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Neuropharmacologic investigation of the serotonin responsiveness in alcohol dependent patients and healthy probands: Behavioral pharmacogenetics with the Selective Serotonin Reuptake Inhibitor Citalopram

Dissertation zum Erwerb des Doktorgrades der Medizin
An der Medizinischen Fakultät der
Ludwig-Maximillians-Universität zu München

Vorgelegt von
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aus
Kuala Lumpur
2008

Mit Genehmigung der Medizinischen Fakultät
Der Universität München

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Tag der mündlichen Prüfung:	13.03.2008

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1 Introduction

Alcoholism is a chronic disorder, with wide-ranging medical, psychiatric, social, economic and global consequences. It is a disease that often starts early and causes high mortality. Generally, the age period of highest prevalence of use and the highest likely quantity of intake for most substances probably occurs between the midteens and mid-20s (Kandel, Yamaguchi et al. 1992). In 2003, per capita consumption of alcoholic beverages in Germany was 147 l. (beer 117.5 l., wine 19.8 l., sparkling wine 3.8 l. and spirits 5.9 l.) (Meyer and John 2005). Sales of alcopops (ready-to-drink bottles of lemonade, cola or fruit juices mixed with liquor) have skyrocketed and alcopops are the most preferred alcoholic beverage among adolescents. The high sugar content in these drinks masks the alcohol content and teenagers perceive alcopops as a product especially made for them, giving them a special allure for teenagers (Hughes, MacKintosh et al. 1997). Alcohol research has shown that the earlier the age at onset of alcoholism, the higher the risks of alcohol dependence, late alcohol-related complications and problems (Grant and Dawson 1997).

John and Hanke (2001) showed that in Germany, altogether approximately 74,000 deaths yearly were caused by alcohol consumption alone or in combination with tobacco, with the majority of deaths from those between 35 and 64 years old (John and Hanke 2002). Deaths attributed to alcohol-related illness calculated as percentage from all causes of deaths, were in 25% of men and 13% of women. Between 1994 and 2003 approximately 4% of all road accidents with bodily injuries were under the influence of alcohol (Albrecht, Lerner et al. 2005). Alcohol was most frequently the cause of road accidents among male drivers aged 21-24 years old, followed by males in the groups 18-20 years old and 25-34 years old. Influence under alcohol is ascertained when the blood alcohol concentration (BAC) exceeds 0.3 promille and breath alcohol concentration exceeds 0.15 mg/l. More than half of these accidents occurred on the weekend, 22.4% on Saturdays and 21.9% on Sundays in 2003. Between 20^{00} hours and 04^{00} hours approximately 52% of alcohol-related accidents occurred, and more than half of these (26%) in the nights from Friday to Saturday or from Saturday to Sunday.

At least 5.5% of all inpatient treatment cases in Germany were attributable to alcohol consumption alone (2.0% women 0.9% and men 3.4%) or the combination of alcohol drinking and tobacco smoking (3.5%: women 1.4% and men 5.7%) (John and Hanke 2003). Data from 707 outpatient treatment centres (with 144,788 patients) and 106 inpatient centres (with 23,768 patients) in 2003 showed illness-related differences between the gender (Welsch and Sonntag 2005). In both centres, three out of four patients were males (73% in outpatient and 78% in inpatient), and the majority of them came for individual substance use disorders.

Both gender were treated in inpatient centres for an average of 12 weeks but in outpatient centres the females were treated longer (18 weeks) compared to the males (15 weeks) (Welsch and Sonntag 2005). 75% of male and 80% of female inpatients completed their treatment regimes, whereas 18% of male and 14% of female inpatients dropped out of treatment. Female inpatients (72%) appeared to have more treatment success compared to male inpatients (57%). Male alcohol-dependent outpatients were on average three years younger than the female ones (42 vs. 45 years) and male inpatients on average two years younger than female ones (44 vs. 46 years). Male patients were more likely than the female ones to be living alone, but unemployment was higher in female patients (43% vs. 33% males). The estimated annual economic costs of alcohol-related diseases were estimated to be €20.2 billion, which approximate 1.1% of the gross national product (Meyer and John 2005).

With this epidemiological background in mind, it is necessary to correctly identify, quantify and treat alcohol abuse or dependence. Given the wide range of alcoholic beverages on the market, a standard measurement to quantify alcohol content in them and the 'pure' alcohol intake the subject has taken is needed. Amount of pure alcohol is calculated with the formula:

$$1 \text{ vol. \%} = 0.79 \text{ wt/vol. \%}$$

Maximum blood alcohol concentration (BAC) after a drinking event can be calculated using Widmark's Formula (named after the pioneering studies by Erik M.P. Widmark of Sweden) (Gilg 1995):

$$\text{For men, BAC} = \frac{\text{Grams alcohol consumed}}{\text{Kg body weight} \times 0.7} \quad (\%)$$

$$\text{For women, BAC} = \frac{\text{Grams alcohol consumed}}{\text{Kg body weight} \times 0.6} \quad (\%)$$

The maximum blood alcohol levels will often not be reached because alcohol is usually not consumed instantaneously and because elimination and distribution across body compartments are not taken into consideration. Under real life drinking conditions, maximum blood alcohol levels were found, on average, to be 10-30% lower than those obtained with Widmark's formula (Zernig G: Handbook of Alcoholism 2000). Other than physiologic and metabolic factors influencing alcohol absorption, alcoholic beverages also contain *congeners* which can affect the rate of absorption and distribution of alcohol, and diverse substances with direct body effects including alterations in sex hormones, and can by themselves have an impact on the brain (Van Thiel. D.H., Galvao-Teles et al. 1991; Roine, Gentry et al. 1993).

1.1 Definition of alcohol dependence

The concept of substance dependence was strongly influenced by the studies and initial description of alcohol dependence syndrome by Edwards and Gross (1976). A *drug of abuse* is any substance, taken through any route of administration, which alters the mood, the level

of perception or brain functioning (Mello and Griffiths 1987; O'Brien 1991), from prescribed medications to alcohol to solvents (Jaffe 1990; Lowinson, Ruiz et al. 1992). All drugs of abuse cause intoxication, all induce psychological dependence and all are self-administered by an individual to change his level of consciousness or to increase his psychological comfort. A hierarchy has been established to help identify the most clinically significant problem first (Schuckit 1995):

1. An *overdose* or a *toxic reaction* is when the patient has taken enough of a drug to seriously compromise his vital signs.
2. *Withdrawal* is when a drug-related clinical syndrome (including confusion or psychotic symptoms) with relatively stable vital signs occurs.
3. An *organic brain syndrome* (OBS) is diagnosed in patients with stable vital signs and no signs of withdrawal, but with levels of drug-induced confusion, hallucinations or delusions.
4. Patients who show stable vital signs, no evidence of clinically significant confusion, and no signs of withdrawal, but who show hallucinations and/or delusions without insight, are regarded as having a *psychosis*.
5. Most remaining patients are expected to be demonstrating a flashback or a drug-induced depression or anxiety state.

The major diagnostic classifications of DSM and ICD have since been continuously developed with regard to criteria for psychoactive substance use disorders (Cottler, Schuckit et al. 1995). Dependence is a more reliable and valid diagnosis than abuse (Friedman and Cacciola 1998; Schuckit 1998). Dependence, also called *habituation* or *compulsive use*, indicates a central role of the substance in an individual's life, with problems relating to controlling intake, and the emergence of physical and psychological difficulties despite which the individual continues to use the substance (Schuckit 1995). Dependence can also be further classified:

1. *Psychological dependence*.
2. *Physical dependence* -Two important aspects to consider in physical dependence are:
    - *Tolerance*
    - *Withdrawal* or an *abstinence syndrome*

The latest versions of the Diagnostic and Statistic Manual of Mental Disorders (DSM IV, (American Psychiatric Association 1994) and the International Classification of Diseases (ICD-10; (WHO 1992) agree on the basic elements of psychoactive substance use disorders,

with the exception of the categories for abuse (DSM-IV) or harmful use (ICD-10) of a substance, which are not comparable between the two systems. For a diagnosis of *dependence* the DSM IV criteria used in this study require a minimum of three of seven substance-related items to occur and cluster together within some 12-month or longer period whereas *abuse* requires evidence of repeated occurrences within a 12-month period of any of four possible social, legal, or interpersonal problems related to the substance.

In psychiatry, there is no single pathognomonic (diagnostic) symptom (Schuckit 1986; Maier, Lichtermann et al. 1994). The following are some of the attempts made to classify alcohol dependence, since treatment and prognosis appear to be related to the type of alcohol-dependence shown (Preuss, Schröter et al. 1997):

- Typology according to Jellinek (1960): α-type, β-type, γ-type, δ-type, ε-type
- Typology according to Cloninger (1981): Type I, Type II
- Typology according to von Knorring (1985): Type I, Type II
- Typology according to Schuckit (1985): primary alcoholism, secondary alcoholism
- Typology according to Lesch et al. (1990): Type I - IV
- Typology according to Babor (1992): Type A, Type B

1.1.1 Criteria ICD 10 (WHO 1992)

The most recent version of the International Classification of Diseases (ICD-10) criteria has similarities with DSM-IV, but includes only six items.

Table 1: ICD 10 criteria of alcohol dependence:

ICD-10 Dependence syndrome

A. Three or more of the following manifestations should have occurred together for at least 1 month or, if persisting for periods of less than 1 month, should have occurred together repeatedly within a 12-month period:

(1) a strong desire or sense of compulsion to take the substance

(2) impaired capacity to control substance-taking behavior in terms of its onset, termination, or levels of use, as evidenced by: the substance being often taken in larger amounts or over a longer period than intended; or by persistent desire or unsuccessful efforts to reduce or control substance use;

(3) a physiological withdrawal state when substance use is reduced or ceased, as evidenced by the characteristic withdrawal syndrome for the substance, or by use of the same (or closely related) substance with the intention of relieving or avoiding withdrawal symptoms;

(4) evidence of tolerance to the effects of the substance, such that there is a need for significantly increased amounts of the substance to achieve intoxication or the desired effect, or a markedly diminished effect with continued use of the same amount of substance;

(5) preoccupation with substance use, as manifested by important alternative pleasures being given up or reduced because of substance use; or a great deal of time being spent in activities necessary to obtain, take, or recover from the effects of the substance;

(6) persistent substance use despite clear evidence of harmful consequences, as evidenced by continued use when the individual is actually aware, or may be expected to be aware, of the nature and extent of harm.

1.2 Aetiology of alcoholism

1.2.1 Psychological theories

Psychological theories compare alcoholics and non-alcoholics on their performance on psychological tests but sometimes neglect the possibility that heavy drinking may be the consequence of a life style and not the original cause (Blane and Leonard 1987). One of these theories is the ‘tension-reduction hypothesis’ which states that alcoholics drink to decrease their level of stress (Young, Oie et al. 1990; Pohorecky 1991). Another states that people begin to drink, drink abusively or remain alcoholic because alcohol somehow rewards or reinforces their behaviour through inducing pleasure, removing discomfort, enhancing social interactions and fulfilling the need to feel powerful or helping them to self-destruct or to abolish unpleasant memories (Hill 1993; Schuckit, Klein et al. 1994).

1.2.2 Socio-cultural Theories

These use observations of similarities and differences between culture groups and subgroups relating to drinking patterns (Heath 1987; Powles, Macaskill et al. 1991) but no factor purported to be important in one culture could be used to generalize to most other cultures.

1.2.3 Environmental Factors

Environmental influences may well contribute to the vulnerability to alcohol dependence in a number of ways. First is the peer pressure or peer influence which is likely to contribute to the beginning of use and patterns of problems with drugs or alcohol (Kandel, Yamaguchi et al. 1992). Higher availability of alcohol and liberal views toward intoxication and drunkenness have been reported to be associated with a greater risk for alcohol-related life problems, especially among males (Reifman, Barnes et al. 1998). Poverty, lower levels of education and high levels of job stress are also hypothesized to contribute to alcoholism risk (Jones-Webb, Snowden et al. 1997; Crum, Ensminger et al. 1998; Schuckit 1998). Interactions within the family may be important when a protective effect appears with higher levels of parental involvement in a child's homework, the establishment of clear rules within the family, and explicit statements within the family that prohibit drinking during adolescence (Crum, Ensminger et al. 1998; Griesler and Kandel 1998). Additional factors include social support system (Kendler 1997) and higher level of religiosity (Kendler, Gardner et al. 1997).

1.2.4 General Biological Theories

Some of these theories include the possibility that alcoholics are seeking relief from an innate hypoglycemia or a cure for abdominal and menstrual distress, that they have allergies to alcohol or to congeners, or that a differential brain responsiveness to alcohol exists in alcoholics (Schuckit 1986; Charette, Tate et al. 1990).

1.2.5 Genetics

In alcoholism, an increasing number of studies have pointed to the importance of genetic factors in the vulnerability for developing alcohol problems including alcohol dependence (Reich, Edenberg et al. 1998). As much as 40-60% of the variance in liability for developing alcoholism may be due to genetic effects (Heath, Bucholz et al. 1997; Reich, Edenberg et al. 1998; McGue 1999). Large, well-constructed and population-based twin studies of alcoholism

and alcohol consumption find that about 50% of the interindividual variation is genetic in origin (Kendler, Davis et al. 1997). Potential genetic markers associated with alcoholism have been found and some biological factors influencing the patterns of alcohol consumption in animals have been identified (Li, Lumeng et al. 1993; Schuckit 1994; Schuckit 1994). Biologic and genetic trait markers for alcohol dependence (Agarwahl 1995; Preuss, Schultz et al. 2004) are:

- Evoked potentials (EEG and ERP)
- Monoamine oxidase (MAO-B)
- Adenylate cyclase (AC)
- Dopamine receptor gene (DR-D_2)
- Dopamine- β-hydroxylase (DBH)
- Endocrine parameters (cortisol, ACTH, prolactin)
- Alcohol dehydrogenase (ADH_2-, ADH_3-genotypes)
- Aldehyde dehydrogenase ($ALDH_1$-, $ALDH_2$-genotypes)
- Gamma aminobutyric acid (GABA, GABRA2 gene)

Several other biologic traits identified are the maximum number of drinks in 24 hours (Saccone, Kwon et al. 2000), a low level of response to alcohol (Schuckit 1994; Schuckit, Edenberg et al. 2001), alcohol factor scores (Dick, Nurnberger et al. 2002) and alcohol symptom severity phenotype (Foroud, Bucholz et al. 1998).

In diseases which follow a non-Mendelian inheritance pattern, such as alcoholism, a direct relationship between phenotype (alcoholism) and genotype is disrupted, i.e. the same genotype may result in different phenotypes or different genotypes may result in the same phenotype (Hesselbrock, Begleiter et al. 2001). Gottesman & Shields (1972; 1973) have suggested the use of intermediate or endophenotypes, and the manifestations of these endophenotypes would be closely linked to gene expression and highly heritable. These characteristics thus allow for endophenotypes to be used to identify persons at genetic risk for the disorder even in the absence of symptoms. Six criteria were specified that must be satisfied for a trait to be identified as an endophenotype (Gottesman and Gould 2003):

- The trait must be present in affected individuals, in both the well state and during the course of the illness.
- The trait must be present in unaffected biological relatives.
- The trait must be present in individuals known to be at high risk for developing the disorder.
- The trait must be predictive of an increased likelihood for developing the disorder.
- The trait must be heritable.

- The trait should have biological manifestations closely linked to gene expression.

