

OPIOID DEPENDENCE:
BRAIN STRUCTURE AND FUNCTION

A magnetic resonance imaging, neuropsychological and electromagnetic study

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Academic dissertation

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List of publications

This thesis is based on the following publications which are referred to in the text by Roman numerals

I Kivisaari R., Kähkönen S., Puuskari V., Jokela O., Rapeli P., Autti T. Magnetic resonance imaging of severe, long-term, opiate-abuse patients without neurological symptoms may show enlarged cerebrospinal fluid spaces but no signs of brain pathology of vascular origin. *Archives of Medical Research* 2004;35, 395-400.

II Rapeli P., Kivisaari R., Autti T., Kähkönen S., Puuskari V., Jokela O., Kalska H. Cognitive function during short-term abstinence from opioid dependence: a comparison to age, gender, and verbal intelligence matched controls. *BMC Psychiatry* 2006;Feb 24;6(1):9.

III Kivisaari R, Lehtinen R, Autti T, Puuskari V, Jokela O, Ahveninen J, Rapeli P, Kähkönen S. Impaired pre-attentive auditory processing in opioid dependence with and without benzodiazepine co-dependence revealed by combined magnetoencephalography and electroencephalography. *Prog Neuropsychopharmacol Biol Psychiatry* 2007 Oct 1;31(7):1378-86. Epub 2007 Jun 12.

IV Kivisaari R, Rapeli P, Van Leemput K, Kähkönen S, Puuskari V, Jokela O, Autti T. Correlations between cerebral measurements and composite cognitive function in opioid-dependent patients. Submitted

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Abbreviations

AEP	auditory evoked potential
ANOVA	analysis of variance
CFIT	Culture Fair Intelligence Test
CSF	cerebrospinal fluid
CT	computed tomography
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders -IV
ECD	equivalent current dipole
EEG	electroencephalography
EOG	electro-oculogram
ERF	event-related fields
ERP	event-related potentials
FLAIR	fluid attenuated inversion recovery
fMRI	functional magnetic resonance imaging
GABA	gamma amino-butyric acid
IV	intra venous
LC	locus ceruleus
MCL	mesocorticolimbic
MEG	magnetoencephalography
MISS	midline internal skull surface
MMN	mismatch negativity
MMNm	magnetic mismatch negativity
MPRAGE	magnetization-prepared rapid acquisition gradient echo
MRI	magnetic resonance imaging
NAcc	Nucleus Accumbens
NMDA	N-methyl d-aspartate
ORL-1	orphan opioid receptor
PASAT	Paced Auditory Serial Addition Task
PET	positron emission tomography
RAVLT	Rey Auditory Verbal Learning Test
RF	radio frequency
RFFT	Ruff Figural Fluency Test
SCID	Structured Clinical Interview
SFR	Sylvian fissure ratio
SPECT	single photon emission computed tomography
SQUID	superconducting quantum interference device
TE	time of echo
TR	time of repetition
VBM	voxel based morphometry
VIQ	verbal intelligence quotient
VTA	ventral tegmental area
WSM-R	Wechsler Memory Scale-Revised

Abstract

Background: Opioid dependence is a chronic severe brain disorder associated with enormous health and social problems. The relapse back to opioid abuse is very high especially in early abstinence, but neuropsychological and neurophysiological deficits during opioid abuse or soon after cessation of opioids are scarcely investigated. Also the structural brain changes and their correlations with the length of opioid abuse or abuse onset age are not known. In this study the cognitive functions, neural basis of cognitive dysfunction, and brain structural changes was studied in opioid-dependent patients and in age and sex matched healthy controls.

Materials and methods: All subjects participating in the study, 23 opioid dependents of whom, 15 were also benzodiazepine and five cannabis co-dependent and 18 healthy age and sex matched controls went through Structured Clinical Interviews (SCID) to obtain DSM-IV axis I and II diagnosis and to exclude psychiatric illness not related to opioid dependence or personality disorders.

Simultaneous magnetoencephalography (MEG) and electroencephalography (EEG) measurements were done on 21 opioid-dependent individuals on the day of hospitalization for withdrawal therapy. The neural basis of auditory processing was studied and pre-attentive attention and sensory memory were investigated.

During the withdrawal 15 opioid-dependent patients participated in neuropsychological tests, measuring fluid intelligence, attention and working memory, verbal and visual memory, and executive functions. Fifteen healthy subjects served as controls for the MEG-EEG measurements and neuropsychological assessment.

The brain magnetic resonance imaging (MRI) was obtained from 17 patients after approximately two weeks abstinence, and from 17 controls. The areas of different brain structures and the absolute and relative volumes of cerebrum, cerebral white and gray matter, and cerebrospinal fluid (CSF) spaces were measured and the Sylvian fissure ratio (SFR) and bifrontal ratio were calculated. Also correlation between the cerebral measures and neuropsychological performance was done.

Results: MEG-EEG measurements showed that compared to controls the opioid-dependent patients had delayed mismatch negativity (MMN) response to novel sounds in the EEG and P3_{am} on the contralateral hemisphere to the stimulated ear in MEG. The equivalent current dipole (ECD) of N1_m response was stronger in patients with benzodiazepine co-dependence than those without benzodiazepine co-dependence or controls.

In early abstinence the opioid dependents performed poorer than the controls in tests measuring attention and working memory, executive function and fluid intelligence. Test results of the Culture Fair Intelligence Test (CFIT), testing fluid intelligence, and Paced Auditory Serial Addition Test (PASAT), measuring attention and working memory correlated positively with the days of abstinence.

MRI measurements showed that the relative volume of CSF was significantly larger in opioid dependents, which could also be seen in visual analysis. Also Sylvian fissures, expressed by SFR were wider in patients, which correlated negatively with the age of opioid abuse onset. In controls the relative gray matter volume had a positive correlation with composite cognitive performance, but this correlation was not found in opioid dependents in early abstinence.

Conclusions: Opioid dependents had wide Sylvian fissures and CSF spaces indicating frontotemporal atrophy. Dilatation of Sylvian fissures correlated with the abuse onset age. During early withdrawal cognitive performance of opioid dependents was impaired. While intoxicated the pre-attentive attention to novel stimulus was delayed and benzodiazepine co-dependence impaired sound detection. All these changes point to disturbances on frontotemporal areas.

1. Literature review

1.1 Opioids

Opium is prepared from the milky juice of the unripe seed capsules of poppy, *Papaver somniferum*, by drying and powdering. Some of the opioids are natural products isolated from opium as morphine or semisynthetic as heroin which is synthesized from morphine. A wide spectrum of synthetic opioid receptor ligands, which are produced mainly for treatment of severe pain, also exists. Methadone and buprenorphine are synthetic opioids used in heroin withdrawal or maintenance therapy. Buprenorphine came to the market in 1980 and the abuse potential was soon recognized (Strang 1985). Buprenorphine -naloxone tablets were introduced to avoid the abuse potential of buprenorphine. Naloxone is an opioid receptor antagonist and it blocks the effects of opioids. A recent study showed that 80% of drug abusers felt the intravenous (IV) use of buprenorphine-naloxone was a 'bad' experience (Alho et al. 2007), demonstrating the effectiveness of the combination.

1.2 Opioid abuse in Finland

In Finland in the year 2002 approximately 16100-21100 substance abusers existed, in which 4200-5900 using opioids. In recent years opioid abuse has become more common, 1997 in Finland was only an estimated 1500-3300 opioid users (STAKES: Huumetilanne Suomessa 2006). Different from other European countries buprenorphine is the main abused opioid in Finland (EMCDDA Annual report 2006; Table TDI 26) and it is typically used IV as 79.3 % of drug abusers in Finland preferred IV route as method of administration. This is among the highest figures in Europe (EMCDDA Annual report 2006; Table TDI 17). Benzodiazepine co-dependence is also very common and probably simultaneous use of buprenorphine and benzodiazepine combined with alcohol is adding the risk of death (EMCDDA Annual report 2006, <http://ar2006.emcdda.europa.eu/en/page011-en.html#10.3>). In the Finnish adult population (15-64 years) approximately 0.5-0.7% use amphetamine or opioids, but among the youngest age group (15-25) the prevalence of abusers is 0.9-1.3% (STAKES: Huumetilanne Suomessa 2006).

1.3 Opioid dependence

1.3.1 Neurobiology

Opioids are compounds that act by binding to specific opioid receptors and mediate their action through the opioid system. Opioid receptors are widely spread throughout the neuroaxis. Three major groups of opioid receptors are mu (μ), kappa (κ), and delta (δ). All divided into subtypes as μ_1 , μ_2 , κ_1 , κ_2 , κ_3 , δ_1 , and δ_2 (Dhawan et al. 1996). Also an additional opioid receptor ORL-1 (orphan opioid receptor) has been identified (Fukuda et al. 1994). All major groups have endogenous opioid ligands β -endorphin have a preference for μ -receptor, enkephalins for δ -receptors, dynorphins for κ -receptors and nociceptin for ORL-1-receptor (Lord et al. 1977, Pugsley 2002). The abused substances, morphine and heroin, are full μ -receptor agonists and buprenorphine is a partial μ -receptor agonist and κ -receptor antagonist (Altman et al. 1996). All opioids induce euphoria, which is the cause of abuse of these substances.

Opioids have very high addictive potential and the reinforcing affects of these drugs are induced by activation of the mesocorticolimbic (MCL) dopamine reward pathway (Bassareo et al 1995, Di Chiara 1995, Di Chiara 2002, Koob 1992, Xi & Stein 2002). This same reward pathway plays a critical role in addiction of any abused substance or addictive behaviour (Nestler 2005, Wise 1996).

The MCL reward system is usually activated by natural sources of pleasure like eating and sex, which are lifesaving acts necessary for the survival of species. The effect of abused substances especially when administered IV is significantly stronger than the effect of natural reinforcers and chronic use of these compounds decrease sensitivity to natural stimuli.

The rewarding and analgesic effects of opioids are mediated mostly by activation of the μ -receptors (Kieffer 1999), which are predominantly located on GABAergic (gamma amino-butyric acid) cells in the ventral tegmental area (VTA) and Nucleus accumbens (NAcc) (Haberstock-Debic et al. 2003, Xi & Stein 2002). Opioid receptor activation generally leads to neuronal inhibition (Xi & Stein 2002) in GABAergic interneurons in the VTA leading to a decrease in GABA release (Solecki et al. 2005), which results in increasing firing of VTA dopaminergic neurons and release of dopamine in the NAcc, amygdala, and orbitofrontal cortex. Opioid peptides probably also modulate neuronal response to excitatory glutamatergic neurotransmission in N-methyl d-aspartate (NMDA) receptors (Giacchino & Henriksen 1998).

Another main target for opioids is the locus ceruleus (LC), where they act as inhibitors, suppressing the neurons reducing sympathetic tonus (McClung et al. 2005). LC produces noradrenalin, which stimulates among other functions breathing, modulates blood pressure, wakefulness, and general alertness. As noradrenalin production is diminished, after administration of opioids respiration is depressed, blood pressure declines, and one becomes drowsy.

It is known that neural adaptation to chronic opioid exposure as a compensatory homeostatic mechanism induced by persistently altered metabolic dynamics of many neurotransmitter systems in brain, causes tolerance and then withdrawal symptoms when the substance is not available. The brain cells become less responsive to opioid stimulation and higher doses of opioids are needed to get a desired rewarding effect. Because tolerance rate varies between drug effects with higher doses a risk of overdose and depression of respiration always exists (Hurle et al. 1982).

After cessation of opioids the μ -receptor activity is decreased causing elevation of GABAergic activity followed by reduced dopamine activity in the mesolimbic system and its projections, but dynorphin, an endogenous opioid peptide release in the striatum and limbic system is elevated. These changes are followed by an increased release of noradrenaline in the LC and bed nucleus of the stria terminalis and also excessive glutamate release in the hippocampus and anterior cingulate cortex. The medial prefrontal cortex dopamine, noradrenalin, and serotonin contents are markedly increased (Espejo et al. 2001). In the hypothalamus and amygdala corticotrophin releasing factor secretion elevates as a sign of brain stress system activation and cortisol secretion is enhanced (Bearn et al. 2001, Cami & Farre 2003). All these neurotransmitter changes induce the clinical withdrawal syndrome; feeling of anhedonia and dysphoria, muscle cramps, diarrhea, palpitation, changes in blood pressure, and runny eyes. Because part of the withdrawal syndrome is induced by noradrenergic hyperactivation the withdrawal symptoms can be ameliorated with α_2 -adrenergic agonists, lofexidine, or clonidine, which reduces the noradrenergic activation.

Based on animal models it has also been hypothesized that the prefrontal cortex noradrenalin has an important role in relapse to opioid abuse, since morphine-induced elevations in the prefrontal cortex noradrenalin release mediate dopamine release in the NAcc (Bossert et al. 2005). The orbitofrontal cortex and anterior cingulate gyrus, which are both activated during intoxication and also during craving, are neuroanatomically connected to limbic structures (Goldstein & Volkow 2002). The amygdala, hippocampus, and dorsal striatum are also activated during craving and these are the areas involved in memory by long-term synaptic potentiation or depression. It is assumed that the relapse is a learned behavior that is activated by various stressors (Volkow et al. 2002).

