DOPAMINE TRANSPORTER IN ALCOHOLISM

A SPET study

PEKKA LAINE

Departments of Psychiatry and
Clinical Chemistry, University of Oulu
Department of Forensic Psychiatry,
University of Kuopio
Department of Clinical Physiology and
Nuclear Medicine, University of Helsinki

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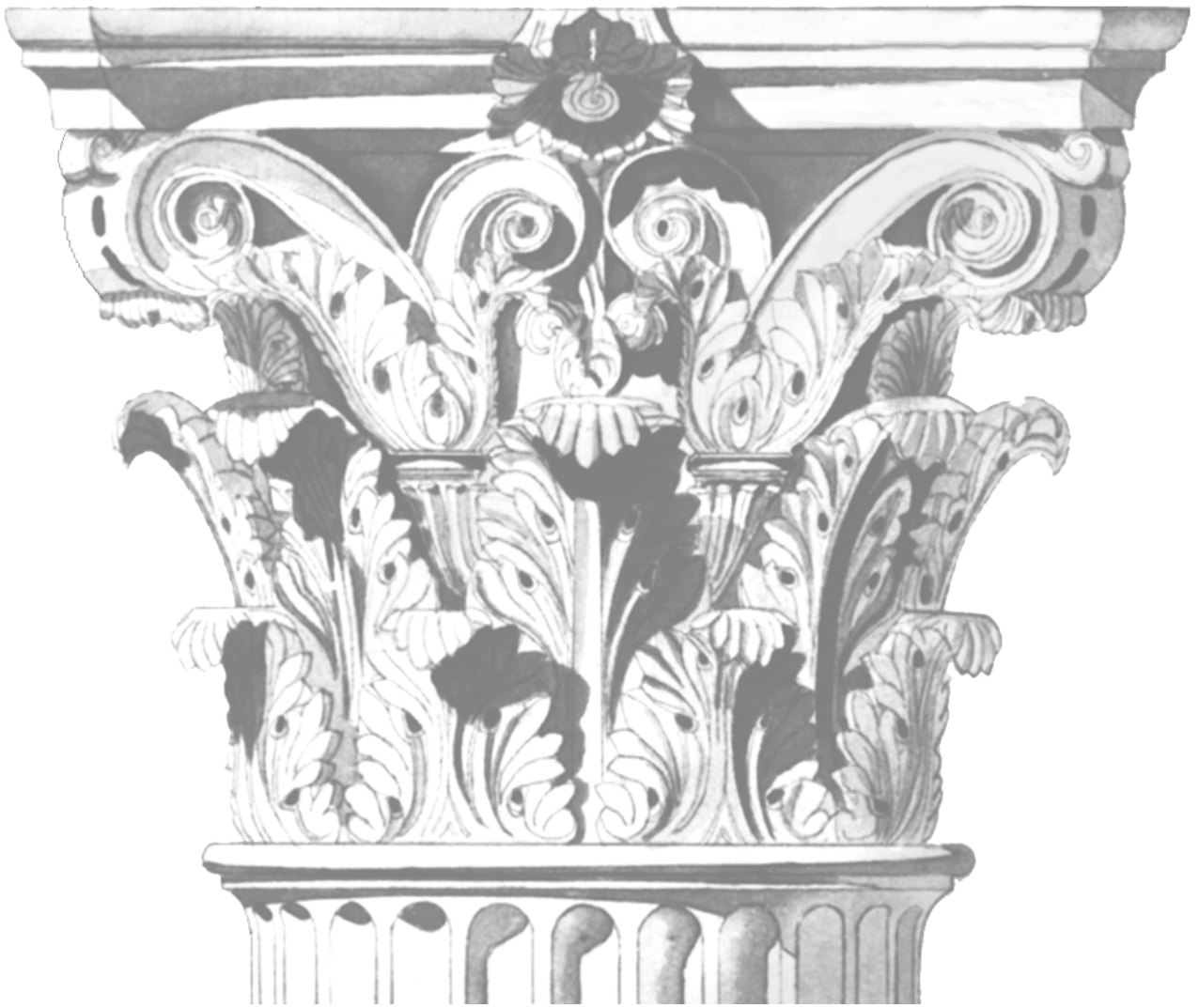

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Department of Clinical Chemistry, Division of Nuclear Medicine, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland, Department of Forensic Psychiatry, University of Kuopio, , FIN-70211 University of Kuopio, Finland, Department of Clinical Physiology and Nuclear Medicine, Division of Nuclear Medicine, University of Helsinki, P.O. Box 340, FIN-00029 Helsinki, Finland, Department of Psychiatry, University of Oulu, P.O.Box 5000, FIN-90014 University of Oulu, Finland

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Abstract

A large body of animal studies indicates that reinforcement from alcohol is associated with dopaminergic neurotransmission in the mesocorticolimbic pathway. However, as most psychiatric phenomena cannot be studied with animals, human studies are needed. Furthermore, because of the fluctuating nature of phenomena regarding the status of abuse and withdrawal, repeated observations of the same study subjects under different situations can elucidate a variety of pathophysiological mechanisms.

In this study 42 alcoholics were monitored during withdrawal and 30 alcoholics after four weeks of abstinence. ^{123}I-β-CIT SPET was used as a method for the semi quantification of their striatal dopamine transporter (DAT) densities reflecting the function and structure of the dopaminergic system.

DAT density was markedly lower during withdrawal among alcoholics as compared to control subjects, but it elevated during abstinence to the level of healthy volunteers. This increases in DAT density during withdrawal and afterwards correlated with the depression scores of alcoholics. DAT density correlated with the Novelty Seeking (NS) personality trait, especially among abstinent alcoholics. After four weeks of controlled abstinence alcoholics with an A1 allele of dopamine receptor D2 were found to have higher DAT densities than alcoholics without it.

The results indicate that striatal DAT density is associated with mood, personality, A1 genotype and the length of the abstinence period after heavy alcohol drinking.

Keywords: depression, A1 allele, alcohol withdrawal, alcoholism, corpus striatum, dopamine, transporter, exploratory behavior, personality, radionuclide imaging, single-photon emission-computed tomography

Nikander: ”Mitäs juot!”
Melartin: ”Alkoholista tulee hyvä olo ja asiat tuntuvat luistavan.”

Aki & Mika Kaurismäki (1986)
Varjoja Paratiisissa.

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The permission of the publishers to reprint the original articles is acknowledged.

Oulu, 9th August, 2001 Pekka Laine

Abbreviations

ALAT	Alanine aminotransferase
ANOVA	Analysis of variance
ASAT	Aspartate aminotransferase
CDT	Carbohydrate deficient transferrin, sialotransferrin
CFT	2 β -carbomethoxy-3 β -(4-[^{18}F]fluorophenyl)tropane
CI	Confidence intervals
CRF	Corticotrophin-releasing factor
DA	Dopamine
DAT	Dopamine transporter
DDA	Daily dose of alcohol
DSM III-R	Diagnostic and statistical manual of mental disorders, revised
DPK	Daily dose of alcohol per kilogram of body weight
EEG	Electroencephalogram
FPCT	[^{18}F]-2-β-carbomethoxy-3 β-(4-chlorophenyl)-8-[-3-fluoropropyl)-nortropane
FWM	Frontal white matter
GGT	Gammaglutamyl transferase
5-HT	5-hydroxytryptamine, serotonin
HA	Harm Avoidance
IBZM	3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl] benzamide
MADRS	Montgomery Åsberg depression rating scale
MAOI	Monoamine oxidase inhibitor
MCV	Mean corpuscular volume
NA	Nucleus Accumbens
NET	Norepinephine transporter
NS	Novelty Seeking
OWM	Occipital white matter
PCP	Phencyclidine
PCR	Polymerase chain reaction

ROI	Region of interest
RD	Reward Dependence
SD	Standard deviation
SERT	Serotonin transporter
SPECT, SPET	Single photon emission (computerized) tomography
SPSS	Statistical Package for the Social Sciences
SSA	Selected severity assessment
STR	Striatum
TCI	Temperament and Character Inventory
TPQ	Tridimensional Personality Questionnaire
TRH	Thyrotropin releasing hormone
VMAT-2	Vesicular monoamine transporter
VTA	Ventral tegmental area
β-CIT	[^{123}I]-2-β-carbomethoxy 3-β-(4-iodophenyl)-tropane
β -CIT-FP	[^{123}I]-N-ω-fluoropropyl-2-β-carbomethoxy 3-β-(4-iodophenyl)-tropane
Δ-DAT	Percental change in DAT density

List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals.

I Laine TPJ, Ahonen A, Torniainen P, Heikkilä J, Pyhtinen J, Räsänen P, Niemelä O & Hillbom M (1999) Dopamine transporters increase in human brain after alcohol withdrawal. Molecular Psychiatry 4:189-191.

II Laine TPJ, Ahonen A, Räsänen P & Tiihonen J (1999) Dopamine transporter availability and depressive symptoms during alcohol withdrawal. Psychiatry Research: Neuroimaging section 90: 153-157.

III Laine TPJ, Ahonen A, Räsänen P & Tiihonen J (2001) Dopamine Transporter Density and Novelty Seeking among Alcoholics. Journal of Addictive Diseases 20: 91-96.

IV Laine TPJ, Ahonen A, Räsänen P, Pohjalainen T, Tiihonen J & Hietala J (2001) The A1 allele of the D2 dopamine receptor gene is associated with high dopamine transporter density in detoxified alcoholics. Alcohol and Alcoholism 36: 262-265.

Some unpublished results are also presented. Reprints are published with a permission of publishers: Stocton Press (I), Elsevier Science (II), The Haworth Press (III) and Oxford University Press (IV).

Contents

1 Introduction

About 10% of the adult males and 14% of the females in Finland are absolutists, the remainder drinking more or less. Half of the total alcohol is consumed by 10% of the people (Hein *et al*. 2000). Heavy drinkers who drink more than 30 g of alcohol daily (Sillanaukee *et al.* 2000) constitute 6-12% of the Finnish people (Hein *et al*. 2000). 3% of the population consumes alcohol on a daily basis (Hein *et al*. 2000).

Alcohol-seeking behavior is a special case of exploratory appetitive behavior and involves different genetic backgrounds than do susceptibility to behavioral tolerance and dependence on the anti-anxiety or sedative effects of alcohol (Cloninger 1987a). The mesocorticolimbic dopaminergic tract from the ventral tegmental area (VTA), *via* the nucleus accumbens (NA) in ventral striatum to the prefrontal neo-cortex, is found to be the crucial pathway mediating reinforcement and addiction to all known substances and drugs of abuse, including alcohol (Wise & Rompre 1989, Koob 1992).

Large psychiatric co-morbidity has been found among alcoholics. Depressive and anxious disorders are the most common psychiatric problems of axis I manifesting itself most seriously during current alcohol problems (Alaja *et al.* 1998). As well antisocial and borderline personality disorders are common among the population with drinking problems (Alaja *et al.* 1998). As circular causalities are common in human behavior, psychiatric problems can predispose to substance abuse disorders and *vice versa*.

Clear and obvious heredity has been found in alcoholism and other substance abuse disorders, and substance abuse problems are common among the relatives of alcoholics. Adoption studies have shown that genes have an important role to play in the development of alcoholism (Cloninger 1995). Gene candidates for alcoholism have been found, for instance, among those regulating dopaminergic system (Noble 2000a,b), but their significance is still poorly understood. There is paucity of data and attempts to bridge the gap in neurobiological models of alcohol abuse between experimental animal and human studies, which is an important task to verify any theories of alcohol addiction. Novel functional imaging studies can be beneficial in mapping unknown areas of knowledge.

2 Review of the literature

2.1 Alcohol dependence

As alcohol consumption is very common also among Finnish people, the boundary to what may be termed harmful alcohol dependence has to be defined. Alcoholics, recruited in this study, fulfilled the following DSM III-R Diagnostic criteria for Psychoactive Substance Dependence (American Psychiatric Association 1987), which were in official use in Finland during the performance of this study.

2.1.1 The DSM III-R criteria for substance abuse

At least three of the following:

1. substance often taken in larger amounts or over a longer period than the person intended
2. persistent desire or one or more unsuccessful efforts to cut down or control substance use
3. a great deal of time spent in activities necessary to get the substance (e.g., theft), taking the substance (e.g., chain smoking), or recovering from its effects
4. frequent intoxication or withdrawal symptoms when expected to fulfill major role obligations at work, school, or home (e.g., does not go to work because hangover, goes to school or work "high," intoxicated while taking care of his or her children), or when substance use is physically hazardous (e.g., drives when intoxicated)
5. important social, occupational, or recreational activities given up or reduced because of substance use
6. continued substance use despite knowledge of having persistent or recurrent social, psychological, or physical problem that is caused or exacerbated by the use of the substance (e.g., keeps using heroin despite family arguments about it, cocaine-induced depression, or having an ulcer made worse by drinking)

7. marked tolerance: need for markedly increased amount of the substance (i.e., at least 50% increase) in order to achieve intoxication or desired effect, or diminished effect with continued use of the same amount

Note: The following items may not apply to cannabis, hallucinogens, or phencyclidine (PCP):

8. characteristic withdrawal symptoms (see specific withdrawal syndromes under Psychoactive Substance-induced Organic Mental Disorders)
9. substance often taken to relieve or avoid withdrawal symptoms
10. Some symptoms of the disturbance have persisted for at least one month, or have occurred repeatedly over a longer period of time.