Since many traits and symptoms are shared across different psychiatric disorders, an endophenotype may not be specific for a particular diagnosis. The presence of an endophenotype could serve as an indicator of increased risk for a disorder or a set of disorders and abnormal behaviors, including alcoholism and suicidal behavior, and assist in the identification of susceptibility.

Alcoholism runs strongly within families (Kendler, Davis et al. 1997), having an alcoholic parent increases the risk of alcoholism four- to five-fold (Preuss, Schuckit et al. 2002), with a higher rate of concordance (two-to-one ratio) in identical twins than in non-identical twins or same-sex siblings. Alcoholism in biologic parents also predicts alcoholism in their adopted-away children (Goodwin 1979; Heath 1995). Separation or adoption-type studies showed that children of alcoholic biological parents separated from their parents early in life and raised without knowledge of their natural parents have markedly elevated rates of alcoholism (three- to four-fold higher risk for alcoholism). But children of non-alcoholics adopted into the homes of alcoholics do not show increased rates of alcohol problems as adults. Studies of sons and daughters of alcoholics showed a decreased intensity of reaction to ethanol in the offsprings which may possibly make it more difficult for them to decide when to stop drinking (Schuckit 1988; Schuckit 1994; Schuckit 1994) and the lower response to alcohol doses help predict who will and who will not develop alcoholism by age 30.

More severe alcoholism may be more heritable (Cloninger, Bohman et al. 1981). Also, heredity of alcoholism is approximately equal in women (0.50–0.60) (Kendler, Heath et al. 1992; Kendler, Neale et al. 1994; Heath, Bucholz et al. 1997; Prescott , Aggen et al. 1999) and men (0.48–0.58) (Kendler, Heath et al. 1992; Prescott , Aggen et al. 1999). Although an Australian twin study found no evidence for sex differences in sources of genetic influence, Prescott et al. in the US found that genetic sources of variability overlap partially, but not completely, in men and women (1999). Another promising endophenotype to be used in alcoholism research is behavioral disinhibition, closely related to impulsive and aggressive behavior (Preuss et al. (2001); Koller et al. (2002).

1.3 Serotonin and alcohol dependence

Serotonin (5-hydroxytryptamine or 5-HT) is a neurotransmitter discovered in 1948 (Rapport, Green et al.) and is now known to play an important role in several aspects of addiction, including reward, craving and relapse. Alcohol, given acutely, increases the release of serotonin within the brain but chronic administration of the drug tends to decrease the amount of serotonin stored in the central nervous system (Virkkunen and Linnoila 1993). Reduced levels of serotonin (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) have been found in alcohol-preferring mice and rats (e.g. AA rats and C57BL6J mice) before ethanol

exposure and after washout, suggesting reduced serotonin synthesis (Benkelfat, Murphy et al. 1991; Li and Mcbride 1995). A specific area of the brain, the dorsal raphe nucleus, has been hypothesized to be especially important in this phenomenon (Allan and Harris 1991; McBride, Murphy et al. 1992). Research using preference and other paradigms consistently reported that a variety of SSRIs reduced alcohol consumption by 50% to 70%, depending on the SSRI dose administered (LeMarquand, Phil et al. 1994). Strong support for the notion of constitutionally low serotonergic neurotransmission in early onset of alcoholism is given by studies of nonhuman primates, which showed that subjects with low CSF 5-HIAA (apart from excessive alcohol consumption) also exhibit behaviors characteristic of type II alcoholism, particularly deficit in impulse control (Higley and Bennett 1999). The genetic influence on central serotonergic neurotransmission is exacerbated by early rearing experiences, particularly parental deprivation (Higley and Bennett 1999).

1.3.1 Serotonin transporter and alcohol dependence

The serotonin transporter (5-HTT) binding site is found on serotonin nerve terminals and platelets (Langer, Moret et al. 1980; Laruelle, Vanisberg et al. 1988), thus it is an ‘index’ of serotonin nerve terminal number or integrity (Laruelle, Vanisberg et al. 1988); (Arango and Mann 1992);. The human 5-HTT is located at chromosome 17q11.1-q12. A 44 base-pair deletion/insertion polymorphism (5-HTTLPR) is present in the 5’ flanking regulatory region that has been reported in differential expression of 5-HTT and V_{max} for serotonin reuptake in transformed lymphoblastoid cell lines (Lesch, Bengel et al. 1996). In these cell-lines, the short form of 5-HTTLPR locus is associated with 40% fewer binding sites in the homozygote (SS) form.

Besides the possibility of a pre-existing reduction in central serotonergic neurotransmission, Heinz et al. (1998) also found about 30% reduction of brainstem serotonin transporters in type I alcohol-dependent individuals and this reduction was associated with lifetime alcohol consumption, suggesting that chronic alcohol intoxication reduces central serotonin transporter density. In a subsequent study Heinz et al. (2000) observed associations of the availability of serotonin transporters both with lifetime alcohol consumption and the genetic constitution of the 5-HTT promoters in alcohol-dependent individuals which suggest homozygous carriers of the long allele of 5-HTT promoters are selectively more vulnerable to the neurotoxic effects on serotonin transporters of long-term excessive alcohol consumption. In the study of Berggren et al.(2002), a negative correlation between the duration of excessive alcohol consumption and the PRL response to D-fenfluramine was found. This suggests that long-term excessive alcohol consumption causes a reduction in central serotonergic

neurotransmission, possibly by a toxic effect of alcohol on serotonin neurons which may in part be reversible after several years of abstinence (Gotjen, Szabo et al. 2002). A high availability of serotonin transporters in the raphe area, the center of origin of the central serotonergic system, was associated with a low response to the acute effects of alcohol intake and with the amount of alcohol intake in a free choice paradigm (Heinz, Jones et al. 2003).

Aggression, impulsivity and alcohol and substance use disorders all carry an elevated risk of suicide and are also associated with serotonergic dysfunction (Mann, Waternaux et al. 1999). Unfortunately, the 5-HTTLPR genotype is not clearly associated with alcoholism, subtypes of alcoholism (Kranzler, Lappalainen et al. 2002), impulsive traits (Lesch, Bengel et al. 1996; Ebstein, Levine et al. 1998) or both (Preuss, Soyka et al. 2000). But suicidal behavior has been found to be significantly increased in alcohol-dependent subjects compared to control groups (Murphy, Wetzel et al. 1992) and the 5HTTLPR polymorphism S-allele has also been found to be significantly associated to suicide attempts in alcohol-dependent patients (Gorwood, Batel et al. 2000). Further evidence came from Mann et al. (2000) who did not find an association between the level of serotonin transporter binding, HTTLPR-genotype and completed suicides in a postmortem sample of 220 subjects, and Russ et al. (2000) who reported an association between suicidal ideation and the HTTLPR-L-allele. In a study from Preuss et al (2001), LS and SS genotypes were significantly more frequent in alcohol-dependent subjects with suicide attempts compared to those without suicide attempts (78.9% vs. 64.0%, χ^2=3.64, df=1, p=0.04) and genotype frequencies between alcohol-dependent subjects with suicide attempts and controls tended to be significantly different (78.9% vs. 65%, χ^2=2.638, df=1, p=0.075). Subsequent analysis showed a significant difference in the number of S-alleles between alcohol-dependent subjects with and without suicide attempts (Mann-Whitney U value = 2353.0, p=0.019) but no significant difference between number of S-alleles in alcohol-dependent subjects with and without a history of depressive disorder (Mann-Whitney U value = 2865.0, p=0.250). Thus, Preuss et al showed the genetic influence of HTTLPR-S-allele on suicidal behavior to be independent of a history of depression.

1.3.2 Findings from treatment studies

Results of animal studies provided a basis for early clinical pharmacologic tests but some difficulties were apparent in extrapolating from rat to human studies. Efficacy in animal studies is dependent on the drug's ability to reduce alcohol consumption but human studies often seek to evaluate the drug's ability to maintain a state of abstinence. Correlational studies of alcohol-dependent individuals suggest that brain serotonergic activity is inversely related to ethanol consumption. Abstinent alcoholics have been shown to have reduced cerebrospinal

fluid (CSF) 5-HIAA levels (Banki 1981); (Borg, Kvande et al. 1985) low platelet 5-HT content (Bailly, Vignau et al. 1993) and low tryptophan availability in plasma (Buydens-Branchey, Branchey et al. 1989) suggesting decreased central serotonergic function. In addition, although results are mixed, blunted neuroendocrine responses have been documented in detoxified alcoholics administered *m*-chlorophenylpiperazine (Krystal, Webb et al. 1996), MK212 (Lee and Meltzer 1991) and fenfluramine (Balldin, Berggren et al. 1994); (Farren and Tipton 1999) suggesting reduced responsivity of the serotonergic system.

Kranzler et al. (1996) examined the hypothesis that individuals with the greatest probability of serotonergic dysfunction may be the most responsive to interventions that increase serotonergic activity. The effect of 12 weeks of fluoxetine or placebo and cognitive-behavioral therapy on drinking was examined in alcohol-dependent subjects who were subdivided on the basis of probable 5-HT dysfunction. Results of this study indicated that type B subjects had poorer outcomes in the fluoxetine condition than in the placebo condition. Kranzler et al. suggest that in this subgroup of type B patients SSRIs, through their agonist effect, may serve as a conditioned stimulus, increasing alcohol consumption instead of decreasing it.

Angelone et al. (1998) performed a 16-week study to test the efficacy of fluvoxamine and citalopram in decreasing relapse and craving in alcoholics. Both SSRIs showed a statistically higher rate of continuous abstinence (63.6 and 60.7% respectively) compared to untreated group (30.4%). Relapse severity did not differ among the three groups and only citalopram showed a significant effect on craving throughout the study period. Similar findings have recently been reported with sertraline (Pettinati, Volpicelli et al. 2000).

1.4 Impulsive behavior

The concept of impulsivity plays a major role in clinical psychiatry as well as in everyday life. Impulsivity is part of the defining characteristics of a number of psychiatric disorders, e.g. borderline and antisocial personality disorders (Stein, Hollander et al. 1993).

Several attempts were made to define impulsivity. In 1938, Murray described impulsivity as the tendency to respond quickly and without reflection (Murray 1938), with difficulty in restraining behavior and being more spontaneous. Douglas described impulsivity as largely related to the inability to sustain attention (Douglas 1972), and Eysenck (1983) described an impulsivity scale as part of extraversion. Barratt and Patton (Barratt and Patton 1983) described impulsivity as acting without adequate reflection, spur-of-the-moment reactions, taking risks and trying to get things done quickly. This concept of impulsivity encompasses elements of cognition and emotion. In subsequent analysis, they found the Zuckerman's Sensation Seeking concept and Eysenck's concept of Extraversion to correlate significantly with their assessment of impulsivity using the Barratt Impulsiveness scale (Barratt and Patton

1983). Lorr and Wunderlich (1985) concluded that there are two major bipolar components of impulsivity: (1) resisting urges versus giving in to urges, and (2) responding immediately to a stimulus versus planning before making a move. The most recent definition (Moeller, Barratt et al. 2001) defines impulsivity as ‘a predisposition toward rapid, unplanned reactions to internal or external stimuli without regard to the negative consequences of these reactions to themselves or to others’ (pg. 1784).

Impulsivity can be expressed as a stable pattern across a variety of situations (trait-dependent) or may be shown transiently (state-dependent) in acute biological or environmental influences (Dougherty, Mathias et al. 2004). Trait-dependent theories emphasize the importance of an interaction between negative life events or psychiatric states with underlying traits of impulsivity (Mann, Waternaux et al. 1999), whereas state-dependent theories emphasize impulsivity as a product of the individual’s diminished capacity or deconstructed state (Baechler 1980); (Baumeister 1990).

1.4.1 Impulsivity and alcohol dependence

Impulsivity is believed to be associated with alcoholism and substance abuse (Knorring. L. von, Knorring et al. 1987). Virkkunen et al. (1994) showed that impulsive behavior such as fire-setting and violent offenses are higher in alcoholics compared to control samples. Quirk and McCormick (1998) investigated 3256 substance abusers using the NEO-PI and found that the more extreme the reported levels of depressive symptoms, aggressive/hostile cognitions, impulsiveness and maladaptive coping styles were, the higher the likelihood of abusing more than one substance.

Lejoyeux et al. (1998) compared two groups of alcohol-dependent subjects with and without impulse control disorders, and healthy controls for their levels of impulsivity and sensation seeking. Significant differences were found in sensation seeking traits between alcohol-dependent individuals with impulse control disorders, compared to alcohol-dependent individuals without, and healthy subjects. However, the difference could not be obtained in BIS-measures of impulsivity. The combination of suicidal behaviors, substance abuse and impulsivity may produce synergistic effects (Putnins 1995; Dalton, Cate-Cater et al. 2003) which makes it all the more imperative to assess impulsivity in alcohol dependent subjects.

1.4.2 Serotonin and impulsivity

Impulsive-aggressive traits and impulsive violence are associated with abnormalities in central nervous system serotonin. An inverse relationship has been observed between indices

of central serotonin function and aggression in humans (Brown, Goodwin et al. 1979; Brown, Ebert et al. 1982; Coccaro, Siever et al. 1989; Virkkunen, De Jong et al. 1989; Limson, Goldman et al. 1991; Manuck, Flory et al. 1998); and nonhuman primates (Higley, Mehlman et al. 1992; Mehlman, Higley et al. 1994; Doudet, Hommer et al. 1995). Trait variation in serotonin function may explain individual differences in the aggression-promoting effect of alcohol (Higley, Mehlman et al. 1992; Allen, Moeller et al. 1998; Phil and LeMarquand 1998).

1.4.3 Measurements of impulsivity

There are primarily three main classes of instruments to measure impulsivity: self-report measures, laboratory behaviour measures, and event-related potentials. The most frequently used self-report measure, Barratt Impulsiveness Scale (BIS-11) (Patton, Stanford et al. 1995), is designed to assess persistent patterns of behaviour through questions regarding acting without forethought (motor impulsiveness), a tendency to make quick non-reflective decisions (attentional impulsiveness) and a failure to prepare for future events (non-planning impulsiveness). Self-report measures of anger and impulsiveness may not discriminate among subjects with very high levels of both traits although they do discriminate between persons with high and low trait scores (Barratt, Kent et al. 1995). Another self-report scale is the Impulse Control Scale (ICS, (Plutchik, van Praag et al. 1989) which has questions regarding spontaneous behaviors and loss of control. The limitations of these self-reports are that they are subjective, require a certain degree of insight and are insensitive to state-dependent fluctuations in impulsivity (Dougherty, Mathias et al. 2003a). They are also unsuitable for repeated use, limiting their usefulness in treatment studies (Moeller, Barratt et al. 2001).

Laboratory behaviour measures for impulsivity are divided into three types (Dougherty, Mathias et al. 2003a):

- *Punishment/extinction* paradigms – impulsivity is defined as the perseveration of a response that is punished or not enforced (Wisconsin Card Sorting Test, Heaton 1981; Gordon Diagnostic System, Gordon 1987).
- *Reward-directed* paradigms – impulsivity is defined as preference, or greater number of choices for a smaller-sooner reward rather than a larger-later reward (Single Key Impulsivity Paradigm, SKIP, Dougherty, Marsh et al. 2003b).
- *Rapid-decision* paradigms – impulsivity is defined as either premature or disinhibited responding (Continuous Performance Test, CPT and go/no-go tasks).