The most important change in the drug use pattern is when abuse, which is an impulsive behavior, changes to dependence, which is a compulsive behavior. During impulsive behavior increasing tension is followed by an act that gives one pleasure and relief (positive reinforcement). Later this impulsive act is regretted and one feels guilty. During compulsive behavior anxiety and stress is followed by an act that gives one relief from anxiety (negative reinforcement). This behavior becomes an obsession that leads to anxiety and a repetitive cycle of compulsive acts (Koob et al. 2004).

The central problem of addiction treatment is not detoxification, but keeping abstinence and avoiding relapses, since months or years after withdrawal symptoms have vanished some stimuli might induce craving and a possible return to opioid abuse. The craving can be induced by exposure to the substance, drug-associated cues (Carter & Tiffany 1999), or stress (Sinha 2001).

1.3.2 Neuropsychological changes

The effects of opioids on neuropsychological functioning have scarcely been studied compared to the volume of research on effects of stimulants or cannabis. Some evidence from neuropsychological studies show that patients with opioid dependence have short-term impairments in attention, concentration, working memory, verbal and visual memory, and executive functions (Mintzer et al. 2005, Verdejo-Garcia et al. 2004). Even a general intellectual decline has been shown while intoxicated or very recently detoxified (Rounsaville et al. 1982). Guerra et al. (1987) showed that after rapid detoxification heroin abusers who had shown a deficit in attention, working and episodic memory, and verbal fluency during abuse did not differ from controls after one to two weeks of abstinence. It seems that opioid abuse induces partially transient alterations of cognitions. On the other hand, after long term abstinence a consistent deficit in executive functioning, especially in impulse control has been found (Davis et al. 2002, Lee & Pau 2002, Ornstein et al. 2000, Pau et al. 2002).

The prefrontal cortex is involved in cognitive functions such as planning, anticipation and establishment of goals, organization and motivation of behaviour, defined as executive functions (Fellows 2007). The functional imaging studies of substance abusers also point to those frontal pathways related to cognition (Volkow et al. 2002, Yucel et al. 2007).

1.3.3 Event-related potentials

Only a couple of electroencephalography (EEG) studies exist in which the event-related potentials (ERP) of opioid dependents have been studied. These studies have evaluated the auditory response P300, which is a late cognitive auditory response, considered a manifestation of active operations since it is elicited during target detection tasks.

These studies have shown that after cessation of opioids the P300 response is attenuated for months (Papageorgiou et al. 2004). In patients with cocaine and opioid dependence the buprenorphine administration enhanced P300 amplitude back to same level as while intoxicated or in controls (Kouri et al. 1996). Attenuation of the response is probably due to physiological abnormalities during the withdrawal.

1.3.4 Structural brain changes

Only few studies of brain structural changes of opioid dependent patients exist. Previous computed tomography (CT) studies have revealed significant ventricular enlargement and cortical volume loss in male opioid-dependent patients (Pezawas et al. 1998, Strang & Gurling 1989). Liu et al. (1998) showed in a magnetic resonance imaging (MRI) study that polysubstance abusers had significantly smaller gray matter volumes than controls, particularly in the prefrontal lobe and to a smaller extent in temporal lobes. Their subjects however, were polysubstance abusers and not all were opioid dependents. In agreement with these former studies Lyoo et al (2006) reported that opioid dependent patients have significantly smaller gray matter densities in the frontal and temporal areas than healthy controls. They used voxel based morphometry (VBM), which can differentiate the densities of different brain tissue types from MRI data. On the other hand, the other VBM study by Schlaepfer et al. (2006) showed diminished white matter volumes on the frontal areas, but could not find any changes in gray matter volumes. Studies also exist that did not find remarkable morphological changes in the brain of opioid dependents among polysubstance abusing addicts (Aasly et al. 1993, Amass et al. 1992).

Most of the functional neuroimaging studies have concentrated on patients using substances other than opioids, such as cocaine. These studies reveal changes in regional blood flow and dopamine D2-receptor availability. Only a couple of studies have been investigating opioid dependents. In a Single Positron Emission Computed Tomography (SPECT) study the significant perfusion deficits were found in the frontal, temporal, and parietal cortices of opioid dependents after a week of abstinence (Rose et al. 1996). But in another study after four months of abstinence only nonsignificant decreases in cerebral blood flow in the frontal, parietal, and left temporal cortex were found (Gerra et al. 1998). A very recent functional magnetic resonance imaging (fMRI) and MRI spectroscopy study showed that patients using opioids showed a normal task-related activation of prefrontal cortex, precisely cingulate cortex, but the activation did not correlate with the cognitive measures as expected. On the other hand, activation of frontal, parietal and cerebellar regions was increased, probably as a compensatory mechanism. Spectroscopy showed that the neuronal substrates n-acetyl aspartate and glutamate were decreased in prefrontal cortex indicating neuronal damage (Yucel et al. 2007). Opioid dependents also seem to have decreased striatal dopamine D2-receptor availability when compared to controls during intoxication or naloxone withdrawal (Wang et al. 1997).

Most of the structural changes seem to be located in the frontal or temporal areas. These areas are also involved in several cognitive deficits especially executive functioning and memory functions, associated with drug abuse.

1.4 Magnetoencephalography-electroencephalography (MEG-EEG)

Positron Emission Tomography (PET), SPECT and fMRI studies measure brain metabolism and hemodynamics. Those operate in the temporal scale limited to seconds, but electromagnetic methods such as magnetoencephalography (MEG) and EEG can measure brain function on millisecond scale, and give valuable information about neuronal abnormalities not detected with other functional methods. MEG and EEG are closely related methods both investigating the electrical activity of the brain. EEG is used to measure the electric field pattern and MEG detects the magnetic field non-invasively outside the head (Hämäläinen et al. 1993). Where an electrical current occurs, also a magnetic field occurs and the primary currents causing the signals measured are the same. MEG is reference free, but EEG is dependent on the position of reference electrodes.

The electrical potentials and magnetic fields produced by neurons are so weak that to be able to detect them outside the skull there must be coherent activity of thousands of cells. It is believed that the measured activity is mainly induced by synchronous pyramidal postsynaptic potentials. Pyramidal cells are predominantly oriented perpendicular to the cortex (Figure 1). MEG can not detect the radial sources because of its physical basis, but the magnetic fields induced by tangential primary currents can be measured. Therefore activity in sulcus, where the current is oriented parallel to the device is better measured with MEG. Superconducting Quantum Interference Device (SQUID) magnetometers are able to measure these very weak signals outside the head and collect information of the cerebral activity. MEG locates the sources accurately in contrast to EEG, because the skull, extracerebral tissues, and cerebrospinal fluid are almost transparent to magnetic field, but they transmit electricity distorting the EEG signal. On the other hand with EEG it is also possible to detect the radial sources invisible to MEG. Therefore, a combined MEG and EEG technique can provide a comprehensive view of brain function with high spatial and temporal accuracy (Virtanen et al. 1996, Virtanen et al. 1997)

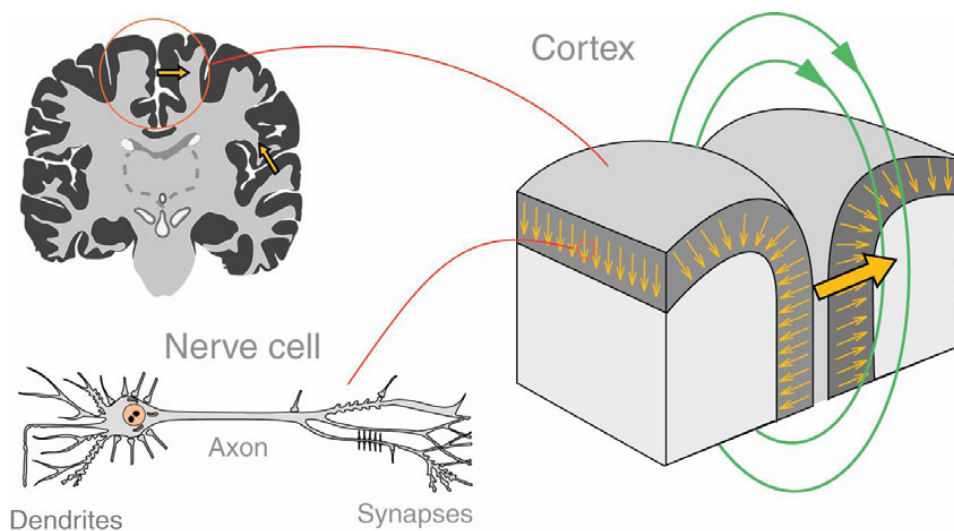


Figure 1. Electrical currents of pyramidal cells (yellow arrows) induce the magnetic field (green arrows) measured with MEG. Courtesy of Elekta Neuromag Oy

The spontaneous activity of the brain can be recorded, but it is also possible to investigate the stimulus-elicited currents, ERPs, and magnetic fields called Event-Related Fields (ERF). The stimulus has to be repeated many times and the responses must be averaged to be able to detect the desired response curve among spontaneous activity. The measured electromagnetic activity outside the head can be explained with numerous different sources in the brain. This so called inverse problem has many mathematical solutions. With the selection of channels over for example the auditory cortex one can limit the number of possible electrical sources and can get a good spatial resolution of brain activity.

These ERPs and ERFs reveal information of the neural basis of perception and cognition providing an objective and high temporal resolution index of auditory processing in the human brain.

1.4.1 Auditory evoked potentials and magnetic fields

The sonic air pressure waves are transformed into neural signals in the auditory pathway. First the external and middle ear filters and amplifies the sound waves and the auditory ossicles transmit the vibrations to the inner ear and cochlear fluids. The movement of the fluid in the cochlea is

transformed to the neural signals. The sensory cells adapted to high frequencies are located in the base and those to low frequencies in the apex of the cochlea. This organized pattern of fibers remains all the way to auditory cortex. The afferent nerve fibers from the inner ear are located in the brain nerve number VIII; nervus vestibulocochlearis. The nerve fibers synapse at ipsilateral cochlear nucleus. The major outputs project into the hemisphere contralateral to the stimulated ear. The second-order neurons ascend into the superior olivary nuclei bilaterally and via lateral lemniscus to the colliculus inferior. From there the pathway continues through the medial geniculate nucleus of the thalamus to the auditory cortex in the temporal lobe. The primary auditory cortex is located in the supratemporal cortex in Brodmans' areas 41 and 42.

The auditory ERP in EEG and ERF in MEG are thought to reflect the processing of the heard information. The cortical responses peak 50-800 ms after stimulus onset and are defined as long-latency components following the earlier brain stem and subcortical auditory responses (Hari et al. 1980). Long-latency ERP and ERF represent a sum of neural activity from several sources. The reflections are classified by the polarity (P positive or N negative) and approximate latency of the peak or succession. The ERF responses have an additional *m* for magnetic. The first of the long latency response P1 (*P1m*) peaks approximately 50 ms after stimulus onset. The most conspicuous of the auditory responses is N1 (*N1m*), a negative reflection that peaks at an average of 100 ms after stimulus onset and reflects the activity of at least three different sources (Näätänen & Picton 1987). The main sources are located on the supratemporal auditory cortex immediately posterior to the primary auditory cortex and two other components in the superior temporal gyrus and around motor cortex (Hari et al. 1980, Näätänen & Picton 1987). These responses can be found in both auditory cortices, on contra- or ipsilateral to the stimulated ear (Figure 2). On the ipsilateral side the latencies are usually slightly longer than on contralateral hemisphere (Pantev et al. 1998).

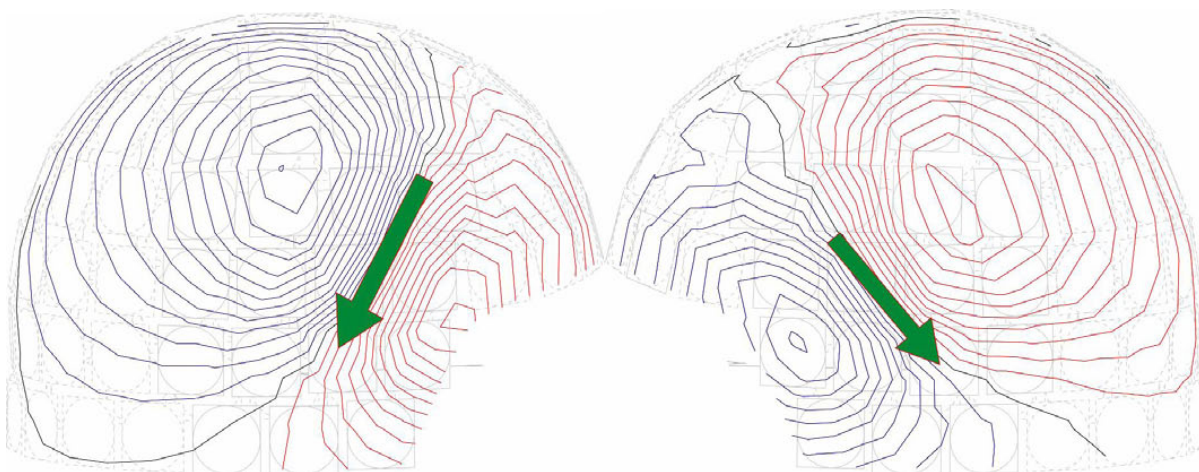


Figure 2. Equivalent current dipoles (green) of *N1m* auditory responses shown on auditory cortices on the contralateral and ipsilateral to the stimulated ear. The magnetic field around the electrical current dipole is also shown.