Criteria for Severity of Psychoactive Substance Dependence:
Mild: Few, if any, symptoms in excess of those required to make the diagnosis, and the symptoms result in no more than mild impairment in occupational functioning or in usual social activities or relationships with others.
Moderate: Symptoms or functional impairment between "mild" and "severe."
Severe: Many symptoms in excess of those required to make the diagnosis, and the symptoms markedly interfere with occupational functioning or with usual social activities or relationships with others.
In Partial Remission: During the past six months, some use of the substance and some symptoms of dependence.
In Full Remission: During the past six months, either no use of the substance, or use of the substance and no symptoms of dependence (American Psychiatric Association 1987).

2.2 Cloninger's classification of alcoholics

The theory of Robert Cloninger about early and late onset alcoholism was based on the Stockholm Adoption Study considering adopted children of alcoholics (Cloninger *et al.* 1981). On the basis of that study and families in the United States with their clinical features and patterns of inheritance, Cloninger defined two groups of alcoholics. These subtypes may be distinguished in terms of distinct alcohol-related symptoms, personality traits, ages of onset, and patterns of inheritance. The late onset, type 1 alcoholism is characterized by anxious (passive-dependent) personality traits and rapid development of tolerance and dependence on the anti-anxiety effects of alcohol. This leads to loss of control, difficulty in terminating binges once they start, guilt feelings, and liver complications following socially encouraged exposure to alcohol intake. This type includes 80% of all alcoholics (Cloninger *et al.* 1988).

In contrast, antisocial personality traits from teenage periods and persistent seeking of alcohol and other substances for their euphoriant effects characterize the early onset, type 2 alcoholism. This type of character leads to early onset of inability to abstain entirely from alcohol, as well as fighting and arrests when drinking (Cloninger *et al.* 1988). In a recent replication study in Stockholm, most of these findings were replicated. Type 1, late onset alcoholism is thought to be, and also called milieu-related. The type 2, early onset alcoholism in Stockholm study was manifested without the effect of the milieu.

However, earlier findings of milieu relation of the two alcoholism types were not replicated, respectively (Sigvardsson *et al.*1996).

Characteristic differences of alcoholic types are crystallized as presented in Table 2.

Table 1. Distinguishing characteristics of two types of alcoholism (Cloninger 1987a, 1995).

Characteristic features	Type of alcoholism	
	Type 1	Type 2
Gender	Both sexes	Males
Usual age of onset (years)	After 25	Before 25
Alcohol related problems		
Spontaneous alcohol seeking (inability to abstain)	Infrequent	Frequent
Fighting and arrests when drinking	Infrequent	Frequent
Psychological dependence (loss of control)	Frequent	Infrequent
Guilt and fear about alcohol dependence	Frequent	Infrequent
Personality traits		
Novelty Seeking	Low	High
Harm Avoidance	High	Low
Reward Dependence	High	Low
Defect	Dopaminergic	Serotonergic

2.2.1 Separating alcoholic types by age of onset

In Cloninger's scenario, the alcoholic subgroups should not be considered as discrete disease entities, because many alcohol abusers have some features of both types (Gilligan *et al.*1988). Rather, the different alcohol related syndromes are associated with the polar extremes of personality traits that vary continuously (Cloninger *et al.*1987b). There has also been a need for a mark, cutting this continuum in two, because there are several alcoholics who do not meet criteria of either types, and a minority meeting them both (Lamparski *et al.*1991). If the biophysiological basis of the two types differs crucially, also the medical cure might be different. Von Knorring *et al.* (1985) and Irwin *et al.* (1990) have suggested that an age of onset of alcohol-related problems before the age of 25 would be the most significant classification criterium between the groups. Johnson *et al.* (2000) found that an onset age of under 20 predisposes to more severe alcoholism.

2.3 Personality disorders and personality traits of alcoholics

There have been several attempts to explain alcoholism in connection with certain alcoholic personalities. Earlier, these explanations have mostly failed to reflect the true habitus of alcoholism (Donovan 1986). Certain personality disorders have been found to be very common among substance abusers. Nace *et al.* (1991) found 57 out of 100 substance abusers to fulfill the criteria of at least one personality disorder. Grilo *et al.* (1997) have found borderline disorders to be significantly more common among substance abusers. Cloninger noticed antisocial personality disorders to be quite typical in early onset alcoholism and passive-avoidant personality in late onset alcoholism (Cloninger 1987a).

2.3.1 Personality traits and neurotransmitters

On the basis of various pharmacological and animal studies, Cloninger proposed that the neurochemical basis of Novelty Seeking (NS), as a behavioral activation system, a heritable tendency toward frequent exploratory activity and intense exhilaration in response to novel or appetitive stimuli, was dopaminergic also in human brains. Dopaminergic cell bodies in the midbrain receive inputs from several sources and then project impulses to the forebrain, thereby possibly acting as a final common pathway for behavioral activation. (Cloninger 1987a)

Parallel to this, Harm Avoidance (HA) as a behavioral inhibitory system, can be seen as a result of the septo-hippocampal system, serotonergic projections from the raphe nuclei in the brainstem, and cholinergic projections to frontal neocortex from the midbrain reticular formation near the ventral tegmental area and the basal nucleus of the amygdala. (Cloninger 1987a)

As a behavioral maintenance system, Reward Dependence (RD) is hypothesized to involve variation in behavioral maintenance or resistance to extinction of previously rewarded behavior. This resistance to an extinction is hypothesized to result from facilitation of paired associate learning by a brain system that is activated primarily at the onset of a reward or the offset of punishment, thereby facilitating formation of conditioned signals of reward or relief from punishment. Norepinephrine seems to satisfy the characteristics required of the major neuromodulator for this system and may play a critical role in the learning of new, paired associations. (Cloninger 1987a)

2.3.2 Tridimensional Personality Questionnaire (TPQ)

To measure the traits mentioned above (e.g. NS, HA and RD), Cloninger created the Tridimensional Personality Questionnaire (TPQ) (Cloninger *et al.* 1991). The TPQ

includes 98 questions divided into 34 items of NS, 34 items of HA, and 30 items of RD (Table 2).

Table 2. The contents of the Tridimensional Personality Questionnaire (TPQ) after Cloninger et al. 1991.

Item	Total number of items	Contents
NS1	9	exploratory excitability vs. stoic rigidity
NS2	8	impulsiveness vs. reflection
NS3	7	extravagance vs. reserve
NS4	10	disorderliness vs. regimentation
HA1	10	anticipatory worry vs. uninhibited optimism
HA2	7	fear of uncertainty vs. confidence
HA3	7	shyness with strangers vs. gregariousness
HA4	10	fatigability and asthenia vs. vigor
RD1	5	sentimentality vs. insensitiviness
RD2	9	persistence vs. irresoluteness
RD3	11	attachment vs. detachment
RD4	5	dependence vs. independence

In Cloninger's theory, the personality traits of the two types of alcoholics are controversial. Type 1 alcoholics are low in NS and high in RD and HA. Type 2 alcoholics, on the other hand, are high in NS and low in RD and HA (Table 1). In sum, Cloninger supposed that type 1 alcoholics are suffering from dopaminergic, and type 2 alcoholics from serotonergic defects (Cloninger 1995).

Later Cloninger supplemented his personality hypothesis to include temperament and character. The questionnaire was broadened to the Temperament and Character Inventory (TCI) by separating persistence from RD and adding items of separate values of character (Cloninger *et al.* 1993). The use of this inventory can be expanded to the diagnostics of the personality disorders (Svaric 1993; Cloninger 1987a, 2000).

2.4 Alcohol withdrawal

The pharmacological effects of ethanol are complex and widespread without a well-defined target. The alcohol withdrawal syndrome according to DSM III R is defined by a set of criteria or symptoms that increase by number and intensity with the syndrome's severity; it can occur without complications, with seizures or with delirium tremens. Abnormalities in underlying neurotransmitter functions are considered the basis for withdrawal symptoms. It is supposed that vegetative symptoms are raised by overactivity of adrenergic/noradrenergic and CRF neurons; hallucinations may be due to hyperdopaminergic states, tremor and seizures are considered to be caused by disinhibition of the gabaergic and/or potentiation of the glutamatergic system, and

emotional and cognitive disturbances were proved to be due to imbalances of the serotonergic and cholinergic system, respectively. (Glue *et al.* 1995)

Since glutamatergic and gabaergic innervations are both dense and diffuse and they account for more than 80% of the neuronal circuitry in the human brain, alterations in glutamatergic and gabaergic function could affect the function of all neurotransmitter systems. In particular, GABA and NMDA-receptors appear to play a central role in alcohol dependence and alcohol withdrawal-induced seizures (Davis & Wu 2001). Alcohol facilitates the function of the inhibitory $GABA_A$ receptor channels and decreases the function of excitatory NMDA receptor function (Korpi *et al.* 1998) These effects explain acute alcohol intoxication and their reversal is taking place during withdrawal. It is not clear how these direct effects of alcohol can affect the dopaminergic system, but changes in receptor functions may largely cause the alcohol intoxication and withdrawal. During withdrawal increased glutamatergic activity has shown to cause neurotoxic effects (Tsai *et al.* 1998). Opiate receptors of the dopaminergic cells in the VTA are found to be sensitive to alcohol influence (McBride *et al.* 1993) possibly by producing gabaergic disinhibition. In addition blocking them with opiate antagonists has also been reported to decrease any reinforcing effects of alcohol (Sinclair 1990).

2.5 Depression among alcoholics

Depressive symptoms are over-represented among alcoholics. In a recent study in general hospitals in Finland, 24% of all the patients with substance use disorders had also mood disorders (Alaja *et al.*1998). Primary major depression has been supposed to be an important predictor of alcoholism, especially among males. Alcohol consumption has been found to occur temporally secondary to other DSM-III disorders (Kessler *et al.*1997). In any case, after detoxification, depressive symptoms persist only as frequently as is common for the average population (Brown & Schuckit 1988).

2.5.1 Secondary depression

Alcohol abuse can cause secondary depressive symptoms, which are similar to symptoms accompanying major depressions. Among male subjects, they are found to improve during a period of 4 weeks of sobriety (Schuckit & Monteiro 1988, Roy *et al.* 1991, Schuckit 1994, Brown *et al.*1995). Depression after detoxification can persist still longer, lasting up to 6 weeks following withdrawal (American Psychiatric Association 1993, Schuckit *et al.*1997). Although short, these depressive situations are very severe when combined with impulsiveness of personal disorders and decreased self-control of drinking. In a general hospital study of 1222 psychiatric patients in Finland, 65% of the attempted suicides were related to substance use disorders (Alaja *et al.*1997)

2.5.2 Monoamine hypothesis of depression

Depression is considered to be associated with decreased activity of monoamines, particularly dopamine, serotonin and norepinephrine (Schildkraut 1965, Van Praag *et al.* 1990, Stahl 1998). The older antidepressants like monoamine oxidase inhibitors (MAOI) inhibited the metabolism of all monoamines (Stahl 1998). In later studies, enhancing the activity of the serotonergic system was found to be the common factor in the function of effective antidepressants, a fact that was utilized when developing selective serotonin re-uptake inhibitors. Up-regulation of serotonin receptors during depression and decreased serotonin flow has been suggested to be one pathological mechanism in depression. Recently also norepinephrine has been found to be important in the socialization of depressive patients. Antidepressants inhibiting norepinephrine or both norepinephrine and serotonin have been developed later in order to enhance also social coping capacity (Stahl 1998).

2.5.3 Dopamine hypothesis on depression

Psychomotor retardation and suicidality in depressed people are related to low dopaminergic activity, reflected as low blood levels of dopamine metabolite homovanillic acid or an effect of dopaminergic agents (Rampello *et al.* 1991, Brown & Gershon 1993). High dopaminergic activity is found among depressive patients with delusions or a history of psychosis (Brown &Gershon 1993). The presynaptic dopaminergic function has also been reported to be decreased in depression with affective flattening and psychomotor retardation (Martinot *et al.* 2001). Some of the antidepressants, like sulpiride (Jenner & Mardsen 1982), amineptin and bupropion affect mainly dopaminergic neurotransmission.

Dopaminergic antidepressants have been claimed to act more rapidly than other antidepressants in depressive symptoms (Freeman 1997, Willner 1997), but see (George & Lydiard 1991). Antidepressants that affect the dopamine system, such as nomifensine, bupropion and MAOIs, are regarded by clinicians to be effective for patients when other antidepressants fail (Nierenberg *et al.* 1998). Freeman (1997) suggested that the dopamine effect might be a straight improvement of the pathophysiological system in depression. He concluded that an effect *via* norepinephrine or serotonin systems could be an indirect way to influence the major mechanisms of depression. There are many studies that concern themselves with serotonin modulation of the dopaminergic system (Carlsson 1992, Campbell & McBride 1995, Tiihonen *et al.* 1996, Fujita *et al.* 1997, Smith *et al.* 1997). The depressive symptoms observed during alcohol withdrawal have been hypothesized to be associated with a down-regulation of net dopaminergic neurotransmissions (Roy *et al.* 1985, 1991).

2.5.3.1 Clinical applications of dopaminergic drugs in depression

The theory of the involvement of the dopaminergic neurotransmission system in dependence has led to only a few practical uses of dopaminergic substances in the treatment of various withdrawal and dependence situations. Street knowledge has for a long time favored the use of any other dopaminergic drug to treat the withdrawal symptoms of other substances and this is one common way how mixed drug abuse has developed. The first reported case was Sigmund Freud's failed attempt to cure his friend's Fleischl-Marxow's morphine dependence with cocaine, producing only the first known mixed drug abuser (Gay 1990).