Research has shown impulsiveness to be inversely correlated to neuropsychology tests (Barratt, Stanford et al. 1997) and impulsive errors of commission using a continuous

performance test (CPT) were correlated significantly with hostile aggression, also defined as impulsive aggression (Atkins, Stoff et al. 1993). The benefits of these tests are that they assess processes which meet an operational definition of impulsivity, are objective, cost-effective, easy to administer and can be administered repeatedly over time to provide more accurate information regarding state-dependent changes in impulsivity within an individual (Dougherty, Mathias et al. 2004) but the disadvantages are that they do not incorporate the social aspects of impulsivity and do not measure long-term patterns of behavior (Moeller, Barratt et al. 2001). Based on these considerations, CPT was chosen for our study.

Impulsiveness has been shown to be related to information processing through proposed differences in attention (Dickman 1993), arousal ((Revelle, Humphreys et al. 1980; Eysenck and Eysenck 1985; Revelle, Anderson et al. 1987), and/or maintaining a cognitive tempo or selected rate of information processing ((Barratt 1983; Barratt and Patton 1983; Barratt 1987). Past research has shown the frontal lobe dysfunction to be implicated in impulsiveness (Barratt 1987; Barratt and Patton 1993; Stein, Towey et al. 1995). Measures of impulsiveness, selected verbal skills including reading, continuous motor performance, and judging time intervals, define a second order factor or temporal information processing according to Stanford and Barratt (1996). A positive waveform (P300) recorded in response to target stimuli during the performance of a wide range of ‘oddball’ tasks has been related to impulsivity and impulse control disorders (Harmon-Jones, Barratt et al. 1997; Sunohara, Malone et al. 1999). The advantage of this type of measure is that it is directly related to brain funtion. Disadvantages are that ERPs also don’t incorporate the social aspects of impulsivity and have been reported to be related to a variety of neurologic and psychiatric conditions (Iwanami, Okajima et al. 2000; Korpelainen, Kaufhanen et al. 2000), thus they are not specific for impulsivity.

1.5 HPA axis

The neuroendocrine stress response is coordinated via a complex, cascading circuitry designed to protect an organism from perceived threat and then return the organism to homeostasis (Van de Kar and Blair 1999). The central nervous system’s (CNS’s) control of this stress response is not completely understood, but multiple brain regions and neurotransmitters are known to be involved (Herman and Cullinan 1997; Lopez, Akil et al. 1999). Afferent signals from various cortical, limbic (i.e., hippocampus, amygdala), and diencephalic regions funnel input to the hypothalamic paraventricular nucleus, leading to the release of corticotropin-releasing hormone (CRH) and arginine vasopressin. These hypothalamic secretagogues then trigger the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary which, in humans, ultimately stimulates the production of cortisol from the adrenal cortex. To complete the circuit and turn off the stress response, a negative

feedback regulatory system involving glucocorticoid receptors at various levels in the cascade is in place (Lopez, Akil et al. 1999).

HPA axis responses to stressors are influenced by age and gender. Kudielka et al. (2004) reanalysed data from five independent studies with a total of 102 healthy subjects (30 older adults, mean age 67.3 y; 41 young adults, mean age 23.5 y; and 31 children, mean age 12.1 y) and found that exposure to the Trier Social Stress Test (TSST) caused highly significant ACTH and total plasma cortisol responses in older and younger male and female adults (all $p<0.0001$) as well as salivary free cortisol responses in all six age and gender groups (all $p<0.0001$). ACTH responses to stress was higher in younger adults compared to older adults (main effect: $p=0.009$, interaction: $p=0.06$). Post hoc analyses showed no age effect in the subgroup of women while younger men had higher ACTH responses compared to older men ($p=0.01$). The researchers concluded that the observed ACTH response patterns in young and elderly adults may suggest a heightened hypothalamic drive in young men decreases with age, resulting in similar ACTH responses in elderly men and women.

Since the HPA axis responses may also differ depending on the time of day, Kudielka et al (2004) conducted another reanalysis with 180 adults (115 younger adults: 49 females and 66 males; 65 older adults: 32 females and 33 males). Stress-related free salivary cortisol, total plasma cortisol and ACTH net increases did not differ according to time of day. However, pre-stress free salivary and total plasma cortisol levels differed significantly between the morning and afternoon group (both $p<0.005$), leading to a significantly higher free cortisol area under the curve in the morning ($p=0.02$). These suggest that the adrenal glands may be more sensitive to ACTH in the morning and higher basal salivary cortisol levels were related to a lower stress-related net increase in ACTH ($p=0.09$).

1.5.1 HPA axis and alcohol dependence

Alcohol dependence has been associated with long-lasting alterations in LHPA axis function which are widespread, beginning with an increase in cortisol secretion during intoxication (Mendelson, Ogata et al. 1971; Stokes 1973; Adinoff, Ruether et al. 2003), and withdrawal (Mendelson, Ogata et al. 1971; Iranmanesch, Veldhuis et al. 1989; Adinoff, Risher-Flowers et al. 1991; Keedwell, Poon et al. 2001), occasionally resulting in a hypercortisolemic state mimicking the clinical and biochemical characteristics of Cushing syndrome (Jeffcoate 1993; Veldman and Meinders 1996). Several investigators have also observed an attenuation of the axis to pharmacological and psychological stressors concurrent with and after this hypercortisolemic state, and suppression of the HPA axis appears to persist for at least several weeks of abstinence (Adinoff, Risher-Flowers et al. 1991). Thus, compared with non-alcohol-

dependent controls, abstinent alcohol-dependent patients have been found to have disturbances in stress hormone and cardiovascular responses to a variety of psychological (Ehrenreich, Schuck et al. 1997; Lovallo, Dickensheets et al. 2000) and physiological stressors (Vescovi, DiGennaro et al. 1997), cold (Errico, Parson et al. 1993), operative trauma (Margraf, Moyer et al. 1967), basal cerebrospinal fluid CRH concentrations (Geracioti, Loosen et al. 1994), exogenous CRH-induced ACTH secretion (Ehrenreich, Schuck et al. 1997); (Loosen, Chambliss et al. 1991), and exogenous CRH-induced (Inder, Joyce et al. 1995); (von Bardeleben, Heuser et al. 1989) or synthetic ACTH-induced release of cortisol (Wand and Dobs 1991). These changes in HPA axis function fluctuate over time, depending on when the alcohol-dependent individual last drank alcohol. Children (Schulz, McKay et al. 1998) and young adults (Wand, Mangold et al. 1999) who are at risk for developing alcoholism also have been found to exhibit signs of LHPA axis dysfunction which might play an important role in the development and maintenance of alcoholism, with evidence from preclinical studies indicating that glucocorticoids and stress affect alcohol self-administration (Fahlke, Hard et al. 1994), alcohol use resumption (Le´ A.D., Poulos et al. 1999), and the mesolimbic dopamine pathways that subserve some of the rewarding effects of ethanol and other drugs of abuse (Piazza, Rouge-Pont et al. 1996).

Alcoholism has also been linked to major disturbances in 5-HTergic function (LeMarquand, Phil et al. 1994). People who are chronically alcohol-dependent are usually reported to have lower amounts of various 5-HT precursors (e.g., tryptophan) and metabolites (e.g., 5-hydroxy-indoleacetic acid) in urine, plasma, and cerebrospinal fluid than non-alcohol-dependent controls (Ballenger, Goodwin et al. 1979; Borg, Kvande et al. 1985; Buydens-Branchey, Branchey et al. 1989), but not all studies agree (Geracioti, Loosen et al. 1994; Geracioti, Goldsmith et al. 1997). Similarly, most studies using platelets from alcohol-dependent individuals and measuring either 5-HT levels (Bailly, Vignau et al. 1990) or various measures of 5-HT uptake (i.e., imipramine binding, V_{max}, K_m) suggest decreased 5-HT turnover (Boismare, Lhuintre et al. 1987; Javors, Blaisdell et al. 1987). Finally, although not directly related to alcohol dependence per se, 5-HT mechanisms seem to play a role in ethanol preference and consumption (McBride and Li 1998), such that drugs that enhance overall CNS 5-HT activity seem to reduce alcohol intake.

1.5.2 HPA axis and impulsivity

The hypothalamus can be conceptualised as the principal ganglion of the autonomic nervous system (Stein, Towey et al. 1995). It receives input from a variety of chemoreceptors and osmoreceptors, and sends efferents to neurons in the pituitary and to motor centers in the

brainstem. The amygdala receives inputs from multiple cortical areas, and has efferents to the extrapyramidal system and the hypothalamus so it may have a role in associating sensory experience with (hypothalamically-directed) affects and behaviors, including anger (Bear 1991). Bilateral temporal lobe damage in humans lead to Klüver-Bucy syndrome characterized by hyperorality, hypersexuality, and loss of aggressive responses, (Klüver and Bucy 1939), with a decrease in regulatory affects and behaviors (Terzian and Ore 1955; Marlowe, Mancall et al. 1975; Isern 1987). Disorders with temporal lobe excitation may cause an increase in aggression as patients with temporal lobe epilepsy demonstrate hyperemotionality and increased aggression (Weiger and Bear 1988; Elliot 1992).

Advance in PET studies has shed new light on the relationship between HPA axis and impulsive/aggressive behavior. The anterior cingulated cortex can be separated into a dorsal 'cognitive' portion and a rostral-ventral 'affective' region (Bush, Luu et al. 2000). The affective subdivision of the anterior cingulated cortex regulates the intensity of response to emotional stimuli, and stimulation of this area in animals increases the latency of attack behavior (Siegel and Edinger 1983). In humans, PET studies of cerebral blood flow demonstrate activation of the ventral anterior cingulated cortex when anger is induced in healthy men using imagery (Dougherty, Shin et al. 1999), when symptoms are provoked in individuals with simple phobia, obsessive-compulsive disorder or posttraumatic stress disorder (Rauch, Jenike et al. 1994; Rauch, Savage et al. 1995; Rauch, van der Kolk et al. 1996), and when men attempted to suppress sexual arousal in response to erotic film excerpts but not in the nonsuppression condition (Beauregard, Levesque et al. 2001). These indicate a left-sided predominance of the abnormality and traumatic brain lesions to the left frontal cortex give rise to aggression and hostility, whereas right-sided lesions lead to anxiety/depression (Grafman, Schwab et al. 1996).

1.5.3 HPA axis and Serotonin

Serotonin (5-hydroxytryptamine [5-HT]) is one of the neurotransmitters involved in regulating the activity of the limbic-hypothalamic-pituitary-adrenal (LHPA) axis (Fuller 1996). Drugs that acutely enhance CNS 5-HT function, including direct agonists, selective serotonin reuptake inhibitors, and releasers, dose-dependently increase CRH, ACTH, and corticosterone/cortisol concentrations in experimental animals (Fuller 1996) and humans (Dinan 1996). In a reciprocal fashion, corticosteroids are involved in a negative feedback loop that extends beyond the pituitary and the hypothalamus to affect limbic structures that receive dense 5-HTergic input (Meijer and de Kloet 1998). Communication between the limbic system structures and the hypothalamic-pituitary-adrenal (HPA) axis is mediated in part by 5-

HT (Lopez, Akil et al. 1999), and evidence exists to support direct and indirect connections between the 5-HTergic and LHPA systems (Dinan 1996). For example, brain corticosteroid receptors and subtypes of 5-HT receptors are collocated in the prefrontal cortex and hippocampus, suggesting a functional interaction between the 5-HT and LHPA systems in humans (Lopez, Akil et al. 1999). Thus, although 5-HT regulates the LHPA axis at various levels, the LHPA axis in turn governs 5-HTergic activity (Dinan 1996; Lopez, Akil et al. 1999).

CRH receptors and nerve fibers have also been found in regions of the raphe nuclei (Price and Lucki 2001), thus providing a circuit wherein the CRH and serotonin systems modulates each other's activity. Thus, alcohol- or tobacco-related disturbances in serotonergic systems that modulate the limbic-HPA axis may manifest themselves as abnormal responses to pharmacologically induced stress.

1.6 Challenge with serotonergic substances

The selective serotonin reuptake inhibitors (SSRIs) are small lipophilic molecules which readily cross biological membranes (Brosen and Rasmussen 1996)(pg. 87). They are generally well absorbed via the gastrointestinal mucosa although time to reach the maximal plasma concentration is relatively long, 4-8 hours. Considerable research has shown that SSRIs do not interact with alcohol in any clinically meaningful way (McClelland and Raptopoulos 1985; Schaffler 1986; Hindmarch and Harrison 1988; Allen and Lader 1989; Schaffler 1989; Hindmarch, Shillingford et al. 1990; Kerr, Fairweather et al. 1992; van Harten, Stevens et al. 1992). The psychomotor impairments caused by alcohol may, in some situations, be antagonized by SSRIs (McClelland and Raptopoulos 1985; Schaffler 1986). Given the proposed role of serotonin in governing ethanol intake, one would expect pharmacological manipulations that decrease serotonergic functioning to increase ethanol intake. The most consistent evidence supporting this comes from studies using 5,6-dihydroxytryptamine (DHT) and 5,7-DHT, neurotoxins that selectively eliminate brain 5-HT neurons and consistently increase ethanol intake (Myers and Melchior 1977). A large number of studies have employed p-chloroamphetamine (pCA), a compound that depletes brain 5-HT by inhibiting tryptophan hydroxylase, the enzyme that concerts tryptophan (TRP) to 5-hydroxytryptophan (5-HTP) and thus 5-HT. This compound, as well as p-chloroamphetamine (pCA: also a central 5-HT depletory) most often decrease ethanol intake. Three temporally related mechanisms appear to be overriding pCPA's central effect of 5-HT depletion, accounting for these results: a short-term accumulation of highly toxic acetaldehyde on alcohol ingestion; a rebound increase in the synaptic activity of 5-HT; and a conditioned taste aversion due to the association of pCPA's noxious side effects with the ingestion of alcohol.

Increasing serotonergic neurotransmission through various means decreases ethanol consumption. Serotonin, 5-HT precursors (TRP and 5-HTP), the L-5-HTP derivative triptosine (possibly a 5-HTP releaser), and 5-HT releasers (fenfluramine), and uptake inhibitors (zimeldine, norzimeldine, alaproclate, fluoxetine, fluvoxamine, indalpine, viqualine, citalopram, and sertraline) consistently decrease ethanol consumption in rats as well as monkeys and chickens. Studies employing 5-HT precursors (TRP/5-HTP) and releaser/uptake inhibitor (fenfluramine/fluoxetine) combinations generally show that precursor pre-treatment enhances the effect of the releaser or uptake inhibitor on ethanol intake (Fischer, Hsu et al. 1991);(Gorelick 1989). The attenuating effect of 5-HTP on ethanol intake is blocked by pre-treatment with the 5-HTP decarboxylase inhibitor RO4-4601 (a compound that inhibits the conversion of 5-HTP to 5-HT) (Geller, Hartmann et al. 1981).

The mechanism of action and specificity of effect of 5-HT uptake inhibitors has been the subject of some speculation. Serotonin agonists, particularly 5-HT1A agonists, effect both presynaptic (somatodendritic autoreceptors), and postsynaptic receptors. Serotonin agonists generally lack specificity, acting at more than one 5-HT receptor subtype as well as at receptors of other neurotransmitter systems. One possible explanation accounting for the results reviewed above is that 5-HT precursors, agonists and uptake inhibitors decrease ethanol intake by increasing serotonergic neurotransmission.

