearson correlation. All statistical tests were two-tailed.

4. Results

4.1 Description of groups

There were no significant differences in demographic and clinical variables between both groups apart from somatic history (p=0.004) and past traumata (p=0.006) (Table 2). Fourty-eight hours after trauma all subjects met the criteria for an acute stress disorder. One month later, four subjects had developed a PTSD (PTSD), in four subjects subthreshold PTSD criteria were met and six subjects were free of symptoms. After six months, only one subject was still suffering from PTSD and thirteen subjects were free of symptoms.

4.2 Psychometric measures

CAPS, HAMD and BDI scores are shown in Table 3. In the trauma group, there were significant differences in CAPS scores over the three points of time (p=0.00, df=2.0, F=39.21 ANOVA). HAMD and BDI scores did not change significantly over the course of time. There were significant differences between the subject group and the control group in CAPS scores at all points of time (p=0.00, df=1.0, F=22.63 ANOVA). HAMD (p=0.004, df=1.0, F=10.25, ANOVA) and BDI (p=0.018, df=1.0, F=6.49, ANOVA) scores did significantly differ

between both groups at fourty-eight hours and one month after trauma. After six months there were no statistically significant differences in HAMD and BDI scores between both groups.

	CAPS	HAMD	BDI	Anandamide [ng/ml]	2-AG [ng/ml]
Subjects					
48 Hours	57 (31.0)	14 (7.6)	10 (11.5)	0.14 (0.1)	13.62 (13.0)
1 Month	37 (35.0)	12 (11.1)	9 (9.3)	0.15 (0.1)	14.11 (10.1)
6 Months	25 (31.2)	9 (10.8)	7 (8.9)	0.15 (0.1)	11.22 (8.60)
Controls					
48 Hours	0 (0)	2 (2.6)	2 (4.0)	0.12 (0.0)	7.76 (5.8)
1 Month	0 (0)	1 (2.7)	3 (3.9)	0.14 (0.1)	6.71 (3.3)
6 Months	0 (0)	2 (3.6)	2 (3.3)	0.11 (0.1)	6.71 (4.3)

Table 3: Synopsis of CAPS and depression scores together with plasma concentration of anandamide and 2-AG. Mean (SD) values are shown.

4.3 Endocannabinoid levels

Table 4 shows 2-AG and anandamide levels of individual subjects and controls.

Group	No.	ANA 48h [ng/ml]	ANA 1 month [ng/ml]	ANA 6 months [ng/ml]	2-AG 48 h [ng/ml]	2-AG 1 month [ng/ml]	2-AG 6 months [ng/ml]
Subject	1	0.33	0.21	0.21	12.10	20.80	11.70
	2	0.14	0.25	0.16	19.80	36.20	21.80
	3	0.22	0.22	na	12.70	15.30	na
	4	0.03	0.06	0.05	2.83	2.27	2.48
	5	0.03	0.10	na	1.03	3.68	na
	6	0.19	0.16	0.06	8.92	13.70	7.50
	7	0.15	0.18	0.10	30.30	12.60	13.00
	8	0.07	0.11	0.20	6.69	5.41	3.29
	9	0.18	0.20	0.20	49.90	30.30	30.30
	10	0.13	0.13	0.08	13.30	5.06	5.93
	11	0.20	0.15	0.14	14.90	15.60	19.00
	12	0.07	0.09	0.21	9.10	19.00	10.60
	13	0.11	0.14	0.35	6.16	13.40	5.26
	14	0.17	0.07	0.06	3.01	4.27	3.77
Control	1	0.12	0.24	na	20.30	13.50	na
	2	0.09	0.14	0.12	15.70	7.06	13.50
	3	0.16	0.10	0.07	3.24	6.14	4.86
	4	0.13	0.10	0.08	7.23	7.13	7.23
	5	0.13	0.08	0.06	5.67	7.14	3.90
	6	0.14	0.13	0.10	9.70	4.23	4.23
	7	0.20	0.15	0.17	14.80	8.92	7.94
	8	na	0.15	0.08	na	4.20	1.78
	9	0.10	0.08	na	2.59	2.02	na
	10	0.08	0.09	0.11	1.25	4.84	1.84
	11	0.08	0.08	0.07	3.36	8.49	11.40
	12	0.10	0.08	0.07	4.34	1.89	3.37
	13	0.07	0.11	0.17	4.95	12.00	6.42
	14	0.18	0.38	0.22	7.69	6.39	14.00

Table 4: Anandamide and 2-AG levels (ng/ml) of individual subjects and controls (na – not assessed)

Regarding endocannabinoid levels over the course of time, an analysis of variance was calculated. On the first level no significant results were found, so an exploratory post hoc analysis was performed. Time effect for 2-AG was not significant (p=0.103, df=2.0, F=2.55, ANOVA, Multivariate Tests). Regarding group x time interaction effect, 2-AG decreased in

the trauma group between one month and six months after trauma (p=0.051, df=2.0, F=3.47, ANOVA, Multivariate Tests). In non-traumatized controls, there were no statistical differences in 2-AG and anandamide levels over the course of time (Table 3, Figure 5 and 6).

As group effects are concerned, 2-AG levels were compared between subjects and controls: there was a statistical significant difference one month after trauma (p=0.046, df=1.0, F=4.49, ANOVA, Tests of between-subjects effects). At fourty-eight hours after trauma and six months after trauma there were no statistical differences in 2-AG levels between both groups (Figure 5). Concerning anandamide levels there were no statistical differences at any point of time between traumatized and non-traumatized (Figure 6).

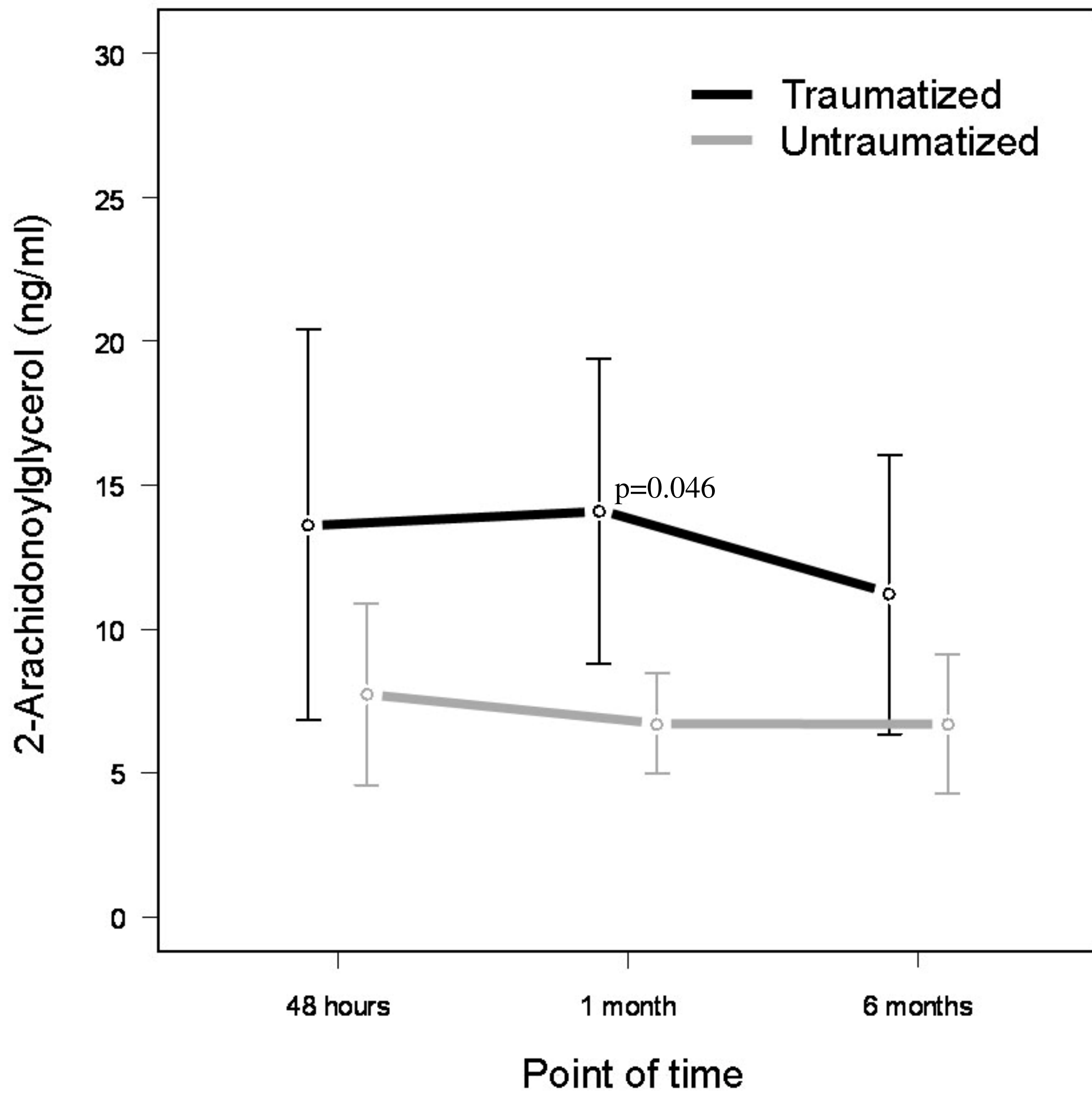


Figure 5: 2-AG over the course of time for both groups (mean, 95% Gaussian confidence intervals)