The dopaminergic D_2 agonist bromocriptine has failed to cure the withdrawal syndrome of rats (Uzbay *et al.* 1994). In a human study, Lawford *et al.* (1995) demonstrated a positive effect of bromocriptine on alcohol withdrawal symptoms of patients with the A1 allele, but this result was criticized by Goldman (1995). Naranjo *et al.* (1997) studied long-acting injectable bromocriptine in treatment of relapsing alcoholism, without positive results. Nadal *et al.* (1996) even found bromocriptine to enhance the alcohol consumption of rats. Mardones & Quintanilla. (1996) found a decrease of alcohol consumption in rats during bromocriptine treatment, but after the treatment the consumption again elevated. Amineptin is a dopamine re-uptake inhibitor used commonly as an antidepressant in France, where it has been developed. The only successful study of treating withdrawal symptoms of amphetamine was completed recently and used amineptin (Jittiwutican *et al.* 1997). Bupropion is another partly dopaminergic antidepressant, commonly administered in the USA. It has been approved to assist smokers to quit smoking under the name Zyban® (Quattrocki *et al.* 2000). In a recent study, Ryynänen *et al.* found an improvement of alcoholism during dopaminergic pergolide medication, but only in a genetic subgroup of alcoholics with the MAE3 allele of dopamine 2 receptor gene (submitted).

2.6 Dopamine

2.6.1 Mesocorticolimbic tract

The reward mechanisms have been a topic of intense interest ever since James Olds and Peter Milner in the year 1954 at McGill University reported the results of their innovative study: Laboratory rats would voluntarily self-administer electrical stimulation delivered through electrodes deep in the brain, mainly in the septal area, ventral tegmentum and gingulate gyrus (Olds & Milner 1954). Several nuclei and tracts have been found to be sensitive to electrical and chemical stimuli and on account of that, seemed involved in reward mechanisms. The ventral tegmental area is the locus sensitive to self-administration of alcohol (McBride *et al.* 1993) and opiates (Jenck *et al.* 1987, Phillips *et al.* 1983), while the nucleus accumbens and the prefrontal cortex are self-administration

loci for amphetamines (Colle & Wise 1988, Phillips *et al.* 1981, Hoebel *et al.* 1983) and cocaine (Volkow *et al.* 1996c). Opioid peptidergic reward neurons project from the NA to the ventral pallidum, thus carrying the neural reward signal one synapse further (Stein 1993, Johnson & Stellar 1994).

Amongst the large body of studies, the most crucial locus for dependence has been found to be the mesocorticolimbic tract that rises from the ventral tegmental area (VTA) to the nucleus accumbens in the ventral striatum and to the frontal cortex (Figure 1). All substances causing dependence and reward, including alcohol but expect for benzodiazepines, are found to activate this tract. Increase in dopaminergic activity in the striatum has been found even during a video game (Koepp *et al.* 1998) but, surprisingly, not during pleasant physical exercise (Wang *et al.* 2000).

2.6.1.1 Influence of alcohol on mesocorticolimbic tract

Alcohol influences on the dopaminergic system take place *via* several mechanisms. In an *in vitro* study, DAT has been found not only to eliminate, but also to release dopamine to the synaptic cleft. Metamphetamine, amphetamine and ethanol enhanced this release, which can be one pathophysiological explanation for alcohol influence (Eshleman 1994). Alcohol releases not only dopamine, but also serotonin in rat nucleus accumbens (Weiss *et al.* 1996). Alcohol may also have many other complicated effects on the networked neuronal systems including various other neurotransmitters (Koob *et al.* 1998).

2.6.2 Dopaminergic synapse

The schematic picture of a dopaminergic synapse is illustrated in figure 2. This structure consists of several components, which have found to be linked with addiction situations, for examples, as follows. MAO activity of synapse cannot be studied in living human brain. MAO activity of platelets in blood samples is shown to be low in alcoholics generally and in alcoholics with the DRD2 A1 allele (Eriksson *et al.* 2000). The levels of this enzyme have been reported to increase during alcohol withdrawal (Berggren *et al.* 2000).

Dopamine receptors are various. Their structure in human brain can be studied with whole hemisphere autoradiography *post mortem* (Tupala *et al.* 2000, 2001a,b) or with functional imaging *in vivo*, see chapter 2.6.3. Receptors genetics have largely been studied, see chapters 2.6.4-2.6.6.

The level of DAT messenger RNA of human cocaine users has been studied in a *post mortem* study by Little *et al.* (1998). Although the striatal DAT binding was found to be increased, the medial DAT messenger RNA levels were decreased.

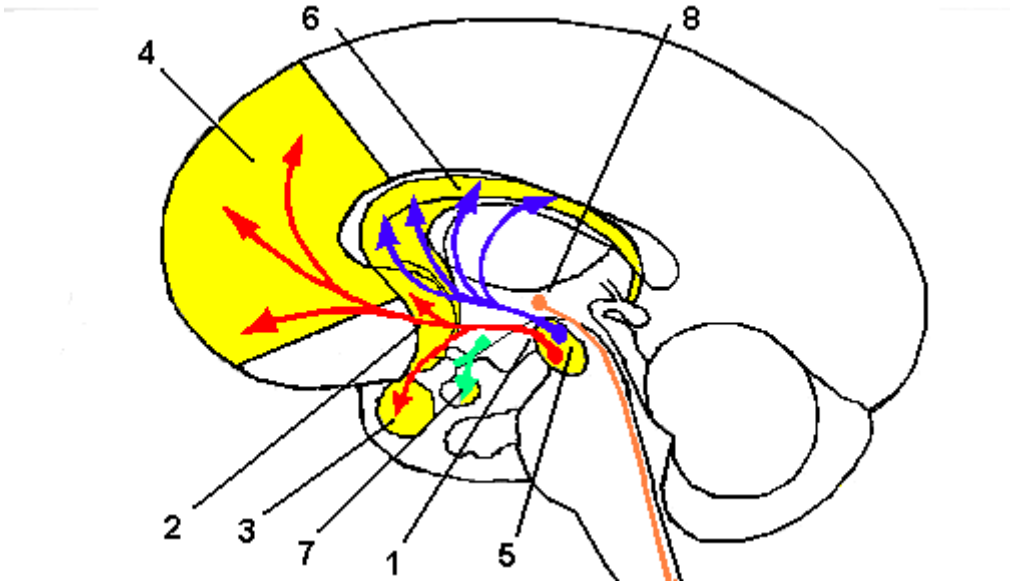

Fig. 1. Main dopaminergic pathways. The mesocorticolimbic pathway rises from the ventral tegmental area (1) to the nucleus accumbens (2), amygdaloid body (3) and prefrontal cortex (4). The nigrostriatal pathway rises from the substantia nigra (5) to the main dorsal part of the striatum (6). The tuberoinfundibular system (7) innervates the pituitary. Dopaminergic neurons of the posterior hypothalamus (8) project to the spinal cord (Kaplan & Sadock 1995(.

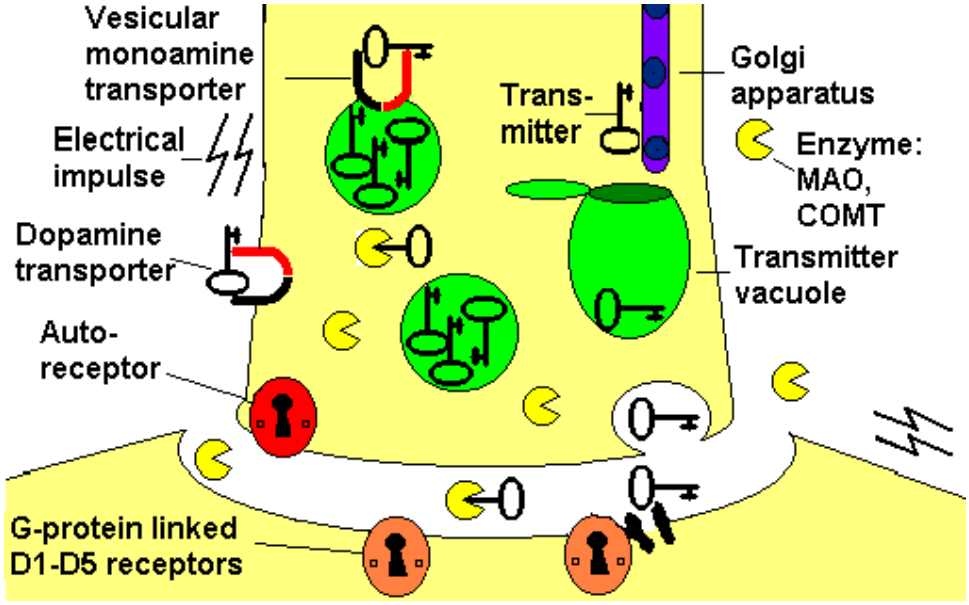

Fig. 2. Dopaminergic synapse. The tracer β-CIT binds to dopamine transporter (DAT).

Sodium dependent dopamine transporters are located within presynaptic plasma membranes of axonal terminals and also somatodendritic spines. The main function of dopamine transporter is to collect and eliminate the released dopamine from the synaptic cleft and perisynaptic areas back into the neuron. This re-uptake process is a primary means of regulating the synaptic concentration of released dopamine and thus the most effective way to terminate its interactions with post- and presynaptic receptors. For example, in brains of mice lacking the DAT gene, the elimination rate of the synaptic dopamine has been found to be 100 times longer than in normal mice (Giros *et al.* 1996). After the re-uptake, the transmitter is packed in vesicles by intracellular transporters or inactivated by metabolizing enzymes. The re-uptake is an energy-demanding process propelled by Na+/K+-ATP-ase. Dopamine can also be released by an action that is carried out by a reversed operation of the DAT. The carrier-mediated release does not require energy and is not associated with neuronal conduction (Levi & Raiteri 1993, Vizi 2000).

2.6.3 Dopamine transporter

Highest levels of axonal DAT are found in the striatum (including putamen, nucleus caudatus and nucleus accumbens) and the tuberculum olfactorium (Donnan *et al.* 1991, Kaufman *et al.* 1991). Dendritic DAT located near the cell bodies of dopaminergic neurons is expressed in intermediate densities in the substantia nigra, and in low densities in ventral tegmental area (Donnan *et al.* 1991, Kaufman *et al.* 1991). A small proportion of striatal DAT is expressed in interneurons using both GABA and dopamine as their transmitters and having both their cell bodies and axons in the striatum (Betarbet *et al.* 1997). DAT affiliates predominantly with axonal membranes and selected dendritic compartments, but not cell soma membranes (Blakely & Bauman 2000, Liu *et al.* 1999). Dopamine transporters are not located in the active synapse but are bounded to perisynaptic areas, meaning that DA diffuses away from the synapse (Vizi 2000). In striatal dopaminergic terminals, which belong to neurons located in the substantia nigra pars compacta, DAT is detected on the varicose and intravaricose plasma membranes, but not in the active synaptic zones. In rats, a very low density of DAT is observed in the prefrontal cortex compared with the striatum, which may explain the finding that extracellular concentrations are higher there and half-lives of dopamine longer (Masson *et al*. 1999).

In the dopaminergic nerve terminals there are also intracellular vesicular monoamine transporters (VMAT-2) transporting various transmitters (serotonin, adrenaline, norepinephine, dopamine and histamine) from the cytoplasm to synaptic vesicles (Masson *et al*. 1999). However, tracers used in PET and SPET do not pass to intracellular vesicular transporters (Bergström *et al.* in press) and vesicular transporters are apparently not regulated by dopaminergic drug treatment (Vander Borght *et al.* 1995).

2.6.3.1 Experimental changes on DAT density

Thyrotropin releasing hormone (TRH) is known to regulate striatal DAT density (Ikegami *et al.* 1988, Prasad 1991). Cocaine and other DAT blockers (Ikegami & Prasad 1988, Wiener *et al.* 1989) have previously been found to up-regulate DAT density by blocking dopamine transporters in human studies (Little *et al.* 1993, 1998, 1999, Malison *et al.* 1998a). In other studies, chronic cocaine use was associated with a reduced level of DAT (Farfel *et al.* 1992, Wilson *et al.* 1996). In an animal study involving rats, the decreased dopamine flow did not decrease DAT density (Moody *et al.* 1996, Scheffel *et al.* 1996), nor has dopaminergic medication, used in Parkinson's disease (Innis *et al.* 1999, Ahlskog *et al.* 1999), or other dopamine 2 receptor stimulants (Little *et al.* 1999) been found to affect β-CIT SPECT imaging. Bergström *et al.* (1998) found the opiate agonist fentanyl to decrease DAT density both in a human subject and in an experimental animal study.

A detailed prescription of other possible agents regulating DAT density has recently been reviewed (Laakso 1999). There is evidence for rapid changes in transporter capacity (1-30 min) following activation of cellular kinases. These studies suggest that transport cannot be considered to present an inert constitutive property of synaptic membranes, but rather an actively regulated element of aminergic signalling (Blakely & Bauman 2000).

2.6.4 Imaging of DAT in vivo

2.6.4.1 Use of radioligands in measuring the DAT density

Location and density of dopamine transporters of dead human brain can be most accurately detected with whole hemisphere autoradiography (Tupala *et al.* 2001a). Although there are several radioligands with sufficient selectivity for *in vitro* studies of DAT, only a few of them are suitable for *in vivo* studies. Metabolism, affinity to plasma proteins, regional blood flow and the blood-brain barrier may all restrict the delivery of the tracer into the brain. Non-specific binding and high affinity for other receptors decrease the signal-to-noise ratio of the detected signal.