1.4.2 Mismatch negativity (MMN)

The electric mismatch negativity MMN (and its magnetic counterpart MMNm) is a cognitive response indexing the neural basis of sensory memory and involuntary attention (Kujala et al. 2006, Näätänen et al. 1993). It is elicited without subjects' attention when the train of standard stimulus is erupted with a deviant stimulus differing from the standard in some respect. It is assumed that the

standard repetitive stimulus is forming a sensory-memory trace in the auditory cortex to which the incoming stimulus is compared. If incoming stimulus differs from the standard in some respect for example by frequency, intensity or duration the change is automatically detected and MMN is elicited. It has also been shown that abstract regularities in a series of sounds can act as standard and a mistake in these regular series can elicit MMN. The response is also found when there is a change in repetitive linguistic stimuli (Jacobsen et al. 2004, Shestakova et al. 2002).

The major MMN generators are located in the auditory cortices on both hemispheres (Alho 1995). The MMN peak on the right hemisphere is stronger than on the left hemisphere when non-linguistic sounds are evaluated by auditory system, while language specific response is stronger on left hemisphere. Frontal MMN generators, on the right hemisphere, also activate a few milliseconds later than temporal sources (Rinne et al. 2000). The temporal activation might indicate a change detection which then triggers the frontal component proposed to be involved in an attention shift to this change (Näätänen et al 1993).

The temporal generators, on the auditory cortex are better detected with MEG, while those generators are located in sulci and activation is tangentially orientated compared to the skull surface. The frontal generators are probably located radially since those are better detected with EEG (Alho et al. 1998). Probably additional parietal (Lavikainen et al. 1994) and subcortical generators exist (Csepe 1995, Kujala et al. 2006, Molholm et al. 2005).

MMN usually peaks at 150-250 ms after stimulus onset and it is followed by a positive peak approximately 300 ms after stimulus. This response called P3a (*P3am*) is believed to be associated with switching attention towards the change in auditory stimulus (Escera et al. 1998).

P3a response is proposed to have sources located in the temporal and frontal lobes. The source responsible for the early peak of P3a is located in the superior temporal cortex (Alho et al. 1998) and the later peak is generated in the prefrontal cortex (Baudena et al. 1995).

1.4.3 Neurochemical features of event-related potentials and fields

The neurochemical basis of auditory responses has been investigated with single-dose drug challenge studies in animals and humans. The generation of N1 response appears to be regulated by GABAergic inhibition. Different GABA agonists have been shown to attenuate N1 amplitude (Meador 1995, Rockstroh et al. 1991, Semlitsch et al. 1995, van Leeuwen et al. 1995) and but apparent counterpart of N1 in monkeys was enhanced by GABA receptor antagonist (Javitt et al. 1996). The role of other receptors is less evident. Haloperidol, which is a dopamine D2-receptor antagonist, did not have any effect on N1 nor *N1m* responses in the study of Kähkönen (Kähkönen et al. 2001).

Neurochemical mechanisms underlying the MMN response of NMDA receptors are most systematically studied. Drug challenge studies have shown that NMDA receptor antagonists block the generation of the MMN response (Javitt et al. 1996, Kreitschmann-Andermahr et al. 2001, Umbricht et al. 2000, Umbricht et al. 2002). Evidence also show that other transmitters modulate MMN; serotonergic (Ahveninen et al. 2002, Kähkönen et al. 2005, Umbricht et al. 2002), dopaminergic (Kähkönen & Ahveninen 2002), cholinergic (Pekkonen et al. 2001, Pekkonen et al. 2005) and GABAergic involvement (Kasai et al. 2002, Nakagome et al. 1998) have been found.

1.5 Magnetic Resonance Imaging (MRI)

MRI is a very useful tool for studying the brain anatomy and structural changes because of the ability of tissue differentiation.

The patient is situated in an imager with a very strong magnetic field (1.5T in this study) generated by the MRI equipment. The protons of the body's hydrogen atoms have a positive electrical charge and these protons are in constant spinning motion. The moving electrical charge is by definition an electrical current. This electrical current induces a magnetic field making the protons little bar magnets and these magnets turn into alignment with the imager's magnetic field, parallel or antiparallel (longitudinal magnetization). A few more protons always stay in the lower energy level, this equilibrium state is disturbed by a radiofrequency (RF) energy pulse the machine emits. The protons with the same frequency as the RF pulse absorb the energy and tilt to the higher energy level in a phenomenon called resonance. This phenomenon is essential for the imaging method that is why it is called magnetic resonance imaging. The protons are spinning around the magnetic field lines like a spin top and this motion is called precession. Another effect this RF pulse has on protons is that they start to precess in a synchronous matter, in phase instead of randomly (transversal magnetization). After the RF pulse the energy is released as protons return to their relaxed position in the magnetic field and lose the precession coherence. The shed energy, as longitudinal and transversal magnetization returns to their relaxed state is collected with sensors and a computer calculates the position of each proton that omits energy. The image is formed from this collected information of proton densities in different sites of the viewed field. The different sequences are induced with different RF pulse repetition time (TR) and signal collecting time (TE). It is possible to get different kinds of images; proton density, T1 and T2 weighted images, or images where fluid is attenuated. (Bushberg et al. 2002)

From MRI images it is possible to measure the areas of structures and calculate indexes of different measures. Voxel (volume element) is a term for a piece of matter where the information is collected and a density of the pixel (picture element) is calculated. The sophisticated computer programs can differentiate brain tissue types from the data. VBM is a technique that allows the determining of different tissue types voxel by voxel and determines the volumes of each tissue compartment.

2. Aims of the study

The general aim was to investigate the brain structural and functional changes in a group of long term opioid dependent patients.

The purposes of the present study were to find:

1. If structural brain changes in long-term opioid dependents exist compared to healthy controls (I,IV).
2. How opioid dependents perform in neuropsychological tests during early opioid withdrawal (II,IV).
3. Are there changes in pre-attentive auditory processing in opioid dependents (III).
4. If any correlation appears between structural brain changes and neuropsychological tests (IV).
5. How the age of abuse onset and the length of abuse history affects these structural and functional changes (I-IV).

3. Materials and methods

3.1 Subjects (Studies I-IV)

Twenty three opioid-dependent patients (Table 1) and 18 healthy controls with no history of drug abuse were attended this study. Opioid dependents were admitted to an in-patient drug detoxification unit in the Helsinki University Central Hospital for withdrawal and evaluation for the methadone maintenance program. The criteria for the methadone maintenance program were a minimum age of 20 years, four years of documented opioid dependence, and the failure of institutional or long-lasting outpatient withdrawal therapy, which also served as criteria for inclusion in the study. Exclusion criteria for methadone maintenance therapy were uncontrolled polysubstance abuse, physical or psychiatric illness that made the routine of therapy impossible, and alcohol dependence. In this study, additional exclusion criteria for both patients and controls were major head trauma, chronic neurological illness or ongoing medication for neurological symptoms, and metallic foreign objects in the body.

All patients had severe long-term opioid dependence (Table 1). They had used opioids from 5 to 26 years (mean 12 ± 8), mainly IV. The self reported daily doses of different opioids were 0.05-1.5 g for street heroin, 2-32 mg for buprenorphine and 250-750 mg for ethyl morphine. Fifteen patients (5 women, mean age 31.5 ± 6.0) also abused benzodiazepines daily (approximate equivalent dose to diazepam 38 ± 21 mg according to Ashton table (Ashton 2005)). Before the study patients gave urine samples twice a week from four to six weeks to exclude other illicit substance abuse than opioids or benzodiazepines.

Patients were in good physical health as determined by a physical examination, laboratory evaluation including a complete blood count, electrolytes, glucose, renal, and thyroid analyses. HIV antibody test was negative in all patients tested (one patient refused). All except two of the patients had positive hepatitis C antibody analysis. Hepatic enzymes were mildly elevated in 7 patients and moderately in 1 patient.

The Structured Diagnostic Interviews (SCID I and SCID II, American psychiatric Association, 1994) (First et al. 1994a, First et al. 1994b) were done on all patients while they were hospitalized by trained psychiatrists to obtain the DSM-IV (Diagnostic and Statistical Manual of mental disorders) diagnosis of axes I and II. Sixteen patients fulfilled the criteria of DSM-IV for antisocial personality disorder and 13 had multiple diagnoses on SCID II. (Table 1)

Controls were healthy volunteers from hospital staff and their friends with no experience of illicit drugs. All had used alcohol in social occasions, but did not meet the criteria of abuse or dependence on alcohol. Patients and controls were age and sex matched and in the subgroup attending the neuropsychological tests verbal intelligence also matched. The control subjects had no DSM-IV axis I or II diagnosis in the SCID evaluation.

The simultaneous MEG-EEG measurement carried out to 21 patients while intoxicated (III) and 15 healthy controls. Neuropsychological tests were done to 15 patients (II, IV) and 15 age, gender and verbal intelligence matched controls. Brain MRI was obtained from 17 patients during the withdrawal therapy in the clinic (I, IV) and 17 controls. Written informed consent was obtained from all subjects and the study had the approval of the local ethical committee.

	sex	age	documented opioid abuse (years)	axis I diagnoses	axis II diagnoses	MRI	MEG/EEG	Neuro-psychology
1	m	21	4	Opioid dependence	antisocial	(x)	x	x
2	m	24	7	Opioid and benzodiazepine dependence	borderline		x EEG only	
3	f	25	8	Opioid and benzodiazepine dependence	antisocial, obsessive-compulsive	x	x	x
4	m	25	8	Opioid and benzodiazepine dependence	antisocial, obsessive-compulsive and borderline	x	x	
5	m	26	7	Opioid and benzodiazepine dependence	antisocial	x	x	x
6	m	28	8	Opioid and benzodiazepine dependence	antisocial	x	x	x
7	m	29	9	Opioid, benzodiazepine and cannabis dependence	antisocial, borderline		x	
8	f	29	12	Opioid, benzodiazepine and cannabis dependence	antisocial, obsessive-compulsive, (features of borderline and dependent)	x	x EEG only	x
9	m	30	7	Opioid and benzodiazepine dependence	antisocial		x	
10	m	30	10	Opioid and benzodiazepine dependence	antisocial, paranoid, borderline, schizotypal, narcissistic and obsessive-compulsive (features of passive-aggressive and depressive)	x	x	x
11	m	32	5	Opioid dependence amphetamine and benzodiazepin abuse	antisocial, obsessive-compulsive, paranoid (features of borderline)	x		x
12	f	33	12	Opioid dependence	antisocial, obsessive-compulsive	x	x	x
13	m	33	10	Opioid and benzodiazepine dependence amphetamine and cannabis abuse	obsessive-compulsive (features of narcissistic and antisocial)	x	x	x
14	m	34	9	Opioid, cannabis and amphetamine dependence	antisocial, passive-aggressive, paranoid	x	x	x
15	m	35	5	Opioid and cannabis dependence	antisocial, borderline (features of obsessive-compulsive, passive-aggressive and paranoid)	x	x	x
16	m	35	20	Opioid and benzodiazepine dependence	antisocial, paranoid (features of depressive, schizotypal and borderline)	x	x	x
17	f	36	6	Opioid dependence amphetamine abuse	antisocial, paranoid, schizoid, borderline, narcissistic, dependent, obsessive-compulsive, passive-aggressive, depressive	x	x	x
18	m	36	8	Opioid and benzodiazepine dependence alcohol abuse	paranoid, borderline, obsessive-compulsive		x	x
19	f	38	20	Opioid and benzodiazepine dependence	antisocial		x	
20	f	41	26	Opioid and benzodiazepine dependence amphetamine abuse	obsessive-compulsive, not otherwise specified (features of passive-aggressive, antisocial, borderline and	x	x	
21	f	43	26	Opioid and benzodiazepine dependence	not otherwise specified (features of antisocial, borderline and narcissistic)	x	x EEG only	
22	m	44	25	Opioid and cannabis dependence	antisocial, obsessive-compulsive, narcissistic (features of schizotypal)	x		
23	f	46	10	Opioid and cannabis dependence	antisocial, obsessive-compulsive, avoidant, depressive	x	x	x

Table 1. The demographic data of patients in the study. DSM-IV axis I and II diagnoses obtained with structural clinical interviews. Attendance of each patient to the measurements is marked.

3.2 Neuropsychological tests (Studies II and IV)

The battery of cognitive tests including the tests measuring working memory, episodic memory, executive function, verbal and visual learning and memory, and fluid intelligence were used.