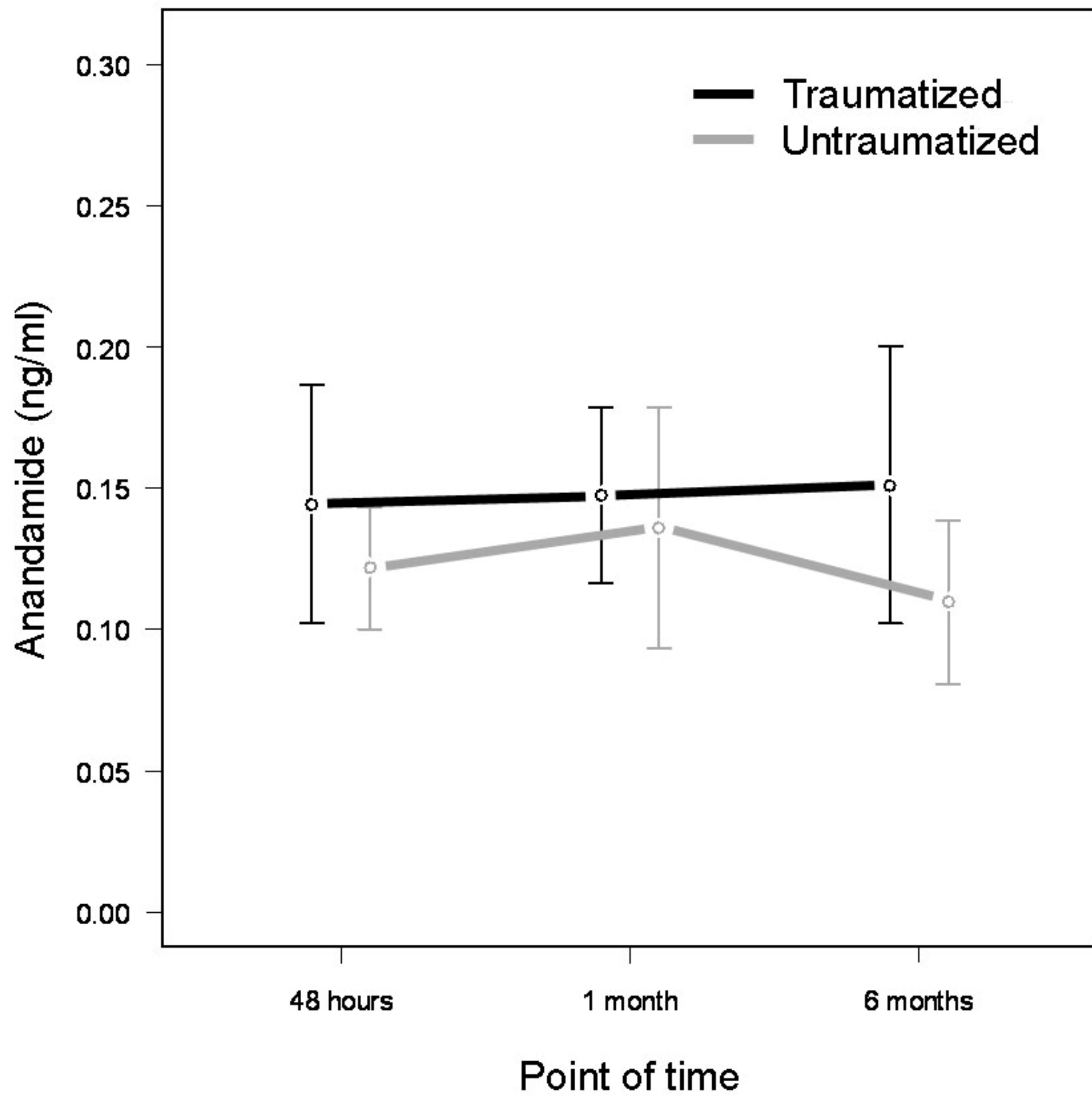


Figure 6: Anandamide over the course of time for both groups (mean, 95% Gaussian confidence intervals)

4.4 Correlations between endocannabinoid levels and Demographics / psychometric scales

No considerable significant correlations were found between endocannabinoid levels and Demographics (Anandamide: Tables 5-9, 2-AG: Tables 10-14) / clinical variables (CAPS, HAMD, BDI) (Anandamide: Table 15, 2-AG: Table 16).

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Gender	Correlation Coefficient	-.252	14	-.308	13
		Sig. (2-tailed)	.384		.306	
	Age	Correlation Coefficient	.010	14	.362	13
		Sig. (2-tailed)	.972		.224	
1 month after trauma	Gender	Correlation Coefficient	-.136	14	-.198	14
		Sig. (2-tailed)	.644		.496	
	Age	Correlation Coefficient	.274	14	.002	14
		Sig. (2-tailed)	.343		.995	
6 months after trauma	Gender	Correlation Coefficient	-.035	12	-.009	12
		Sig. (2-tailed)	.915		.977	
	Age	Correlation Coefficient	.022	12	.043	12
		Sig. (2-tailed)	.946		.895	

Table 5: Correlations between anandamide plasma levels and gender / age during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Past traumata	Correlation Coefficient	.482	14	-.070	13
		Sig. (2-tailed)	.081		.820	
	Somatic injury	Correlation Coefficient	-.456	14	a	13
		Sig. (2-tailed)	.101			
1 month after trauma	Past traumata	Correlation Coefficient	.340	14	.121	14
		Sig. (2-tailed)	.234		.680	
	Somatic injury	Correlation Coefficient	-.663**	14	a	14
		Sig. (2-tailed)	.010			
6 months after trauma	Past traumata	Correlation Coefficient	.311	12	.184	12
		Sig. (2-tailed)	.325		.568	
	Somatic injury	Correlation Coefficient	-.516	12	a	12
		Sig. (2-tailed)	.086			

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).
a. Cannot be computed because at least one of the variables is constant.

Table 6: Correlations between anandamide plasma levels and past traumata / somatic injury during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Psychiatric history	Correlation Coefficient	-.225	14	a	13
		Sig. (2-tailed)	.440			
	Somatic history	Correlation Coefficient	.496	14	.374	13
		Sig. (2-tailed)	.071		.208	
1 month after trauma	Psychiatric history	Correlation Coefficient	-.073	14	a	14
		Sig. (2-tailed)	.805			
	Somatic history	Correlation Coefficient	.255	14	.110	14
		Sig. (2-tailed)	.378		.707	
6 months after trauma	Psychiatric history	Correlation Coefficient	.257	12	a	12
		Sig. (2-tailed)	.420			
	Somatic history	Correlation Coefficient	.212	12	-.393	12
		Sig. (2-tailed)	.508		.207	

a. Cannot be computed because at least one of the variables is constant.

Table 7: Correlations between anandamide plasma levels and psychiatric history / somatic history during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Intake of any medication	Correlation Coefficient	-.012	14	.050	13
		Sig. (2-tailed)	.969		.871	
	Substance abuse history	Correlation Coefficient	.181	14	-.305	13
		Sig. (2-tailed)	.536		.311	
1 month after trauma	Intake of any medication	Correlation Coefficient	-.139	14	.244	14
		Sig. (2-tailed)	.636		.401	
	Substance abuse history	Correlation Coefficient	-.237	14	.114	14
		Sig. (2-tailed)	.415		.698	
6 months after trauma	Intake of any medication	Correlation Coefficient	.188	12	-.252	12
		Sig. (2-tailed)	.559		.429	
	Substance abuse history	Correlation Coefficient	-.467	12	.244	12
		Sig. (2-tailed)	.126		.444	

Table 8: Correlations between anandamide plasma levels and intake of any medication / substance abuse history during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Marital status	Correlation Coefficient	-.261	14	-.461	13
		Sig. (2-tailed)	.368		.113	
	Psychiatric family history	Correlation Coefficient	.179	14	-.060	13
		Sig. (2-tailed)	.540		.846	
1 month after trauma	Marital status	Correlation Coefficient	-.014	14	-.138	14
		Sig. (2-tailed)	.962		.639	
	Psychiatric family history	Correlation Coefficient	.049	14	.196	14
		Sig. (2-tailed)	.867		.502	
6 months after trauma	Marital status	Correlation Coefficient	-.266	12	.192	12
		Sig. (2-tailed)	.403		.549	
	Psychiatric family history	Correlation Coefficient	-.341	12	.323	12
		Sig. (2-tailed)	.278		.305	

Table 9: Correlations between anandamide plasma levels and marital status / psychiatric family history during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Gender	Correlation Coefficient	-.233	14	-.053	13
		Sig. (2-tailed)	.422		.863	
	Age	Correlation Coefficient	-.152	14	.271	13
		Sig. (2-tailed)	.604		.370	
1 month after trauma	Gender	Correlation Coefficient	-.218	14	-.428	14
		Sig. (2-tailed)	.455		.127	
	Age	Correlation Coefficient	.029	14	.007	14
		Sig. (2-tailed)	.922		.982	
6 months after trauma	Gender	Correlation Coefficient	-.161	12	-.026	12
		Sig. (2-tailed)	.618		.937	
	Age	Correlation Coefficient	-.135	12	.093	12
		Sig. (2-tailed)	.677		.773	

Table 10: Correlations between 2-AG plasma levels and gender / age during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Past traumata	Correlation Coefficient	-.041	14	.027	13
		Sig. (2-tailed)	.889		.930	
	Somatic injury	Correlation Coefficient	-.475	14	a	13
		Sig. (2-tailed)	.086			
1 month after trauma	Past traumata	Correlation Coefficient	-.029	14	-.036	14
		Sig. (2-tailed)	.921		.902	
	Somatic injury	Correlation Coefficient	-.574*	14	a	14
		Sig. (2-tailed)	.032			
6 months after trauma	Past traumata	Correlation Coefficient	-.153	12	-.039	12
		Sig. (2-tailed)	.634		.905	
	Somatic injury	Correlation Coefficient	-.440	12	a	12
		Sig. (2-tailed)	.152			

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).
a. Cannot be computed because at least one of the variables is constant.

Table 11: Correlations between 2-AG plasma levels and past traumata / somatic injury during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Psychiatric history	Correlation Coefficient	-.310	14	a	13
		Sig. (2-tailed)	.281			
	Somatic history	Correlation Coefficient	.051	14	.145	13
		Sig. (2-tailed)	.863		.637	
1 month after trauma	Psychiatric history	Correlation Coefficient	.025	14	a	14
		Sig. (2-tailed)	.933			
	Somatic history	Correlation Coefficient	.085	14	-.457	14
		Sig. (2-tailed)	.774		.100	
6 months after trauma	Psychiatric history	Correlation Coefficient	-.148	12	a	12
		Sig. (2-tailed)	.645			
	Somatic history	Correlation Coefficient	.007	12	.021	12
		Sig. (2-tailed)	.982		.948	

Table 12: Correlations between 2-AG plasma levels and psychiatric history / somatic history during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Intake of any medication	Correlation Coefficient	-.167	14	.130	13
		Sig. (2-tailed)	.568		.673	
	Substance abuse history	Correlation Coefficient	-.332	14	.233	13
		Sig. (2-tailed)	.246		.444	
1 month after trauma	Intake of any medication	Correlation Coefficient	-.242	14	-.298	14
		Sig. (2-tailed)	.405		.300	
	Substance abuse history	Correlation Coefficient	-.303	14	.049	14
		Sig. (2-tailed)	.292		.867	
6 months after trauma	Intake of any medication	Correlation Coefficient	-.096	12	.291	12
		Sig. (2-tailed)	.767		.358	
	Substance abuse history	Correlation Coefficient	-.215	12	.136	12
		Sig. (2-tailed)	.501		.673	

Table 13: Correlations between 2-AG plasma levels and intake of any medication / substance abuse history during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Demographic variable		Trauma	N	Controls	N
48 hours after trauma	Marital status	Correlation Coefficient	.155	14	-.052	13
		Sig. (2-tailed)	.596		.866	
	Psychiatric family history	Correlation Coefficient	.239	14	-.044	13
		Sig. (2-tailed)	.410		.887	
1 month after trauma	Marital status	Correlation Coefficient	-.064	14	.197	14
		Sig. (2-tailed)	.829		.500	
	Psychiatric family history	Correlation Coefficient	-.046	14	-.011	14
		Sig. (2-tailed)	.876		.969	
6 months after trauma	Marital status	Correlation Coefficient	.147	12	.648*	12
		Sig. (2-tailed)	.649		.023	
	Psychiatric family history	Correlation Coefficient	.231	12	-.101	12
		Sig. (2-tailed)	.470		.755	

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).