Most DAT tracers are tropane derivates structurally related to cocaine. Radioisotope labeled cocaine itself has been used, but considerable affinity to other monoamine transporters, a poor specific-to-non-specific binding ratio *in vivo* and a low statistical quality of images caused by fast kinetics make it less than ideal as a quantitative DAT tracer (Fowler *et al.* 1989). Other tropane analogues, such as β-CIT (Bergström *et al.* 1994), β -CIT-FP (Lavalaye *et al.* 2000), RTI-32 and FPCT (Goodman *et al.* 1997) also suffer from considerable binding affinities to serotonin transporter (SERT) and norepinephine transporter (NET). Thalamic uptake of these ligands is displaceable with SERT and NET ligands, whereas striatal uptake is sensitive only to DAT ligands, meaning that striatal binding is reasonably selective (Laruelle *et al.* 1993, Farde *et al.* 1994).

The β-CIT is a tracer with a high rate of specific versus non-specific binding. It has the highest binding rate to dopamine transporters. It has also affinity to 5-HT transporters and in vitro also norepinephrine transporters. Given a degree of neuroanatomic knowledge of the dopaminergic system (Farde *et al.* 1994), we can use it as a radioligand in investigating the striatal dopamine system. The DAT density, determinable with radioligand β -CIT, is relatively stable and a good indicator of the dopaminergic system (Kuhar *et al.* 1990, Bergström *et al.* 1994, Kuikka *et al.* 1995, Seibyl *et al.* 1996). For a detailed prescription of the preparation of β-CIT, see Bergström *et al.* (1994).

2.6.4.2 Age and DAT

Several studies have described decreases of DAT density with age (Meng *et al.* 1999, Pirker *et al.* 2000), ranging from 4% (Kuikka *et al.* 1999) to 6.6% *per decade* (Volkow *et al.* 1996b). In one study, the decline was found to be nonlinear in a population of 27 men and 28 women. The rate of decline was significantly faster in young adults than in older subjects. The brake-point age in this study was 36 years, after which the rate of change became more stable (Mozley *et al.* 1999).

2.6.4.3 Disorders with altered DAT densities

The cell death of dopaminergic neurons, resulting in a decrease of DAT density, in Parkinson's disease has been known for long time (Ehringer & Hornykiewicz 1960). As β-CIT was developed as a SPET tracer for DAT imaging (Laruelle *et al.* 1993, Brücke *et al.* 1993, Bergström *et al.* 1994) it has been recruited for everyday practice in diagnoses of Parkinson's disorder (Innis *et al.* 1993) and its severity (Rinne *et al.* 1995). Malison *et al.* (1995) found a 30% increase of striatal DAT densities in patients with Tourette's syndrome, using [^{123}I]β-CIT SPET. Ginovart *et al.* (1997) even reported a 50% decrease of [^{11}C]β-CIT binding in patients with Huntington's disease in a PET study.

Tiihonen *et al.* (1997) detected a statistically significant decrease of striatal DAT densities in patients with social phobia in a [^{123}I]β-CIT SPET study and Laasonen-Balk *et al.* (1999) have demonstrated increased DAT densities among patients with major depression.

2.7 Dopamine in alcoholism

2.7.1 Experimental animal studies

The homovanillic acid and dihydroxyphenylacetic acid has been studied in animal model (Fadda *et al.* 1990). The levels of these dopamine metabolites were increased after acute alcohol administration. In another experimental animals, decreased brain DA levels have been observed after abrupt cessation of alcohol administration (Rossetti *et al.* 1992, Diana *et al.* 1993, 1996). Mash *et al.* (1996) found chronic alcohol consumption to down-regulate also the DAT densities in alcohol preferring vervet monkeys. This effect was reversed by acute withdrawal. Microinjections of amphetamine to the nucleus accumbens (NA) of ethanol-initiated rats increased their total behavioral responding. On the contrary, the dopamine receptor blocker raclopride caused a dose-related decrease in the total response. These phenomena were seen in the caudate striatum, but not in NA (Samson *et al.* 1993). Cohen *et al.* (1998) found the selective D_3 agonist 7-OH-DPAT to be effective in reducing ethanol self-administration of rats, while the D_2 agonist bromocriptine was not.

Alcohol has found to cause several biochemical changes in the NA, such as increased levels of tyrosine hydroxylase, NMDA R1 and Glutamate R1 receptor subunits and decreased levels of subunit α1 of the $GABA_A$ receptor complex (Ortiz *et al.* 1995). The metabolism of the dopamine precursor thyrosine has been studied *via* the enzyme tyrosine hydroxylase in animal model (Beitner-Johnson D & Nestler 1991). The same workgroup has also examined neurofilament proteins of mesolimbic dopamine system (Beitner-Johnson *et al.* 1992). They reported that cronic morphin and cocaine treatment decreased the level of this rate limiting enzyme (opposite to alcohol effect) and levels of some of the neurofilament proteins.

2.7.2 Human laboratory studies

Plasma homovanillic acid, the major metabolite of DA, has been found to be present in low concentration in abstinent alcoholics (Fulton *et al.* 1995). Heinz *et al.* (1996) found higher dopamine plasma levels and higher apomorphine-induced growth hormone releases on the first day of alcohol withdrawal and even higher levels during the 8th day compared with healthy volunteers. The latter can represent higher dopaminergic basal activity in alcoholics compared with healthy volunteers.

2.7.3 Human imaging studies

Previous studies in at least five days abstinent alcoholics have indicated decreased dopamine D_2 receptors and normal striatal DA transporter binding when comparing alcoholics as a group with an age-matched group of healthy control subjects (Volkow *et al.* 1996b). Heinz *et al.* (2000) studied the role of the DAT gene. They found alleles of this gene to influence on DAT density, but they did not find any connections between alcoholism and DAT density or allele variants.

2.7.3.1 Effect of subgrouping alcoholics

In late onset alcoholics, striatal DA transporter binding has been reported to be decreased, when compared with age and sex-matched control subjects in SPET and *post mortem* autoradiography studies (Tiihonen *et al.* 1995, Repo *et al.* 1999, Tupala *et al.* 2000). In the study of Repo *et al.* (1999) D_2 receptor occupancy ratios did not differ between alcoholics and controls. On the other hand, in a post-mortem autoradiography study, D_2/D_3 binding of epidepride was lower in the nucleus accumbens of type 1 alcoholics when compared with controls (Tupala *et al.* 2001b).

Among violent offenders and early onset alcoholics striatal DA transporter binding with β-CIT-SPECT has been reported to be normal or slightly increased, compared with age and sex matched control subjects (Tiihonen *et al.* 1995, Heinz *et al.* 1998). Higher DAT densities among antisocial early onset alcoholics rather than late onset alcoholics have been described in a *post mortem* study (Tupala *et al.* 2000). The tracer uptake distribution of violent offenders was found to be significantly more heterogeneous in the right than left *striata* in one SPET study sample (Kuikka *et al.* 1998).

2.7.3.2 Imaging studies of dopamine in depression

With IBZM (3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl] benzamide) -SPET D'haenen (1994) found the D_2 receptor density to increase during a major depression, but to decrease again after recovery from the depression. This was also investigated in un-medicated depressive patients, which were re-investigated after treatment with tricyclic antidepressants, and compared with healthy control subjects (Ebert *et al.* 1996). The only change noticed was the decrease of IBZM-binding in those psychomotorically-retarded patients, whose retardation had improved. In another study, IZBM-binding to D_2 receptors had correlations with reaction time and verbal fluency in depressed patients (Shah *et al.* 1997). Hietala *et al.* found that depressive symptoms in schizophrenic patients were associated with a decrease in dopamine synthesis studied with 6-[^{18}F]fluorodopa-PET (1999).

Malison *et al.* (1998a) discovered an inverse correlation between DAT densities and scores on the Hamilton Depression rating scale of cocaine abusers, measured with β-CIT

SPECT. Laasonen-Balk *et al.* (1999) have reported elevated DAT density among patients with major depressions.

2.7.3.3 Findings on Cloninger's personality traits

There is a growing body of data on links between the findings of TPQ or TCI ratings and those of chemical or neuroimaging studies. Patients with Parkinson's disease have been found to have both low Novelty Seeking and low striatal [^{18}F]DOPA uptake (Menza *et al.* 1995). On the other hand, the theory of Cloninger was based on the earlier findings of destruction of the dopaminergic system and rigid, stoic personality of Parkinson patients. Both social phobia and detachment can be seen as negations of Novelty Seeking. Tiihonen *et al.* (1997) found decreased DAT densities among patients with social phobia and Farde *et al.* (1997) have reported an inverse correlation between detachment scores of the Karolinska Scales of Personality and D_2 receptor density. Breier *et al.* (1998) replicated the result of this study. Laakso *et al.* (2000) have found detachment to be negatively correlated with DAT binding in a PET study using [^{18}F]CFT as a radioligand.

2.7.4 The DRD2 gene, candidate gene for alcoholism

It has been estimated from twin and other studies that in severe substance use disorders 60% are genetically determined and 40 % environmentally determined. Of this genetic diathesis 27% has been thought to attribute to the DRD2 gene and 33% is attributed to other genes (Uhl *et al.* 1993).

Genes determining the components of the mesocorticolimbic dopaminergic reward system have been studied widely. Most evidence is available concerning the role of D_2 dopamine receptor (DRD2) minor (A1) allele T*aq*I A in relation to alcoholism (Noble 2000a,b). The A1 allele, however, does not represent functional gene variation *per se*, but endophenotypic studies link this gene variation to low D_2 receptor density *in vivo* (Pohjalainen *et al.* 1998, Jönsson *et al*. 1999). The first studies of this reward gene were very encouraging, suggesting it's over representation among alcoholics (Blum *et al.* 1990). Later this connection has strongly been criticized and a large number of original articles and reviews have been published, with positive or negative conclusions on connections between the A1 allele and alcoholism, other substance abuse or even behavioral dependence like gambling. Recently, after some methodological problems had been solved, the interest in the A1 allele has again been aroused. A1 allele have recently been linked to the severity of alcoholism (Lawford *et al.* 1997, Gorwood *et al*. 2000, Noble *et al.* 2000) For the latest review and detailed meta-analysis, see Noble (2000a,b). Still, some of the authorities conclude negative association between alcoholism and A1 allele (Gelernter *et al.* 1993, Schuckit 1999).

2.7.5 Other gene candidates for alcoholism

The genetic variant producing low catechol-O-methyltransferase levels is found to be higher among late onset alcoholics compared with the general population (Tiihonen *et al*. 1999). Oppositely, this was not found among early onset alcoholics (Hallikainen *et al.* 2000). Functional polymorphism in the promotor region of the X-chromosomal monoamine oxidase A (MAO_A) gene is shown to be related to antisocial and anxious-depressive traits in alcoholics (Schmidt *et al.* 2000). Also genes determining serotonergic and GABA-ergic systems have been under a current interest of addiction studies (Schuckit *et al.* l999, Heinz & Goldman 2000, Loh & Ball 2000).

2.7.6 Genes of dopamine receptors and NS

Genes of dopamine receptors and NS have been studied with the following results: the Dopamine 3 receptor (DRD3) might have an important role in alcoholism. Thome *et al.* (1999) found alcoholics with A1/A2 alleles of the DRD3 gene to have significantly lower NS scores than patients with the genotype A1/A1. Also Staner *et al.* (1998) found carriers of the A1 allele of the DRD3 gene to have lower NS values compared with other bipolar patients without this allele. Dopamine D_4 receptor (DRD4) exon III genes have been suggested to correlate with NS (Benjamin *et al.* 1996, Ebstein *et al.* 1996) but this has not been confirmed in several replication studies (e.g. s.c. Jönsson *et al.* 1997). Boys with DRD2 receptor genes A1, B1 and intron 6 1 alleles as well as boys with the DRD4 receptor gene 7R allele have been found to have higher NS than boys without any of them. NS is highest in subjects with all of these alleles (Noble *et al.* 1998). Attention deficit hyperactivity syndrome, with high NS scores (Faraone *et al.* 2001), is linked with antisocial personality disorder in adulthood.

2.7.7 Genetic studies referring to imaging studies

The A1 allele has been found to correlate with low D_2 receptor availability among alcoholics (Noble *et al.* 1991) as well as healthy volunteers (Thompson *et al.* 1997, Pohjalainen *et al.* 1998), although not all studies are in agreement with each other (Laruelle *et al.* 1998). D_2 receptor densities have been found to be lower among alcoholics than among controls (Noble *et al.* 1991) Among alcoholics, D_2 receptor densities are low in relation to the DAT densities (Volkow *et al.* 1996b). Heinz *et al.* (2000) reported that alleles of the DAT gene correlated with DAT densities, but they failed to find any connections between alcoholism and DAT.

2.8 Conclusions based on the literature

In 1995, when this study was planned the novel SPET tracer β-CIT became available with good kinetics and excellent target-background ratio. It was found to be valid in studying the permanent quantitative changes of Parkinson's disease. To study alcoholics, repeated measurements were needed to determine, whether the possible changes were inherited or acquired, and if acquired, whether they were permanent or transient or, in cases of short-term changes, if they were detectable as changes in DAT density. In all earlier studies DAT densities were measured only in comparisons of alcoholics with healthy volunteers. The study comparing DAT densities of same alcoholics twice - during the withdrawal and after abstinence - was lacking.

The monoamine hypothesis on psychopharmacology of depression also includes the usually ignored dopamine, but on the other hand, dopamine has for a long time been known to be a key transmitter in substance abuse. Although alcohol-related depressive symptoms arise during withdrawal, and dopaminergic activity is putatively down-regulated simultaneously, it was not known, if this subtype of usually male-type depression was connected with the dopaminergic system.