Working memory performance was tested with the Digit Span subtest from the Wechsler Memory Scale-Revised (WSM-R) and a computerized version of Paced Auditory Serial Addition task (PASAT) from the FORAMENRehab software package. During the Digit Span Test one has to repeat the series of numbers in a same order as heard. Numbers are given one per second. This test measures verbal working memory storage. During the PASAT test one is presented a number between 1 and 9 every 1.6 seconds and they have to add the two previous numbers heard and say out loud the result. The PASAT test performance requires continuous storage of incoming numbers, rapid arithmetic processing, and executive control of interference of heard and calculated numbers. This requires complex working memory function.

The immediate verbal learning was studied with the Rey Auditory Verbal Learning Test (RAVLT) in which three learning trials of 15 presented words were given to all participants. The sum of the results of the three tries was calculated to form a result for the test. Visual memory was measured by the Benton Visual Retention test. In this test 10 drawings, one at the time, are shown and these designs have to be reproduced onto plain paper from memory as exactly as possible.

Executive function was measured with a modified Stroop task. In the first part of this test there were 50 words, names of different colors written with black ink, and in the second part the same words were written with different ink colors. The color of the ink was different from the written word. The result was the time of naming the ink colours in the second part subtracted with the reading time of the first list. Inhibition of not reading the words in the second part and the time of naming the colors affect the test result. The Ruff Figural Fluency (RFFT) test, which forms from two parts of unique designs and preservative errors, measures the executive function, planning and fluency of action. The squares with five dots in each are presented and one has to draw as many different figures as possible drawn with connecting at least two dots with straight lines in each square.

Culture Fair Intelligence Test (CFIT), includes a group of visuo-spatial reasoning tasks that are sensitive to fluid intelligence deficit due to various origins. The performance of the test reflects fluid and general intelligence needed in highly demanding novel problem solving situations.

The composite cognitive function was calculated as a sum of the z-score of all test results. Only one score per tests was used if the test yielded more than one score. The result of each test was first standardized to z-scores which were calculated from the individual's test score by subtracting the group's mean score and dividing the difference by the group's standard deviation. These z scores were summed up to obtain person's composite cognitive function.

3.3 Combined MEG-EEG (Study III)

3.3.1 Measurements

MEG and EEG recordings were carried out a 306-channel MEG, consisting of 204 planar gradiometers and 102 magnetometers (Vectorview, Neuromag TM) and a 60-channel EEG. The position of the subjects' head relative to the recording instrument was determined by measuring the magnetic fields produced by marker coils attached to the scalp. The locations of these coils in

relation to cardinal points on the head (nasion, left and right pre-auricular points) were determined before the experiment using an Isotrak 3D-digitizer (Polhemus, Colchester, VT, U.S.A.). During the measurement the subject sat in a comfortable chair in a magnetically and electrically shielded room (Euroshield, Finland) and watched a silent video. Auditory stimulus was presented monaurally to the left ear through a plastic tube and an earpiece and the subject was instructed to ignore the tones. The stimulus block consisted of the standard (80%) stimulus (700 Hz with 60 ms duration, including 5 ms rise and fall times) randomly embedded with infrequent (6.6% for each type) deviant tones differing in frequency (larger deviant 400 Hz and smaller deviant 600 Hz) and novel sounds (10 different complex sound bursts in random order). The interstimulus interval was 599 ms. Each two-channel sensor unit measured two independent magnetic field gradient components $\partial B_z/\partial x$ and $\partial B_z/\partial y$, with the z -axis being normal to the local helmet surface. The recording band-pass was 0.03–172 Hz and the sampling rate was 600 Hz. Epoch was averaged from 150 ms prestimulus until 600 ms poststimulus.

AEPs were recorded with an electrode cap (Virtanen et al. 1996) and an amplifier (Virtanen et al. 1997) specifically designed and built for simultaneous EEG and MEG measurements. The nose electrode was used as a reference. Vertical and horizontal electro-oculograms (EOG) were recorded. First responses in the train, and all the epochs coinciding with EOG or MEG changes exceeding 150 μ V or 3000 fT/cm, respectively, were omitted from averaging. At least 100 artefact-free standard and deviant responses were recorded and averaged.

3.3.2 Analysis of MEG-EEG

Digital band-pass filtering was performed off-line at 2-40 Hz for P1/P1*m*, at 1-30 Hz for N1/N1*m*, at 2-15 Hz for MMN/MMN*m* and P3a/P3a*m*. The analysis period was 500 ms. Distinct ERP/ERF peaks were obtained from latency ranges of 30-70 ms for P1/P1*m*, 60-150 ms for N1/N1*m*, 100-250 for MMN/MMN*m* and 170-500 ms for P3a/P3a*m*. The responses were judged significant when they were two standard deviations (SD) larger than the pre-stimulus noise.

MEG analysis was done with a source modelling program with software provided by Neuromag Ltd. (Hämäläinen et al. 1993). The dipole modelling was performed using a subset of 64 channels separately over each auditory cortex using one's own brain MRI image if available. In the four cases MRI images were not obtained the sphere model was used.

For EEG analysis, the peak latencies of P1 and N1 were determined from standard responses at the channel Cz. The peak latencies of the MMN and P3a were determined from subtraction curves at the electrode sites Fz or FCz depending on which channel a given deflection was larger. Subtraction curves were calculated by subtracting the standard AEP from the deviant. The P1, N1, MMN, and P3a amplitudes were determined from the averaged amplitude of 15 channels in groups of three (prefrontal; AF1, AFz, AF2, frontal; F1, Fz, F2, frontocentral; FC1, FCz, FC2, central; C1, Cz, C2, centroparietal; CP1, CPz, and CP2).

3.4 Brain MRI (Studies I and IV)

3.4.1 Imaging

Brain MRIs were acquired with a 1.5 T Siemens Magnetom imager. After scout images, axial and coronal T2- and proton density weighted images with a spin echo sequence, 3000/14-85 (TR/TE) with a slice thickness of 5.0 mm, axial fluid attenuated inversion recovery (FLAIR) 9999/105 (TR/TE) with a slice thickness of 5.0 mm, and a three dimensional (3D) magnetization-prepared

rapid acquisition gradient echo (MPRAGE 9.7/4.0 (TR/TE) with a slice thickness of 1mm were obtained. The 3D-series were used in MEG analysis to localize the equivalent current dipoles (ECD).

3.4.2 Analysis of MRI

The visual analysis was done by two radiologists (RK and TA) to detect the anatomical pathology of the brain or changes in gray and white matter signal intensity. Sizes of ventricles, cortical cerebrospinal fluid spaces and Sylvian fissures were evaluated by using a series of standard FLAIR images demonstrating the upper limits of each of the three lower grading categories, the fourth category being the widest. (Figures 3 and 4)

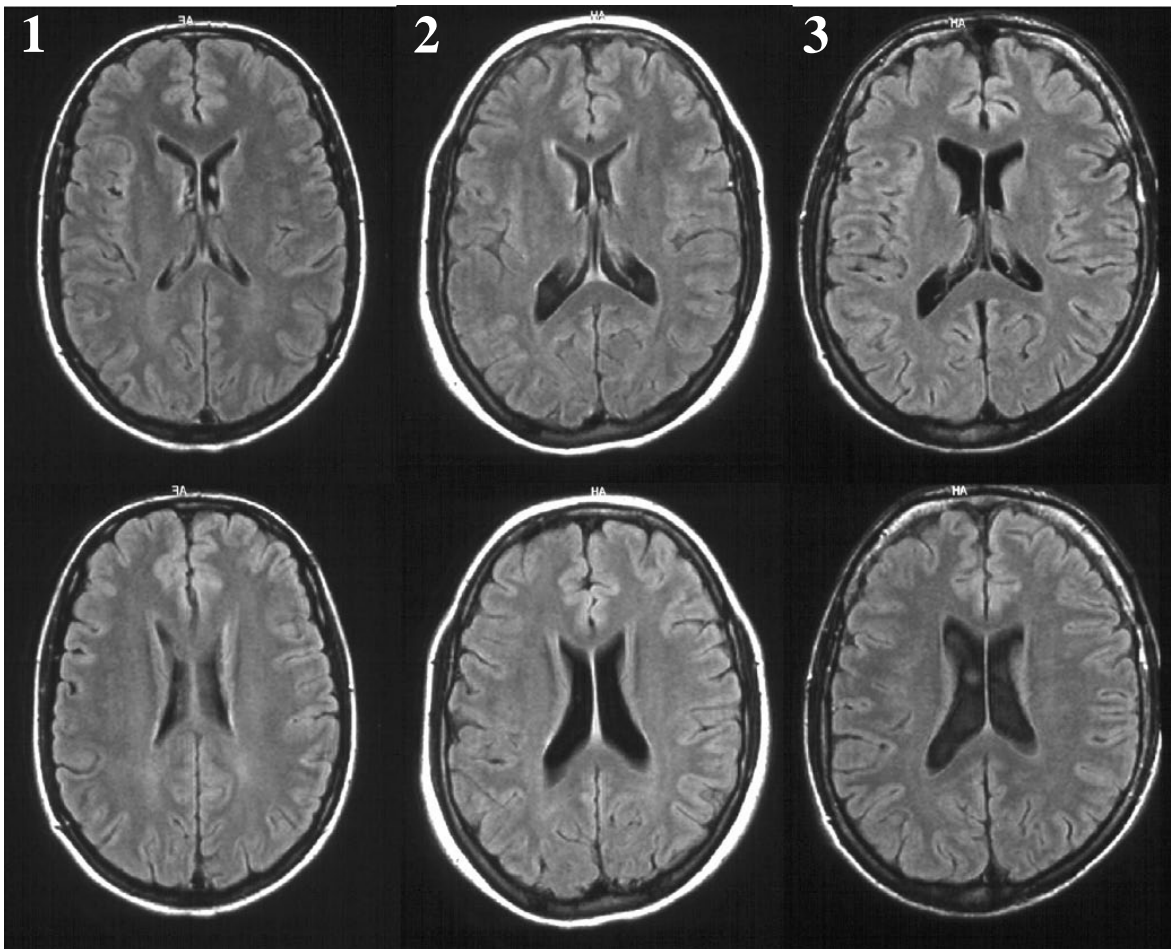


Figure 3. Standard images for grading the size of lateral ventricles. Two axial slices are shown for each category. The images show the upper limits of each grade. Grade 1 showing narrow lateral ventricles; grade 2 shows slightly enlarged ventricles; grade 3 shows moderately enlarged ventricles. Every image showing larger ventricles than in grade three was graded four indicating severely enlarged lateral ventricles. (I)

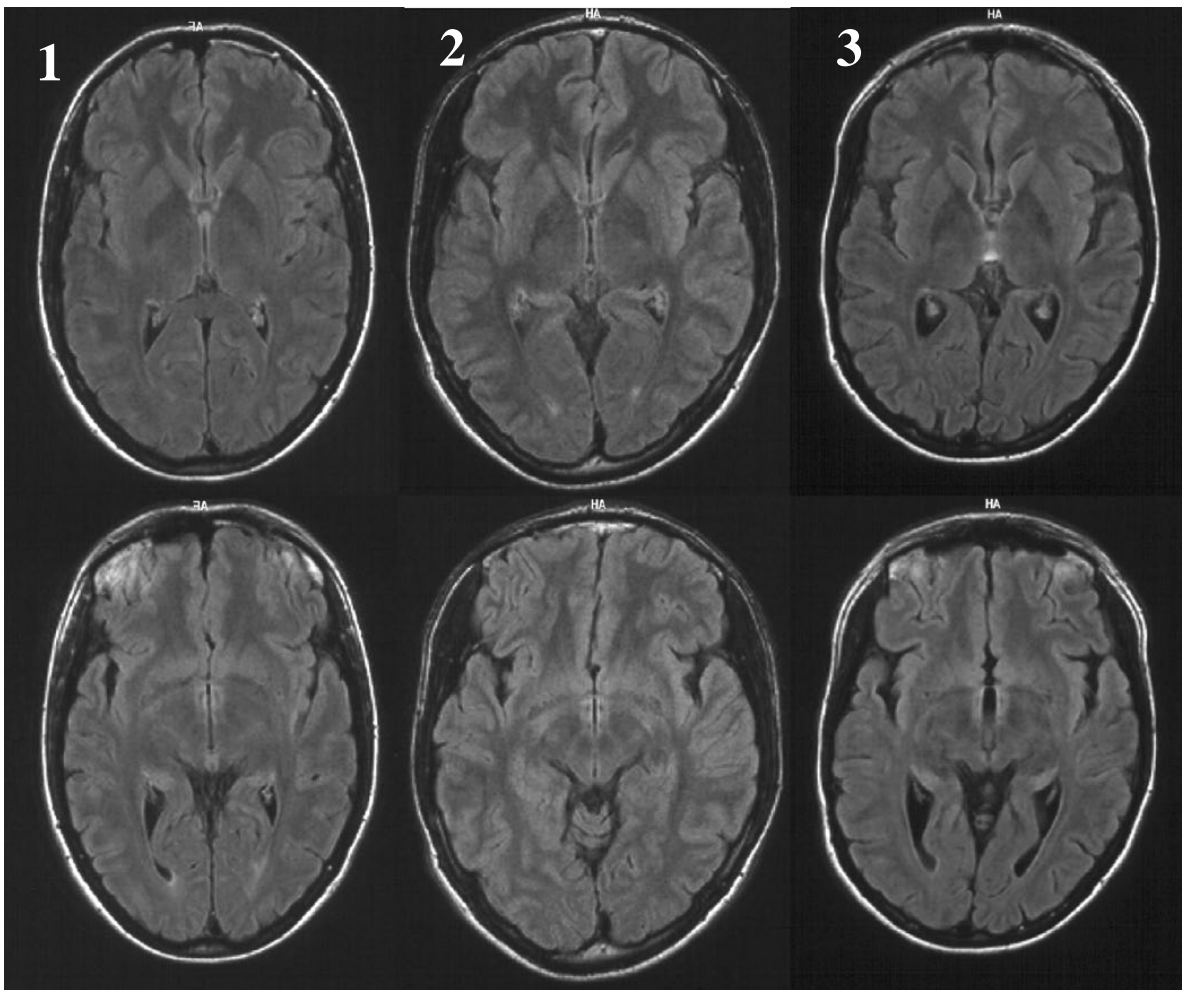


Figure 4. Standard images used grading Sylvian fissures. Axial slices through Sylvian fissures show maximal sizes of the spaces in grades 1-3. Grade four is not shown. Grade 1 showing narrow Sylvian fissures; grade 2 shows slightly enlarged temporal parts of Sylvian fissures; grade 3 shows enlarged frontal and temporal parts of Sylvian fissures. Every image which showed larger Sylvian fissures than in grade three was graded four. (I)