Table 14: Correlations between 2-AG plasma levels and marital status / psychiatric family history during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Clinical variable		Trauma	N	Controls	N
48 hours after trauma	CAPS	Correlation Coefficient	-.512	14	a.	13
		Sig. (2-tailed)	.061			
	HAMD	Correlation Coefficient	.041	14	.062	13
		Sig. (2-tailed)	.889		.841	
	BDI	Correlation Coefficient	.122	14	-,147	13
		Sig. (2-tailed)	.677		.632	
1 month after trauma	CAPS	Correlation Coefficient	-.351	14	a.	14
		Sig. (2-tailed)	.219			
	HAMD	Correlation Coefficient	-.550*	14	-.142	14
		Sig. (2-tailed)	.042		.628	
	BDI	Correlation Coefficient	-.300	14	.175	14
		Sig. (2-tailed)	.297		.549	
6 months after trauma	CAPS	Correlation Coefficient	-.241	12	a	12
		Sig. (2-tailed)	.450			
	HAMD	Correlation Coefficient	-.198	11	.075	11
		Sig. (2-tailed)	.560		.826	
	BDI	Correlation Coefficient	-,429	9	.102	12
		Sig. (2-tailed)	.250		.753	

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).
a. Cannot be computed because at least one of the variables is constant.

Table 15: Correlations between anandamide plasma levels and CAPS / HAMD / BDI during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Clinical variable		Trauma	N	Controls	N
48 hours after trauma	CAPS	Correlation Coefficient	-.364	14	a.	13
		Sig. (2-tailed)	.201			
	HAMD	Correlation Coefficient	.126	14	.400	13
		Sig. (2-tailed)	.668		.176	
	BDI	Correlation Coefficient	.375	14	,105	13
		Sig. (2-tailed)	.186		.733	
1 month after trauma	CAPS	Correlation Coefficient	-.244	14	a.	14
		Sig. (2-tailed)	.401			
	HAMD	Correlation Coefficient	-.517	14	.354	14
		Sig. (2-tailed)	.058		.214	
	BDI	Correlation Coefficient	-.259	14	.476	14
		Sig. (2-tailed)	.371		.086	
6 months after trauma	CAPS	Correlation Coefficient	-.281	12	a	12
		Sig. (2-tailed)	.377			
	HAMD	Correlation Coefficient	-.199	11	.429	11
		Sig. (2-tailed)	.558		.188	
	BDI	Correlation Coefficient	-,425	9	-.103	12
		Sig. (2-tailed)	.254		.751	

a. Cannot be computed because at least one of the variables is constant.

Table 16: Correlations between 2-AG plasma levels and CAPS / HAMD / BDI during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

4.5 Correlations between endocannabinoid levels and brain volume changes

In order to relate changes of endocannabinoid levels to brain plasticity in key regions following trauma, correlations between peripheral anandamide and 2-AG levels were calculated.

Significant negative correlations were found for anandamide levels and brain volumes (Table 17). Left hippocampal volumes at six months were significantly negatively correlated with anandamide levels at six months after trauma (r=0.90, p=0.001, Pearson correlation) (Figure 7). Right hippocampal volumes at six months correlated statistically significant with anandamide levels at six months after trauma (r=0.74, p=0.02, Pearson correlation) (Figure 8). Fourty-eight hours and one month after trauma, no statistically significant correlations for anandamide levels and hippocampal volumes were observed. In the control group, anandamide levels were not correlated with hippocampal volumes.

For 2-AG levels, no correlations with hippocampal volumes were found in any group (Table 18).

Time point	Brain region		Trauma	N	Controls	N
48 hours after trauma	Vol. left hippocampus	Correlation Coefficient	-.03	11	-.35	13
		Sig. (2-tailed)	.93		.25	
	Vol. right hippocampus	Correlation Coefficient	-.18	11	-.38	13
		Sig. (2-tailed)	.60		.20	
1 month after trauma	Vol. left hippocampus	Correlation Coefficient	-.48	12	-.04	14
		Sig. (2-tailed)	.11		.90	
	Vol. right hippocampus	Correlation Coefficient	-.49	12	-.07	14
		Sig. (2-tailed)	.10		.81	
6 months after trauma	Vol. left hippocampus	Correlation Coefficient	-.90**	9	.08	12
		Sig. (2-tailed)	.001		.80	
	Vol. right hippocampus	Correlation Coefficient	-.74*	9	.15	12
		Sig. (2-tailed)	.02*		.65	

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).

Table 17: Correlations between anandamide plasma levels and hippocampus volumes during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

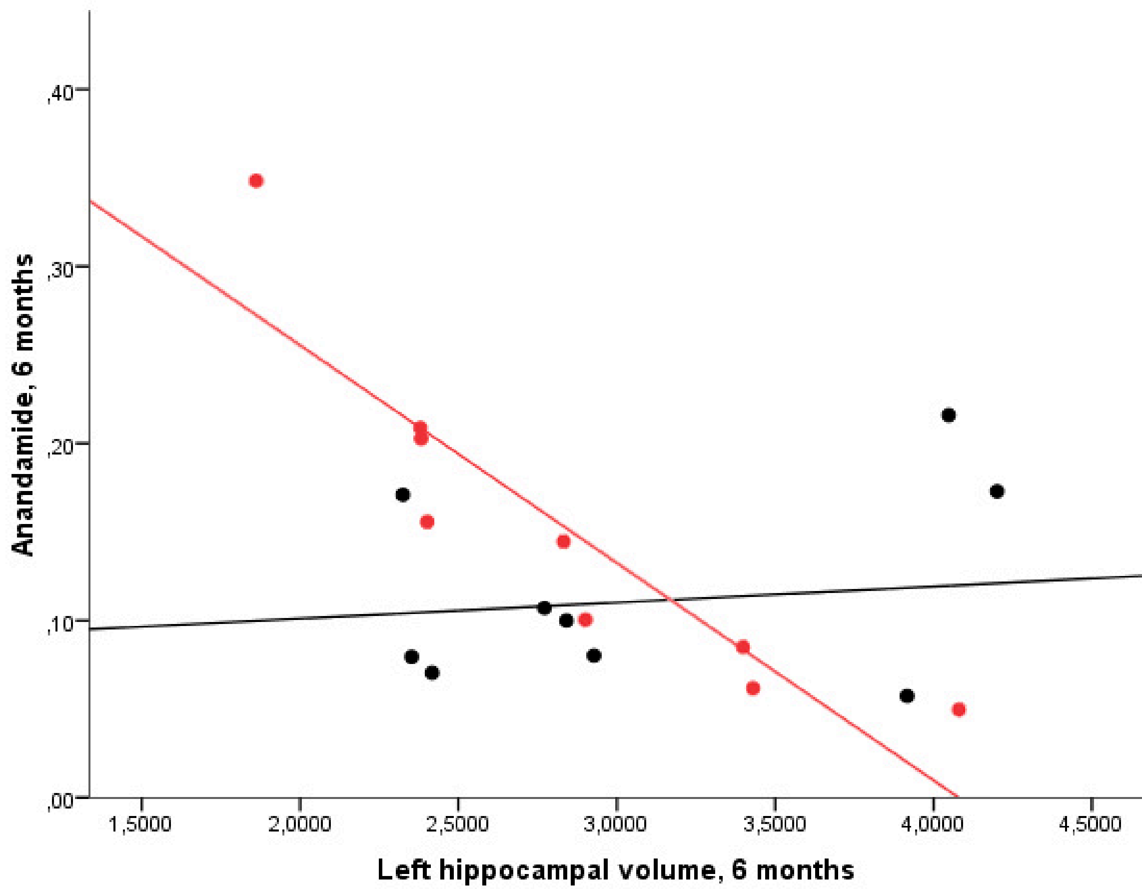


Figure 7: Scatterplot of anandamide and left hippocampal volume six months after trauma – traumatized (red; r = 0.90, p = 0.001), untraumatized (black; r = 0.15)

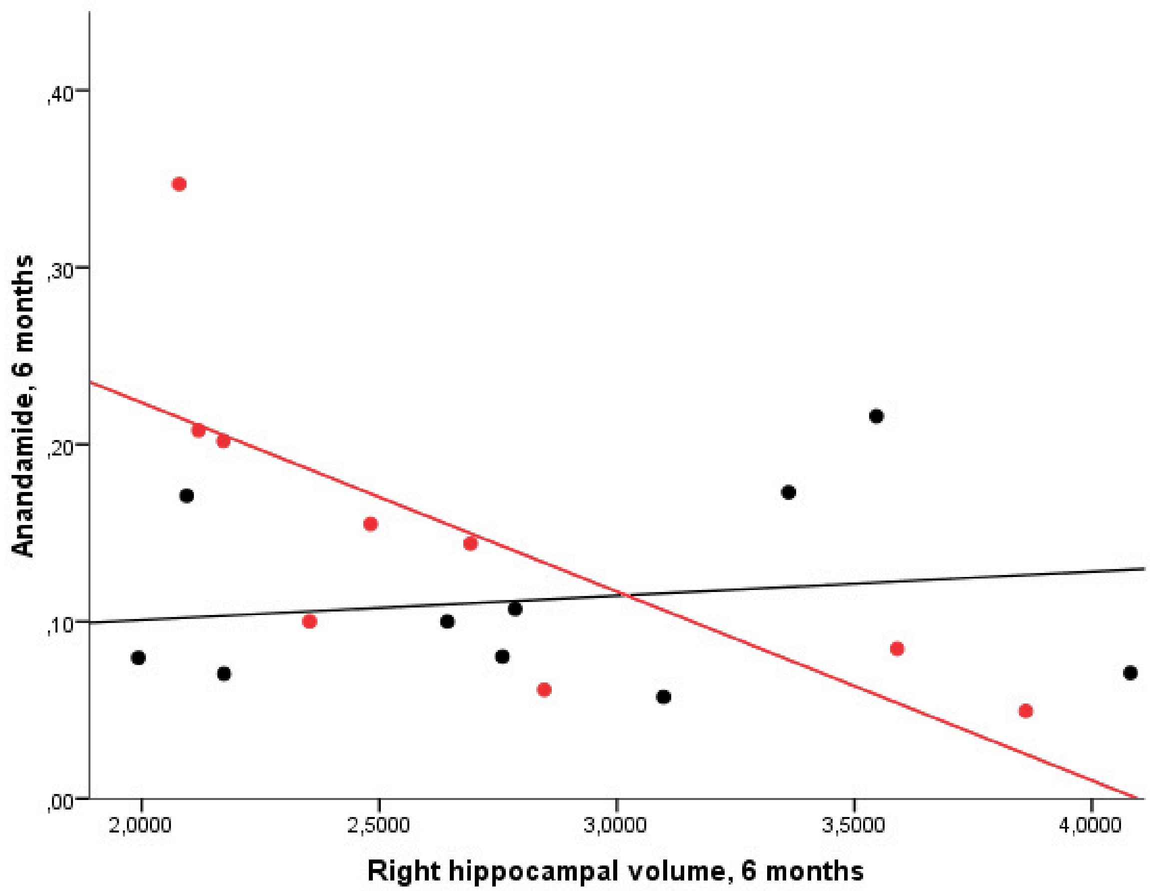


Figure 8: Scatterplot of anandamide and right hippocampal volume six months after trauma - traumatized (red; r = 0.74, p = 0.02), untraumatized (black; r = 0.17)

Time point	Brain region		Trauma	N	Controls	N
48 hours after trauma	Vol. left hippocampus	Correlation Coefficient	-.12	11	-.08	13
		Sig. (2-tailed)	.73		.80	
	Vol. right hippocampus	Correlation Coefficient	-.31	11	.02	13
		Sig. (2-tailed)	.36		.95	
1 month after trauma	Vol. left hippocampus	Correlation Coefficient	-.33	12	-.04	14
		Sig. (2-tailed)	.30		.89	
	Vol. right hippocampus	Correlation Coefficient	-.38	12	-.12	14
		Sig. (2-tailed)	.23		.68	
6 months after trauma	Vol. left hippocampus	Correlation Coefficient	-.30	9	.11	12
		Sig. (2-tailed)	.44		.73	
	Vol. right hippocampus	Correlation Coefficient	-.31	9	.25	12
		Sig. (2-tailed)	.42		.43	

Table 18: Correlations between 2-AG plasma levels and hippocampus volumes during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

One statistically significant negative correlation between left amygdala volume at six months and anandamide levels at six months after trauma was found in traumatized subjects (r= -0.78, p=0.01, Pearson correlation) (Table 19). In non-traumatized controls, for 2-AG levels and amygdala volumes at one month follow-up following results were found (Table 20): left amygdala volumes at one month correlated statistically significant with 2-AG levels at one month after trauma (r=0.55, p=0.041, Pearson correlation) (Figure 9). Right amygdala volumes at one month correlated statistically significant with 2-AG levels at one month after trauma (r=0.55, p=0.042, Pearson correlation) (Figure 10).