C. Robert Cloninger's theory of temperament and character is one attempt to solve the body-mind connection concerning monoamines and detectable characteristics of temperament. Lack of human studies with straight, clear and unambiguous findings of these connections was the basis of the interest in whether β-CIT could reliably reflect the personality trait of the NS.

The A1 allele of the DRD2 receptor was the first studied candidate gene of the dopaminergic system, which might have some connection with alcoholism. As there were no studies dealing with associations between this gene and DAT density, it was unclear, if this gene predisposing to severe alcoholism was also connected with altered DAT densities.

3 Aims of the study

The aims of this study were:

1. To explore possible temporary decreases in DAT densities during alcohol withdrawal. (I)
2. To explore the effect of excessive ethanol consumption on dopaminergic transmission by measuring DA transporter binding with [^{123}I]β-CIT SPET after alcohol withdrawal and after a 4-week period of abstinence. (I)
3. To test the hypothesis that the depressive symptoms observed during alcohol withdrawal could be associated with a down-regulation of DAT density measured with [^{123}I] β -CIT SPET. (II)
4. To investigate the possible association between the personality trait of Novelty Seeking (NS) and DAT density measured with [^{123}I] β -CIT SPET in the human brain. (III)
5. To study the effects of the genetic polymorphism of the D_2-receptor gene TaqI A on DAT density measured with [^{123}I] β -CIT SPET in the human brain in relation to the status of detoxification. (IV)

3.1 Main hypotheses

The main hypotheses of this study were:

I The dopaminergic system of human brain is down-regulated during alcohol withdrawal and recovers after abstinence. (I)

II The depressive symptoms during alcohol withdrawal are associated with altered DAT density, reflecting a disturbed dopaminergic system. (II)

III The personality trait of Novelty Seeking has a positive correlation with DAT density if DAT density reflects the overall dopaminergic activity. (III)

IV The genetic polymorphism of the dopamine receptor gene variant T*aq*I A is connected with DAT density *in vivo*. (IV)

4 Patients and methods

4.1 Ethical statement

Though β-CIT is an active cocaine analogue, the amount of 100 μg of the tracer that is used in a SPET experiment, is too small to cause any pharmacological cocaine-related effects (Balster *et al.* 1991). The radioactive radiation of iodine 123 is relatively harmless since the strength of the radiation can be compared to one thorax X-ray scan. Alcoholics were helped to stay without alcohol during the 4-week follow-up period by frequent meetings with a psychiatrist. No money was given to the subjects. All the study subjects gave their written informed consent after having received detailed information of the study: See appendix 1. The Ethical Committee of the Oulu University Hospital approved the protocol of this study.

4.2 Inclusion criteria

Inclusion criteria for this study were the following: The study subjects had at least a 14 day drinking bout immediately before detoxification. The amount of alcohol consumed was at least 140g of absolute ethanol daily. Detoxification was begun, and the first SPET scan was carried out 1-4 days after the cessation of alcohol drinking.

4.3 Exclusion criteria

Diseases of the central nervous system including psychotic disorders other than alcohol delirium, Parkinson's disease, epilepsy, and major head trauma in the history were exclusion criteria as well as the use of neuroleptic or antidepressant medication currently taken or given during the last 6 months. Head MRIs were performed to exclude any

structural brain pathology. Patients with a history of abuse of any illegal drugs or a dependence on anxiolytics were excluded. This was verified through with personal familiarity of the staff of the detoxification clinic and case records of the patients. Use of illegal drugs was additionally screened with urine tests at the beginning of the study and randomly during the study. SPET-results from the first scanning session were excluded from one patient because of gamma-camera failure, and from two patients because of changed alcohol anamnesis: These latter two were revealed to have been drinking less than 140 g daily before the first examination. Their data were not used in papers I and II, in which we used data comparing first and second measurements. As they fulfilled the other inclusion criteria, their data of the second imaging was used in papers III and IV.

4.4 Alcoholic subjects

A total of 42 alcoholics were recruited for the first SPET scan. forty of them came from the Kiviharju detoxification clinic, Oulu, Finland and two from the departments of Psychiatry, Oulu University Hospital. Of the subjects recruited (age ranging from 22 to 70 years, mean 40.7, SD 9.9) 35 were men and 7 women. They had all been drinking alcohol daily in large amounts until admission. Most of them were periodic heavy drinkers. The self-reported daily alcohol intake (mean ±SD) was 3.3 ± 1.2 g/kg/d or 245.5 ± 85.6 g/d.

4.5 Dropouts

Ten of the subjects failed to participate in the second cluster of measurements, possibly because of a new drinking bout. One subject, with a DRD2 genotype A1/A1 failed to participate in the SPET studies despite consuming disulphiram during the control period. One was excluded later because of a very short and mild drinking bout before detoxification. One of the subjects was found to have been drinking more during the "sobriety" period than before the study, and his results were excluded. One subject was excluded for mechanical failure in the gamma camera during the first SPET scan.

Other data were also missing for various reasons. MADRS, TPQ and SCID II were not in use in the five first studied subjects. Only one of them appeared to the second SPET scan. Not all alcoholics filled the given scales and some failed to appear to the heavy measurements. These facts can be found in variations of n-values of the results.

4.6 Healthy control subjects

The controls were 29 healthy Caucasians subjects, 12 men and 17 women, 19 to 73 years of age (mean 37.7, SD 13.4), who volunteered for the study. They were recruited at a time when no alcoholics were available or an alcoholic did not appear at cluster II measurements. Healthy control subjects were mostly physician colleagues, other healthy workers and/or students. All of them were social drinkers who had fully abstained from alcohol for at least one week before brain imaging. Their age, weight and other characters were as near as possible to the study subjects. Sociodemographic situation of the control subjects was better. Control subjects performed the SPET procedure once. They also filled in the TPQ questionnaire.

5 Study protocol

The study protocol is presented as a time schedule in Table 3.

Table 3. The study protocol as a time schedule.

Time axis						
> 2 week	0-4 d	0 week	1 week	2 week	3 week	4 week
		Cluster 1				Cluster 2
Drinking bout	Detoxifi-cation	Recruitment Informed consent Sociodemo-graphic data SPET scan Quantitative EEG MADRS Laboratory tests ASAT ALAT GGT CDT Albumin Thrombocytes Urine drug analysis TaqI-allele	Weekly meetings SCID-II TPQ			Head MRI SPET scan Quantitative EEG MADRS Laboratory tests ASAT ALAT GGT CDT Albumin Thrombo-cytes Urine drug analysis

5.1 Recruitment

Recruitment of patients from the detoxification clinic usually occurred on Tuesday afternoons, because the tracer was provided from Tikkakoski on Wednesday mornings. Time for the measurements was limited because of the short activity of the tracer The half-life of ^{123}I is approximately 13 h. The sociodemographic data was collected during the recruitment. The intensity of the withdrawal symptoms was collected on SSA (Selected Severity Assessment Gross *et al.* 1973) while the depressive symptom scores were evaluated with MADRS (Montgomery-Åsberg Depression Rating Scale, Montgomery & Åsberg 1979). Possible neurological symptoms were evaluated clinically.

5.2 Detoxification

The patients were allowed to take only benzodiazepines used for detoxification. Routine detoxification was used for 20 of the subjects and included chlordiazepoxide 25 mg 1-3 x 3, and 20-60 mg temazepam or 7.5 mg zolpidem for insomnia. Saturation treatment with diazepam was used in two patients with the heaviest withdrawal symptoms. One of the subjects was detoxified with lorazepam, which is the only benzodiazepine studied with raclopride PET that apparently does not to influence the dopaminergic system (Hietala *et al.* 1997, Volkow *et al.* 1997). The amount of the benzodiazepines and zolpidem given preceding the first SPET scan was calculated in diazepam equivalents. Twenty mg of temazepam, 10 mg of zolpidem, 2 mg of lorazepam and 25 mg of chlordiazepoxide were counted as the equivalent of 10 mg of diazepam (Kaplan & Sadock 1995).

5.3 The first cluster of measurements

Every Wednesday morning, one recruited subject was guided to the isotope laboratory for measurements. Blood samples were collected for laboratory tests and genetic analyses. Liver function tests [aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT)] and laboratory markers of alcohol abuse (mean corpuscular volume (MCV), carbohydrate deficient transferrin (CDT), gamma-glutamyl transferase (GGT)) were used to monitor sobriety during the follow-up. All these parameters had a significant trend to normalize indicating true abstinence during the follow-up. Urine analyses were performed to determine presence or absence of illegal drugs either in the laboratory or during the recruitment at detoxification. Drug test Triage®, Merck, included following items: methadon, benzodiazepines, cocaine, amphetamines, tetrahydrocannabinol, opiates, barbiturates and tricyclic antidepressants. Pregnancy was excluded among fertile women by urine test. The SPET scan was performed 1, 4 and 24 hours post-injection. Quantitative EEG studies were performed on the same day.

5.4 Follow-up period

During the following 4 weeks, the investigator met his subjects weekly in the detoxification clinic to collect psychiatric data of the study subjects. The SCID-II (Structured Clinical interview for DSM-III-R Personality Disorders, Spitzer *et al.* 1989) form was filled in according to self-reporting. Subjects also filled in the TPQ (The Tridimensional Personality Questionnaire, Cloninger *et al.* 1991) scale. The second MADRS was filled in four weeks after detoxification.

5.5 The second cluster of measurements

The second SPET scan was performed on Tuesday, 27 days after the first scan. SPET scans were repeated for each alcoholic patient by exactly the same technique that had been used four weeks earlier and the difference of the binding in each individual between the two measurements of striatal DA transporter binding was calculated. The quantitative EEGs were repeated. Also MRI scans were performed at this stage when the patients were in a better psychophysical condition. Blood samples were collected repeatedly.

5.6 SPET procedure

SPET studies were performed using a dual head gamma camera (ADAC Vertex) equipped with high-resolution fan beam collimators 1, 4 and 24 h after the injection of [^{123}I]β-CIT, obtained from MAP Medical Technologies Inc (Tikkakoski, Finland). The specific radioactivity of the ligand was >180 GBq/μmol and the radiochemical purity was >98%. No-carrier-added [^{123}I] was purchased from PSI, Switzerland and from Medgemix, Belgium. The ligand was synthesized as described previously in detail (Bergström *et al.* 1994).

After blocking of thyroid uptake with 400 mg potassium perchlorate, which was administered orally 30 min before tracer application, the subjects received a dose of 120-185 MBq [^{123}I]β-CIT diluted in 10 ml of physiological saline by a slow (30 s) intravenous injection.

For brain imaging, the head of each subject was positioned in a head holder using a crossed laser beam system for repositioning. Raw data were obtained from photo peak counts within a 20% symmetric energy window centered around 159 KeV. Parallel to the cantomeatal plane, 4.6 mm thick cross-sections were reconstructed by filtered back projection in 128x128 matrix using a Butterworth filter (power factor 5, cut-off 0.22 Nq). Attenuation correction was subsequently performed using Chang zero order correction based on an ellipse fitted to the brain using a linear attenuation factor (=0.09 cm^{-1})

The physicist who performed regions-of-interest analyses was ignorant of the sociodemographic data of the subjects. Transaxial slices oriented along the orbitomeatal

line were reconstructed, and the two slices corresponding to the highest striatal uptake were summed digitally, yielding a final slice of 9.3 mm (pixel size 4.64 mm, voxel volume 99.9 mm^3). The regions of interest were drawn over the right and left striatum (STR) using a color scale with about 60% isocontour cut-off boundaries for delineation. The size of the average striatal area of interest was about 20 pixels ≈ 431 mm^2, corresponding to a volume of 4.0 cm^3. Frontal white matter (FWM) regions of interest were drawn on the slice about 60 mm superior to the orbitomeatal line. FWM values were used for reference (non-displaceable activity), because post-mortem studies have revealed a very low density of DA transporters in this region (Günther *et al.* 1997). Occipital white matter (OWM) values were also determined and were not significantly different from those of the FMW values, but we did not use them, because of their greater variability and content of possible artifacts. The cerebellar region was not taken as a reference for non-displaceable activity, because this region is situated close to the bed surface and could thus cause artifacts for raw SPET data.

Striatal DAT binding was calculated as the ratio of the total binding in STR minus the non-displaceable binding in FWM to the non-displaceable binding in FWM, i.e. (STR-FWM)/FWM. Because it has been shown that a state of equilibrium exists in striatal and occipital areas 24 hours after the injection, the ratio at this time point can be used as an estimate of the binding potential.

5.7 DNA restriction and *Taq*I A RFLP

For DNA analyses, blood samples were collected from each subject and frozen at -70ºC in glass tubes. DNA was extracted from 10 ml of the peripheral blood samples by standard procedures (Vandenplas *et al.* 1984). Subjects were genotyped for the *Taq*I A RFLP located in the 3' flanking region of the dopamine D_2 receptor gene as described by Grandy *et al.* (1993). PCR (polymerase chain reaction) was carried out on a total volume of 10 µl containing 1x reaction buffer supplied with Pfu polymerase (Stratagene, La Jolla, USA), 100 ng of genomic DNA, 40 pmol of each primer, 200 µM of each deoxynucleotide and 0.15 U of Pfu polymerase. PCR conditions were denaturation at 94ºC for 3 min followed by 35 cycles at 94ºC for 45 s, 64ºC for 45 s, 72ºC for 45 s, and a final extension at 72ºC for 5 min. The digested fragments, separated on a 3% agarose gel containing 0.5 µg/ml ethidium bromide, were then visualized and photographed. The A1 allele remained intact while the A2 allele was cut into one 180 bp and one 130 bp piece.