The measurements of different brain areas were done in the slice of the 3D-series where the aqueduct was best seen defined as the midsagittal image. The areas of the midline internal skull surface (MISS), vermis and corpus callosum were obtained (Laissy et al. 1993). FLAIR images were used while measuring the Sylvian fissure ratio (SFR) defined as the average width of both Sylvian fissures divided by the transpineal temporal brain width (van Zagten et al. 1999). Bifrontal ratio was measured at the level of third ventricle from T2 axial images. Bifrontal ratio was defined as a distance between the most lateral tips of the anterior horns of lateral ventricles divided by the brain width in the same line and level (Aylward et al. 1991). (Figure 5)

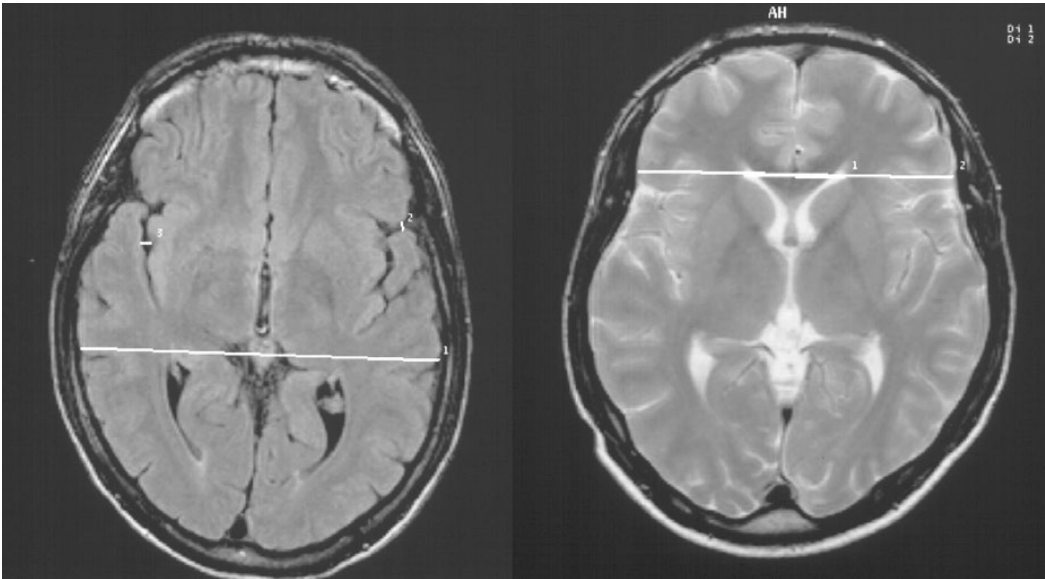


Figure 5. The Sylvian fissure ratio was calculated as an average width of the Sylvian fissures divided by transpineal width of the brain. The bifrontal ratio was calculated as a measure between the tips of the frontal horns divided by the brain width at the same level. (I)

Volumetric analysis with VBM was done using the SPM2 software package and Matlab program (<http://www.fil.ion.ucl.ac.uk/spm>). The total absolute volumes of CSF, white matter, and gray matter were computed by explicitly segmenting these structures in the MPRAGE images using a fully automated image-processing procedure. First, a digital brain atlas, containing information about the expected location of the major tissue types, was aligned with each image under study (Evans et al. 1993). To this end, a nonlinear registration technique was employed that minimizes the residual squared difference between an image under study and a template associated with the atlas, while simultaneously maximizing the smoothness of the deformations (Ashburner & Friston 1999, Ashburner & Friston 2000). After alignment, the atlas information was fed into an automated segmentation algorithm that iteratively estimates the tissue classification in each voxel while simultaneously training a Gaussian mixture model classifier and correcting for MR intensity inhomogeneity artifacts (Van Leemput et al. 1999).

The relative volume percentages were calculated by normalizing the absolute volumes of white and gray matter and CSF with the total absolute cerebral volume.

3.5 Statistical analysis

The SPSS statistical PC program version 11.0 (I, II) and version 12.0 (III, IV) were used. In MRI studies the patients and controls were compared with the Mann-Whitney U test (I, IV). The one-way analysis of variance (ANOVA) test was used to compare the raw scores of cognitive tests (II, IV).

The statistics in the MEG-EEG studies were done with one-way and repeated-measures of ANOVA (III). In the MEG-EEG measures for one-way ANOVA group membership (patients or subgroups of opioid-dependents with and without benzodiazepine co-dependence and healthy controls) was entered as a factor and latencies, amplitudes (EEG), dipole strengths (MEG) or locations (MEG) were entered as dependents. For repeated-measures ANOVA between group factors were group

membership and within subject variables were electrode location (prefrontal, frontal, frontocentral, central, centroparietal) in EEG or hemisphere in MEG measurement analysis. Differences between the subgroups were calculated with the Fisher's LSD test. Statistical significance was set at 0.05 (two-tailed).

Correlation between different measurements and age of abuse onset, years of opioid abuse and days of abstinence were calculated with the Pearson correlation coefficient. (IV)

4. Results

4.1 Cognitive performance (Studies II and IV)

In neuropsychological tests the opioid-dependent patients had inferior performance compared to healthy controls in working memory tests (PASAT) ($F(1,28)=12.0$, $p=0.002$), executive function (RFFT unique designs) ($F(1,28)=5.22$, $p=0.03$) and fluid intelligence (CFIT) tests ($F(1,27)=7.97$, $p=0.009$) (Table 2) A positive correlation emerged between PASAT and CFIT test results and the days of abstinence ($r^2=0.39$, $r=0.63$, $p=0.01$ and $r^2=0.43$ $r=0.65$, $p=0.009$) showing better test results with longer abstinence. (Figures 6 and 7) None of the results of neuropsychological tests correlated with the years of abuse or the age of abuse onset. (II)

The composite cognitive function evaluated with the z-score sum was inferior ($p=0.005$) in opioid dependents (mean -0.25 , SD 0.43) compared to controls (mean 0.25 , SD 0.36). In controls the z-score sum correlated positively with gray matter volume ($r^2=0.35$, $r=0.59$, $p=0.032$), but this correlation was not found in opioid-dependents in early abstinence. (IV) (Table 3)

Cognitive tests	Patients	Controls			
Fluid intelligence	Mean (SD)	Mean (SD)	F	df	p
CFIT	30.4 (4.2)	34.0 (3.8)	7.97	1,27	0.009
Attention, working memory					
PASAT	36.1 (10.1)	47.5 (7.8)	12.00	1,28	0.002
WMS-R Digit Span	14.9 (2.7)	15.4 (3.8)	0.15	1,28	ns
Executive function					
Stroop, modified interference time	25.1 (8.8)	24.5 (12.0)	0.30	1,28	ns
RFFT, unique designs	68.1 (21.2)	86.3 (22.6)	5.22	1,28	0.03
RFFT, perseverative errors	3.4 (2.9)	2.8 (2.6)	0.34	1,28	ns
Memory					
RAVLT, learning trials 1-3	28.9 (6.0)	32.3 (6.1)	2.64	1,28	ns
RAVLT, delayed recall	9.0 (2.8)	10.7 (2.8)	2.65	1,28	ns
WMS-R Logical memory, immediate	22.3 (7.3)	25.1 (6.6)	0.72	1,28	ns
WMS-R Logical memory, delayed recall	22.3 (7.3)	25.1 (6.6)	1.20	1,28	ns
BVRT number of right figures	6.8 (1.6)	7.4 (1.3)	0.88	1,28	ns

Table 2. The results of the neuropsychological tests (ANOVA) CFIT=Culture Fair Intelligence Test, PASAT=Paced Auditory Serial Addition Task, WMS-R=Wechsler Memory Scale-Revised, RFFT=Ruff Figural Fluency Test, RAVLT=Rey Auditory Verbal Learning, and BVRT=Benton Visual Retention Test.

Correlations	Composite cognitive function (z-score sum) controls	Composite cognitive function (z-score sum) patients	abuse onset age patients	duration of opioid dependence patients
Gray matter	p=0.032 r=0.594	ns	ns	ns
White matter	ns	ns	ns	ns
Cerebrospinal fluid spaces	ns	ns	ns	ns
Sylvian fissure ratio	ns	ns	p=0.036 r=-0.585	ns

Table 3 Correlations between cognitive performance, cerebral measurements and opioid abuse history.

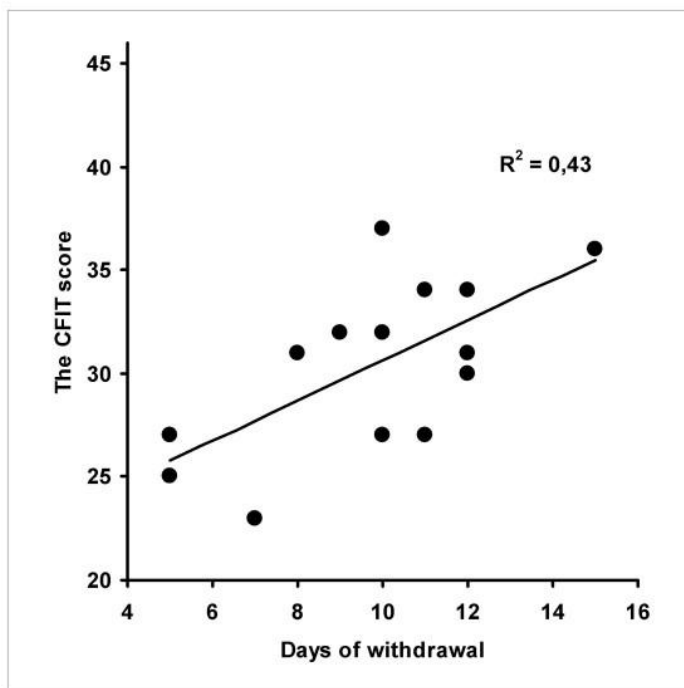


Figure 6. A positive correlation was found between the days of abstinence and the test score of the fluid intelligence test CFIT. (II)

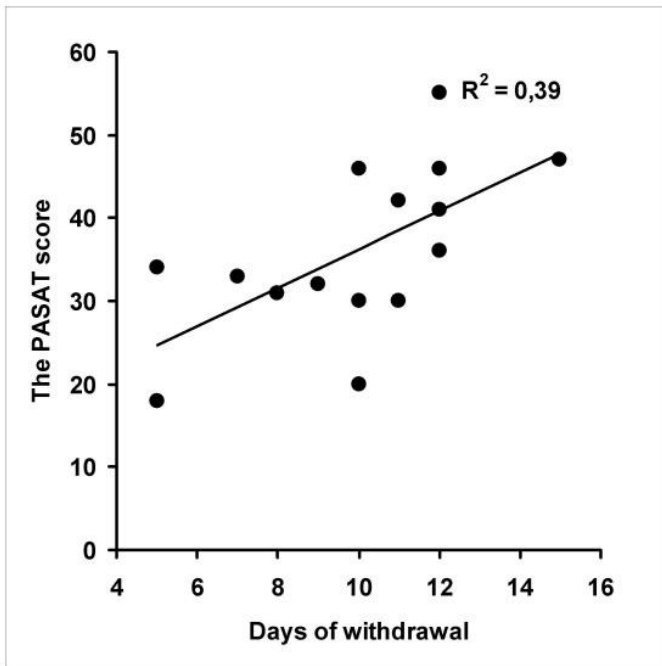


Figure 7. PASAT test results measuring attention and complex working memory correlated positively with the duration of withdrawal. (II)

4.2 MEG (Study III)

While intoxicated the repeated measures ANOVA revealed a significant main effect on *N1m* dipole strength ($F(2,26)= 5.9, p=0.008$). The post hoc analysis showed that this effect was due to significantly stronger source activity in opioid-dependent patients with benzodiazepine co-dependence than in opioid-dependent patients without benzodiazepine co-dependence ($p=0.005$) or in controls ($p=0.013$). *N1m* group X hemisphere interactions were not significant.