As the serotonin pathways are related to dopaminergic function which mediates alcohol-induced reward associated with its abuse liability, SSRIs have been considered for the treatment of alcoholism. Johnson (2004) recently showed that buspirone, a serotonin 5-HT1A partial agonist, was not effective in the treatment for late-onset alcoholics without comorbid disease but may have some utility for treating alcoholics with comorbid anxiety disorder. Ritanserin, a 5-TH2 antagonist, was also not effective in the treatment but ondansetron, a 5-HT3 antagonist, is an efficacious and promising medication for treatment of early-onset alcoholism. Combining the *mu* opiod receptor antagonist, naltrexone, with the 5-HT3 antagonist ondansetron promises to be more effective for treating alcoholism than either alone.

Habitual cigarette smoking has also been linked to alterations in LHPA axis function (Frederick, Reus et al. 1998). Chronic smokers with (Coiro and Vescovi 1999) and without (Kirschbaum, Scherer et al. 1994) a history of alcohol dependence were found to have disturbances in their stress hormone response to cigarette smoking. Anthenelli et al. (2001) explored the effects of cigarette smoking on hormonal responses in a pharmacological challenge with D,L-fenfluramine and found no significant effect in 109 alcohol dependent men. In our study, we controlled for the confounding effects of cigarette smoking by asking the subjects (both patients and controls) to abstain from smoking from 2100 the day before

examination to the end of examination the next day, i.e. no smoking for approximately 11 hours prior to testing.

1.6.1 Studies with Fenfluramin

The reasons for the reduced central serotonergic metabolism and neurotransmission in alcohol-dependent individuals are not fully elaborated. In a recent study of Berggren et al (2002), a positive correlation was found between the age at onset of excessive alcohol consumption in alcohol-dependent individuals and central serotonergic neurotransmission. This, together with the earlier findings of Virkkunen and Linnoila (1990) and Fils-Aime et al. (1996) lend support to the notion that individuals with early onset of excessive alcohol consumption have a pre-existing reduction in central serotonergic neurotransmission.

Anthenelli and Maxwell (2000) challenged this notion and reported no difference in the PRL responses to D,L-fenfluramine (used as an index of central serotonergic neurotransmission) between controls, type I alcoholics, type II alcoholics, and alcoholics with antisocial personality disorder. Their notable finding was that of reduced PRL response in current cigarette smokers in comparison to non-smokers. The reduced PRL response in the smokers appeared to be influenced both by pharmacodynamic and pharmacokinetic factors related to smoking and possibly more specifically to nicotine. Therefore, the reduced central serotonergic neurotransmission in alcohol dependence may be attributed to the effects of smoking and nicotine rather than to alcoholism or its subtypes.

1.6.2 Studies with m-CPP

M-Chlorophenylpiperazine (m-CPP) is a metabolite of the antidepressant trazodone that possesses agonist properties at some 5-HT receptors and antagonist properties at others. It has highest affinity for the $5HT_{2C}$ receptor, where it acts as a partial agonist (Sanders-Bush and Breeding 1990). It also binds to the $5HT_{3}$ and $5HT_{2A}$ receptors, where it acts as an antagonist. In addition, mCPP binds to the $5HT_{1A}$, $5HT_{7}$, and $5HT_{6}$ receptors but with an affinity more than an order of magnitude lower than its affinity for the $5HT_{2C}$ receptor (Hoyer 1988; Hamik and Peroutka 1989; Schoeffter and Hoyer 1989). Despite its non-specific pharmacology m-CPP has been used extensively in psychiatric research to gauge the sensitivity of the serotonin (5-HT) system of the brain.

Benkelfat et al. (1991) and Krystal et al. (1994) reported that when mCPP is administered to alcoholics it induces a “craving” for alcohol. This effect appears most dramatically among early onset or type II alcoholics. In addition, both George et al. (1997) and Krystal et al.

(1996) found blunted cortisol and adrenocorticotrophic hormone (ACTH) responses to m-CPP among alcoholics. The blunted ACTH and cortisol responses seem consistent with a reduced sensitivity to 5HT2C agonists among alcoholics.

However, not all studies in which m-CPP was administered agree (Handelsman, Holloway et al. 1996; Buydens-Branchey, Branchey et al. 1997). In addition, alcohol-dependent patients were found to have a transiently decreased ACTH response to m-CPP administration as compared with the response of non-alcohol-dependent individuals (George, Benkelfat et al. 1997). It also has been hypothesized that only subgroups of alcohol-dependent patients with impulsive, aggressive personality traits (i.e., type 2 or early-onset alcohol-dependent patients) might exhibit such 5-HTergic dysfunction. In support of this theory, type 2 alcohol-dependent patients were found to exhibit differential subjective responses from those of type 1 alcohol-dependent patients after 5-HTergic stimulation (George, Benkelfat et al. 1997).

1.6.3 Studies with Citalopram

Much of the treatment efficacy research use “change in mean alcohol consumption” as the primary dependent variable. Using these measures, overall reductions of 15% to 20% from baseline drinking levels are consistently reported (Kranzler, Amin et al. 1999). Inter-individual variability in response to SSRIs is large, with reductions in alcohol consumption ranging from 10% to more than 70% (Naranjo, Brenner et al. 1997). In addition, gender may affect response to citalopram treatment, with men exhibiting larger reductions in alcohol consumption than women (Naranjo, Knoke et al. 2000). The extent of drinking also appears to affect response. For example, Balldin et al (1994) found no significant overall effect of 40 mg/day of citalopram in a 5-week trial but an analysis of responders revealed that citalopram significantly reduced alcohol consumption in a subgroup of heavy drinkers who had lower baseline drinking values (between 60 and 100 g of alcohol/day).

In a series of studies (Naranjo, Sellers et al. 1987; Naranjo, Poulos et al. 1992; Naranjo, Brenner et al. 1995) citalopram (40 mg/day) produced short-term reductions in alcohol consumption and alcohol craving in subjects with mild to-moderate dependence. In another study of severely alcohol-dependent subjects, 12 weeks of citalopram treatment (titrated up to 40 mg/day) produced significant reductions in alcohol consumption, as measured by subject and relative report as well as by γ-glutamyl-transferase levels (Tiihonen, Ryyanen et al. 1996). However, in a 12-week treatment study of mildly to moderately dependent men and women, no significant benefit of citalopram on alcohol consumption or craving was evident beyond the first week of treatment (Naranjo, Brenner et al. 1995). Angelone et al. (1998) administered citalopram (20 mg/day, N = 81) or fluvoxamine (150 mg/day, N = 81) to relapse

prevention therapy for 16 weeks to detoxified male and female alcoholics, and showed that increased rate of continuous abstinence were found in both drug groups as already mentioned earlier. Despite these positive results with fluvoxamine, the frequency of adverse events may limit the usefulness of this medication (Kranzler, Del Boca et al. 1993).

2 Summarizing recent research and open questions

Considering that many different aspects of alcohol dependence are still unclear yet they can have a significant impact on the diagnosis and treatment of alcohol dependence, we ventured to conduct a study with pharmacologic challenge to investigate the effects, if any, on impulse control or behavior inhibition as measured with CPT, HPA axis response measured with ACTH levels, influence of serotonin transporter polymorphism (5HTTLPR genotypes) on the HPA axis response and subjective feelings of craving for alcohol, anxiety and intoxication.

2.1 Rational for this study

The aim of this study is to improve the understanding of the biological, psychical and behavioral processes which cause or influence this psychiatric illness. This study is a step further in researching alcohol dependence with regard to functionally relevant alleles of a candidate gene, changes in the serotonergic system and their significance in alcohol dependence, as well as impulsive behavior, in the context of a neuropharmacologic model.

Impulsive behavior, which probably occurs more frequently in alcohol dependent patients and in terms of the course of disease, presents an increased risk for relapses and is at least partially mediated through the serotonergic system. This study aims to document in parallel and in detail, the biological and psychical changes which can be induced by stimulation of the serotonergic system, and clarify their relationships. By examining the interaction between alcohol-dependent patients, their impulsive behavior and functionally relevant alleles of candidate genes, the genesis of impulsive behavior in alcohol dependent patients may be traced back. The interaction between genetic disposition and behaviour is also of importance.

3 Hypothesis

The following parameters were investigated after an intravenous administration of 0.4 mg/kg body weight of either citalopram or placebo (natrium chloride), on 11 age-matched alcohol-dependent patients and 12 healthy controls:

3.1 Primary hypothesis

- Would impulsive behavior in alcohol-dependent patients and healthy controls be reduced by a challenge with citalopram 0.4mg/kg body weight (assessed with CPT)?

3.2 Secondary hypotheses

- Can citalopram increase the endocrine responsivity, using serum ACTH levels in alcohol-dependent patients compared to controls?
- Is there any relationship between the genotype of serotonin transporter (5HTTLPR), and endocrine responsivity (ACTH levels) under citalopram in alcohol-dependent patients and healthy controls?
- What are the subjective experiences, in particular anxiety, craving and subjective feeling of intoxication, under citalopram (using Visual Analogue Scales) in both groups?

The effects would be compared between verum and placebo, between alcohol-dependent patients with healthy controls on the other hand, and between the different genotypes of serotonin transporters.

4 Materials and methods

This study was a double-blind cross-sectional examination. The investigator administering the intravenous infusion, or the patient/control receiving the infusion, will not know if the infusion contained the verum citalopram or placebo. 20 male alcohol-dependent patients and 14 healthy male controls were screened with the German version of semi-structured interview for assessment of genetics in alcoholism (SSAGA)(Bucholz, Cadoret et al. 1995) and recruited in a case-control-group design. Patients' recruitment was conducted among inpatients of the addiction ward in the Munich Hospital psychiatric clinic. This ward admits ca. 400-450 patients each year (main diagnosis alcohol- or medication dependence), for a qualified detoxification course spanning an average of 23 days. Male control subjects were recruited from the local community, and were age-matched to patients. To ensure sufficient number of patients and controls after the initial examination could be recruited, this project was planned to run for at least 2 years.

4.1 Patient or Control Sample

For the evaluation, inclusion and exclusion criteria as well as questionnaires, individual clinical examinations, history and routine physical and psychiatric examinations were conducted. To control for possible confounding factors from age and gender, only male alcohol dependent patients and male healthy controls were recruited, all young adults and all informed to abstain from smoking from 21oo hours the day prior to examination to the end of examination the next day.

4.1.1 Inclusion criteria

- Alcohol-dependence (according to DSM-IV and ICD-10) or healthy controls (defined as having at most moderate alcohol consumption < than 2 standard drinks/day).
- 12 to 26 days after the last consumption of alcohol
- Male
- Written informed consent

4.1.2 Exclusion criteria

- Age <18 years
- Inability to give consent
- Additional heavy abuse or dependence on other substances other than nicotine in the last year
- Treatment with medication (SSRI or antipsychotics)
- Additional other significant psychiatric diagnoses (psychoses, depression)
- Significant liver damage
- Significant medical diseases, especially liver cirrhosis, known hypertension and asthma, known allergy to citalopram
- Past history of Serotonin Syndrome
- Acute suicidality

4.2 Study procedures

The patients were informed of the study according to the "Guidelines to Patient Information of Hospital Inpatients over expected measures" in Helsinki Declaration with amendments from Tokyo 1975, Hong Kong 1989, and Somerset West 1996. Time of examination was fixed in the mornings on weekdays, and the schedule of blood taking for ACTH and genetic testing, VAS and CPT was kept to ensure as little variation in the procedures as possible to reduce any possible influence from the time of day and the degree of hypoglycemia from fasting on the HPA axis response, subject's comfort and level of tiredness which could influence his CPT performance, and to speed up the fasting period so that subjects can continue their daily routine and eat again after examination.

4.2.1 Pre-Examination

- Suitable patients or healthy controls were informed of the study in detail and given time to ask questions before they signed a written informed consent to participate in the study.
- Patients were instructed the day before examination, to fast after 9pm till the end of examination the next day, and not to smoke during this whole period.
- If possible, a training session with the Continuous Performance Test-München was conducted to ensure patients understood the test procedures. This is to avoid any bias from misunderstanding and hesitation on the first day of examination, which may be less on the second day of examination.

- Questionnaires used were shown to the patients/controls to ensure they understood the scoring procedure and the medical terms used.

4.2.2 Examination Day 1

- Examination started between 7:30 and 8:30 in the mornings. Patients sat on a comfortable stool and were given some time alone to relax.
- Personal and familial addiction history was obtained using the German version of SCID, routine physical examination was conducted.
- A CPT-M training session was conducted if one was not done the day before. CPT-M was tested 90 mins prior to i.v infusion (time point –3)
- Placement of an i.v. canula for blood testing and for i.v. challenge in non-dominant arm when possible.
- 8 blood tests on each examination day: 60 and 30 mins prior to administration of i.v.-medication/placebo (i.e. time points –2 and –1), and 30, 60, 90, 120, 150 and 180 mins after i.v.-medication/placebo (i.e. time points +1 to +6). The tubes were labelled and frozen accordingly.
- Administration of medication/placebo (0.4 mg citalopram/kg body weight in NaCl) or 0.9% Na-Cl as placebo intravenously over 10-15 minutes by investigator. Both solutions are identical in appearance and prepared by a research assistant in another room to ensure double-blinding.
- Measurements of subjective effects with VAS, side effects with Serotonin Syndrome Scale and impulsivity with CPT-M according to plan.
- At the end of examination, the investigator examined the patient or control to ensure he was well, removed the i.v. canula before the patient or control went back.

4.2.3 Examination Day 2

Examination Day 2 is identical in its course to Day 1, the examinations under (parallel) placebo or verum-conditions followed at least 72 hours later.

4.3 Blood tests

4.3.1 Serum ACTH levels

400 µl plasma (EDTA) were collected by canula in vacutainers (siliconized glass or plastic tubes) at each of the time points ie 8 times during the examination, from -2 to +6, and repeated tests were done on the second examination day. After the collection, the tubes were frozen. For determination of ACTH levels, the tubes were thawed in the lab, 200µl were pipetted into another tube and carefully mixed with 100 µl antibodies. The remaining 200 µl sample was processed in the same way. A small avidine-coated ball was put in each tube and the samples were incubated in a horizontal rotator for 4 hours at 180±10 Upm in room temperature (15-25°C). The balls were washed with 2 ml salt solution in an automated station and within 2 hours after washing, counted in Nichols Institute Diagnostic luminometer for 2 seconds.

4.3.2 Serotonin transporter promoter (5-HTTLPR)

For genetic analyses, 5 mL blood samples (EDTA) were collected by canula once at the beginning of the examination and DNA was extracted using standard isolation methods. The biallelic polymorphism in the 5-regulatory region of the 5-HTT gene was amplified by polymerase chain reaction (PCR) using the primers described by Cook et al (1997). PCR products were separated on a 3% agarose gel (FMC NuSieve 3:1, from Biozym, Hess Oldendorf, Germany), and visualized by ethidium bromide staining with fragment sizes determined by comparison with molecular length standards. Without knowledge of the subjects' clinical history, the 484bp fragment of the biallelic polymorphism in the 5-regulatory region of the 5-HTT gene was designated as short allele and the 528bp fragment as long allele, or the S-allele was 0.41 and 0.59 for the L-allele, respectively.