5.8 Statistical analyses

5.8.1 Study variables

The variables used in statistical analysis were age, sex, duration of the last drinking bout, amount of alcohol consumed daily during the last drinking bout, number of days of abstinence preceding the first SPET scan, withdrawal symptom score in the SSA scale and total amount of benzodiazepines given to the patient preceding the first scan. TPQ questionnaires and SCID II scores were collected during the follow-up period. The following measurements were performed twice: MADRS scores, laboratory values of alcohol related studies: GGT, CDT, ASAT, ALAT, MCV, S-albumin, thrombocytes and specific striatal β-CIT binding.

5.8.2 Statistical methods

In the first paper 95% confidence intervals (CI) and/or repeated measures ANOVA –tests were used. Stepwise linear regression analyses were performed to test, which factors significantly influenced the change in DA transporter binding during the 4-week period of abstinence (I).

In later papers, means with standard deviations (SD) were used in descriptions of the continuous variables. Paired samples t-tests were used to test the statistical significance of the difference between two measurements of continuous variables of alcoholics. For normally distributed continuous data 2-tailed independent-sample t-tests were used to compare the means of separate groups. For correlation analyses, Spearman's two-tailed rank correlation coefficients were calculated due to the non-normal distribution of the studied variables (II-IV).

Mann-Whitney's U-test was used for non-parametric data to compare the means between independent groups in first three papers (I-III).

To adjust the effect of age on DAT-density and to test differences between the hemispheres, the repeated measures ANOVA –tests were used in the fourth paper (IV).
Statistical analyses were made using the Statistical Package for the Social Sciences (SPSS), version 6.1, for Microsoft Windows.

6 Results

6.1 DAT

6.1.1 Increase of DAT density during abstinence (I)

The DAT densities were compared during the withdrawal and after the four weeks of abstinence in the 27 alcoholics whose data were available from both scans. The mean specific striatal DA transporter binding [(STR-FWM)/FWM] was 6.42 during the withdrawal state. The DAT density increased significantly ($p<0.0001$, $t= 5.4$, paired samples t-test) during the four weeks of abstinence. The mean (±SD) value at the end of that period was 7.25 (±1.23; 95% CI 7.1-7.5) (Figure 3). The DAT density during alcohol withdrawal correlated positively with the increase during the abstinence ($r=0.42$, $n=30$, $p=0.20$ Spearman's correlation coefficient). The regression analysis showed that the number of days elapsed between the last drink and the first SPET scan was the most significant predictor of the change in DAT binding during the subsequent period of abstinence ($p=0.0021$, stepwise linear regression analysis), the correlation being an inverse one (Figure 4). Age, sex, amount of alcohol consumed and duration of the last drinking bout were not significant predictors. Careful checks for alcohol drinking were carried out among the subjects thorough the abstinence period by means of laboratory markers. All the parameters measured (Table 5) indicated abstinence during the follow-up in all those patients included in this study.

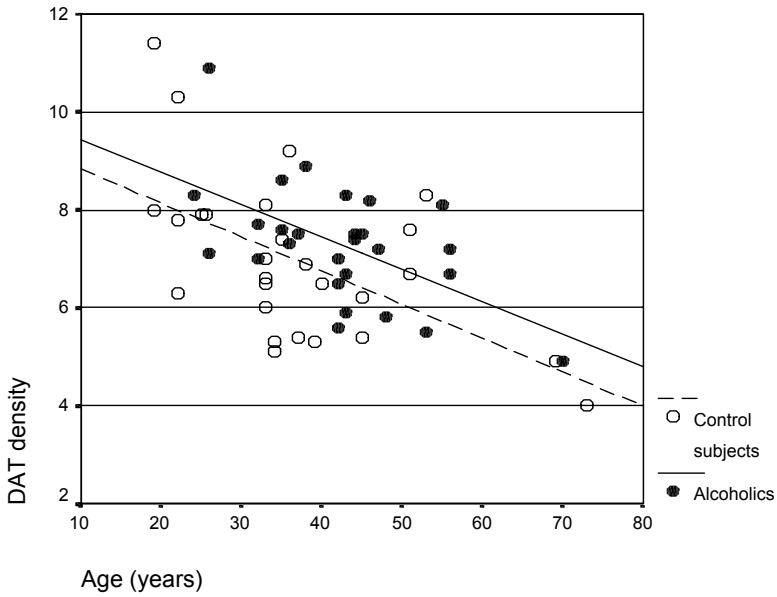

Fig. 3. DAT density among alcoholics and control subjects with age.

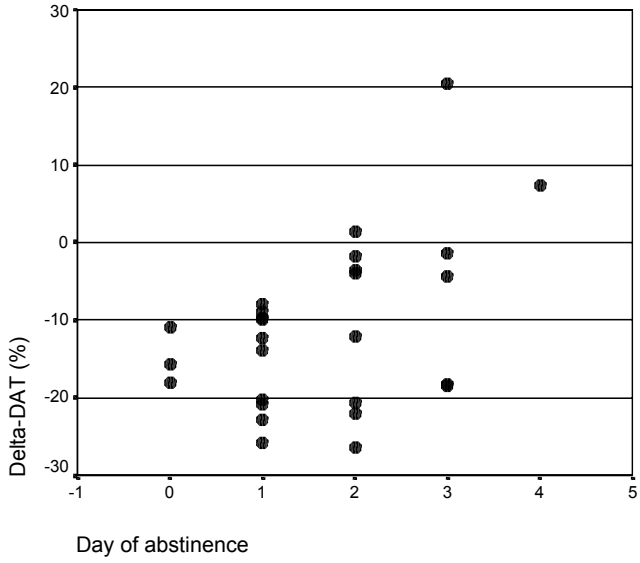

Fig. 4. Individual DAT decreases (Delta-DAT or Δ-DAT = (DAT2-DAT1) x DAT2/100%) compared with the abstinence period before the first SPET scan.

6.1.2 Adjusting the effect of medication (I)

The dosage of benzodiazepines given before the first scan correlated positively with the change (p=0.0032, Spearman's correlation coefficients). Those patients who had needed large amounts of benzodiazepines at the beginning of the detoxification period seemed to show greater increases in striatal DA binding during the 4-week period of abstinence. There were five patients who were not treated with benzodiazepines. They showed a similar increase in DA transporter binding during the abstinence period (from 6.4 ±1.1 to 7.3 ±1.2, p=0.002, t= -7.6, paired samples t-test) as the group of alcoholics who were treated with benzodiazepines. After omitting these five patients from the linear regression analysis, the amount of benzodiazepines no longer appeared to predict the increase in DA binding. The use of benzodiazepines correlated with DDA (r=0.42, p=0.029, n=27) and SSA (r=0.27, p=0.18, n=27).

Table 4. Results of the first cluster measurements compared with the second cluster (p value for paired sample t-test). Also results of the dropouts are compared with those of the other alcoholics when both groups are in withdrawal (the statistical significance of difference tested for independent sample t-test).

Variable	Results during the withdrawal (mean±SD), n=27	Results after four weeks of abstinence (mean±SD), n=27	Change (mean difference, p value)	Dropouts (mean, p value) n=12
DAT	6.42±1.11	7.25±1.23	0.83 (<0.0001)	6.857 (0.51)
MADRS	21.8±12.9	8.6±12.6	-13.2 (<0.001)	22.0 (0.87)
Age (years)	42.2±10.1			37 (0.089)
Age of onset	29.7±12.3			24.0 (0.066)
Diazepam eq. (mg)	107±81			114 (0.68)
DDA (g/kg)	3.4±1.3			3.14 (0.41)
DDA (g)	250±94			237 (0.68)
SSA	11.0±5.5			12.1 (0.39)
GGT (U/l)	413±717	105±137	-308 (0.055)	95.6 (0.031)
CDT (U/l)	31.6±16.9	14.7±5.3	-16.9 (0.01)	32.1 (0.84)
ASAT (U/l)	100.4±106.3	30.1±11.3	-70.3 (0.018)	66.3 (0.52)
ALAT(U/l)	132.6±274.6	37.2±21.6	-95.4 (0.19)	59.7 (0.41)
MCV (fl)	95.0±4.5	94.9±4.5	-0.06 (0.92)	94.3 (0.29)
S-Alb (g/l)	37.9±3.0	40.8±2.8	2.9 (0.10)	35.0 (0.18)
Trom (E9/l)	186.9±80.2	223.1±43.4	36.1 (0.056)	301 (0.10)

6.1.3 Control subjects (I)

The mean DAT density of the healthy controls was 6.96±1.68. It did not significantly differ from the DAT density of the alcoholics when they had been four weeks without alcohol (6.96 vs. 7.29, p= 0.41, t= 0.83, independent samples t-test). Their DAT density and NS values (r= -0.49, p=0.009, Spearman's correlation coefficients) decreased with age (Figure 3). Women had slightly higher DAT densities than men (7.4 ±1.9 vs. 6.4 ±1.2, p= 0.09, t= 1.7).

6.1.4 Dropouts

Twelve of the recruited study subjects failed to participate in measurements of the second cluster. The striatal β-CIT binding of the dropout group in alcohol withdrawal was 6.92 ±0.95, which did not differ statistically significantly from the other alcoholic subjects (p=0.187, t= -1.36, independent samples t-test) (Figure 5). NS was available only from three dropout subjects but it was found to be higher than that of other subjects (mean 23.3, p= 0.012, t= -2.98 independent samples t-test). Otherwise, the dropout group did not significantly differ from the other alcoholics, despite the GGT (Table 1).

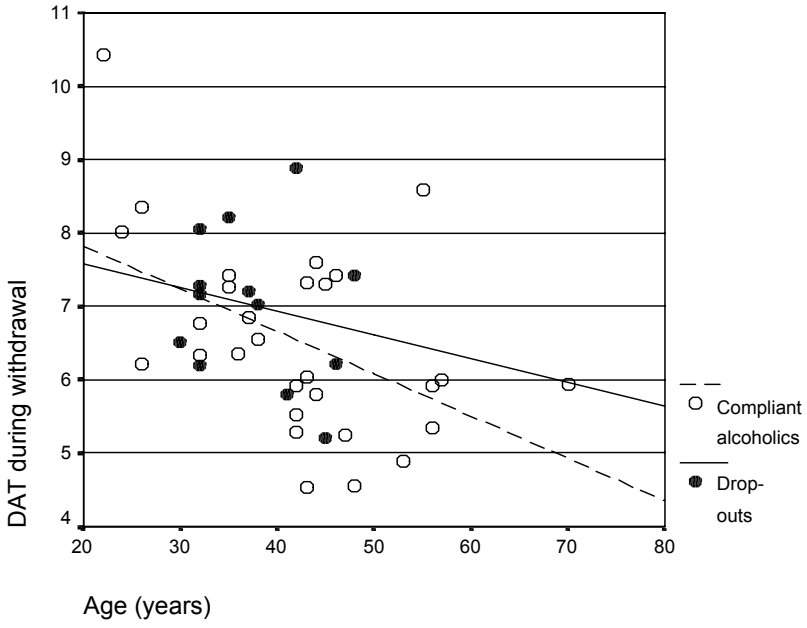

Fig. 5. The dropout population did not significantly differ from the other alcoholics during the withdrawal.

6.2 Depression and DAT (II)

The difference between DAT densities during withdrawal and after four weeks of abstinence, the percent DAT-variance, ΔDAT = (DAT2-DAT1)*DAT2/100%, was selected to represent the change in DAT density for each individual. The ΔDAT was compared with MADRS at the beginning and after four weeks of abstinence. MADRS scores decreased significantly during the four week period of abstinence ($p<0.001$, $t= 6.2$, paired samples t-test; Table 1). The association between ΔDAT and depressive symptoms score was statistically significant during both withdrawal ($r= 0.43$, $p= 0.03$, $n=25$, Spearman's correlation coefficients) and sobriety measurements ($r= 0.55$, $p= 0.006$, $n =23$) (Figure 6). However, ΔDAT failed to correlate with differences between MADRS scores ($r= 0.16$, $p= 0.47$, $n= 24$), even when use of benzodiazepines was parsed out ($r= 0.087$, $p= 0.69$, $n= 21$).

There were no statistically significant correlations between DAT-densities and depressive symptoms during withdrawal ($r= 0.25$, $p= 0.22$, $n= 26$,) or during abstinence ($r= -0.23$, $p= 0.29$, $n= 24$). The ΔDAT did not correlate with DDA ($r= 0.02$, $p= 0.9$ $n= 27$) or SSA-scores ($r= 0.30$, $p= 0.12$, $n= 27$), even when the effect of age was parsed out (DDA vs. DAT: $r= 0.063$, $p= 0.78$, $n= 20$), although DDA did correlate with SSA ($r= 0.42$, $p= 0.029$, $n= 27$).

The use of benzodiazepines did not correlate significantly with ΔDAT or MADRS score during withdrawal, or in the later measurement.