MEG	contralateral hemisphere				ipsilateral hemisphere			
	all patients	patients with BZ dependence	patients without BZ dependence	controls	all patients	patients with BZ dependence	patients without BZ dependence	controls
Source activity (nAm)								
<i>P1m</i>	12.3 (7.6)	13.2 (8.4)	9.7 (4.0)	9.5 (5.2)	7.8 (4.1)	9.2 (3.8)	4.7 (3.1)	9.9 (6.1)
<i>N1m</i>	20.3 (10.3)	23.3 (10.8)*	14.5 (6.6)	15.9 (7.9)	13.3 (8.2)	16.9 (7.4)*	5.5 (1.9)	10.1 (8.6)
MMN larger deviant	26.7 (19.5)	29.8 (20.9)	19.9 (15.6)	35.5 (24.1)	19.5 (13.1)	21.8 (14.4)	12.5 (4.6)	18.2 (8.2)
MMN smaller deviant	20.1 (10.9)	20.7 (10.3)	17.1 (18.4)	31.3 (30.2)	19.5 (13.1)	20.9 (14.8)	14.7 (2.1)	15.3 (7.2)
MMN novel	34.4 (15.5)	37.3 (15.4)	22.8 (10.8)	57.4 (48.8)	21.2 (11.8)	22.1 (13.9)	19.6 (7.7)	24.8 (13.1)
<i>P3a</i>	20.6 (16.3)	24.6 (17.8)	10.1 (1.3)	37.7 (36.7)	22.7 (13.8)	25.3 (13.6)	14.6 (13.7)	20.8 (11.0)

Table 4. The mean strength of equivalent current dipoles of auditory responses in both hemispheres in patients and controls. Also the results of patient subgroups with or without benzodiazepine co-dependence are shown. BZ benzodiazepine

*Patients with benzodiazepine co-dependence compared to patients without benzodiazepine co-dependence $p=0.005$ and controls $p=0.013$. (Repeated measures ANOVA)

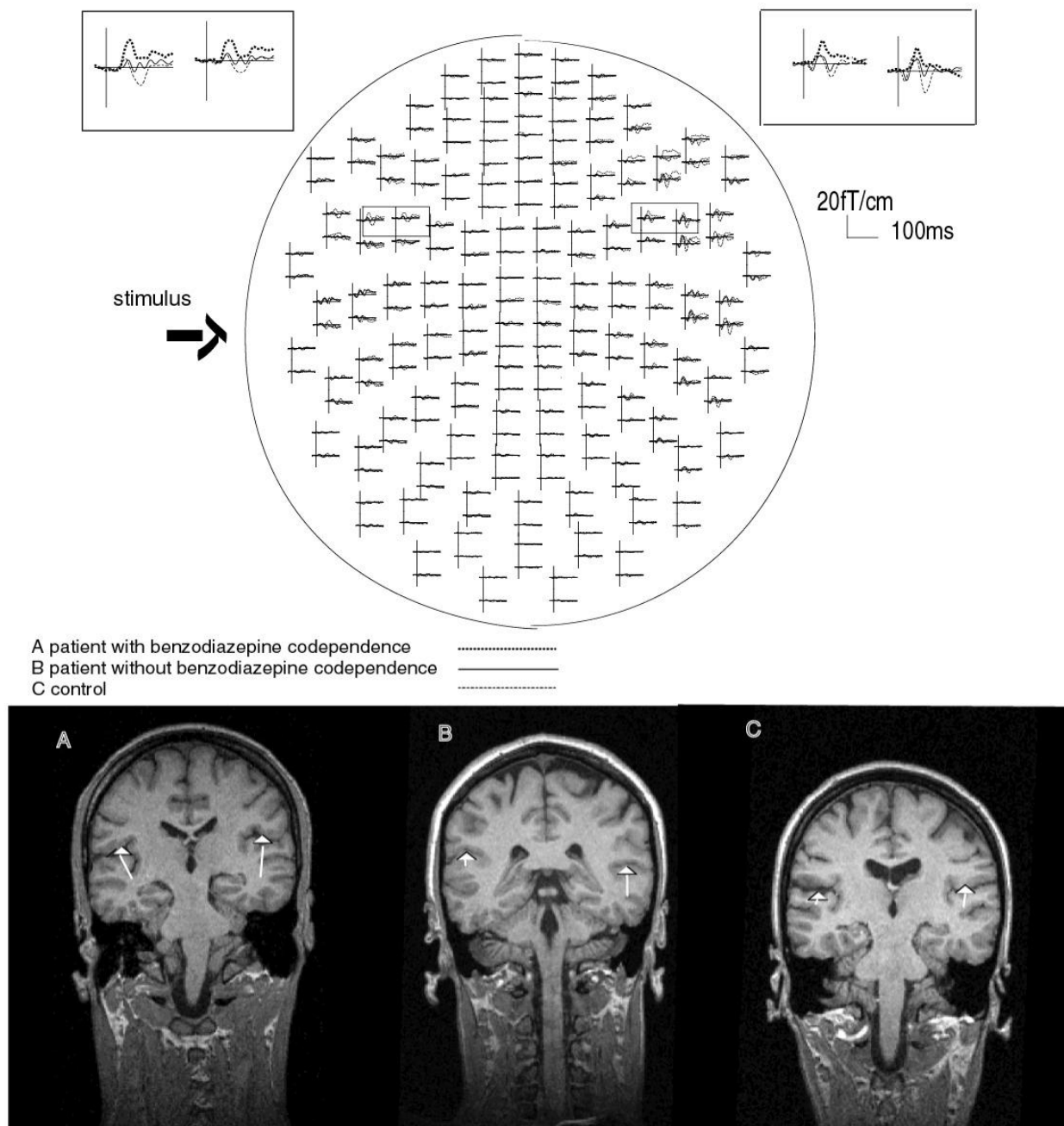


Figure 8. The $N1m$ response in opioid dependents (A) with benzodiazepine co-dependence, (B) without benzodiazepine co-dependence, and (C) healthy control shown as response curves and equivalent current dipoles in ones MRI image. (III)

Latencies of the $P3am$ on the contralateral hemisphere to the stimulated ear were longer in patients with opioid dependence compared to healthy controls (one-way ANOVA) $F(1,23) = 4.3, p=0.049$, but no differences were observed in latencies of $P1m$, $N1m$ and $MMNm$ ($p>0.05$). (Table 5) The strengths or locations of the $P1m$, $N1m$, $MMNm$ and $P3am$ ECD did not differ significantly between patients and healthy controls ($p>0.05$)

MEG latency ms mean (SD)	contralateral hemisphere		ipsilateral hemisphere	
	patients	controls	patients	controls
P1	46.9 (11.1)	45.6 (14.3)	63.8 (8.0)	62.5 (14.8)
N1	88.6 (18.0)	88.9 (17.1)	109.4 (17.7)	117.2 (27.1)
MMN (dev1)	148.0 (26.3)	139.7 (19.9)	153.0 (23.7)	160.1 (18.3)
MMN (dev2)	151.6 (24.5)	157.1 (24.0)	159.8 (26.8)	163.9 (17.0)
MMN (novel)	136.0 (19.6)	125.1 (14.1)	151.1 (31.7)	140.0 (14.4)
P3a	223.4 (42.0) *	198.5 (10.1) *	230.0 (24.6)	212.0 (19.0)

Table 5. Latencies of auditory responses in MEG measurement.

* ≤ 0.05 , one-way ANOVA

4.3 EEG (Study III)

Latency of MMN to novel sound ($F(1,34)=4.9$, $p=0.033$, one-way ANOVA) was longer in patients than in controls, but no differences were observed in P1, N1, MMN or P3a latencies ($p>0.05$). No statistically significant changes emerged in P1, N1, MMN and P3a amplitudes ($p>0.05$). (Table 6 and Figure 7)

EEG Latency ms mean (sd)	patients	controls
P1	51.9 (14.2)	47.3 (9.9)
N1	98.8 (12.6)	91.2 (12.7)
MMN to large deviant	147.9 (29.5)	146.8 (24.1)
MMN to small deviant	152.9 (33.0)	166.8 (28.6)
MMN to novel sound	159.0 (45.8) *	130.0 (22.2) *
P3a	260.4 (55.4)	235.9 (23.9)

Table 6. Latencies of auditory responses in EEG.

* ≤ 0.05 , one-way ANOVA

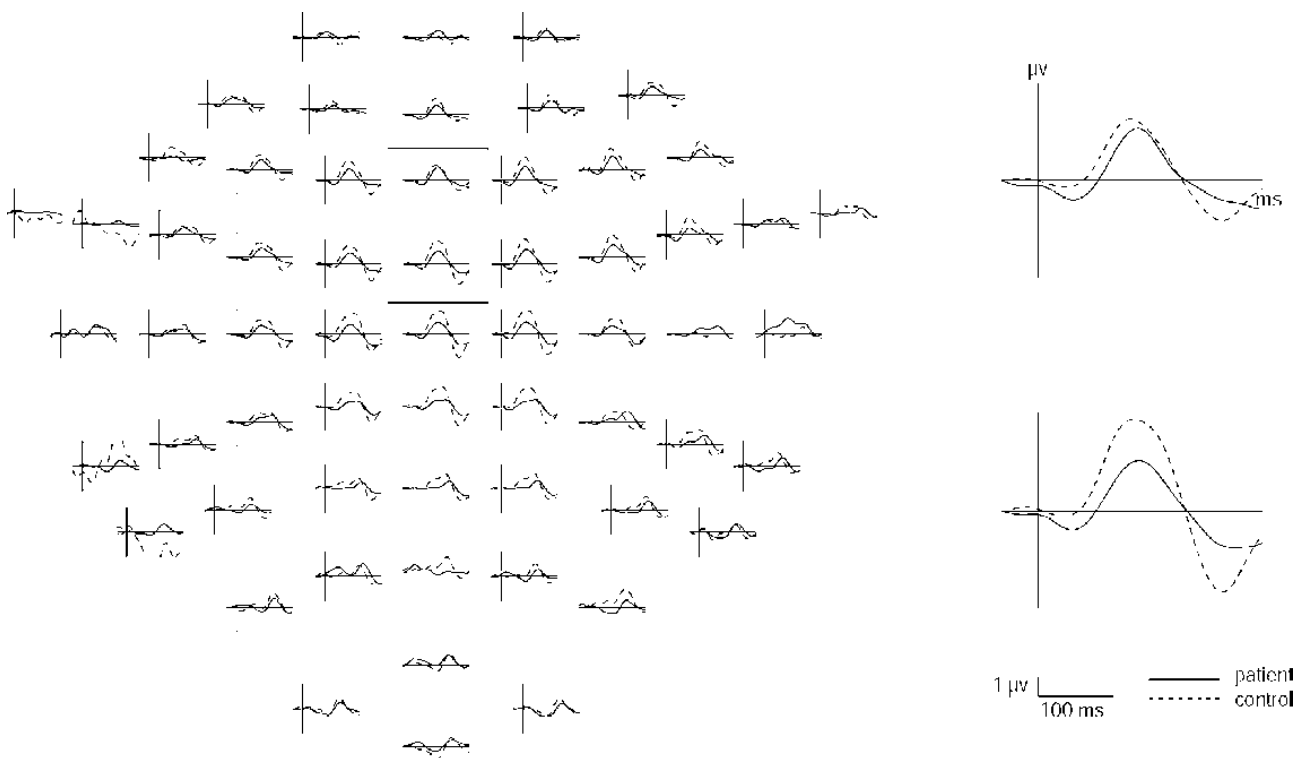


Figure 9. The grand average of MMN to novel sound EEG of patients and controls. (III)

4.4 Brain imaging (Studies I and IV)

No signs of ischemic changes appeared in the visual analysis. The gray and white matter intensities were otherwise normal, but one patient had a small post-traumatic subcortical lesion on the parietal cortex. Visual analysis with a standard set of reference images showed that Sylvian fissures and lateral ventricles were wider in patients than controls ($p=0.008$ and $p=0.04$ respectively, Mann-Whitney U-test) (Table 7) The calculated SFR and the bifrontal ratio were larger in patients ($p=0.005$ and $p=0.013$, respectively) (I) (Table 8) A significant negative correlation emerged between SFR and the age opioid abuse had started; the younger the substance abuse had started the wider the Sylvian fissures were ($p=0.017$, $r=-0.569$, $n=17$). (Table 3) Also some of the opioid-dependents had smaller vermian areas than controls, even though the difference between the groups was not significant ($p=0.109$). Only one control, but 5 out of 17 patients had area of vermis -1 SD or below. The measured area of MISS and of corpus callosum did not differ between the groups ($p=0.125$ and $p=0.277$, respectively) (I).