In traumatized subjects, no correlations existed between 2-AG levels and amygdala volumes (Table 20).

Time point	Brain region		Trauma	N	Controls	N
48 hours after trauma	Vol. left amygdala	Correlation Coefficient	.07	11	-.48	13
		Sig. (2-tailed)	.84		.10	
	Vol. right amygdala	Correlation Coefficient	-.05	11	-.06	13
		Sig. (2-tailed)	.88		.84	
1 month after trauma	Vol. left amygdala	Correlation Coefficient	-.00	12	-.12	14
		Sig. (2-tailed)	.99		.68	
	Vol. right amygdala	Correlation Coefficient	.13	12	.03	14
		Sig. (2-tailed)	.70		.93	
6 months after trauma	Vol. left amygdala	Correlation Coefficient	-.78*	9	-.13	12
		Sig. (2-tailed)	.01		.69	
	Vol. right amygdala	Correlation Coefficient	-.23	9	.18	12
		Sig. (2-tailed)	.56		.57	

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).

Table 19: Correlations between anandamide plasma levels and amygdala volumes during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Brain region		Trauma	N	Controls	N
48 hours after trauma	Vol. left amygdala	Correlation Coefficient	.33	11	.05	13
		Sig. (2-tailed)	.32		.88	
	Vol. right amygdala	Correlation Coefficient	-.12	11	.26	13
		Sig. (2-tailed)	.74		.40	
1 month after trauma	Vol. left amygdala	Correlation Coefficient	.11	12	.551*	14
		Sig. (2-tailed)	.74		.041	
	Vol. right amygdala	Correlation Coefficient	.21	12	.550*	14
		Sig. (2-tailed)	.52		.042	
6 months after trauma	Vol. left amygdala	Correlation Coefficient	.07	9	-.10	12
		Sig. (2-tailed)	.86		.75	
	Vol. right amygdala	Correlation Coefficient	.07	9	.29	12
		Sig. (2-tailed)	.85		.37	

* .Correlation is significant at the 0.05 level (Pearson, 2-tailed).
**. Correlation is significant at the 0.01 level (Pearson, 2-tailed).

Table 20: Correlations between 2-AG plasma levels and amygdala volumes during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

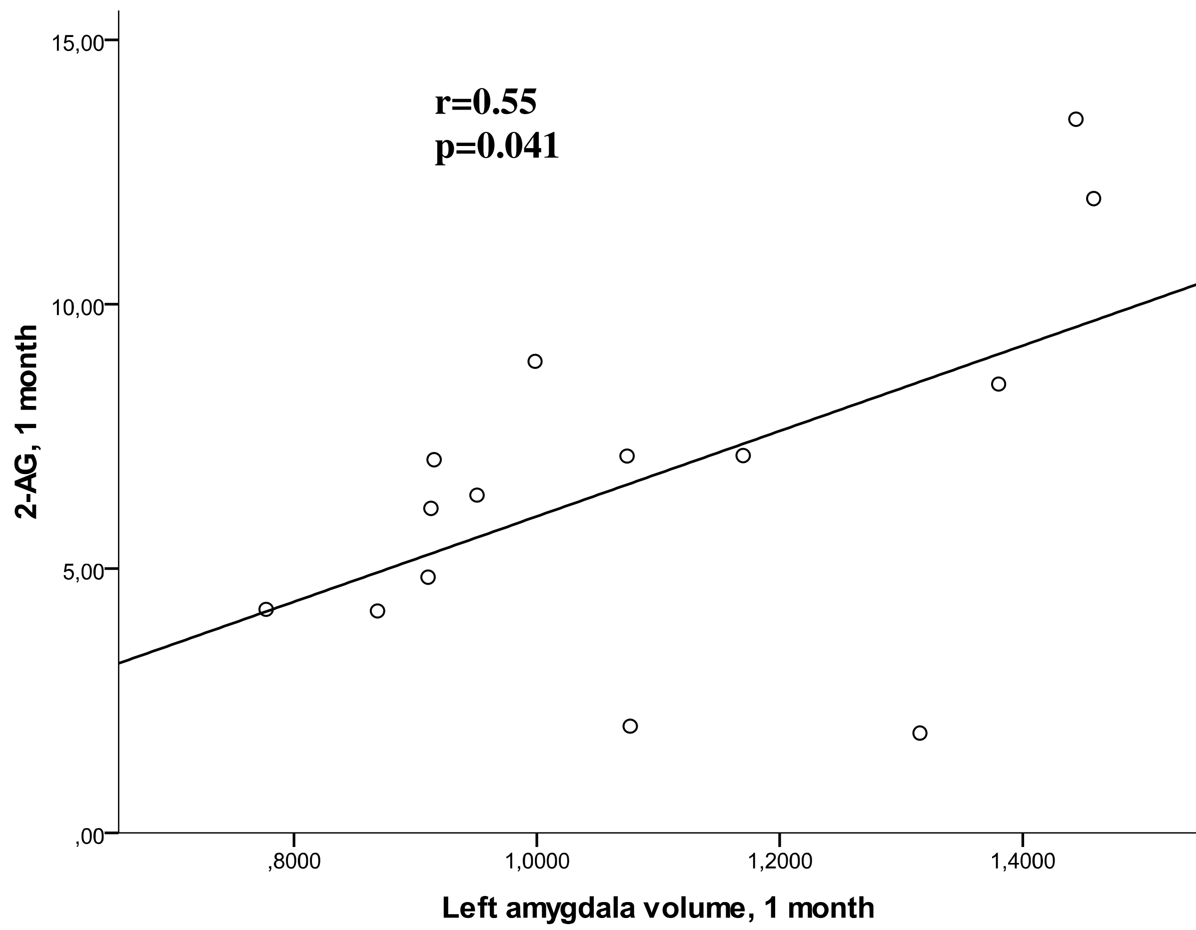


Figure 9: Scatterplot of 2-AG and left amygdala volume one month after trauma (controls)

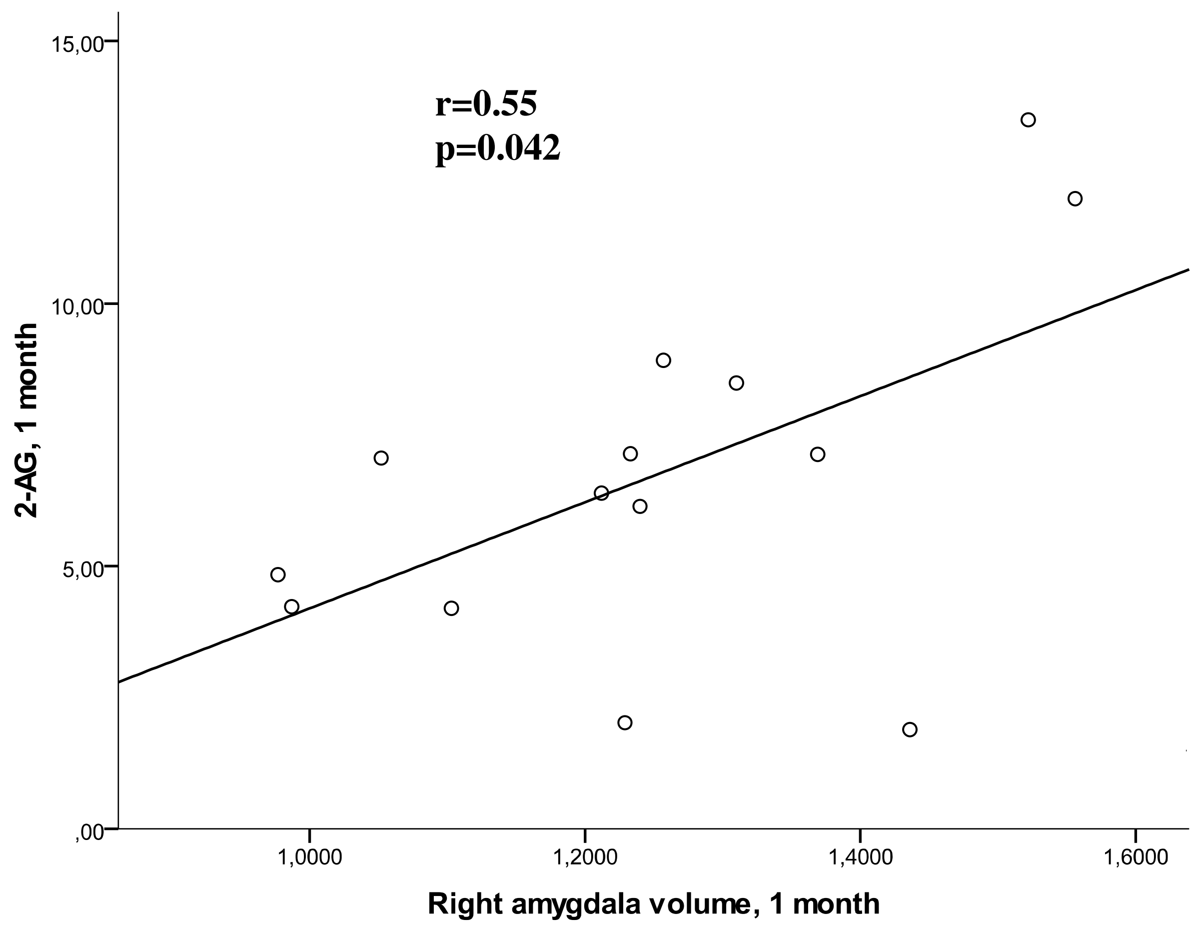


Figure 10: Scatterplot of 2-AG and right amygdala volume one month after trauma (controls)

No significant correlations were detected between plasma endocannabinoid levels and cingulum volumes (Table 21 + 22).