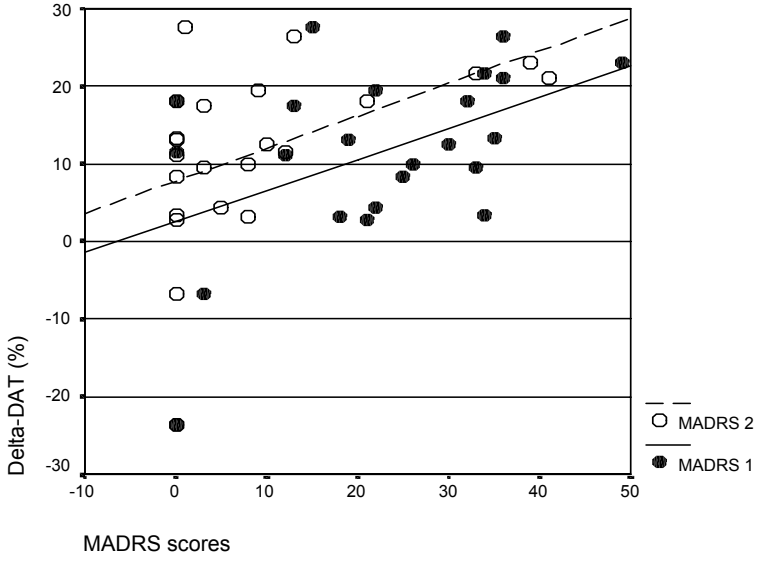

Fig. 6. The MADRS scores correlated negatively with (Delta DAT or Δ-DAT = (DAT2-DAT1) x DAT2/100%) both during withdrawal and after four weeks of sobriety.

6.3 Novelty Seeking and DAT (III)

Analyses of the data showed that the mean NS scores were 18.8 (±5.7) among abstinent alcoholics and 17.8 (±5.1) among controls, without a significant difference between the groups (t= 0.73, p=0.47, independent samples t-test). HA scores of alcoholics were statistically significantly higher among alcoholics than among control subjects (17.3 vs. 12.4, t=2.9, p= 0.007, independent samples t-test). This difference considered items HA3 (shyness with strangers; 4.0 vs. 2.5, t= 2.6, p= 0.012) and HA4 (fatigability & asthenia; 4.1 vs. 2.1, t= 2.9, p= 0.005). Otherwise RD was lower among alcoholics (17.0 vs. 19.0, t= -0.21, p= 0.045) especially RD4 (dependence; 2.5 vs. 3.4, t= -2.4, p= 0.023).

Statistical analyses revealed a significant correlation between the DAT density and NS among abstinent alcoholics (r= 0.46, p= 0.011, Spearman's correlation coefficients; Figure 7), but not among control subjects (r= 0.18, p= 0.36). DAT density correlated negatively with age in both groups (r= -0.50, p= 0.005 among alcoholics and r= -0.49, p= 0.009 among controls). NS correlated negatively with age among abstinent alcoholics (r= -0.46, p= 0.011) but not among the controls (r= 0.15, p= 0.47).

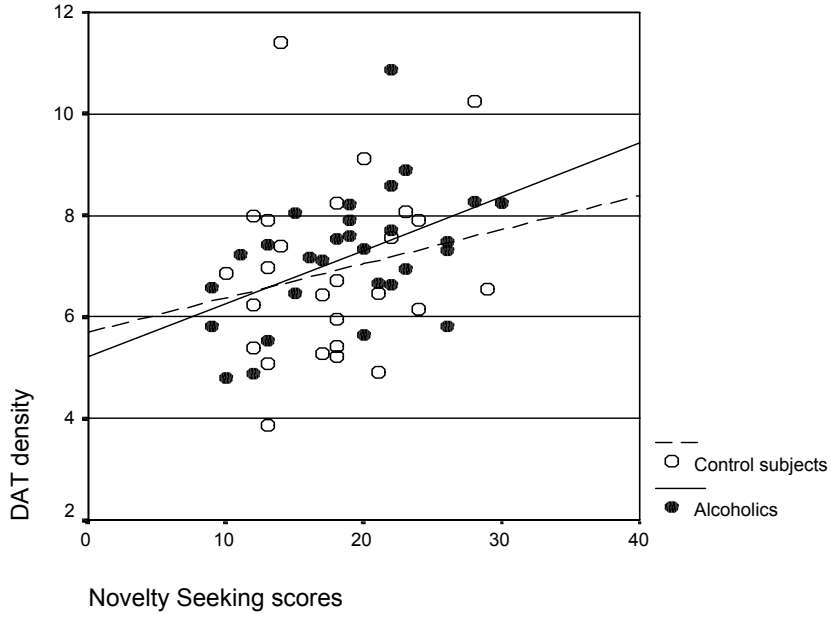

Fig. 7. DAT density correlates significantly with Novelty Seeking in alcoholics.

6.3.1 Subgroups of alcoholism (III)

The alcoholics were divided into subgroups depending on the age of onset of alcoholism either before or after the age of 25. NS-scores were statistically significantly higher among early-onset alcoholics than for late-onset alcoholics (mean 21.3, ±4.2 vs. 17.2, ±6.0, p= 0.041, t= -2.15, independent samples t-test). Among abstinent early onset alcoholics, DAT-density was slightly elevated (7.6 vs. 7.0, p= 0.26, t= -1,16, independent samples t-test). Further, the abstinent early-onset alcoholics with antisocial personality disorder (n=8) had higher NS scores (mean 23.6, ±4.5 vs. 17.1, ±5.1, p= 0.004, t= -3.41, independent samples t-test) and slightly higher DAT densities (mean 7.71, ±1.66 vs. 7.09, ±1.06,p= 0.35, t= -1.0, independent samples t-test) than alcoholics without antisocial personality disorder (n= 22).

6.3.2 Gender differences

The statistical analysis revealed a significant correlation between DAT density and NS in the male population taken together (r=0.47, p=0.004 Spearman's correlation coefficients), which was stronger in abstinent alcoholics (r=0.51, p=0.016, n=22) than in control subjects (r=0.30, p=0.32). Two females of the control subjects were found to have been in a phase of major depression during the SPET scan. Unaware of this, we used their DAT density data in publication I, but excluded their data concerning DAT density and Novelty Seeking from analyses of this chapter. Their NS scores were low (5 and 3) and DAT densities fairly high (10.2 and 7.5).

In female populations a positive correlation between DAT densities and NS scores was not found (total: r= -0.019, p= 0.93; abstinent alcoholics: r= -0.051; p= 0.94; control subjects: r= 0.013; p= 0.96).

When correlating NS to DAT density at an early withdrawal state, the resulting correlation was not significant in the male population (r= 0.42, p= 0.054, n= 22) or in females (r= 0.56, p= 0.32, n= 5).

6.3.3 Aging, DAT and NS (I-IV)

DAT density correlated negatively with age among both alcoholics (r= -0.50, p= 0.005 Spearman's correlation coefficients) and control subjects (r= -0.49, p= 0.009; Figure 3).
NS correlated negatively with age only among alcoholics (r= -0.46, p= 0.011) but not among the controls (r= 0.15, p=0.47). NS scores correlated negatively with anamnesis at the age of onset of alcoholism (r= -0.46, p= 0.015).

In order to adjust the effect of age and to avoid reducing the number of the subjects in the separate groups too much, we combined the results of the alcoholics during the later measurement and control subjects and categorized subjects according to age groups.

Significant correlations between NS and DAT densities were found in the younger age groups 20-30 years (r=0.73, p=0.04, n=8) and 30-40 years (r=0.50, p=0.03, n=19), but not in the older age groups, 40-50 years (r=-0.00, p=0.99, n=16) or 50-60 years (r=0.45, p=0.27, n=8).

6.4 A1 allele (IV)

We studied the polymorphism of the A1 and A2 alleles of T*aq*I A genes, to measure their influence on the dopaminergic phenotype. In our database we found 10 subjects with the A1/A2 genotype (1 female) and 19 with the A2/A2 genotype (4 females). There was one subject with the A1/A1 genotype in our original sample, but he failed to complete this study. Neither did the dose of daily-consumed alcohol (A1/A2: 245 ±104 g/d vs. A2/A2: 253 ±94.1 g/d; p= 0.85, t= -19, independent samples t-test) or the benzodiazepine amount needed for detoxification (109.5 ±83.2 mg vs. 93.9 ±79.9 mg diazepam equivalents, p= 0.63, t= 0.48, independent samples t-test) differ between the two subgroups.

Statistical analyses revealed higher DAT densities after 4 weeks of abstinence in subjects, who were heterozygote with regard to the A1/A2 allele rather than in the homozygotes with the A2/A2 alleles (mean 8.0 ±1.2 vs. mean 6.9 ±1.1, t= 2.3, p= 0.036, independent samples t-test, figure 8). Differences in mean age between the subgroups did not reach statistical significance (A1/A2: 37.5 ±11.2 vs. A2/A2: 44.4 ±10.4 years, t= 0.13, p= 0.13, independent samples t-test). The Novelty Seeking of patients with the A1 allele was not significantly higher (20.2 vs. 19.2, t= 0.52, p= 0.61, independent samples t-test).

6.4.1 Ages of allelic subgroups

We used the Repeated Measures Analysis of Variance to adjust for the reported decreasing effect of age on the DAT density (Volkow *et al.* 1996a), and used DAT density of left and right hemispheres as repeated measurements with age as a covariant. In this analysis, age had a highly significant effect on the DAT density (p=0.002). Statistical significance regarding the effect of the A1 allele on the DAT-density was confirmed (p=0.026).

When the ratio between left and right hemisphere was calculated they did not differ from each other among abstinent alcoholics(p=0.834). Subjects with the A1 allele had lower binding in the left striatum and others in the right. The difference between groups was statistically significant (0.98 vs. 1.02 p=0.47, independent samples t-test).

The reported lengths of the drinking bouts of the subjects with A2/A2 alleles were longer than those of the subjects with the A1/A2 genotype, but the difference was not statistically significant (mean 641, median 120, SD 1262 vs. mean 41, median 120 SD 52 days, p=0.31, independent samples t-test). Withdrawal symptoms of the A1/A2 -subjects were not significantly worse than those of the A2/A2 subjects (SSA mean scores 12.9 SD 5.8 vs. 9.6 SD 5.4, p=0.15, t=1.5, independent samples t-test).

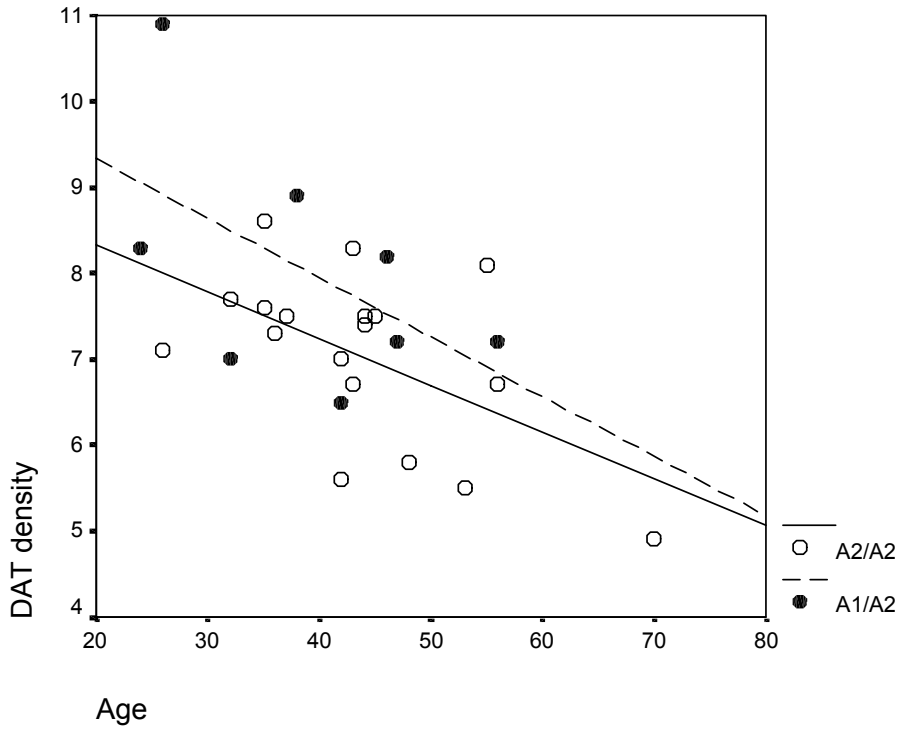

Fig. 8. Figure 8. DAT densities were higher in alcoholics with the A1/A2 genotype.

6.4.2 A1 allele and depressive symptoms during withdrawal

Subjects with the A1/A2 genotype had significantly more depressive symptoms during withdrawal than A2/A2 patients (mean MADRS scores 28.5 ±11.2 vs. mean 17.8 ±12.3, p= 0.012, t= 2.7, independent samples t-test). The former also exhibited more recoveries from depressive symptoms studied with MADRS scores under sobriety (mean 20.2 ±8.7 vs. mean 11.2 ±12.8, p= 0.041, t= 2.6, paired samples t-test). After four weeks of sobriety, two patients with the A1/A2 and two with the A2/A2 alleles were suffering from clinical depression (MADRS score > 17; Mittmann *et al.* 1997).

7 Discussion

7.1 Increase of DAT density

7.1.1 Changes in DAT density

DAT density had been earlier suggested to be a relatively stable indicator of the dopaminergic function reflecting the distribution and density of dopaminergic nerve terminals (Kuikka *et al.* 1995, Moody *et al.* 1996, Scheffel *et al.* 1996, Nurmi *et al.* 2000). This was also shown in repeated measurements (Seibyl *et al.* 1996). The role of the dopamine transporter is to eliminate dopamine from the synaptic cleft. When decreased DAT density is found in an imaging study involving patients with a disorder compared with healthy volunteers, there are theoretically following explanations:

1. The dopaminergic system is structurally weak and genetically determined inborn weaknesses e.g., genetic defects, such as point mutations affecting ligand affinity could predispose to various disorders.
2. The neurons of the presynaptic cell terminals are damaged in the pathophysiological process.
3. The DAT is occupied by endogenous dopamine competing with a low affinity tracer.
4. The DAT is down-regulated, possibly because of reduced axoplasmic transport of DAT towards the axon terminals, or internalizing DAT into the cytoplasm of a neuron, possibly not reachable for the ligand.