The volumetric analysis carried out with 16 patients (excluding one patient with subcortical posttraumatic lesion) and 16 controls showed that the total cerebral volume was smaller in patients $p=0.043$. The relative CSF volume normalized with the total cerebral volume was significantly larger in patients ($p=0.021$). (Table 8)

In controls the composite cognitive function (z-score sum) correlated positively with gray matter volume ($r^2=0.35$, $r=0.594$, $p=0.032$), but this correlation was not found in opioid-dependents in early abstinence. ($p=0.017$, $r=-0.569$). (Table 3) This correlation was calculated in those 13 patients who had both neuropsychological tests and MRI volumetric analysis done.

Visual analysis		Grade 1	Grade 2	Grade 3	Grade 4	p
Size of the Sylvian fissures	patients	4	10	2	1	0.008
	controls	12	4	1	-	
Size of the ventricles	patients	7	4	6	-	0.04
	controls	12	4	1	-	
Size of the cortical sulci	patients	3	8	5	1	0.26
	controls	3	12	2	-	

Table 7. The size of the cerebrospinal fluid spaces graded with standard images. (Mann-Whitney U test)

	patients	controls	
Measurements (n=17)	mean (SD)	mean (SD)	p
Vermis (cm ²)	12.2 (1.30)	13.0 (1.47)	0.1
MISS (cm ²)	156 (10.92)	162 (11.14)	0.1
Corpus callosum (cm ²)	7.12 (0.79)	7.47 (1.00)	0.3
Distance between tips of the lateral ventricle anterior horns (cm)	3.5 (0.18)	3.2 (0.35)	0.01
Width of the frontal brain (cm)	10.9 (0.41)	10.8 (0.41)	0.7
Bifrontal ratio	0.317 (0.015)	0.296 (0.030)	0.01
Average of the width of the Sylvian fissures (cm)	0.315 (0.023)	0.174 (0.003)	0.005
Width of the temporal brain (cm)	13.2 (0.48)	13.2 (0.53)	0.8
Sylvian fissure ratio	0.024 (0.011)	0.013 (0.004)	0.005
Volumes (n=16)			
total volume (ml)	1360 (0.116)	1436 (0.113)	0.04
Gray matter relative to total cerebral volume	0.537 (0.009)	0.541 (0.007)	0.34
White matter relative to total cerebral volume	0.320 (0.006)	0.327 (0.011)	0.067
Cerebrospinal fluid spaces relative to total cerebral volume	0.142 (0.013)	0.132 (0.013)	0.021

Table 8. Cerebral measurements in patients and controls. MISS=midsagittal internal skull surface. (Mann-Whitney U test)

4.5 Correlations between brain structure, opioid abuse and neuropsychological performance (Studies I and IV)

SFR had a negative correlation with the opioid abuse onset age. The Sylvian Fissures were wider in the individuals who started the opioid abuse younger ($r^2=0.32$, $r=-0.569$, $p=0.017$, $n=17$). (Figure 10) Correlations between age of the subject or the length of the opioid abuse history and SFR were not found. The CFIT and PASAT test results correlated with the days of abstinence. In opioid-dependents in early abstinence the composite cognitive function (z-score sum) did not correlate with an abuse history or any cerebral measurements but in controls the positive correlation between gray matter volume and composite cognitive function was found ($r^2=0.35$, $r=0.594$, $p=0.032$). (Table 3) Electromagnetic measures did not correlate with opioid abuse history, cerebral measurements, or neuropsychological performance.

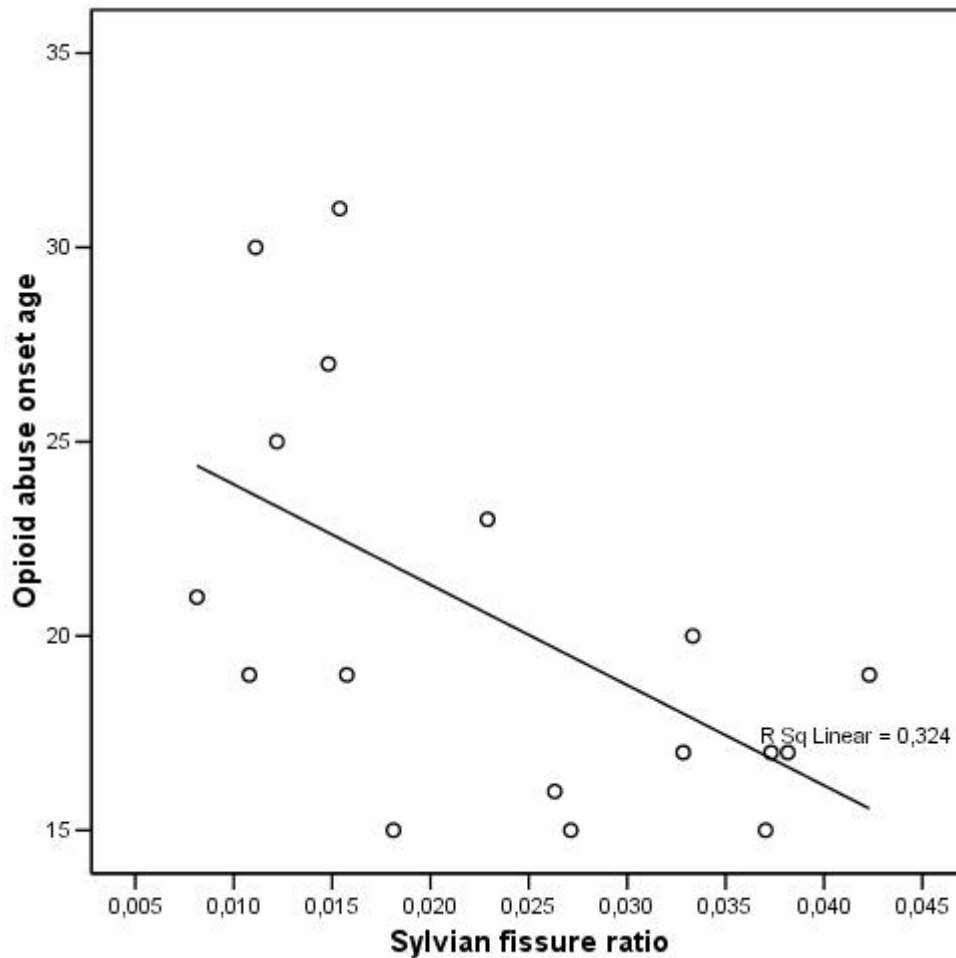


Figure 10. Correlation between the age of opioid abuse onset and the Sylvian fissure ratio. (IV)

5. Discussion

We studied the neurocognitive performance and auditory evoked potentials and magnetic fields as well as the structural brain changes in opioid dependents. The auditory responses MMN and P3am were delayed compared to controls indicating changes in neuronal circuits involved in pre-attentive auditory processing and involuntary attention. The benzodiazepine co-dependence seemed to affect the N1 response, which was enhanced in patients with opioid and benzodiazepine dependence. In early withdrawal the composite cognitive performance was debilitated in opioid dependents compared to controls. Complex working memory and fluid intelligence were particularly impaired and the duration of abstinence had a positive correlation with the test results. The test scores of executive function were also inferior in patients with no correlation to the withdrawal period. The composite cognitive function correlated with relative gray matter volume in controls but not in patients in early abstinence. The CSF spaces especially the Sylvian fissures were enlarged in opioid dependents indicating frontotemporal atrophy. All the changes found in these patients point to impaired frontotemporal neural circuits and brain areas.

5.1. Changes in neural basis of pre-attentive attention

MMN and P3a are suggested to reflect the operation of auditory sensory memory and attention switching to irrelevant tones in ones' surrounding (Escera et al. 1998, Grillon et al. 1990, Sams et al. 1985, Woods 1992), indexing the simplest form of working memory. Both responses seem to have temporal and frontal subcomponents reflecting different states of detection and orienting of sound change.

While intoxicated the patients with opioid dependence showed delayed latencies of MMN response to novel sounds in EEG and delayed P3a_m at the contralateral hemisphere to the stimulated ear in MEG. The temporal MMN subcomponent is first elicited indexing the sound change detection in a train of repetitive series of similar tones and the subsequent involuntary attention shift to this sound change is probably reflected by the later frontal MMN subcomponent (Alho et al. 1998, Rinne et al. 2000, Takegata et al. 2001). Since MMN latencies to novel sounds were increased in EEG, but not in MEG, it can be assumed that the frontal MMN component is specifically affected the frontal MMN is probably radially orientated and invisible to MEG (Rinne et al. 2000).

P3a is also proposed to have temporal and frontal located sources. The source responsible for the early peak of P3a is located in the superior temporal cortex (Alho et al. 1998) and the later peak is generated in the prefrontal cortex (Baudena et al. 1995). The delayed P3a_m activity mainly reflected the earlier superior temporal source on the contralateral hemisphere to the stimulated ear. This thus suggests that impairment of attention shifting in opioid dependent patients also involves superior temporal regions, in addition to the presumed frontal MMN generator abnormalities. The change in frontotemporal interactions has been found in these same patients when the spontaneous activity was studied (Fingelkurts et al. 2006a, Fingelkurts et al. 2006b). This finding of changes in both temporal and frontal generators probably also reflects the impairment of these connections. These results suggest that patients with opioid dependence have some impairment in processing of novel sounds at different phases of pre-attentive auditory processing.

5.1.1 Neurochemical modulation of pre-attentive attention

The clearest evidence exists of the role of NMDA receptors involved in MMN generation. It is shown in animal and human studies that NMDA antagonists block MMN elicitation (Javitt et al. 1996, Kreitschmann-Andermahr et al. 2001, Umbricht et al. 2000, Umbricht et al. 2002). In opioid dependence evidently some changes also occur in the excitatory system as a NMDA subunit expression and density is enhanced in NAcc and decreased in the forebrain in opioid dependent rodents (Murray et al. 2007), but exact role of glutamatergic neurotransmission at the NMDA receptor site in opioid dependence is not clear. It might be that the NMDA system has some role in the changes found in auditory pre-attentive processing.

Dopamine has a crucial role in development of opioid dependence in humans (Volkow et al. 2002). Some evidence shows that chronic opioid use changes the D₂-receptor binding in the cortical areas, hippocampus, and midbrain (Elwan & Soliman 1995). Previous studies in healthy individuals showed MMN and P3a amplitude changes after a single dose of dopamine D₂-receptor antagonist haloperidol (Kähkönen et al. 2001, Kähkönen et al. 2002), so it can be assumed that dopamine is in part related to involuntary attention change. Interestingly, dopamine in the NAcc has been shown to mediate novelty responding in rats (Saigusa et al. 1999).

Benzodiazepines are known to impair active and passive attention, studied by MMN and P3 in healthy subjects in single-dose studies (Javitt et al. 1996, Lucchesi et al. 2005, Rockstroh et al.

1991, Rosburg et al. 2004), but the effects of chronic benzodiazepine abuse have not been studied yet. We did not find differences in MMN or P3a in patients with and without benzodiazepine co-dependence. Even though benzodiazepine co-dependence did not affect these responses, we clearly had two subgroups, which appeared in the N1 response.

5.1.2 N1 response in patients with benzodiazepine co-dependence

Opioid dependents with benzodiazepine co-dependence demonstrated stronger N1m source activity compared to patients without benzodiazepine co-dependence or healthy controls, suggesting that auditory processing is changed in opioid dependence when patients also abuse benzodiazepines. It is not surprising that N1m is changed, since GABAergic system is modulated in both opioid and benzodiazepine dependence. Increased N1m source activity at the auditory cortex may be related to reduced inhibition and increased excitability of the cortical neurons reflecting neural adaptation to chronic simultaneous benzodiazepine and opioid effects.

Previous single dose drug challenge studies in animals have shown controversial results since administration of a GABA_A antagonist enhanced exogenous auditory responses resembling the N1 whereas it was attenuated by several different GABA_A agonists (Javitt et al. 1996, Meador 1995). Also in humans GABA_A agonist lorazepam, decreased N1m source activity in MEG study (Rosburg et al. 2004). As the N1m has been suggested to reflect the sound detection (Parasuraman and Beatty, 1980), this function may be impaired in these subjects chronically using GABA_A agonists and opioids.