4.4 Instruments

4.4.1 Visual Analogue Scale (VAS)

The VASs used were for Anxiety, Craving for Alcohol and Subjective Feeling of Intoxication, as shown below:

VAS for Anxiety

Not at all Very

_____

Feeling anxious

VAS for Craving for Alcohol

Not at all Very

_____

I'd like to drink alcohol now

VAS for subjective feeling of intoxication

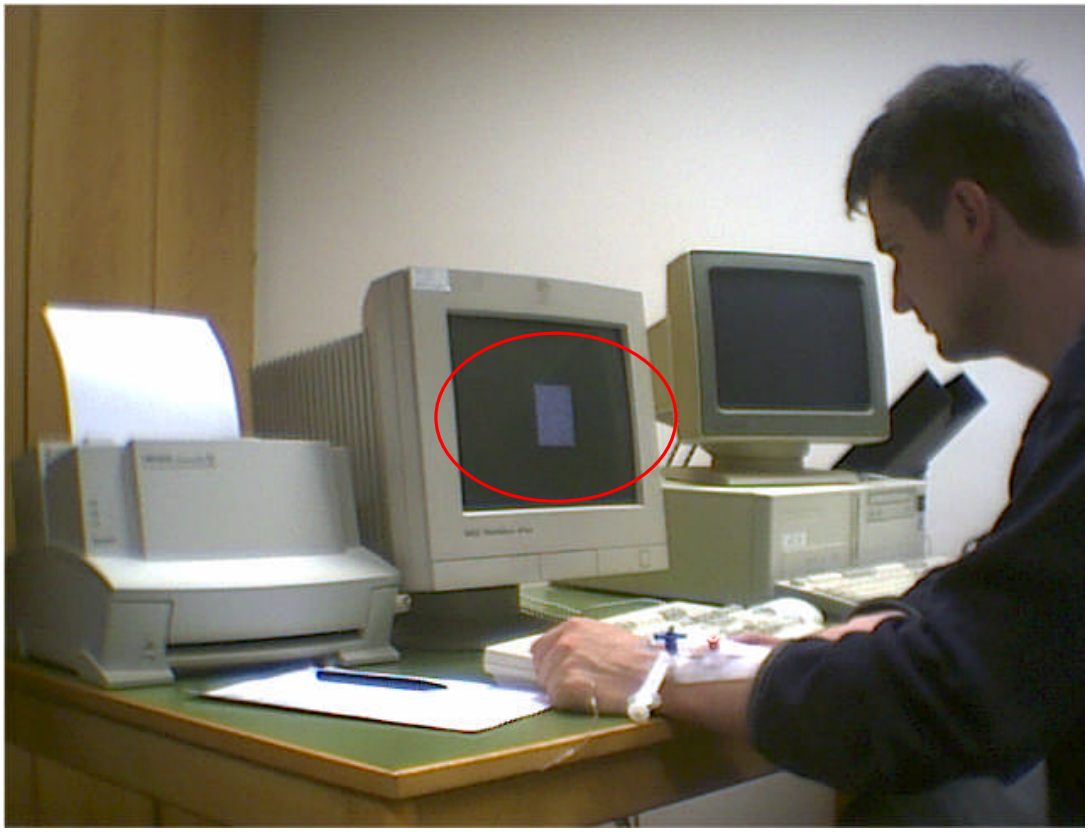
Not at all Very

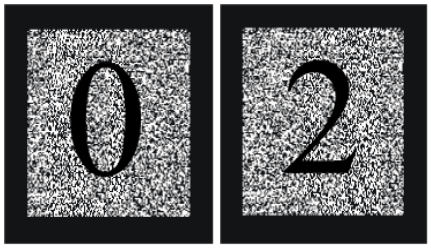
_____

I feel intoxicated

4.4.2 Continuous Performance Test-München (CPT-M)

The Continuous Performance Test-München (**CPT-M**) used to test attention and ability to suppress impulsive behavior was generated on a PC-Monitor in pseudo-randomised order 480 times the numbers 0, **2**, 4, 6 and 8, which could hardly be distinguished from their background due to the low contrast difference. Only one number appeared on the screen at any one time and time between each individual appearance was 1.1 seconds. The patient‘s task was to identify the **target number 0** and press a key as quickly as possible. The probability of the target appearing was p=0.25. Performance errors included responding before the target appeared (commission errors), not responding within 1000 ms (misses or omission errors) or responding to numbers other than the target 0 (commission errors). The following parameters were obtained: number of hits (commission errors) and misses (omission errors), and median reaction latency. In increased impulsive behavior, an increase in commission errors is expected. In the study, both errors are counted together.





4.4.3 Serotonin Syndrome Scale (SSS)

Since the SSRIs came into use, serotonin-related side effects have been documented and the serotonin syndrome represents the severest form (Sternbach 1991; Lejoyeux, Ade´s et al. 1994). This potentially fatal syndrome can develop when drugs with different serotonin agonistic effects are combined (e.g. monamineoxidase inhibitors and SSRI) but also under monotherapy with a serotonin agonist (Fischer 1995; Sporer 1995). A proposed explanation for the clinical symptoms is an enhanced activity of both the central and peripheral serotonin systems (Brown, Skop et al. 1996). Serotonin syndrome has the following criteria proposed by Sternbach (1991): 1) coincident with the addition of or the increase in a known serotonergic agent to an established medication regimen, with at least three of the following clinical features – mental state changes, agitation, myoclonus, hyperreflexia, diaphoresis, shivering, tremor, diarrhea, incoordination and fever; 2) other etiologies have been ruled out; 3) a neuroleptic had not been started or increased in dosage prior to the onset of the features above. Hegerl et al. (1998) modified Sternbach's diagnostic criteria and developed a nine-item scale, rated from 0=not present to 3=severe, for the operationalized assessment of the presence and the severity of the serotonin syndrome. This scale includes most of the items proposed by Sternbach and validated the scale with a study using both paroxetine plasma levels and loudness dependence of the auditory evoked potentials (LDAEP).

A SSS score of >6 is the cutoff point where mild and subacute forms of the serotonin syndrome can be included in the serotonin syndrome group. SSS scores of ≤ 6 could be called serotonergic reactions (Fischer 1995);(Brown, Skop et al. 1996). Mild to moderate SS usually resolves completely when the drug is withdrawn in 24 to 72 hours. Rarely, SS can lead to rhabdomyolysis, myoglobinuria, renal and hepatic failure, disseminated intravascular coagulation, respiratory distress syndrome, and death (Simpson and Warner 1999). Laboratory tests show non-specific changes such as increased total white blood count, and creatine phosphokinase and decreased bicarbonate levels. There are no diagnostic tests available. Since the most common diagnostic confusion occurs in distinguishing SS from the neuroleptic malignant syndrome, patients on antipsychotics or other psychiatric medications are excluded from the study (Simpson and Warner 1999).

Table 2: The german version of Serotonin Syndrome Scale

Grading: 0=no symptom available, 1= mild, 2=moderate, 3=severe

1. Orientation disturbances: ☐
   (items: time, place, situation, to oneself)
   The highest severity of each item will be assessed. Should two items simultaneously be assessed as moderate, a "3" would be given.

2. Internal restlessness ☐

3. Agitability ☐
   (motor: restlessness also resting restlessness)
   0=no agitability
   1=occasional agitability observed
   2=obvious agitability (hand-twisting, can't sit still)
   3=severe agitability, patient can hardly sit still for a minute, gets up and down constantly

4. Daytime lethargy ☐

5. Loss of appetite ☐

6. Increased appetite ☐

7. Nausea ☐
   0=no nausea
   1=mild nausea or abdominal discomfort, occurs occasionally
   2=almost constant nausea, urge to vomit
   3=nausea and vomitting

8. Constipation ☐

9. Diarrhea ☐
   0=no diarrhea
   1=stool has reduced consistency (cr), normal frequency (f)
   2=stool cr loose and/or f 1-3/day
   3=cr and/or f>3, watery stool

10. Micturition inhibition ☐

11. Mouth dryness ☐

12. Increased salivation ☐

13. Sweating ☐
   0=no sweating
   1=subjective feeling, increased sweating
   2=skin definitely moist, solitary beads of sweat
   3=beads of sweat and clothes or bedsheets wet through

14. Accomodation disturbances ☐

15. Headache ☐
   0=no headache
   1=occasional mild headache
   2=frequent headache on many days
   3=headache for which the patient needs additional medication

16. Dizziness ☐
   (every form of dizziness is meant here)
   0=no dizziness
   1=mild dizziness, the patient sometimes feels as if he is drunk
   2=moderate dizziness, constant feeling of drunkenness, but no disturbance in function (stands straight, walk etc)
   3=constant dizziness, with functional disturbances or one time severe functional disturbance (eg. fall)

17. Orthostatic hypotension ☐

18. Hypokinesia ☐

19. Rigor ☐

20. Tremor ☐
   0=no tremor
   1=tremor with small amplitude, no functional disturbances
   2=tremor with obvious amplitude, daily activities limited (hold cup, write)
   3=tremor with large amplitude, constant functional disturbances

21. Hyperreflexia ☐
   0=no increase reflexes
   1=increased reflexes but normal reflex range
   2=increased reflexes with increased reflex zones or exhaustible clonus
   3=increased reflexes with increased reflex zones or non-exhaustible clonus

22. Myoclonus ☐
   Definition: short, jerky, clonic movements of solitary muscles ± small movements.
   Myoclonus in sleep should not be counted.
   0=no myoclonus
   1=patient reports solitary muscle jerks
   2=myoclonus occurring on many days and can also last longer (many minutes)
   3=obvious myoclonus which are almost constantly available

23. Tongue tremors ☐
   Assessed with stretched-out tongue

4.4.4 Flowchart of the study plan

Time points	Lab examination	Questionnaires (self-rated)	Questionnaires (examiner-rated)	Instrument
90 mins prior to infusion (-3)	i.v. canula inserted			CPT-M
60 mins prior to infusion (-2)	ACTH levels Blood specimen for genotyping	VAS for craving, anxiety and intoxication		
30 mins prior to infusion (-1)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	
Citalopram 0.4mg/kg or placebo infusion (0)		VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	CPT-M done at the end of infusion
30 mins after infusion (+1)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	
60 mins after infusion (+2)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	
90 mins after infusion (+3)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	
120 mins after infusion (+4)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	
150 mins after infusion (+5)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	
180 mins after infusion (+6)	ACTH levels	VAS for craving, anxiety and intoxication	Serotonin Syndrome Scale	CPT-M

5 Analyses

All variables were tested for normal distribution using Kolmogorov-Smirnov test. Besides descriptive statistics, group differences were tested according to scale characteristics of the dependent variables using χ^2-statistics for nominal variables, Kruskal-Wallis-χ^2 for not normally distributed continuous variables and ANOVA for normally distributed continuous variables. Post-hoc single comparisons were computed with corrected α-levels (Bonferroni). Depending on scale distributional characteristics, Student's t-tests for normally distributed variables and Mann-Whitney-U tests for not normally distributed variables were used. To control group statistics for potential confounders, only male patients and male control subjects were investigated. All of them must abstain from smoking, starting from 9pm on the day before examination till the examination was over the next day, in order to reduce any confounding influence from cigarette smoking. Statistical comparisons of the influence of HTTLPR-alleles between subgroups and time points for hormonal values, questionnaires and CPT-data were carried out using MANOVA repeated measurement statistics.

6 Ethical Aspects

6.1 Characterisation of patients

These were male alcohol-dependent patients who were admitted for detoxification and motivational support as inpatient treatment in the addiction ward in the Ludwig-Maximilians University Psychiatric Clinic, Munich. The average stay in the ward was 23 days (not purely detoxification but qualified detoxification with accompanying therapy). During treatment, craving for alcohol and subjective alcohol-like effects may develop. For most patients the alcohol craving after withdrawal is one of the problems they have to face daily. Learning to handle craving is the aim of therapy. The eventual necessary confrontation with craving or alcohol-like experiences, given the experimental context and available empirical knowledge, was not disadvantageous. Patients were given the possibility to work out these experiences during therapy, which have been shown to be therapeutically helpful. Furthermore, exposition with alcohol-relevant stimuli (so-called Cue Exposure) is recognised nowadays as behavioral therapy for relapse prevention.

A positive vote for this project was obtained (034/2000) on 5.5.00 from the ethic commission, Ludwig-Maximilians University.

6.2 Patient Information

Patients were informed according to "Guidelines for Patient Information of Hospital Inpatients over expected measures" in Helsinki Declaration with amendments in Tokyo 1975, Hong Kong 1989, and Somerset West 1996.

6.3 Adherence to German Drug Laws

Citalopram is registered as an oral antidepressant in Germany. In Belgium and in Switzerland, and recently in Germany, it is registered in intravenous form. In the planned challenge investigation, conditions set by the German Drug Laws (Arzneimittelgesetz, AMG) and principles of proper conduct of clinical studies with pharmaceuticals in "Good Clinical Practice" in its current version were adhered to (ICH-GCP, status 1996).

6.4 Security profile of the challenge substance

This test substance, Citalopram, is registered in Germany and other European countries for treatment of mainly anxiety and affective disorders. The choice of dose, 0.4mg/kg, was determined by considerations of safety and tolerability in acute intravenous infusion over 10-15 minutes. Intravenous citalopram was chosen over oral application to speed up the time to reach maximal plasma concentration and avoid the problem of protein binding (about 50-75% for oral citalopram). Citalopram is metabolised in the liver by cytochrome P450 and alcohol-dependent patients may well have some liver damage, thus prolonging the elimination of citalopram. Previous observations and reports showed a favorable security profile and no significant interaction with alcohol, a consideration to keep in mind in case the alcohol-dependent patients relapsed during inpatient treatment and drank again before the test period. In fact, the psychomotor impairment caused by alcohol may even be antagonized by citalopram, as already elucidated in the sections above. Therefore, significant risk to participants in this study was not expected. Still, possible side-effects were continuously documented with the Serotonin Syndrome Scale and vital signs; had it been necessary, the study would have been discontinued and medical interventions given.

Citalopram has a half-life of about 12-36 hours, so an interval of ≥72 hours between the two examination days was set to allow the study subjects to recover fully from citalopram application before placebo was applied. To minimize the risk of drug-drug interactions to subjects even more, subjects who take any other psychiatric medications or SSRIs are excluded from the study.

6.5 Ethical Aspects of the genetic investigation

The challenge study and questionnaires took about 2 days and the assessments could be distributed over 4 appointments on 4 examination days. If the plan was kept, the burden for patients was altogether minimal. Patients and controls were informed extensively about the test substance's possible side-effects. Application of the test substance or placebo and blood testing (total 9x9.5ml) presented no difficulties for the patients.

6.6 Patient's Consent

Prerequisite for the challenge investigation, questionnaires and blood testing to obtain genetic material and hormone levels was an informed, written consent from the patient. Patient Information was conducted orally and later in written form separately (see attached separate Information Sheet). Special emphasis was placed on exact information about the conservation and use of genetic information in the form of lymphocyte cultures. In the investigation, recommendations from the World Medical Association (Revised Helsinki Declaration by WMA in its 41 General Assembly in September 1989 in Hongkong) were adhered to (Dt. Ärzteblatt 88 (50), 1991).

6.7 Capability to consent

Blood test to obtain genetic material was conducted only in patients who were capable of consent. For patients with legally appointed guardians, the signature of their legal guardians was additionally obtained.

Separate information for the challenge investigation as well as for the investigation of genetic material was attached. Both information sheets were distributed to possible participants and control persons for an extensive, informative dialog for the purpose of obtaining consent. Thereby, the voluntary participation in the study as well as the possibility to withdraw from it at any time without having to give a reason, were especially emphasized on.

6.8 Information regarding the Privacy Act

Data storage: All data were stored strictly in anonymized form on storage discs and kept separately. Data could not be traced back to patients.