Following cell death, dopaminergic cells have not found to be regenerated. The amphetamine derivate MDMA causes damage mostly to serotonergic nerve terminals and possibly not to whole neurons (Ricaurte & McCann 1992, Ricaurte *et al.* 2000). A possible regeneration after the destruction has been discussed but similar phenomena have not been studied among dopaminergic nerve terminals.

7.1.2 Rapid recovery of DAT function

Methamphetamine induces rapidly reversible decrease of DAT function (Sandoval *et al.* 2001). A rapid decrease and recovery of DAT function has been observed to follow metamphetamine injection. The mechanism is, unfortunately, still unknown. It could be caused by blockade rather than down-regulation of dopamine transporters (Metzger *et al.* 2000). β-CIT has a very high affinity to DAT and, therefore, cannot be displaced by endogenous dopamine (Laruelle *et al.* 1993, Malison 1998b).

7.1.3 Impulse flow regulation

Choline uptake in cholinergic nerve terminals has been found to be sensitive to impulse-flow regulation i.e. higher impulse-flow causes higher transmitter-release, producing higher re-uptake-site density (Simon & Kuhar 1975). Studies concerning impulse-flow related up-regulation of DAT density has not been published.

7.1.4 Findings of increase of DAT

To the best of our knowledge, this study is the first in which an acute increase of the DAT density has been shown in human brains with a setting of repeated measurements. Increased DAT densities in human studies have been described among cocaine users compared with healthy subjects (Little *et al.* 1993, Malison *et al.* 1998a). This could show the effect of cocaine to up-regulate DAT densities by blocking dopamine transporters. Otherwise people with high DAT densities can be vulnerable to cocaine addiction. In a study involving experimental animals, e.g. rats, decreased dopamine flow did not have a decreasing effect on DAT density (Moody *et al.* 1996, Scheffel *et al.* 1996). Dopaminergic medication, used in Parkinson's disease, was not found to affect β-CIT uptake in SPET (Innis *et al.* 1999, Ahlskog *et al.* 1999).

7.1.5 Rate of recovery

The majority of the change in β-CIT binding in our study occurred within a few days after cessation of alcohol consuming. Recent studies have shown the change in some conditions to occur within a few minutes (Pristupa *et al.* 1998, Blakely & Bauman 2000). A rapid decrease of DAT density has been shown to follow metamphetamine injection (Metzger *et al.* 2000).

7.1.6 Possible weaknesses in imaging

Bergström *et al.* (1995) have described a possible role of lipophilic metabolites of β-CIT in SPECT imaging. Changes in non-selective binding to the reference area or changed metabolism of tracer in alcohol-induced liver could have affected the results. A modified perfusion of the brain could theoretically have an effect on the binding of the tracer. As the time after the injection of the tracer in SPET studies is relatively long, the tracer reaches a balance in various tissues. This should compensate the possible effect of perfusion differences between brain areas. In a study by Volkow *et al.* (1997), no robust changes were seen in the perfusion of the basal ganglia during alcohol withdrawal.

7.1.7 Medication

Alcohol is known to influence on various neurotransmitter systems (Harris 1999), but in this study we wanted to observe the dopaminergic system, especially. Benzodiazepines blunt component of the withdrawal symptoms mediated *via* reduced GABA -ergic activity and probably significantly reduces also glutamatergic symptoms (Glue *et al.* 1990). Medications used for detoxification could also have affected the observed change in DAT density. The dopaminergic system is regulated with GABA-ergic inhibitory synapses (Cornish *et al.* 2001). Therefore GABA-ergic regulation through benzodiazepines may have an influence on the dopaminergic system.

Those patients who had needed large amounts of benzodiazepines seemed to show greater increases in striatal DA binding, suggesting that either benzodiazepines or the severity of the withdrawal state were responsible for the effect. As significant changes in DAT density were also observable among those subjects who were not medicated, benzodiazepines alone cannot be responsible for the change. As these patients had the mildest withdrawal symptoms, we can speculate rather that as the benzodiazepines effectively alleviate withdrawal symptoms, they might also blunt other changes to be measured.

Detoxification medication was essential, because severe withdrawal symptoms of alcohol, like delirium tremens, can cause damage to the brain. Benzodiazepines as potent detoxification medication can protect against some of the worst symptoms, and allowing voluntary patients to suffer unpleasant symptoms would be against ethical principles. Hietala *et al*. (1997) have described a lack of effect of a small dose of lorazepam medication on D_2 receptor occupancy of raclopride in PET. Therefore, we assumed that other benzodiazepines might not affect the dopaminergic tract more than lorazepam.

7.1.8 Use of illicit drugs

Use of other illegal drugs was very uncommon in the secluded district of Oulu during the time of our study. No positive findings were found in urine screening analyses with regard to any illegal drugs among the study subjects and therefore the results cannot be attributed to, for example, amphetamines.

7.2 Aging, gender and DAT

Age had a decreasing effect on DAT density among both alcoholics and control subjects. Many authors have described this phenomenon (Van Dyck *et al.* 1995, Volkow *et al.* 1996a, Kuikka *et al.* 1999, Meng *et al.* 1999, Pirker *et al.* 2000, Lavalaye *et al.* 2000). It has been found to be due to cellular loss of dopaminergic cells of the substantia nigra (Ma *et al.* 1999). It has also been found that the decrease is not linear (Mozley *et al.* 1999). In our data, DAT density decreased linearly before the age of 30 and remained at a relatively stable state until the age of 60 (Figure 2).

In Lavalaye's work (2000), women had significantly higher DAT densities. This phenomenon was not found in the study by Van Dyck *et al.* (1995), or in our healthy control subjects.

7.3 Alcoholic subtypes

In this study, dividing alcoholics up into two subtypes according to age of onset, Novelty Seeking or DAT density was not possible. No two-humped scatter of two types of alcoholics was observed. Rather, the two types of alcoholics present also in our study two edges of continuity like Cloninger *et al.* have described (1988). This study sample was selected as our study subjects represent only those alcoholics, who volunteered for detoxification. Also the group of dropouts can cause a certain bias in our study. Probably, some of the most antisocial alcoholics cannot participate in a study with several time schedules and meetings with engagements. As well, patients with the most robust guilt feelings may have failed to continue with the measurements. This could have ruled out the most extreme ends of the continuity of alcoholics. We also had difficulties to get reliable information on our alcoholics' age of onset of alcoholism before or after 25 years of age. For these reasons, we used a correlation co-efficient test to analyze correlations between DAT and NS as continuing variables. Antisocial personality disorders could be seen in our study as predictors of higher Novelty Seeking, which is in line with the data indicating that type 2 alcoholics have higher DAT densities when compared with type 1 alcoholics (Tiihonen *et al.* 1995, Tupala *et al.* 2001b).

7.4 Depressive symptoms

Numerous studies have described depression to be connected with alcoholism and *vice versa*. Dramatic recoveries have been described from depression after a cessation of drinking (Schuckit *et al.* 1997). Changes in the DAT density and depression scores were associated in our study. The causal relationship between these two categories is unclear, even if changes would have happened simultaneously. Previously, an inverse correlation between DAT-density and Hamilton Depression Rating Scale scores has been reported in cases of acute cocaine abstinence, as measured by β-CIT SPECT (Malison *et al.* 1998a). Therefore, we expected that DAT density would have correlated with MADRS scores. We also expected that Δ-DAT would have correlated with Δ-MADRS. That was not the case, possibly due to different recovery time curves for DAT and depressive symptoms, as we found DAT to recover in only a few days while recovery from alcohol related depression may last several weeks (Schuckit *et al.* 1997).

However, also during antidepressive medication, the acute biochemical changes are followed by very slow improvements in mood, and reasons for this phenomenon are still hypothetical. Although there is literature in favor of the dopaminergic theory of withdrawal depression, we cannot rule out that they might have a common source (i.e., heavy crisis of alcohol withdrawal) without mutual causality. The correlation between ΔDAT and MADRS scores after four weeks of sobriety may indicate fragility in the dopaminergic system among seriously depressed patients. The lack of correlation between MADRS scores and DAT-densities may be due to the large inter-individual variation in the DAT density (Seibyl *et al.* 1996).

Two control subjects with primary major depression had elevated DAT densities compared with their NS scores. Laasonen-Balk *et al.* (1999) have described this phenomenon earlier suggesting that major depression is associated with high DAT density.

7.5 Novelty Seeking

In this study NS correlated statistically significantly with DAT density in the total population, alcoholics, males and young subjects. Relationships between dopaminergic system and temperament are probably quite complex, and the capacity of the brain to adjust is powerful (Zuckerman 1996). Schooling, role expectations and environment could widely adjust an individual's behavior.

High HA scores including especially high "Shyness with Strangers" and "Fatigability & Asthenia" scores among alcoholics are typical for late onset alcoholism, covering the majority of the alcoholics. Low RD scores and its subscale low "Dependence" scores among alcohol dependent subjects in our study sample are complicated and difficult to explain.

Both NS and DAT decreased with age. It is possible that degenerations of the dopaminergic cell structures might be the reason for the DAT decrease. A connection between cellular loss involving the dopaminergic system resulting from decreased DAT

density and NS has previously been described by Menza *et al.* (1995) among patients with Parkinson's disease.

7.6 A1 allele and DAT

In this sample, alcoholics with the dopamine DRD2 A1/A2 genotype, independent of age, had statistically significantly higher striatal DAT densities after four weeks of sobriety than alcoholics with the A2/A2 genotype. Their age of onset was slightly lower and they were also slightly younger than alcoholics with theA2/A2 genotype.

The mechanism linking the A1 allele to increased DAT densities is unclear. TaqI A RFLP does not represent functional gene variation *per se*. The A1 allele may have other unknown effects on alcohol dependence and dopaminergic neurotransmission for instance, through a disequilibrium with other close mutation (Gorwood *et al.* 2000). Another recent finding has shown the platelet MAO-B activity was significantly lower in individuals with the DRD2 A1 allele (n = 8), compared to those without it (n = 29). (Eriksson *et al.* 2000). As some authors suggest that A1 allele would be a marker of early onset alcoholism (Kono *et al*. 1997), we expected it to be related with higher NS. The difference between groups was anyhow not significant.

However, endophenotypic studies link this polymorphism to low D_2 receptor density *in vivo* (Pohjalainen *et al.* 1998, Jönsson *et al.* 1999) In principal, low postsynaptic D_2-receptor density among patients with the A1 allele may result in low net dopamine neurotransmission. This might cause a compensatory increase of presynaptic dopamine firing *via* a yet unknown feedback mechanism.

7.7 Applications of these results and call for further studies

The results of this study indicated, that there are certain changes in DAT density during alcohol withdrawal. Some changes in dopaminergic system are already known from earlier studies, which have been made with animals or in humans with other laboratory or imaging methods than used in our study. Our study, more or less, confirms these preliminary findings in living human with a method of novel functional imaging. We found also a link between depression, as a withdrawal symptom, and change in DAT density. This finding should be taken on account when planning research for medication for alcohol withdrawal.

Alcoholics as a population consist of subgroups with varying personality traits and genetic background. In our study we found link between DAT density and NS in alcoholics. This finding supports Cloninger's theories concerning the biochemical background of human temperament. This theory has shown its usefulness also in everyday practice with alcoholics. The alcoholic subgroup with DRD2 A1 allele has been found to suffer from more severe alcoholism than alcoholics without it. In our study, they had also significantly higher DAT densities. The mechanism of this variation

remains unclear, but it shows one biochemical change in this population, which might be important in their reactions to alcohol consumption. This might be important to be taken account in their treatment.

Further research is needed to clarify if individual changes can also be detected in less robust and life-threatening situations than the alcohol withdrawal. During the last years, radioligands have been developed that make possible more specific binding to their receptors and higher signal-to-noise ratios. SPET and PET technologies have also developed, and the improved resolution of pictures now gives detailed information of small-sized neuronal structures in the brain.

8 Summary and conclusions

The aim of this study was to measure, with β-CIT SPET, whether striatal DAT density changes during alcohol withdrawal and if DAT density is linked with NS and depression symptoms. We found a marked decrease in DAT density during a withdrawal and an increase on DAT density during a four-week long period of abstinence starting from acute withdrawal. We also obtained results suggesting quite a rapid increase, occurring within a few days.

When searching for connections between mind (psychological representations such as Novelty Seeking and depression) and body (DAT densities and A1 alleles of T*aq*I A gene), we made the following discoveries: There is a significant correlation between the change in DAT density and depression symptoms measured as MADRS scores, and also between the DAT density of abstinent alcoholics and their Novelty Seeking personality trait. The NS scores were especially high among alcoholics with antisocial personality disorder.

We found the alcoholics with an A1/A1 genotype of the DRD2 receptor T*aq*I A gene to have higher DAT densities than alcoholics with the A1/A2 genotype when abstinent. They also had more depressive symptoms during alcohol withdrawal.

We conclude that alcohol abuse causes transient decreases of DAT density. Comparing results of repeated measurements can detect the state-associated changes of dopaminergic activity. The results collected in this way can be related to behavioral variables, which can be detected with structured and semi-structured scales.

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