Time point	Brain region		Trauma	N	Controls	N
48 hours after trauma	Vol. left ant. cingulate	Correlation Coefficient	.003	11	-.152	13
		Sig. (2-tailed)	.992		.621	
	Vol. right ant. cingulate	Correlation Coefficient	-.007	11	.-.120	13
		Sig. (2-tailed)	.984		.696	
	Vol. left med. cingulate	Correlation Coefficient	-.209	11	-.246	13
		Sig. (2-tailed)	.537		.418	
	Vol. right med. cingulate	Correlation Coefficient	.332	11	-.143	13
		Sig. (2-tailed)	.319		.642	
	Vol. left post. cingulate	Correlation Coefficient	.173	11	-.112	13
		Sig. (2-tailed)	.612		.715	
	Vol. right post. cingulate	Correlation Coefficient	.370	11	-.157	13
		Sig. (2-tailed)	.262		.609	
1 month after trauma	Vol. left ant. cingulate	Correlation Coefficient	.000	12	-.105	14
		Sig. (2-tailed)	.999		.722	
	Vol. right ant. cingulate	Correlation Coefficient	-.064	12	-.126	14
		Sig. (2-tailed)	.844		.668	
	Vol. left med. cingulate	Correlation Coefficient	.169	12	-.150	14
		Sig. (2-tailed)	.599		.608	
	Vol. right med. cingulate	Correlation Coefficient	.221	12	-.103	14
		Sig. (2-tailed)	.491		.727	
	Vol. left post. cingulate	Correlation Coefficient	.462	12	-.259	14
		Sig. (2-tailed)	.131		.371	
	Vol. right post. cingulate	Correlation Coefficient	.153	12	-.351	14
		Sig. (2-tailed)	.634		.219	
6 months after trauma	Vol. left ant. cingulate	Correlation Coefficient	-.347	9	-.029	12
		Sig. (2-tailed)	.360		.929	
	Vol. right ant. cingulate	Correlation Coefficient	-.160	9	.-.093	12
		Sig. (2-tailed)	.680		.774	
	Vol. left med. cingulate	Correlation Coefficient	-.354	9	-.134	12
		Sig. (2-tailed)	.350		.678	

Vol. right med. cingulate	Correlation Coefficient	-.085	9	-.008	12
	Sig. (2-tailed)	.828		.981	
Vol. left post. cingulate	Correlation Coefficient	-.235	9	-.228	12
	Sig. (2-tailed)	.543		.476	
Vol. right post. cingulate	Correlation Coefficient	.096	9	-.361	12
	Sig. (2-tailed)	.807		.248	

Table 21: Correlations between anandamide plasma levels and cingulate volumes during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

Time point	Brain region		Trauma	N	Controls	N
48 hours after trauma	Vol. left ant. cingulate	Correlation Coefficient	-.158	11	.170	13
		Sig. (2-tailed)	.642		.578	
	Vol. right ant. cingulate	Correlation Coefficient	-.285	11	.125	13
		Sig. (2-tailed)	.396		.684	
	Vol. left med. cingulate	Correlation Coefficient	.016	11	.126	13
		Sig. (2-tailed)	.964		.682	
	Vol. right med. cingulate	Correlation Coefficient	.158	11	.148	13
		Sig. (2-tailed)	.642		.629	
	Vol. left post. cingulate	Correlation Coefficient	.128	11	.150	13
		Sig. (2-tailed)	.708		.625	
	Vol. right post. cingulate	Correlation Coefficient	.082	11	-.077	13
		Sig. (2-tailed)	.811		.802	
1 month after trauma	Vol. left ant. cingulate	Correlation Coefficient	-.011	12	.230	14
		Sig. (2-tailed)	.972		.428	
	Vol. right ant. cingulate	Correlation Coefficient	.033	12	.272	14
		Sig. (2-tailed)	.920		.346	
	Vol. left med. cingulate	Correlation Coefficient	.128	12	.259	14
		Sig. (2-tailed)	.691		.372	
	Vol. right med. cingulate	Correlation Coefficient	.060	12	.446	14
		Sig. (2-tailed)	.852		.110	
	Vol. left post. cingulate	Correlation Coefficient	.401	12	.293	14
		Sig. (2-tailed)	.197		.310	
	Vol. right post. cingulate	Correlation Coefficient	-.007	12	.046	14
		Sig. (2-tailed)	.982		.876	
6 months after trauma	Vol. left ant. cingulate	Correlation Coefficient	.042	9	.092	12
		Sig. (2-tailed)	.915		.776	
	Vol. right ant. cingulate	Correlation Coefficient	.233	9	.047	12
		Sig. (2-tailed)	.546		.886	
	Vol. left med. cingulate	Correlation Coefficient	.113	9	.012	12
		Sig. (2-tailed)	.772		.971	
	Vol. right med. cingulate	Correlation Coefficient	.596	9	.175	12
		Sig. (2-tailed)	.090		.586	
	Vol. left post. cingulate	Correlation Coefficient	.103	9	.025	12

		Sig. (2-tailed)	.792		.938	
	Vol. right post. cingulate	Correlation Coefficient	.195	9	.002	12
		Sig. (2-tailed)	.616		.996	

Table 22: Correlations between 2-AG plasma levels and cingulate volumes during the 6-months follow-up period after trauma. Traumatized subjects are compared to non-traumatized controls.

5. Discussion

A summary of the results as well as the method is discussed summarizing the statistically significant data. We will show the limitations of our study. We will point out future research perspectives which appear to be particularly interesting in relation to the role of the endocannabinoid system in the pathophysiology of psychic trauma, coping and resilience.

5.1 Brief summary of results

A traumatized and a non-traumatized group of 14 subjects were investigated at three time points following type I trauma. One month after trauma six subjects were free of symptoms and after six months thirteen subjects were free of symptoms. A statistically significant difference between the traumatized and the non-traumatized group was found for 2-AG one month after trauma. Statistically significant negative correlations existed between hippocampal volumes and anandamide levels of traumatized subjects after six months, as well as significant positive correlations between amygdala volumes and 2-AG levels of non-traumatized one month after trauma.

5.2 Discussion of methods

5.2.1 Number of participants and types of trauma

All traumatized subjects had experienced a type I trauma, although the trauma types were heterogeneous. It is important to differentiate the type of stress investigated - whether somatic, psychic or somato-psychic stress was investigated. Eleven cases were sole psychic traumata, whereas three cases consisted of traumata involving physical injuries in addition. Three of the traumatized subjects had sustained mild to severe physical injuries additionally to the psychic stress they underwent. This might have influenced endocannabinoid levels, thus restricting the expressiveness of our results. A completely homogeneous study sample, based on sole psychic traumatized subjects would be more desirable for future studies of the endocannabinoid system after psychic trauma. It is known that the endocannabinoid system is independently activated as a result of traumatic injury itself (Garcia-Ovejero et al. 2009; Kaufmann et al. 2009). Seven of our traumatized subjects had a history of past trauma, which might have of course influenced reactions of the endocannabinoid system, since past trauma can modify the endocannabinoid response.

The non-traumatized control group did not statistically differ from the traumatized group in terms of age, sex, psychiatric history, family history or history of addiction. For some of the investigations at different points of time, N varies between 9 and 14; as the participants did not appear at some of the appointments, although they were reminded of their appointments by phone calls shortly before. In future investigations, reasons for not-appearing of the subjects should be analyzed. Moreover, the comparison with the controls can be discussed in relation to environmental ascendancies on the endocannabinoid system which could not be identified, as the endocannabinoid system is involved in the regulation of multiple physiological responses.
We regard our study as a pilot experiment, hence the small number of subjects, which needs to be studied on greater sample numbers in future.

5.2.2 Endocannabinoid measurement

Endocannabinoid measurement was conducted by using high performance liquid chromatography-tandem mass spectrometry (HPLC / MS-MS), which was developed by Vogeser et al (Vogeser et al. 2006). Pitfalls in measuring 2-AG in human plasma, arising from the circumstance that 2-AG undergoes rapid non-enzymatic isomerization to biologically inactive 1-AG were circumvented as recommended by Vogeser and Schelling (Vogeser et al. 2007). Future endocannabinoid measurements should be accomplished at the same time of the day since there is a relationship between endocannabinoid signalling and circadian processes (Vaughn et al. 2010). Vaughn et al. described tissue-specific diurnal changes in the amounts of endocannabinoids, their degradative and synthetic enymes as well as their receptors. Two patterns were found with regard to anandamide concentrations. In hippocampus (Valenti et al. 2004), among other regions, anandamide concentrations are higher during active periods of rats than during inactive periods. An opposite pattern has been observed in CSF and hypothalamus, where anandamide concentrations are higher in inactive periods (Murillo-Rodriguez et al. 2006). Remarkably, tissue contents of 2-AG was found to be in reverse relation to anandamide tissue contents; 2-AG concentrations were higher during inactive periods in hippocampus among others (Valenti et al. 2004). In a small pilot study, Vaughn et al. explored the circadian rhythms of circulating endocannabinoids in humans. Plasma was obtained in the late evening, early morning and early evening from five healthy humans who had consistent sleep / wake cycles for at least five days prior to sampling. These results indicated that lack of normal sleep produces a significant dysregulation of circulating AEA

(Vaughn et al. 2010). Thus, the circadian rhythm of endocannabinoids should certainly be taken into account in future studies.
Regarding the validity of the peripheral (*plasma)* endocannabinoid levels with regard to effects of endocannabinoids in the *central* nervous system it is difficile to assess this, as no papers dealing with this topic were found. Studies so far have solely measured peripheral endocannabinoid levels. Naturally, this would be an important issue for further research.

5.2.3 Data processing for volumetric data analysis

Discussion of data processing for volumetric data analysis will be treated in the doctor´s thesis of the colleague Dino Santangelo de Souza.

5.3 Discussion of results

To our knowledge, this is the first prospective study investigating endocannabinoid levels after type I trauma in relation to the psychological sequelae and structural changes in brain candidate regions involved in the formation of traumatic memories.
Objective of this study was to investigate the influence of one-time psychic stress on the human endocannabinoid system over the course of time. It should be envisioned that research in animal models up to date has investigated the endocannabinoid system after stress solely at one point of time. Our findings span three points of time after a single psychic trauma.

Diagnoses at the three points of time were distributed as expected. All traumatized subjects exhibited an acute stress disorder immediately after trauma. After one month there were four traumatized with a fully blown PTSD, whereas six months post trauma only one subject remained diagnosed of PTSD.
Similarly, CAPS, HAMD and BDI scores turned out as anticipated. In traumatized subjects, scores decreased with increasing time after the trauma. For controls, scores amounted to no pathological results.
Concerning endocannabinoid levels, there was a statistically significant difference in 2-AG levels between the traumatized group and the non-traumatized group one month after trauma. Furthermore, a difference was observed when 2-AG levels of traumatized subjects were compared one and six months after trauma. Another significant correlation existed between 2-AG levels and left / right amygdala volumes in controls.

5.3.1 Implications for our endocannabinoid results

2-AG levels were found to be significantly higher in traumatized subjects compared to controls one month after trauma, whereas anandamide levels showed no statistic differences between groups. Interestingly, this pattern is consistent with the result of Hill et al. in women with major depression (Hill et al. 2009) after social stress. Social stress was administered by the Trier Social Stress Test, which consists of a public speaking task (mock job interview), after subjects have been told that their session will be video- and audiotaped for subsequent analysis of paraverbal and nonverbal signs of stress. This paradigma has been shown to induce significant and consistent endocrine responses (Kirschbaum et al. 1995). It is self-evident that the type of social stress in the Trier Social Stress Test cannot be compared to the stress of our subjects, who experienced a psychic trauma. Still it is remarkable that results in these two investigations are so congruent, which are the only studies on this topic in humans. Further studies examining different types of stress in humans are required.