6.9 Information about cell cultures and conservation of genetic material

The rapid developments in addiction research and their respective growing knowledge regarding causes and background of addiction have reduced the screening of further unknown candidate genes. To justify scientific developments, long-term conservation of genetic information in the form of lymphocyte cultures was sensible. The lymphocyte cultures were kept in strictly anonymized form. Data on the molecular genetics and biochemical results were stored anonymized in the Neurochemistry Department. Clinical data was obtained and stored in the respective wards. Data in anonymized form was only brought out for the purpose of analysis.

7 Insurance

Insurance was obtained for all the studies conducted in the Psychiatric Clinic of the Ludwig-Maximilians University, Munich, from AXA-Colonia Versicherung, Insurance Policy-Nr. 80 23 20 18965.

8 Results

8.1 Sample Characteristics and Citalopram Dose

20 alcohol-dependent patients and 14 control subjects were screened and recruited from 2001 to 2003, of these 11 alcohol-dependent patients and 12 control subjects completed the study on two separate examination days. As depicted in Table 3, the alcohol-dependent patients and healthy controls were all males aged between 18-65 years. Since they were age-matched within a range of 5 years, there were no significant differences between them with regard their ages at the time of testing. As expected, citalopram application was well tolerated by both alcohol-dependent patients and healthy controls.

Table 3: Healthy controls and illness-related variables in alcohol-dependent patients

Mean ± SD	Alcohol-dependent Patients	Healthy Controls	t-Value; significance
Age at testing (years)	36.5 ± 7.7	32.5 ± 6.4	-1.35; 0.19
Age at onset (years)	27.6 ± 7.8	-	-
Mean alcohol intake (g/day)	326.4 ± 220.8	32.5 ± 41.4	-4.35; <0.001
No. of DSM-IV criteria fulfilled	5.7 ± 1.2	-	-
Duration of illness (years)	8.9 ± 3.4	-	-
Body weight (kg)	80.0 ± 11.2	85.6 ± 19.0	0.85; 0.41
Citalopram dose (mg)	31.96 ± 4.45	34.22 ± 7.65	0.85; 0.41

8.2 Genotyping

Genotyping revealed the distribution of L-allele to be slightly more than the S-allele in both groups but the difference was not significant. Homozygosity for the LL-genotype was also the highest in both groups, and the SS-genotype the lowest, but again genotype frequencies were not significantly different between alcohol-dependent patients and controls (χ^2 =0.42, df=2, p= 0.81). The data followed the Hardy-Weinberg equilibrium for both alcohol-dependent patients and controls (presented in Table 4).

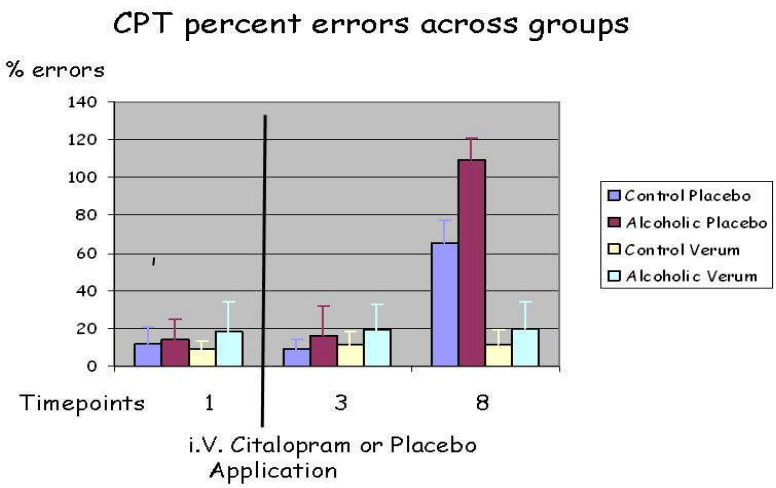
Table 4: Results of the genotyping of 5HTTLPR:

5-HTT Polymor-phism	Genotype frequencies			Allele Frequencies		Hardy-Weinberg Eq. Statistic. F value and p (Exact Test)	Alc. vs. Contr.
5-HTTLPR	**LL**	**LS**	**SS**	**L**	**S**		
Alcoholics	57.1%	28.6%	14.3%	61.4%	38.6%	0.30; 0.44	χ^2 =0.42; p= 0.81
Controls	44.4%	44.4%	11.1%	66.6%	33.4%	0.00; 1.00	

8.3 CPT Results

Regarding the first hypothesis, impulsive behaviour as assessed with the Continuous Performance Test-München (CPT-M) revealed that after citalopram applications, both alcohol-dependent patients made significantly less errors than healthy controls (p=0.002), at time point +6 (180 minutes after iv verum/placebo application). Comparing the verum vs. placebo applications, it was shown that the citalopram application significantly reduced the number of errors made by both groups but more so in the patients (p<0.001) than in healthy controls. There is also a significant statistical interaction between percentage of errors made by both groups over time (p<0.001).

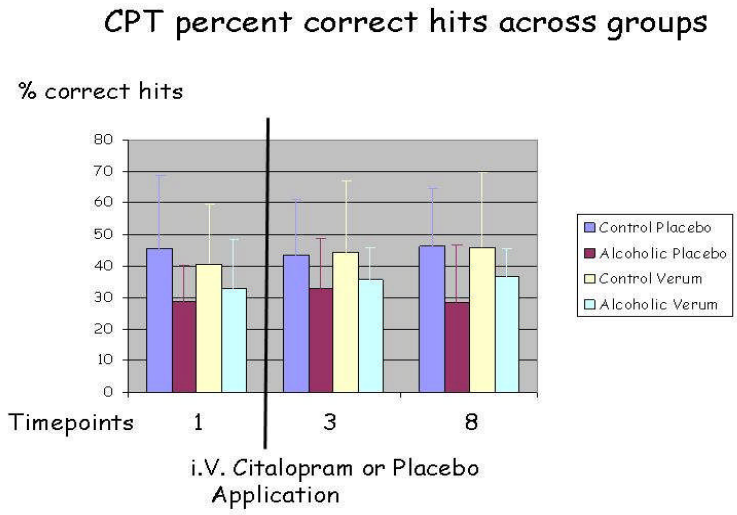
Fig. 1



Verum vs. Placebo: F-value 168,35; df 1; p < 0.001
Alc vs. Controls: F-value 12,89; df 1; p= 0,002
Time : F-value: 283,70; df 2; p < 0.001

In terms of correct hits, there was no significant difference between alcohol-dependent patients and controls under iv verum or placebo applications. There was also no significant statistical interaction between percentage of correct hits and status of patient or control over time.

Fig. 2

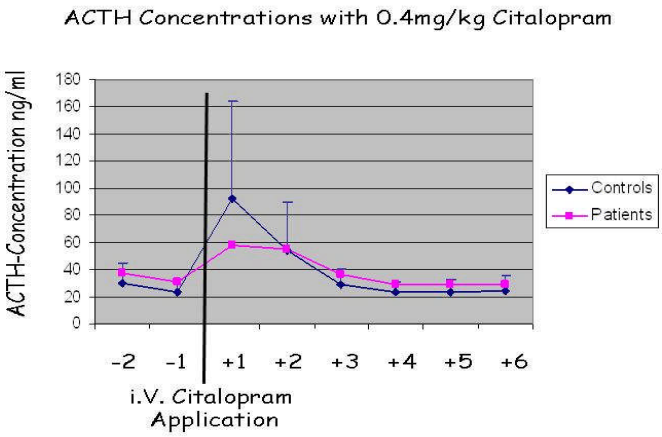


Verum vs. Placebo: F-value 0,38; df 1; p= 0.55
Alc vs. Controls: F-value 3,32; df 1; p= 0,08
Time : F-value: 1,84; df 2; p= 0.18

8.4 ACTH Results

As for the secondary hypotheses, serum ACTH levels before and after application of verum / placebo in patients vs. controls are shown in Fig. 3-6. No significant changes were observed between patients and controls regarding serum ACTH levels before and after application of citalopram 0.4mg/kg body weight. Furthermore, no significant statistical interaction was observed between patient or control status and ACTH changes over time.

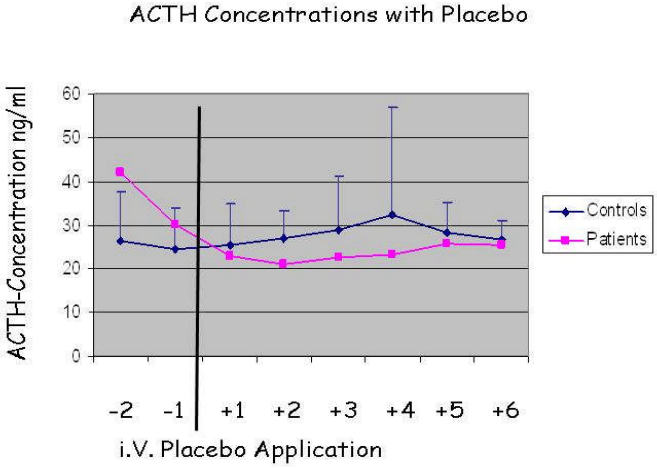
Fig. 3



Time: F-value: 4.20; df 7, p=0.12
Time x Status (patients vs. contols): F-value: 0.41; df 7; p= 0.33

Similar to the verum run, no significant differences were observed in serum ACTH levels between patients and controls after the placebo application. No significant statistical interaction was observed between patient or control status and ACTH changes over time.

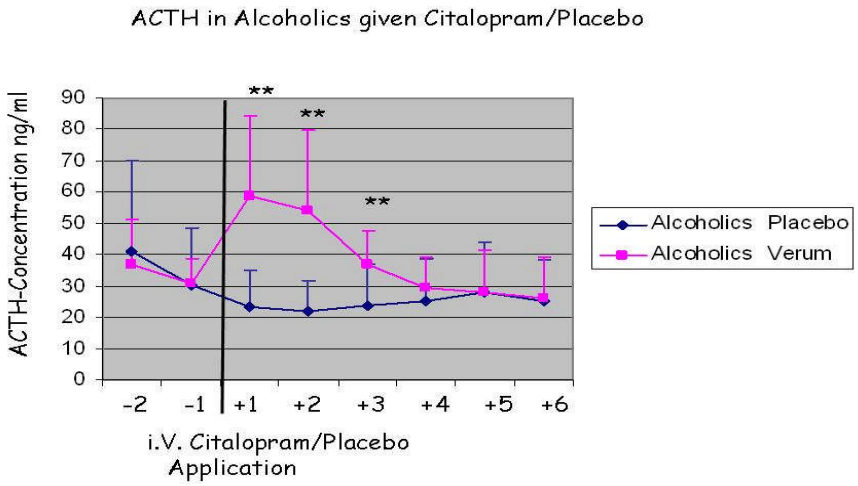
Fig. 4



Time: F-value: 1.12; df 7; p= 0.41
Time x Status (patients vs. contols): F-value: 1.35; df 7; p= 0.31

Fig. 5 shows the comparison between Citalopram vs. placebo runs in alcohol-dependent subjects only, and significant differences at time points +1 to +3 (30, 60 and 90 minutes after verum/placebo application) were detected.

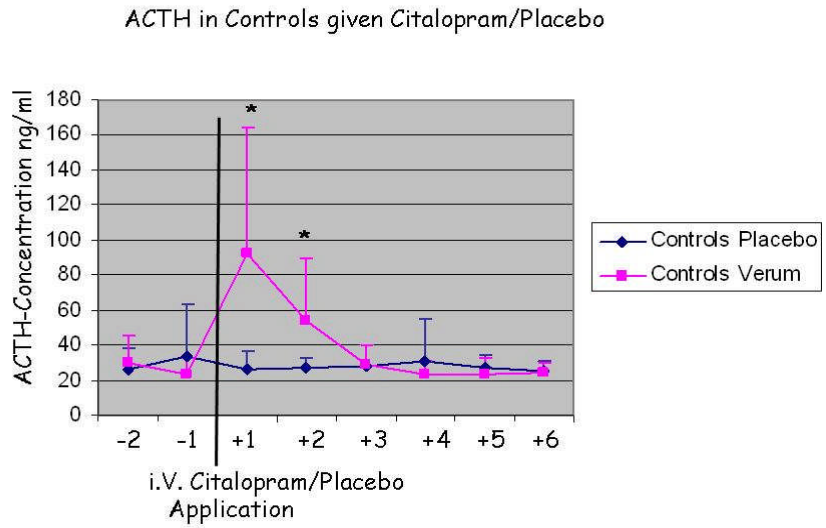
Fig. 5



*p < 0.05; ** p < 0.01; *** p < 0.001

Comparing the Citalopram vs. placebo runs in healthy controls only, significant differences at time points +1 to +2 (30 and 60 minutes after verum/placebo application) were also detected as shown in Fig. 6.

Fig. 6

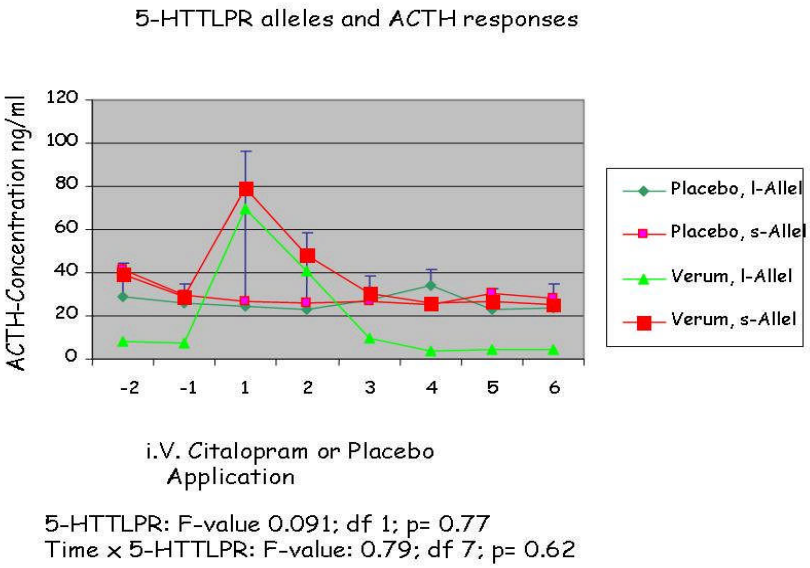


*p < 0.05; ** p < 0.01; *** p < 0.001

8.5 5-HTTLPR Results

Comparing ACTH response under both verum and placebo conditions, no significant statistical interactions with 5-HTTLPR alleles (s or l -allele) was detected for both alcohol-dependent individuals and controls. (Fig. 7).

Fig. 7

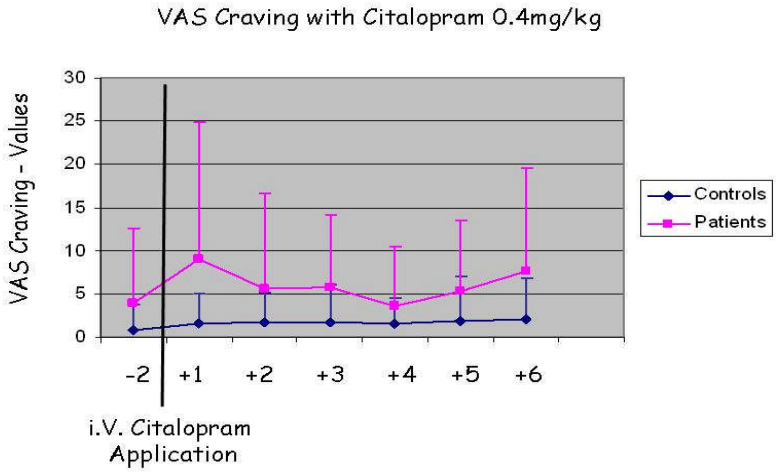


8.6 VAS Craving for Alcohol Results

Subjective experiences of craving for alcohol, anxiety and intoxication were measured with Visual Analogue Scale (VAS).