5.2 Cognitive function during early withdrawal

5.2.1 Cognition and neural pathways

The auditory pre-attentive response MMN and P3a delay in MEG-EEG measurement indexes changes in perception and cognition. Also the neuropsychological tests showed that composite cognitive efficiency was inferior in patients in early abstinence and they especially had problems in complex working memory, executive function, and fluid intelligence (II). As mentioned, opioid dependence modulates the mesolimbic dopamine pathway which projects to the NAcc and prefrontal cortex (Gerra et al. 2004, Volkow et al. 2002). This same monoamine pathway has a crucial role in cognitive functions (Buhot et al. 2000, Coull 1998) and it is linked to cognitive impairments in opioid addicts (Volkow et al. 2002). Transient dysfunction of the prefrontal dopamine system is also found under chronic stress (Izzo et al. 2005). During early opioid abstinence the high stress system activation shown as elevated cortisol level is common, but starts to normalize during the second week of abstinence (Harris & Gewirtz 2005). High cortisol levels are especially pronounced among individuals with antisocial personality disorder (Gerra et al. 2003), which was also diagnosed in most of our patients. It is also known that high cortisol levels may associate with a working memory deficit (Elzinga & Roelofs 2005, Roozendaal et al. 2004). It has been suggested that the stress system induced episodic memory impairment needs a more chronic stress system abnormality than working memory impairment (McEwen 2007, Wolf et al. 2001). Working memory and fluid intelligence test results seem to be better with longer abstinence so the stress induced by withdrawal might play a role in cognitive impairment in early abstinence. On the other hand, the more permanent deficiency in executive function is in line with previous studies showing deficits even in late abstinence (Lee & Pau 2002, Pau et al. 2002).

5.2.2 Dissociations between the neuropsychological tests

We found that patients had an inferior performance in the PASAT test, which is a complex working memory task, but an equal or almost equal performance with controls in episodic memory performance and simple working memory Digit Span tests. This dissociation might be explained by the need for both storage and central executive components of working memory in the PASAT (Audoin et al. 2003, Audoin et al. 2005), but the Digit Span task especially demands storage of several items and the central executive is involved to a lesser degree. Because PASAT test performance was deficient it is suggested that central executive component of working memory is impaired during early opioid abstinence while storage is intact. Episodic memory studied with immediate or delayed free recall tasks on the other hand is dependent on hippocampal function, which despite the neural dysregulations did not seem to affect the test results significantly. Here also the elevated stress system activation may be related to the dissociation between complex working memory and episodic memory functions.

During the early withdrawal patients were inferior to VIQ matched controls in the fluid intelligence task. According to functional neuroimaging studies this indicates deficiencies in the frontoparietal networks needed in several demanding cognitive task (Duncan & Owen 2000).

5.2.3 Effect of abstinence duration on neuropsychological function

Some evidence suggest that the complex working memory deficit is a transient phenomena. Individuals with current opioid use (methadone), or under early opioid abstinence, have shown a working memory deficit, whereas individuals who have reached late opioid abstinence with nine months of opioid abstinence did not show a similar deficit (Mintzer et al. 2005). During early opioid abstinence neural dysregulations are pronounced and probably affect more cognitive performance than possible neural damage (Mintzer et al. 2005, Robinson & Kolb 2004). If neurocognitive deficits are considered a reflection of the permanent neurotoxicity of opioid abuse test performance should not depend so much on the duration of abstinence or current abuse. Our results support the recovery of cognition, at least to some extent by high positive correlations found between fluid intelligence performance or complex working memory performance and abstinence. Executive function, studied with the figural fluency test, did not correlate with the days of abstinence, which is well in line with previous studies (Lee & Pau 2002, Pau et al. 2002). Thus, it is possible that executive function deficit may be more permanent than complex working memory or fluid intelligence deficit.

5.2.4 Neurocognitive and structural correlations

The composite cognitive function correlated with gray matter volume in controls but not in patients (IV). It is known that gray matter volume correlates with intelligence (Frangou et al. 2004), so it could also be assumed that patient's composite cognitive function would correlate with their gray matter volume without some disturbances in their test performance. It is unclear whether each tissue compartment has unique relationships with various neuropsychological abilities, but myelinated white matter for example is essential for rapid transfer of information required for novel working memory and complex attention tasks (PASAT) (Lockwood et al. 2004). The frontotemporal atrophy seen in these opioid-dependents might in part affect the results of these quite complex global tests, but the negative influence of withdrawal on test performance was probably more pronounced. Interestingly Pezawas et al. (1998) has found that the small frontal volume is associated with higher rates of relapses back to opioid abuse.

5.3 Changes in brain structure in opioid dependents

5.3.1 Brain atrophy

Opioid dependent patients showed dilatation of lateral ventricles and in cortical CSF spaces the Sylvian fissures were enlarged. Volumetric analysis confirmed that the relative CSF volume percentage was larger in opioid dependents than in age and sex matched controls. This is indicative of brain atrophy, usually assessed as enlarged sulci and ventricles. This finding is in line with the previous CT studies showing significant ventricular enlargement in opioid dependents (Pezawas et al. 1998, Strang & Gurling 1989).

The dilatation of Sylvian Fissures is probably reflecting the atrophy of frontal and temporal cortical and subcortical areas (Guo et al. 2001, LeMay 1984). This is well in agreement with Lyoo et al (2006) as they showed that opioid dependents have diminished gray matter volumes in frontal and temporal areas. Another MRI study also showed that polysubstance abusers had significantly smaller gray matter volumes than controls, particularly in the prefrontal lobe (Liu et al. 1998). Only a few of these patients were opioid dependents though. MRI spectroscopy of long-term heroin users have shown decreased N-acetylaspartate levels in gray matter in frontal areas, which is considered a sign of neural damage (Haselhorst et al. 2002). A recent human study showed a widespread axonal damage in autopsy specimens of the polydrug abusers' brain and this change was more pronounced in young subjects, but only a few patients showed changes in myelin (Buttner et al. 2006). Also an animal study has shown the decrease of neuronal size in the ventral tegmental area (VTA) in rats after morphine administration (Skclair-Tavron et al. 1996). Decreased frontal white matter volumes in chronic substance abusers have also been reported (Schlaepfer et al. 2006).

Jankovic et al (1991) showed abnormally increased serum autoantibodies to brain antigens, S100 protein (a calcium-binding protein localized to astroglial cells), neuron- specific enolase (a glycolytic enzyme that is localized primarily in the neuronal cytoplasm) and myelin basic protein in the patients with opiate abuse. Furthermore, they found that the incidence of autoantibodies was positively related to the duration of drug abuse. These markers have been considered to be associated with neuronal death as well as with abnormal myelin.

5.3.2 Brain maturation

Very interestingly enlargement of Sylvian fissures had a negative correlation with the age of opioid abuse onset even though it did not correlate with the duration of opioid abuse. In normal development, these dorsolateral frontal cortex and superior parts of the temporal lobes are the last to mature in early adulthood and frontotemporal white matter even later (Gogtay et al. 2004, Sowell et al. 2004, Yakolev et al. 1967). Most of our patients had begun their substance abuse in adolescence or early adulthood, which might have in part disturbed this late maturation process of brain.

Brain maturation is a complex sequence of development of gray and white matter regulated by genetic codes. This process is influenced positively or negatively by environmental factors. Brain maturation starts during fetal life, but the volume of the new born baby's brain is only a quarter or a third of the volume of adult brain.

The mature white matter consists of large groups of myelinated axons interconnecting brain areas. Myelin is an electrically insulating phospholipid fatty sheet around neurons and axons affecting the

speed of neural transmission between different brain regions and it is essential for proper cognitive, motor and sensory functions. Myelination of the brain begins during the 5th fetal month with myelination of the cranial nerves, and is most rapid during the first 2-3 years of life. Historical histological studies by Yakolev and Lecours showed that myelination continues in association areas of frontal lobes into the third and maybe even fourth decade of life (Yakolev et al. 1967). This finding was confirmed later by Benes et al showing that myelination is continuing well into the third decade (Benes et al. 1994). The maximum volume of white matter in temporal and frontal lobes is reached in the mid forties (Bartzokis et al. 2001).

The gray matter development consists of the growth of cortical and subcortical gray matter nuclei, cell proliferation and organized cell migration. Branching dendrites are making synaptic connections between neurons. First an overproduction of these synapses occurs followed by the elimination of connections while myelination of neurons and axons proceed. Overproduction of the synapses is probably a tool for plasticity of the neuronal network in early childhood. Also stimulation and experiences modulate the dendrite branching of neurons and the numbers of synaptic connections and these connections are remodeled throughout life (Toga et al. 2006, Toga & Thompson 2007). The development of the prefrontal cortex; an area of the brain involved in executive, attentional and regulatory function, peaks at 1-2 years with substantial decline from preschool age to mid-to-late adolescence (Huttenlocher 1979). Gray matter volume reaches its maximum at the age of 12 years in the frontal lobes and 16 years in the temporal lobes (Gogtay et al. 2004).

5.3.3 The effects of polysubstance abuse

We could not find changes in gray and white matter volumes, but gray matter and white volumes were measured in the whole cerebrum and the patient group was relatively small, which both affect detection of small local differences. The total cerebral volume was smaller in the patient group, which probably indicates that both white and gray matters are at least somewhat diminished. Several factors may contribute to brain atrophy including other abused substances, additional substances such as adulterants in injected substances, and possible overdoses of drugs.

In cocaine dependent subjects age-related expansion of white matter volume occurring in normal subjects was absent (Bartzokis et al. 2002). Also the fosfomonoester and fosfodiester concentrations were lower in the central white matter of cocaine dependent polysubstance abusers compared to controls most likely reflecting altered synthesis or breakdown of myelin phospholipids (MacKay et al. 1993). In contrast methamphetamine abusers are reported to have an increase of white matter volumes which was speculated to be due to altered myelination and adaptive glial changes (Thompson et al. 2004). To our knowledge no reports correlate the myelination process and opioid abuse, neither in animals nor patients.

Our patients had a history of long term heavy opioid abuse, thus we were expecting to find more remarkable alterations on brain MRIs. Although recreational drug abuse is one of the most important risk factors for stroke in young adults (Sloan et al. 1998), we did not find any signs of ischemia. Also in polydrug abusers non-specific white matter alterations has been found (Aasly et al. 1993), but our patients did not show any unspecific focal changes. Only one patient had a subcortical post traumatic lesion.

We found a tendency to a smaller area of the vermis in the midsagittal image as five of the 17 patients had relatively small vermes and wide vermian sulci, which related to their age could be considered slightly atrophic. It is well known that heavy alcohol consumption may cause cerebellar

and cerebral atrophy (Hayakawa et al. 1992), which are more severe with increasing age (Pfefferbaum et al. 1997). On the other hand, after abstinence, some of these changes may be reversible (Pfefferbaum et al. 1995). In this study those four patients who admitted to heavy alcohol use in youth, showed no reduction of the area of vermes, but the ones with small vermian areas did not have a history of alcohol abuse.

5.4 Limitations

A limitation of our study is that most of our patients fulfilled DSM-IV criteria for antisocial personality disorder. The P3 amplitudes during the active task have been shown to be reduced in subjects with antisocial personality disorder (Bauer et al. 1994). To our knowledge, it is not known whether antisocial personality disorder may change pre-attentive auditory processing measured with MMN and P3a. The previous MRI imaging studies of patients with personality disorders have shown frontotemporal atrophy and ventricular enlargement (Dolan et al. 2002, Raine et al. 2000). Some evidence indicates that personality disorders may be associated with lower prefrontal gray matter, lower posterior hippocampal volume, and higher callosal white matter volume or some reduction in gray matter volumes (Brambilla et al. 2004, Pridmore et al. 2005).

Previous cannabis abuse was common among our patients as well as benzodiazepine dependence and long-term abuse was diagnosed in many. Long-term cannabis abuse and benzodiazepine abuse both have an adverse effect on cognitive function (Barker et al. 2004, Solowij et al. 2002). Also long-term benzodiazepine use may lead to some minor morphological changes, such as slight ventricular dilatation, also found in our patients (Lader et al. 1984, Moodley et al. 1993).

Current benzodiazepine medication during withdrawal and at the time of cognitive testing was common. In the normal population benzodiazepines have adverse effects on several cognitive functions, but the acute effect on opioid dependents and opioid dependents with benzodiazepine co-dependence is not known. On the other hand, the α_2 -adrenergic receptor agonist, lofexidine, which was given to (Barch 2004) the patients of this study, may improve reduced working memory performance.

In the neuropsychological test groups patients and controls were verbal intelligence quotient (VIQ) matched whereas in most other studies the matching is based on education. Since substance abuse typically onsets at a young age, which results in skipping school and dropping out it is most probable that these individuals do not achieve the level of education they could. Some other opioid studies have also matched the groups by VIQ or premorbid IQ (Davis et al. 2002, Ornstein et al. 2000).

5.5 Conclusion

Our results of structural and functional changes in opioid dependents appear in the frontotemporal areas where higher order cognition such as executive function, working memory, and fluid intelligence are situated. Thus, higher order cognition disturbance, interruption of anticipation and establishment of goals, and impulsive behaviour during early opioid abstinence are likely to be associated. This on the other hand may indicate relapses back to substance abuse.

Changes in auditory perception were found since pre-attentive auditory processing was disturbed in the opioid dependent individuals and benzodiazepine co-dependence further modulated the auditory response indicating changes in the frontotemporal neural pathways.

Opioid dependents also showed the dilatation of cerebrospinal fluid spaces especially in frontotemporal areas, indicating brain atrophy. The structural changes seen in opioid dependents correlated with the age of opioid abuse onset, which indicates the vulnerability of adolescents to drug abuse.

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