In this context it is an important question whether the reaction of the endocannabinoid system to stress is *specific,* depending on the type of stressor, or if it is a *generalized* reaction, independent of the type of stressor. In other words, if different patterns of changes in endocannabinoid levels occur depending on the type of stressor, or if one pattern of changes occurs independently of the kind of stressor. Naturally, the hypothesis of a stressor-dependent *specific* reaction is much more likely. One explanation for the partly inconsistent results described in literature may lie in this aspect – the stressors applied were not homotypic, but mostly differed from study to study.

In our study, endocannabinoid levels were measured in peripheral blood (plasma). Endocannabinoids play a crucial role in the central nervous system and are also synthesized and metabolized in the central nervous system. For comparable results it might be of importance whether peripheral or central nervous system levels were measured and the location of the sampling for the endocannabinoid determinations in the brain.

The difference observable between 2-AG levels in traumatized subjects one and six months after trauma is consistent with the hypothesis that endocannabinoids act on-demand as a protective mechanism, being released after a stressful stimulus to inhibit its consequences (Resstel et al. 2009). This corresponds to the findings that six months after trauma there was no difference in 2-AG levels between traumatized subjects and controls anymore, and thirteen out of fourteen traumatized had fully recovered after six months. Resilience to stress-related disease and dysfunction may depend, at least in part, on the physiological integrity and proper functioning of the endocannabinoid system, as described in previous studies (Finn 2010).

5.3.2 Endocannabinoids levels, neuroplasticity within hippocampus / amygdala and trauma

So far, mainly animal studies are available for interpreting our results concerning the relation between anandamide / 2-AG levels and hippocampal / amygdala plasticity,. Study results of the endocannabinoid system and its role for regulating processes in hippocampus, amygdala as well as in depressive disorders will be propounded as a theoretical foundation for interpreting our results.

An impact of endocannabinoids for neurogenesis is assumed by the finding that diacylglycerol lipase (diacylglycerol lipase synthesizes 2-AG) knock-out mice show reduced neurogenesis, including neurogenesis in the hippocampus (Gao et al. 2010). There is data demonstrating that the endocannabinoid system in the hippocampus is sensitive to environmental change and suggesting that this system serves as a mediator for experience-induced plasticity (Hill et al. 2010). Further data of Hill et al. imply that endocannabinoids may play a complex role in the regulation of neurogenesis via cell proliferation in the hippocampus (Hill et al. 2006).

Concerning hippocampal plasticity after psychic traumatization, smaller hippocampal volumes are discussed either as a susceptibility factor for PTSD or as a consequence of trauma. A recent study dealing with this problem in a mouse model of PTSD showed in summary that traumatic experience in mice causes a reduction in hippocampus volume (Golub et al. 2011). Similarly, a study with 21 participants with PTSD and 17 trauma-exposed controls revealed a significant negative correlation between right hippocampal volume and PTSD duration, implying that duration of PTSD predicts hippocampal grey matter loss (Felmingham et al. 2009). In Vietnam veterans with PTSD, hippocampal volume was directly negatively correlated with combat exposure; implying that traumatic stress might lead to hippocampus atrophy (Gurvits et al. 1996).

On the other hand, there is data of an identical twin study supporting the contrary notion, in which disorder severity in PTSD patients was negatively correlated with the hippocampal volume of both the patient and the patient´s non-traumatized identical twin (Gilbertson et al. 2002). Similarly, Bonne et al. observed that a smaller hippocampal volume was not a necessary risk factor for developing PTSD and did not occur within six months during the course of PTSD (Bonne et al. 2001). A recent meta-analysis concluded that hippocampal volume reduction is associated with trauma exposure, independent of PTSD diagnosis (Woon et al. 2010).

The brain seems to have capacity for plasticity in the aftermath of traumatic stress. Antidepressant treatment and changes in environment can reverse the sequelae of stress on hippocampal neurogenesis, this was investigated on humans with PTSD showing increased hippocampal volume after treatment with both paroxetine or phenytoin (Bremner et al. 2008). In an animal study, long-term treatment with tricyclic antidepressants lead to an increase of CB1 receptor density in the region of hippocampus and hypothalamus (Hill et al. 2006). In CB1 knockout mice, the lack of CB1 receptors was associated with an enhanced response to stress and deficiency in neuronal plasticity by decreasing brain derived neurotrophic factor (BDNF) levels in the hippocampus (Aso et al. 2008). Recent literature also report cannabinoid agonists and enhancers to increase serotonin release in the hippocampus besides enhancing noradrenergic neuronal firing and activity promoting neurogenesis (Bambico et al. 2009).

Regarding the connection of hippocampus with emotional stress, particularly childhood stress, a study by Frodl et al. compared 43 patients with major depression and 44 age as well as gender matched healthy controls using high-resolution MRI (hippocampus, whole brain voxel-based morphometry) and assessment of childhood stress. Results were inter alia that left hippocampal white matter was smaller in depressive patients, who had experienced emotional neglect, compared to those without neglect. Especially interesting, childhood stress and brain structure volumes independently predicted a non-favourable course of depression (Frodl et al. 2010). Considering depressive episodes as one separate form of stress, another investigation of Frodl et al. is noteworthy: 38 patients with major depression and 30 healthy controls were examined with high-resolution MRI at baseline and three years later. In depressive patients, grey matter density of the hippocampus was significantly reduced compared to controls. Patients who had a remission during the three year period had less volume reduction than nonremitted patients in the left hippocampus. Thus, these results point to neuroplastic stress-related processes that occur in the hippocampus during depressive episodes (Frodl et al. 2008). There is also data supporting the hypothesis that mechanisms resulting in reduced hippocampal volumes could be related to the development of major depression (Frodl et al. 2006) and that the hippocampus with its connections within limbo-cortical networks may play a crucial role in the pathogenesis of major depression (Frodl et al. 2002).

Regarding the role of the endocannabinoid system in depressive disorders, changes in the hippocampal endocannabinoid system after stress in relation to the onset of treatment in depression were found in rats: McLaughlin et al. implanted bilaterally cannulae directed at the dentate gyrus of the hippocampus and administered infusions of either CB 1 receptor agonist,

fatty acid amide hydrolase inhibitor, or CB 1 receptor antagonist. Afterwards assessment during the forced swim test was conducted. Results indicate that activation of CB 1 receptors in the dentate gyrus of the hippocampus results in an antidepressant-like response (increase in swimming behaviour) (McLaughlin et al. 2007). In an animal model of chronic unpredictable stress, downregulation of endocannabinoid signalling in the nucleus accumbens was measured and contributed towards the pathophysiology of depression (Wang et al. 2010). In summary, the majority of animal models proposes reduced endocannabinoid levels in depressive disorders.

The majority of studies report a hyper-reactivity in PTSD patients (Damsa et al. 2005) for the amygdale. A meta-analysis of Woon et al. came to the conclusion that amygdala volumes are asymmetrically lateralized after trauma exposure and in PTSD (Woon et al. 2009). A mediating role for CB1 receptors in the right basolateral amygdala has been suggested regarding short-term extinction of conditioned aversive behaviour (Roche et al. 2007). They demonstrated that repeated stress elevates 2-AG levels and enhances short-term endocannabinoid signalling at inhibitory synapses in basolateral amygdala in mice (Patel et al. 2009). Akin, the central amygdala was found to be an important neural substrate subserving the interactions between cannabinoids and environmental stress (Patel et al. 2005).
With regard to the influence of the endocannabinoid system on brain structures, a neuroprotective effect of endocannabinoids was described following traumatic brain injury in rodents (Mechoulam et al. 2002), as well as an essential role of CB1 proteins in the central nucleus of the amygdala for acute fear adaptation of mice (Kamprath et al. 2011).

5.3.3 Implications of brain volume and endocannabinoid results

Anandamide plasma levels showed strong negative correlations with left and right hippocampal volumes in traumatized subjects six months after trauma which was absent in controls. To our knowledge, this is the first study investigating the interaction between endocannabinoid levels and the hippocampal volume after psychic trauma. The fact, that correlations were found exclusively in the traumatized group, and not in the non-traumatized controls, suggests that a psychic trauma may initiate changes in both systems which may possibly be interrelated. This interpretation is consistent with the data from animal studies presented above.

Significant positive correlations were found between amygdala volumes and 2-AG levels of non-traumatized one month after trauma. This can be interpreted in two variants: either 1. this is an incidental finding, as the the correlative analyses were not Bonferroni corrected and false positive findings are possible, or 2. this is the manifestation of an anyway existing nexus between endocannabinoid levels and amygdala volumes, which is either attributable to circumstances not known to us with effect on the endocannabinoid system or is not detected at other points of time due to the high variance in the small sample size. Supporting the second assumption is the fact that correlations have been found bilaterally for right and left amygdala. In our sample no correlations were found between endocannabinoids and anterior, medial and posterior cingulate volumes. Perhaps the small sample size might be the reason why no correlations between peripheral endocannabinoid levels and cingulum volumes were found. Remarkably little is known about the relationship of the endocannabinoid system and the cingulate cortex.

One study regarding this topic describes decreased CB 1 receptor immunopositive glial cells in the grey matter of postmortem major depression patients. (Koethe et al. 2007). The study in CB 1 receptor knockout mice shows evidence of the involvement of CB 1 receptors in the control of GABAergic responses suggests a role of the endocannabinoid system in the modulation of anxiety-related disorders (Uriguen et al. 2011).

Another aspect which needs to be taken into account is the circumstance that the animal studies conducted are based on partly mouse or rat models, as mentioned above. Species differences have been established for endocannabinoid level changes and effects for rats and mice. Therefore, results of animal studies should only be extrapolated with caution to humans. Finally, the dose-dependent impact of endocannabinoids has to be postulated when discussing endocannbinoid levels and brain volume changes. A dose-effect relationship for endocannabinoid levels and anxiety was suggested by Rubino et al. (Rubino et al. 2008) with even contrary effects depending on the dose of endocannabinoids. Similarly, it is possible that a concentration dependent association exists between endocannabinoid levels and changes of hippocampus and amygdale volumes.

5.4 Future perspectives

In summary, this study investigating changes of peripheral endocannabinoid levels and volumes of key regions involved in the pathophysiology of traumatic memories during six months after a single psychic trauma yields interesting results, which are mainly in line with

the conclusions of animal models. In view of the fact that to our knowledge this is the first study exploring the endocannabinoid system in healthy humans after psychic stress, future studies with larger samples and an assessment of further clinical variables (e.g. aversive memories), other endocrine stress responses (e.g. HPA axis), additional brain areas, separate investigations of brain volumes (MRI) versus function (fMRI) and different types of traumatic events (e.g. type 1 vs. type 2 trauma) or stressful life-events (e.g. single, repeated or chronic) would be worthwhile.

In future studies, endocannabinoid levels should probably be measured at a standardized time of each day, taking the putative circadian rhythm of endocannabinoid levels into account (Vaughn et al. 2010). Moreover, there is evidence of an influence of gender on endocannabinoid mediated effects (Atkinson et al. 2010). Hence, results of males and females should perhaps be interpreted separately. Furthermore, the question of *peripheral* endocannabinoid measurements versus validity for effects in the *central* nervous system should be incorporated in future studies.