When craving for alcohol was measured in both groups, there was no significant difference between alcohol-dependent subjects and healthy controls after citalopram application. There was also no significant statistical interaction observed between patient or control status and VAS for craving over time.

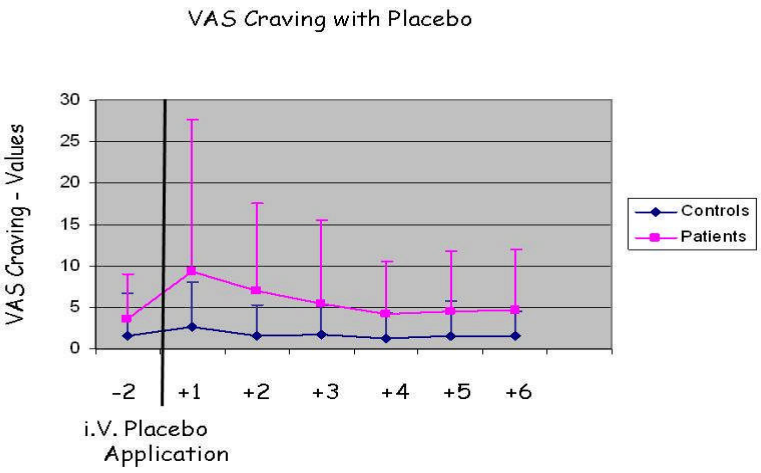
Fig. 8



Time: F-value: 1.22; df: 6; p= 0.35
Time x Status (patients vs. contols): F-value: 0.75; df 6; p= 0.66

Similar to the verum run, VAS for craving after placebo application did not differ significantly in both alcohol-dependent patients and healthy controls. Again, there was no significant statistical interaction between patient or control status and VAS for craving over time.

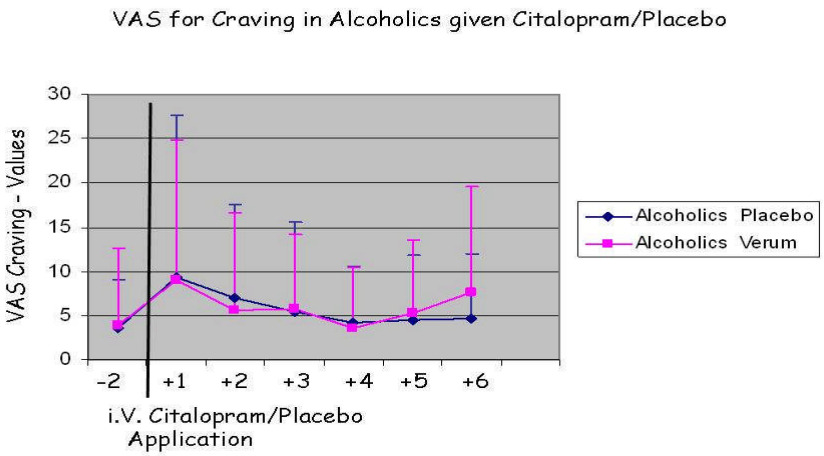
Fig. 9



Time: F-value: 1.10; df 6; p= 0.40
Time x Status (patients vs. contols): F-value:1.46; df 6; p= 0.25

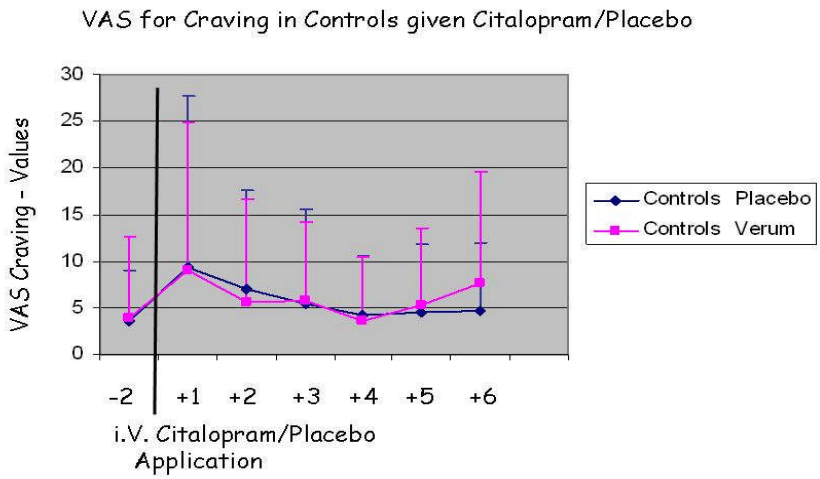
Comparing the verum vs. placebo runs in alcohol-dependent subjects only, there was no significant difference in VAS for craving as shown in Fig. 10.

Fig. 10



Similarly, comparing the verum vs. placebo runs in healthy controls only, there was no significant difference detected in VAS for craving.

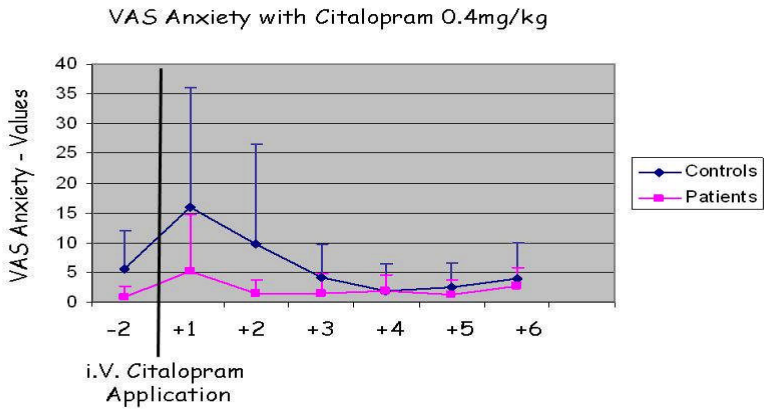
Fig. 11



8.7 VAS Anxiety Results

VAS for anxiety was not significantly different between alcohol-dependent patients and healthy controls after iv. citalopram application. Here too, there was no significant statistical interaction between patient or control status and VAS for anxiety over time.

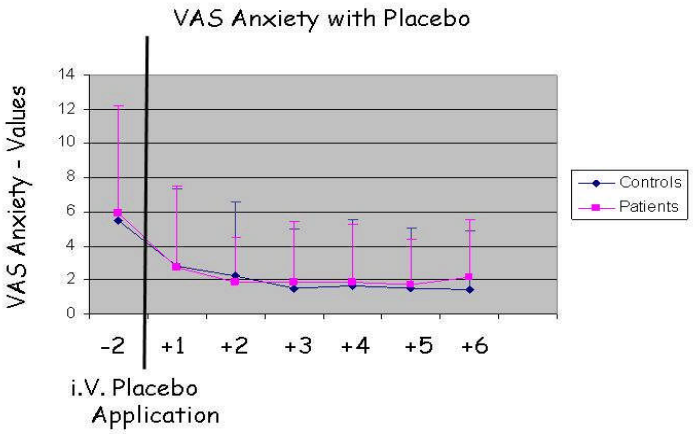
Fig. 12



Time: F-value 2.17; df 6; p= 0.10
Time x Status (patients vs. contols): F-value: 1.60; df 6; p= 0.21

Similarly, there was no significant difference in VAS for anxiety between patients and controls after placebo application, and no significant statistical interaction between patient or control status and VAS for anxiety over time.

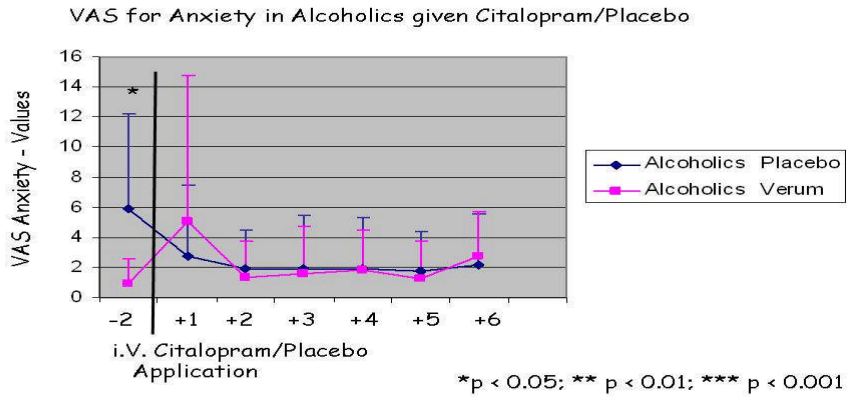
Fig. 13



Time: F-value: 2.53; df: 6; p= 0.07
Time x Status (patients vs. contols): F-value: 0.92; df 6; p= 0.50

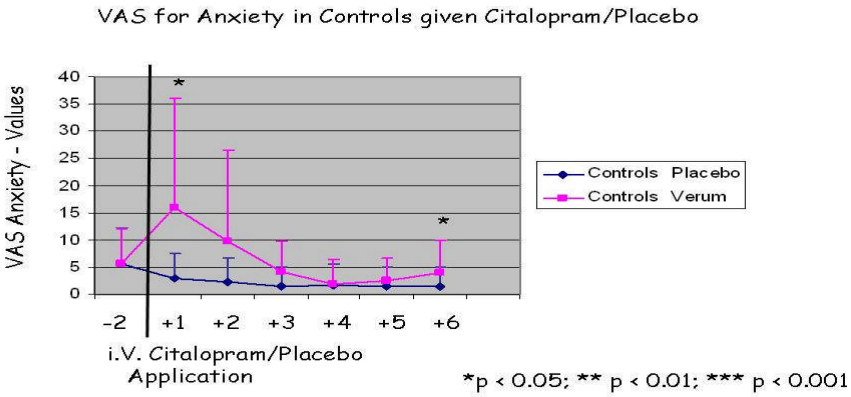
When comparing the verum vs. placebo runs in alcohol-dependent patients only, VAS for anxiety was significantly different at time point -2 (60 minutes prior to verum/placebo application).

Fig. 14



Comparing verum vs. placebo runs in healthy controls only, there were significant differences at time points +1 (30 minutes after application) and +6 (180 minutes after application) detected.

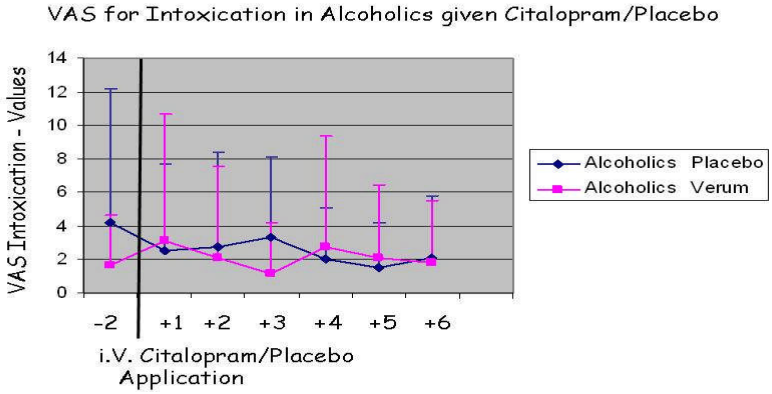
Fig. 15



8.8 VAS Subjective Feeling of Intoxication Results

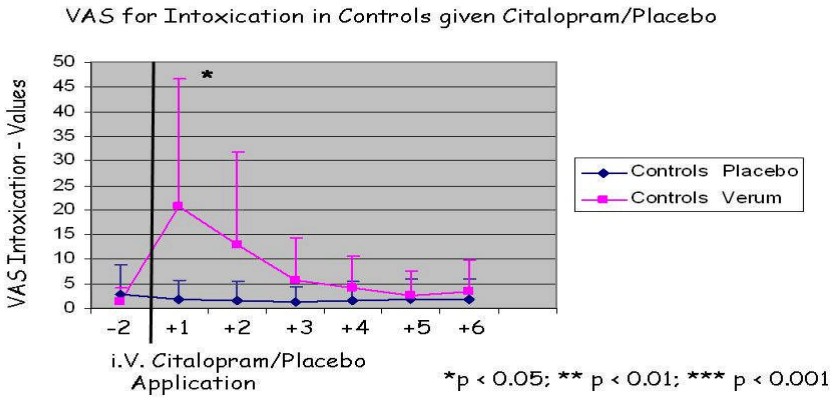
When subjects were asked how intoxicated they felt during the infusion with citalopram/placebo, alcoholics felt no difference during verum or placebo administration throughout the whole period of administration.

Fig. 16



By contrast, healthy controls felt more intoxicated during verum administration compared to placebo and this was significant at time point +1 (30 mins after verum administration) ($p<0.05$).

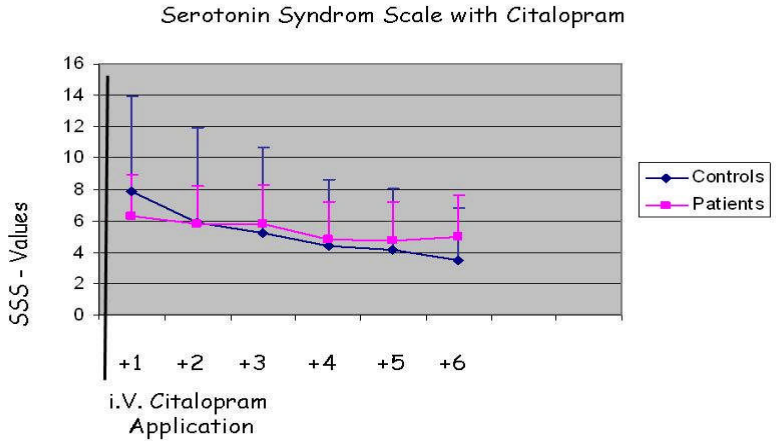
Fig. 17



8.9 Serotonin Syndrome Scale Results

Considering the side-effects as measured with Serotonin Syndrome Scale, there was no significant difference between alcohol-dependent patients and controls after iv. citalopram application. There was also no significant statistical interaction between patient or control status and side-effects over time.

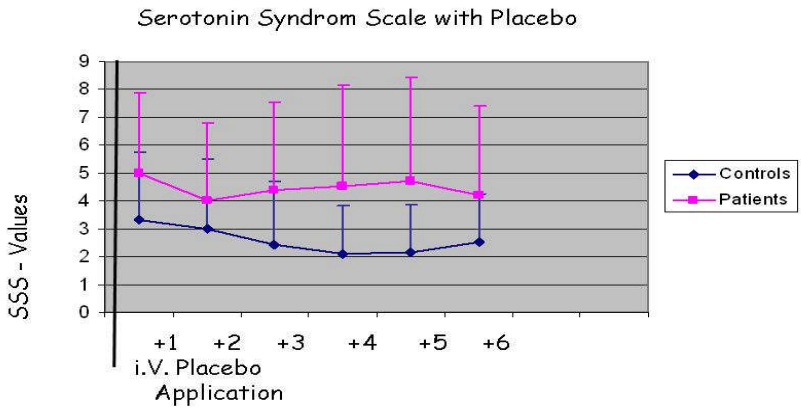
Fig. 18



Time: F-value 6.00; df 5; p= 0.002
Time x Status (patients vs. contols): F-value: 2.03; df 5; p= 0.126

Similarly, in the placebo run there was no significant difference between patients and controls after placebo application, and there was no significant statistical interaction between patient or control status and side-effects over time.