Furthermore, the type of stressor should be standardized and as homotypic as possible with regard to somatic versus psychic stress, intensity, predictability and duration.

It would also be interesting to correlate endocannabinoid levels with the degree of extinction of aversive memories in future studies. In order to scrutinize existing and future related studies for the treatment of PTSD, further clinical trials are necessary which investigate the potential therapeutic efficacy of drugs that enhance endocannabinoid levels in anxiety disorders including PTSD (Finn 2010).

6. Conclusion

As a concluding remark it can be stated that the endocannabinoid system is an intriguing regulatory system, which is still in its infancy regarding the theoretical knowledge about its nature and interaction with other neurotransmitters, brain regions and behavioral consequences. Anyhow it appears to play a pivotal role in the neural response and plasticity after stress, and in consequence it modifies pathophysiological processes which occur in conditions like PTSD, anxiety, depression as well as it may exert a neuroprotective function. Most probably it can contribute to a psychophysiological equilibrium only as long as it works within a physiological range. Regarding research data, it is salient that studies are preponderantly based on animal models. Our investigation provides first data about changes in endocannabinoid regulation after psychic stress in humans, and future studies should aim at revealing more detailed information about different endocannabinoids, their homeostatic role and specifity. Potential implications for prophylactic or therapeutic use after stress respectively trauma seem promising.

7. Summary

The endocannabinoid system has emerged as one of the most important facilitators of stress adaptation in the body. So far, little to no research took place concerning the endocannabinoid response to stress in humans. In this study, we investigated the influence of a type I trauma on endocannabinoid plasma levels in humans over a period of 6 months, compared with non-traumatized controls. We measured endocannabinoid plasma levels (using high performance liquid chromatography-tandem mass spectrometry) as well as hippocampal and amygdala volumes in fourteen participants who had experienced a psychic trauma and developed an acute stress disorder. Fourteen healthy non-traumatized age- and gender-matched controls were studied in comparison over a 6 months period. Data were collected at three points of time: within 48 hours after the traumatic event, after one month and after six months. At each point of time a psychiatric interview was conducted and the Clinician Administered PTSD Scale (CAPS), HAMD and BDI were rated. When 2-Arachidonoylglycerol (2-AG) levels were compared between traumatized subjects and non-traumatized controls, there was a statistical significant difference one month after trauma ($p=0.046$). Regarding endocannabinoid levels over the course of time, 2-AG decreased in the trauma group between one month and six months after the initial trauma. This finding was in line with the hypothesis that endocannabinoids act on-demand as protective mechanism, being released after a stressful stimulus to inhibit the development of posttraumatic sequelae. In traumatized subjects, anandamide levels after six months showed a strong negative correlation with hippocampal volumes at this time point. A statistically significant negative correlation between left amygdala volume at six months and anandamide level at this time point in traumatized subjects was found. In non-traumatized controls, remarkably, there were positive correlations of 2-AG levels and amygdala volumes one month after trauma, which were not detected at the other time points. In contrast, endocannabinoid levels were neither correlated with any demographic and clinical variable, nor with volumes of cingulate regions.

In conclusion, the results of our study point to a delayed and possibly regulatory response of the endocannabinoid system after a single psychic trauma over the course of six months in humans. Moreover, the data point to a genuine relation of peripheral endocannabinoid levels possibly reflecting central endocannabinoid activity and neuroplasticity in brain key regions involved in the generation of traumatic memories, i.e. hippocampus and amygdale.

8. Zusammenfassung

Das Endocannabinoidsystem hat sich als eines der bedeutendsten Systeme hinsichtlich der Anpassung an Stress in biologischen Systemen erwiesen. Bislang liegen wenig bis keine Forschungsergebnisse zu Veränderungen des humanen Endocannabinoidsystem nach psychischem Stress vor. In vorliegender Studie untersuchten wir im Vergleich mit untraumatisierten Kontrollprobanden über einen Zeitraum von sechs Monaten den Einfluss eines Typ–I-Traumas auf menschliche Endocannabinoidkonzentrationen im Plasma. Wir bestimmten die Endocannabinoidwerte im Plasma (mittels einer Kombination von Hochdruck-Flüssigkeitschromatographie und einem massenspektrometrischen Tandemdetektionsverfahren) als auch Hippocampus- und Amygdalavolumina bei 14 Probanden, die ein psychisches Trauma innerhalb 48 Stunden vor Rekrutierung erlebt hatten, und eine akute Belastungsreaktion entwickelt hatten. Zum Vergleich wurden 14 untraumatisierte alters- und geschlechtsgematchte Kontrollprobanden über einen Zeitraum von sechs Monaten untersucht. Die Daten wurden zu drei Unterschungszeitpunkten erhoben: innerhalb von 48 Stunden nach dem Trauma, einen Monat später und sechs Monate später. Zu jedem Untersuchungszeitpunkt wurde ein „Clinician Administered PTSD Scale“ Interview (CAPS) durchgeführt, sowie HAMD und BDI Skalen erhoben. Es gab einen statistisch signifikanten Unterschied zum Zeitpunkt 1 Monat nach Trauma bei den 2-AG Werten zwischen den traumatisierten Probanden und untraumatisierten Kontrollprobanden (p=0,046). Bezüglich der Endocannabinoidwerte über den Zeitverlauf kam es zu einem Abfall von 2-AG in der Gruppe der traumatisierten Probanden zwischen einem Monat und sechs Monaten nach dem initialen Trauma. Dieser Befund stimmte mit der Hypothese überein, dass Endocannabinoide bedarfsorientiert nach dem Auftreten eines Stressstimulus als protektiver Mechanismus freigesetzt werden, um die Entwicklung posttraumatischer Folgen zu begrenzen. Bei den traumatisierten Probanden korrelierten die Anandamidwerte zum Zeitpunkt sechs Monate nach Trauma hochsignifikant negativ mit den Hippocampusvolumina zu diesem Zeitpunkt. Eine statistisch signifikante negative Korrelation fand sich bei den traumatisierten Probanden zwischen linkem Amygdalavolumen nach sechs Monaten und Anandamidwerten zu diesem Zeitpunkt. Bei den untraumatisierten Kontrollprobanden gab es bemerkenswerterweise positive Korrelationen zwischen 2-AG Werten und den Amygdalavolumina nach einem Monat, die zu den anderen Zeitpunkten nicht festgestellt wurde. Im Gegensatz dazu gab weder Korrelationen von Endocananbinoidwerten mit den demographischen bzw. klinischen Variablen, noch mit den Cingulumvolumenwerten.

Abschließend lässt sich sagen dass die Ergebnisse unserer Studie über den Zeitraum von sechs Monaten auf eine verzögerte und möglicherweise regulierende Antwort des Endocannabinoidsystems auf ein einmaliges psychisches Trauma beim Menschen hinweisen. Zudem deuten die Daten auf einen echten Zusammenhang der peripheren Endocannabinoidspiegel hin, der möglicherweise die Beteiligung der Aktivität des Endocannabinoidsystems im ZNS und der Neuroplastizität in Schlüsselhirnregionen, sprich in Hippocampus und Amygdala an der Formation traumatischer Erinnerungen, widerspiegelt.

9. Appendix

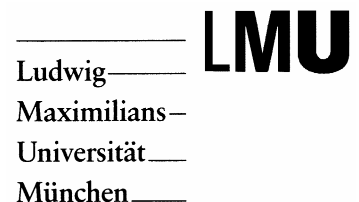
9.1 Information sheet for participants and informed consent

Klinikum der Universität München

Klinik und Poliklinik für Psychiatrie und

Psychotherapie-Innenstadt

Direktor: Prof. Dr. med. Hans-Jürgen Möller



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Probanden-Informationsblatt

für die Teilnahme an der Studie:

Akute Belastungsreaktion und psychische Traumatisierung – Untersuchungen zur Neurobiologie und neuronalen Konnektivität im Verlauf

Sehr geehrte Probandin, sehr geehrter Proband,

Sie haben sich zur Teilnahme an unserer Probandenstudie entschieden. Im folgenden Abschnitt wollen wir Sie über Fragestellung, Ablauf und mögliche Nebenwirkungen der geplanten Untersuchung ausführlich informieren.

1. Fragestellung der Studie:

In der vorliegenden Studie geht es darum, strukturelle Veränderungen im Gehirn von Patienten darzustellen, die ein traumatisches Ereignis erlebt haben. Hierbei gilt das Hauptinteresse dem Mandelkern und dem Hippokampus. Dies sind Hirnregionen, die für die Verarbeitung emotionaler Inhalte wichtig sind und die durch das körpereigene Hormon Cortisol beeinflusst werden. Es gibt bereits Hinweise darauf, dass Patienten die in Folge

eines traumatischen Ereignisses an einer so genannten posttraumatischen Belastungsstörung erkranken, Auffälligkeiten in diesen Hirnarealen aufweisen.
Zum anderen gibt es Hinweise, dass erhöhte Cortisol-Spiegel im Blut unmittelbar nach einem traumatischen Erlebnis das Risiko vermindern, später eine posttraumatische Belastungsstörung (Post Traumatic Stress Disorder, PTSD) zu entwickeln.
Anfang der neunziger Jahre wurde eine neue Gruppe körpereigener Botenstoffe, die so genannten Anandamide oder auch Endocannabinoide, entdeckt. Diese Botenstoffe spielen bei körpereigenen Regulationsprozessen der Kognition, der Schmerzwahrnehmung, der Stimmungsregulation und dem Gedächtnis eine Rolle. Die bisherige klinische Erfahrung deutet auf einen Zusammenhang dieses körpereigenen Botenstoffes bei der Verarbeitung traumatischer Erfahrungen hin.
In der vorliegenden Studie sollen nun diese körpereigenen Prozesse zum einen mittels einer bildgebenden Technik und zum anderen mittels Bluttests untersucht werden. Die in den Untersuchungen gewonnenen Erkenntnisse sollen dabei helfen, psychische Belastungsfolgen zukünftig differenzierter zu erkennen und therapeutische Ansätze zu entwickeln.

2. Ablauf der Untersuchung:

ZUR BILDGEBENDEN TECHNIK: Bei der sog. DTI-Untersuchung werden die oben angeführten Hirngebiete dargestellt. Diese Untersuchung ist eine Weiterentwicklung der Ihnen möglicherweise bekannten Kernspintomographie (MRT) die mit Hilfe von magnetischen Feldern durchgeführt wird und somit ohne Strahlenbelastung für Sie ist.
Es werden insgesamt maximal 3 DTI-Untersuchungen durchgeführt. Die erste soll innerhalb von 48 h nach dem stattgefundenen Trauma und die zweite nach vier Wochen durchgeführt werden. Sollte es bei Ihnen zur Ausbildung einer posttraumatischen Belastungsstörung kommen, so wird nach einem halben Jahr (6 Monate) die dritte Untersuchung durchgeführt.
Die DTI - Untersuchung wird jeweils etwa 30 Minuten dauern. In der Zeit dazwischen werden verschiedene psychologische Tests durchgeführt werden. Diese dienen dazu, begleitende psychiatrische Erkrankungen zu erkennen, Ihren momentanen psychischen Zustand zu erfassen und eine evtl. familiäre Belastung darzustellen.
ZUM BLUTTEST: Hierbei sollen mit Hilfe der Untersuchung der Stimulierbarkeit des Streßhormons Cortisol weitere Informationen über gestörte Regelsysteme bei psychiatrischen Erkrankungen erhalten werden. Zu diesem Zweck wird zusätzlich zu den klinisch notwendigen Routineuntersuchungen eine Hormonuntersuchung, der so genannte Low dose Dexamethason Suppressionstest (abgekürzt Low-dose DEX-Test) durchgeführt.
Im Rahmen dieser Untersuchung, die seit vielen Jahren an einer großen Zahl von Patienten in psychiatrischen Kliniken durchgeführt wurde, wird Ihnen am Vortag der Untersuchung

zunächst um 8.00 Uhr 10 ml Blut entnommen. Um 23.00 Uhr nehmen Sie eine Tablette 0,5mg Dexamethason einmalig ein. Dexamethason ähnelt einem körpereigenen Hormon, dem Streßhormon Cortisol. Nach einer einmaligen Einnahme sind keine Nebenwirkungen zu erwarten. Am folgenden Tag werden Ihnen um 8.00 Uhr und um 16.00 Uhr jeweils 10ml Blut entnommen. Diese Untersuchung kann Ihnen im weiteren Behandlungsverlauf noch ein weiteres Mal vier Wochen und gegebenenfalls sechs Monate nach dem Trauma empfohlen werden.
Um die Rolle und die Funktion der körpereigenen Anandamide genauer zu untersuchen werden Ihnen im Rahmen der ersten Blutentnahme für den Low-dose DEX-Test und dann nochmals zu den Nachsorgeterminen während der ersten vier Wochen und ggf. erneut nach sechs Monaten jeweils 5 ml Blut entnommen.
Unabhängig von der Studienteilnahme werden die in unserem Hause üblichen Laboruntersuchungen einschließlich Drogenscreening bei Aufnahme und im Behandlungsverlauf durchgeführt. Ihre behandelnde Ärztin / ihr behandelnder Arzt wird Ihnen diese gerne näher erläutern. Die Ergebnisse dieser Untersuchung haben auf die Behandlung Ihrer Erkrankung keinen unmittelbaren Einfluß.

3. Mögliche Nebenwirkungen und Risiken:

Die bildgebende Untersuchung ist für Sie mit keiner Strahlenbelastung verbunden. *Schädigende Wirkungen der Kernspintomographie sind nach dem derzeitigen Stand der Wissenschaft nicht bekannt. Eine Gabe von Kontrastmittel ist nicht nötig. Einige Patienten können mit dieser Methode nicht untersucht werden. Dies betrifft Patienten die einen Herzschrittmacher haben, in deren Körper sich Metallteile befinden, die spezielle Tätowierungen oder permanentes Make-up haben. Auch bei Schwangeren sowie bei Patienten die an schwerer Platzangst leiden sollte diese Untersuchung nur nach Rücksprache mit dem Arzt durchgeführt werden.* Die Bluttests sind für Sie mit keinem Risiko verbunden, dass über das Risiko einer normalen Blutentnahme hinausgeht. Falls Ihr klinischer Zustand sich verschlechtert oder andere schwerwiegende Beschwerden auftreten sollten, wird die Studie umgehend abgebrochen.
Falls bei Ihnen körperlicher Erkrankungen oder eine Schwangerschaft besteht, teilen Sie dies bitte umgehend dem Prüfarzt mit. Teilen Sie dem Prüfarzt bitte umgehend mit, ob sie dauerhaft oder auch nur vorübergehend ein Medikament eingenommen haben.

4. Versicherungsschutz

Es muss für diese Studie keine Versicherung für die Probanden abgeschlossen werden, da die Studie nicht unter das Arzneimittelgesetz fällt und die Risiken im Rahmen der Studie nur gering sind. Für sonst übliche Risiken wie z.B. Wegeunfälle besteht für Sie

Versicherungsschutz bei der Firma Gerling (Adresse) unter der Versicherungsnummer 22-7271666. Im Schadensfall können Sie sich direkt an den Versicherer wenden und Ihre Ansprüche selbständig geltend machen. *Bitte unterziehen Sie sich während der Dauer der Studie einer anderen medizinischen Behandlung nur im Einvernehmen mit den Untersuchungsleitern; ausgenommen davon sind Notfälle*. Dies gilt auch für die zusätzliche Einnahme von Medikamenten. Auch müssen Sie eine Gesundheitsschädigung, die als Folge der klinischen Prüfung eingetreten sein könnte, den Untersuchungsleitern und der oben genannten Versicherungsgesellschaft unverzüglich mitteilen. Falls Sie während der Teilnahme an der Studie eine Gesundheitsschädigung erleiden, wird die studiendurchführende Klinik jegliche medizinische Behandlung sowie eine eventuelle Überweisung an geeignete medizinische Einrichtungen sicherstellen.

5. Vertraulichkeit/Datenschutz:

Die im Rahmen der Studie erhobenen Daten zu Ihrer Person und zu Ihrem Befund werden in verschlüsselter Form gespeichert und streng anonym ausgewertet. Dazu wird Ihren Daten eine Studiennummer zugeteilt. Eine Rückverfolgung der Daten zu Ihnen beispielsweise über Ihre Initialen oder Ihr Geburtsdatum ist nicht möglich. Nur die Prüfer sowie autorisierte Personen der Gesundheits- bzw. Strahlenschutzbehörden haben im Rahmen der entsprechenden gesetzlichen Vorschriften Zugang zu den vertraulichen Daten, in denen Sie namentlich genannt werden. Diese Personen unterliegen der Schweigepflicht und sind zur Beachtung des Datenschutzes verpflichtet. Die Weitergabe der Daten im In- und Ausland erfolgt ausschließlich zu statistischen und wissenschaftlichen Zwecken, und Sie werden ausnahmslos darin nicht namentlich genannt. Auch in etwaigen Veröffentlichungen der Daten dieser klinischen Prüfung werden Sie nicht namentlich genannt.

Die Therapieempfehlung Ihres behandelnden Arztes erfolgt unabhängig von den gerade beschriebenen Untersuchungen. Diese dienen ausschliesslich der Grundlagenforschung und haben keine Bedeutung für die individuelle Therapieplanung. Es könnte sich evtl. die Möglichkeit für Ansatzpunkte für eine Pharmakotherapie bieten. Durch den Vergleich der Ergebnisse der Untersuchungen mit den Ergebnissen anderer Patientengruppen und von gesunden Probanden sollen sich Hinweise zum genaueren Verständnis einer psychischen Traumatisierung, auf den Therapieverlauf sowie auf den weiteren Verlauf einer eventuell daraus entstehenden Erkrankung gewinnen lassen.
Ihre Teilnahme an der Studie ist freiwillig und Sie haben das Recht, sie jederzeit zu beenden, ohne dass Ihnen irgendwelche Nachteile daraus entstehen. ***Sollten Sie Ihr Einverständnis zur Untersuchung widerrufen, werden die bis dato erhobenen Daten unter den weiter***

oben angeführten Einschränkungen weiterhin genutzt. Die Untersuchung kann auch jederzeit ohne Angabe von Gründen vom Untersuchungsleiter beendet werden.

Klinikum der Universität München

Klinik und Poliklinik für Psychiatrie und

Psychotherapie-Innenstadt

Direktor: Prof. Dr. med. Hans-Jürgen Möller

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Probandeneinverständniserklärung für die Teilnahme an der Studie

Akute Belastungsreaktion und psychische Traumatisierung – Untersuchungen zur Neurobiologie und neuronalen Konnektivität im Verlauf

Das Informationsblatt wurde mir ausgehändigt und ich habe es sorgfältig gelesen. Zu der mir vorgeschlagenen Studie habe ich noch folgende Fragen:

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Es bestand genügend Zeit, um meine Fragen zu beantworten.

Ich bin über den Ablauf und mögliche Nebenwirkungen der obengenannten Untersuchung aufgeklärt worden und gebe meine Zustimmung zur Teilnahme an der Studie. Ich kann mein Einverständnis zu jedem Zeitpunkt, ohne Angabe von Gründen, zurücknehmen.

Ich erkläre mich freiwillig mit der Teilnahme an der Studie einverstanden. Ich wurde über den Untersuchungsablauf unterrichtet. Meine zusätzlichen Fragen wurden beantwortet.

München, den ____________

______________________ ______________________

Name und Unterschrift des Probanden   Name und Unterschrift des aufklärenden Arztes

Die Angaben zur Verwendung und Verschlüsselung meiner Daten habe ich gelesen. Ich erkläre mich freiwillig mit der Speicherung und Verwendung meiner Daten im Rahme der Studie einverstanden.

München, den _______________

____

Name und Unterschrift des Probanden

9.2 Exclusion criteria

Ausschlusskriterien

Eine Studienteilnahme war nicht möglich, wenn ein oder mehrere der folgenden Kriterien erfüllt waren:

- Bestehen einer Betreuung/ Geschäftsunfähigkeit
- Bestehende Schwangerschaft
- Implantierte Metallteile die eine MRT-Untersuchung unmöglich machen
- Drogen-, Medikamenten- oder Alkoholmissbrauch zum Zeitpunkt der Studie
- Zerebrovaskuläre Erkrankungen
- Demenz (DSM IV/ICD 10-Kriterien)
- Wiederholte Schädel-Hirn-Traumata in der Anamnese
- Hinweise auf strukturelle Schädigung der Basalganglien oder des Hirnstamms
- Schwere neurologische Erkrankungen (wie Diskusprolaps in den letzten 6 Monaten, Polyneuropathien, Parkinson-Syndrom, Epilepsie, Demenz, systemische neurologische Erkrankungen, zerebrovaskuläre Erkrankungen, Schlaganfall in der Anamnese, wiederholte zerebrale Ischämien mit einer stufenweisen Verschlechterung, erhöhter Hirndruck, Normaldruckhydrozephalus)
- Schwere psychiatrische Erkrankungen (mit Ausnahme der Depression)
- Akute Suizidalität oder Suizidversuch in der Vorgeschichte
- Schwere internistische Erkrankungen (wie manifeste arterielle Hypertonie, schwere Herzerkrankungen, Herzschrittmacher, respiratorische Insuffizienz)
- Maligne Erkrankungen jeglicher Art
- Schwere aktive Infektionskrankheiten
- Knochenerkrankungen (wie M. Paget, Osteoporose mit Spontanfrakturen, frische Frakturen)
- Andere Umstände, die nach Meinung des Prüfarztes gegen eine Teilnahme des Patienten an dieser Studie sprechen

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CINP (Poster):

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07/2006 Krauseneck T, Krähenmann O, Severus E, Cerovecki A, von Heimendahl J, Riedel M, Padberg F
Akute Belastungsreaktion und psychische Traumatisierung – Untersuchungen zur Neurobiologie und neuronalen Konnektivität im Verlauf

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Morphologische Veränderungen der Amygdala im Verlauf einer akuten psychischen Traumatisierung

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Der Einfluss psychischer Traumatisierung auf das Endocannabinoidsystem im Verlauf