Fig. 19



Time: F-value 1.34; df 5; p= 0.29
Time x Status (patients vs. contols): F-value: 1.22; df 5; p= 0.34

9 Discussion

This study aimed to shed light on how a pharmacological challenge on the serotonin system affects behaviour and endocrine response in alcohol-dependent subjects compared to age- and sex-matched healthy controls. Furthermore, it was intended to investigate the influence of functional genetic variants of the serotonin transporter (5HTTLPR) on ACTH response in both groups since the serotonin transporter is the site of action of citalopram.

Regarding the **primary hypothesis** whether impulsiveness may be influenced by citalopram administration, using CPT it was found that both alcohol-dependent patients and healthy controls made significantly more errors after placebo application at time point +6 (180 minutes post placebo application) than after citalopram application. At time point +1 (30 mins post application) there was no difference between the groups. This abrupt change in the results may be a late effect of citalopram occurring 180 mins after application. Comparing the verum vs. placebo applications in each group, it was shown that the citalopram application significantly reduced the number of errors made by both groups but more so in alcohol-dependent patients than in healthy controls. There is also a significant statistical interaction between percentages of errors made by both groups over time. In between these two time points there were no CPT measurements possible due to the complexity of the test and time required. These results may be explained by the improvement in vigilance or concentration after citalopram application but not after placebo application in both groups, or the influence of citalopram on impulsivity. The fact that both control subjects and patients have similar correct hits after placebo and citalopram applications but both subject groups made more errors after placebo application showed that their attention was most likely not affected, and the results lend more support to the primary hypothesis that citalopram influenced impulsive behavior.

Impulsiveness has typically been measured with self-report scales like the Barratt Impulsivity Scale. Since these scales are designed to measure long-standing behavioural tendencies, they are not amenable to repeated assessment over short periods of time or across experimental manipulations (Dougherty, Marsh et al. 2002) so performance measures like the CPT was chosen for this study. Performance measures are sensitive to conditional manipulations and provide quantitative measure of the elemental behavioural tendencies that constitute this trait. The CPT, like the go/no-go task, was developed based on the *rapid-decision* paradigm, and performance on CPT requires behavior inhibition or suppression of response (Dougherty, Mathias et al. 2003a).

Despite the small sample size, this result is highly significant and was expected from previous studies. SSRIs like citalopram are known to enhance 5-HT neurotransmission and modulate 5-

HT concentrations in certain brain regions. CPT is one of the neuropsychological tests which requires *behavior inhibition* or *response suppression*, a component of impulsivity shown to activate widespread prefrontal and temporal cortical areas in patients more than healthy controls (Casey, Castellanos et al. 1997; Völlm, Richardson et al. 2004). The lateral orbitofrontal cortex (OFC), particularly on the right, is a common area activated by 'no-go' compared with 'go' conditions and appears to be specifically involved in the inhibition of responses (Liddle, Kiehl et al. 2001; Menon, Adleman et al. 2001; Aron, Fletcher et al. 2003; Rubia, Smith et al. 2003). The right middle frontal gyrus, including dorsolateral prefrontal cortex (DLPFC) also appears to be an important component of behavioural inhibition circuitry (Menon, Adleman et al. 2001; Garavan, Ross et al. 2002), whereas error detection and processing have been associated with the activation of more medial areas of the prefrontal cortex and the anterior cingulated cortex (Liddle, Kiehl et al. 2001; Menon, Adleman et al. 2001; Garavan, Ross et al. 2002; Rubia, Smith et al. 2003; Hester, Fassbender et al. 2004). In a study by Del-Ben et al. (2005) on 12 healthy volunteers, during behavior inhibition there were significant neuronal responses after small volume correction in the right DLPFC, OFC bilaterally and right middle frontal area. Additional responses, significant at $p<0.001$ uncorrected, were observed in right temporal cortex, bilateral supramarginal gyri, right precuneus, and right thalamus. Citalopram enhanced activation of DLPFC, right lateral OFC and middle temporal gyrus. Attenuations were in OFC, more on the left, bilateral supramarginal gyri and right superior temporal gyrus.

Thus, in line with Del-Ben et al. (2005), these findings are consistent with a role for 5-HT in the effective inhibition of unwanted behaviors and suggest a direct involvement in the circuitry involved in withholding responses. As suggested by these researchers, activation of right OFC supports a modulatory role for 5-HT in this region, probably 5-HT_{2C} receptor-mediated.

In terms of correct hits, there was no significant difference between alcohol-dependent patients and controls after i.v. citalopram or placebo applications although a statistical trend could be considered when comparing alcohol-dependent patients and controls. There was also no significant statistical interaction between percentage of correct hits and status of patient or control over time. Overall, these findings corroborate in part with recent findings (Bjork, Hoffman et al. 2004) also obtained from 130 detoxified alcohol-dependent patients using a variety of laboratory measures of impulsivity. The researchers found that alcohol-dependent patients, compared to control subjects, demonstrated 1) increased rates of commission errors, but not omission errors, in CPT; 2) a more severe devaluation of delayed reward; 3) increased rates of risky responses in a new risk-taking paradigm; 4) higher psychometric scores of impulsivity and aggression. In comparison with patients with late onset of problem drinking

and no problem-drinking parent, those alcohol-dependent patients with earlier age of problem drinking and had a problem-drinking father (type 2-like alcohol dependence) demonstrated faster response latencies and more responses to non-target stimuli (commission errors) in CPT, as well as higher psychometric aggression. They concluded that alcohol-dependent patients are more impulsive in several dimensions, with elevated impulsivity in a working memory task as well as aggressiveness characteristic of alcohol-dependent men with type 2-like features.

The significance of impulsivity in alcohol dependence should not be overlooked. Even if one argues whether impulsivity is the cause or the consequence of alcohol dependence, elevated impulsivity may lead to lowered ability to cope with early school problems and higher drop-out rates and shorter school careers. Longitudinal research in children has also revealed impulsive temperamental traits to be predictive of both early substance abuse and scholarly problems (Tarter, Kirisci et al. 2004). Most recent research data (Dom, Hulstijn et al. 2006) confirm this. Dom et al. studied early-onset alcoholic (EOA) and late-onset alcoholic (LOA) inpatients to compare the severity of their substance abuse and related problems. They found the symptom severity of EOAs' alcohol-use disorder and related problems to be higher than that of the LOAs. Furthermore, EOAs had higher levels of impulsivity, sensation seeking and aggression relative to the LOAs. The differences in impulsivity remained after an analysis controlling for the effect of aggressiveness. The researchers concluded that active screening for impulsive traits in treatment-seeking alcohol-abusing populations is recommended to improve treatment planning and prevent early drop-out. A recent review (Moeller, Barratt et al. 2001) supported the relationship between substance abuse and impulsivity with the following conclusions:

- Substance abusers are more impulsive on self-report and laboratory behavioral measures.
- Impulsive groups have higher incidence of substance abuse.
- Impulsivity is both a risk factor for the development of substance abuse and a resulting consequence of substance abuse.
- Impulsivity is a significant predictor of quitting drug treatment.
- Treatments for impulsivity improve outcome in substance abuse.

Pertaining to the **secondary hypothesis, part 1**, whether citalopram administration could raise ACTH levels in both alcohol-dependent patients and controls, no significant difference between the two groups could be found. Placebo administration also did not raise the ACTH levels significantly in both groups. In alcohol-dependent patients, it was found that ACTH response was markedly and significantly raised after citalopram administration at time points

+1 to +3 (30, 60 and 90 mins post infusion) ($p<0.01$) compared to placebo, whereas the same significant rise in ACTH in healthy controls ($p<0.05$) occurred only at time points +1 to +2 post infusion. When compared to healthy controls, the ACTH response in alcohol-dependent patients was blunted both during citalopram and placebo infusions but this was not significant. The study design (with i.v. citalopram as the pituitary stimulation/exogenous CRF analogue) and findings are similar to the one from Ehrenreich et al (1997).

They (1997) showed that intravenous human CRF (pituitary stimulation/exogenous CRF) compared to multifaceted stress test (hypothalamic activation/endogenous CRF) caused a fall in mean arterial pressure, most pronounced in abstinent alcoholics, with a sustained increase in ACTH, cortisol and norepinephrine after hCRF in both groups although the ACTH response in alcoholics were blunted. By contrast, stress testing elevated norepinephrine in both groups while increasing plasma ACTH and cortisol only in controls. Because the normalization of ACTH response to stress, but not to i.v. CRF, occurred after 12 weeks of abstinence they concluded that other ACTH secretagogues may be compensating for CRF dysfunction in alcoholics. Despite the dramatically lowered plasma NE/E ratio in alcoholics, the NE response to stimuli was unaffected. The exaggerated hypotensive reaction and blunted ACTH response to i.v. CRF may reveal a long-term dissociative dysregulation of CRF actions in alcoholics.

A possible ‘other’ ACTH secretagogue compensating for CRF dysfunction may be AVP (arginine-vasopressin-peptide). The study by Inder at al. (1995) showed that in twelve non-alcoholic volunteers given oral ethanol (1.1mg/kg of 95% ethanol or placebo), those who had gastrointestinal side effects from ethanol also had a rise in ACTH and cortisol ($p<0.0001$), and a synchronous rise in AVP ($p<0.02$). Those who had no GI side effects had no significant fall in AVP the first half hour following ethanol administration but a significant fall following placebo administration ($p<0.05$). Furthermore, plasma renin activity was increased by ethanol ($p<0.05$). They concluded that intoxicating levels of ethanol per se do not result in activation of the hypothalamic-pituitary-adrenal axis in humans. However, GI side effects induced by ethanol do result in such activation, which appears to be mediated by AVP as the dominant ACTH secretagogue.

Similar significant ACTH rise in alcoholics have been found after naltrexone infusion compared to placebo ($p<0.005$) where post-hoc analysis showed the significant difference to be between placebo and 25 mg dose ($p<0.01$) and 50 mg dose ($p<0.005$) but not the 100 mg dose ($p=0.1$) (Farren, O'Malley et al. 1999). Another study with fenfluramine also showed a significant increase of mean plasma ACTH (+85%) and cortisol (+129%) levels as well as urinary free cortisol secretion (+44%) with high dose (60 mg) fenfluramine (Schurmeyer, Brademann et al. 1996). The blunted ACTH response in alcoholics to subjective stressors like

social stressors or alcohol exposure is important in that it is predictive of a return to early drinking (Adinoff, Junghanns et al. 2005).

Blunted ACTH response also means impaired cortisol response to stress. In a study by Errico et al (2002), forty-eight male alcoholics who were abstinent were tested. Their verbal memory deficits were found to be more severe in alcoholics who had more withdrawals and ingested a higher typical quantity of alcohol during the prior year ($p<0.05$), and higher cortisol levels during withdrawal were associated with more errors on the Wisconsin Card Sorting Test. Alcoholics also had lower cortisol levels after stress and this post-stress cortisol levels were associated with poorer logical memory on the Wechsler Memory Scale and more errors on the Wisconsin Card Sorting Test ($p<0.05$). Errico et al. concluded that alcoholics have poorer cognitive performance which is related to more alcohol withdrawals, heavier alcohol consumption, and higher cortisol levels during a recent withdrawal.

As for **part 2** of the **secondary hypothesis**, 5HTTLPR short and long alleles were found to have no significant influence on the ACTH levels in both alcohol-dependent patients and healthy controls. Although there was a rise in ACTH levels after citalopram administration compared to placebo in both the short and the long allele carriers, this was not statistically significant.

Even though suicide attempts in alcohol-dependent subjects compared to healthy controls were not investigated in this study, the relationship between 5HTTLPR polymorphism to suicide attempts in alcohol-dependent patients has been shown (Gorwood, Batel et al. 2000; Russ, Lachman et al. 2000; Preuss, Koller et al. 2001). There is also evidence of decreased serotonin transporter binding in suicide victims (Arango, Underwood et al. 1995), considered a specific form of self-directed impulsive/aggressive behavior. There was no evidence found supporting any significant influence from 5HTTLPR polymorphism on ACTH levels in both subject groups under citalopram and placebo administrations. Thus, this functional polymorphism of the serotonin transporter may not be significantly involved in generating an endocrine response following a pharmacological challenge with serotonergic substances Though it is beyond the scope of this study, the relationship between 5HTTLPR polymorphism and cognitive performance with CPT, subjective craving, anxiety and intoxication could also be investigated further.

Part 3 of the **secondary hypothesis** dealt with subjective experiences under citalopram administration. Alcohol-dependent patients in this study were found to have higher level of craving for alcohol during both citalopram (time alone: $p=0.35$; time X status: $p=0.66$) and placebo (time alone: $p=0.40$; time X status: $p=0.25$) administrations compared to the healthy

controls, but the difference was not significant. This study also showed no significant reduction in craving after citalopram application in both alcohol-dependent patients and controls throughout the whole study period. This shows that alcohol-dependent patients basically crave alcohol more than healthy controls and this craving is not influenced by citalopram.

Surprisingly, the alcohol-dependent patients in this sample felt more anxious 30 mins after citalopram administration compared to control subjects, but this difference was not significant. This may be attributed to the side effect of SSRI which might acutely increase nervousness and irritation. However, both groups felt more anxious 60 mins prior to placebo administration but there was no significant difference between them ($p=0.50$). Alcohol-dependent patients were significantly more anxious prior to placebo administration (60 mins prior, $p<0.05$) compared to citalopram administration, during which they were more anxious 30 mins after administration, but this was not significant. The healthy controls were significantly more anxious 30 mins and 180 mins after citalopram administration (both $p<0.05$) compared to placebo. Thus, it appears that citalopram administration did not reduce anxiety in alcohol-dependent patients; instead it increased the anxiety in them. This finding can be explained by the laboratory setting or external surroundings causing anxiety, or citalopram/placebo application alone being an external stressor for the both groups of subjects.

Alcoholics did not feel intoxicated during citalopram or placebo administration whereas the healthy controls reported more intoxication during citalopram administration compared to placebo, and significantly intoxicated 30 mins after citalopram administration ($p<0.05$). This may be explained through activation of the P450 system in alcoholics as a consequence of chronic alcohol intake, which lets them metabolize various pharmacological substances much faster than controls and thus causing less intoxication. This indicated that alcohol-dependent subjects, even when they were abstinent, have low sensitivity to or have achieved a certain tolerance to the effects of intoxication such that they didn't feel any difference anymore, unless perhaps, when the effects were more with greater amounts of alcohol. This low sensitivity to the intoxicating effects of acute alcohol intake was also found in young men with a positive family history of alcoholism (Newlin and Thompson 1990; Pollock 1992; Schuckit and Smith 1996), and individuals with this low level of response to acute alcohol intoxication are at risk to consume excessive amounts of alcohol and to develop alcohol dependence (Schuckit and Smith 1996). Alcohol intake may be more rewarding if there are only a few negative effects of alcohol intake such as ataxia or sedation. Brain imaging studies in non-human primates indicate that a low alcohol response may be associated with serotonergic dysfunction (Heinz, Higley et al. 1998). Alcohol seems to exert some of its

sedative effects by binding in a 'pocket' within the transmembrane regions of the GABA-A-receptor (Mihic, Ye et al. 1997). In non-human primates, a low serotonin turnover rate was correlated with reduced effects of GABAergic inhibition on frontal glucose turnover (Doudet, Hommer et al. 1995) and this indicates that the acute GABAergic, sedative effects of alcohol may be reduced in subjects with a low central serotonin turnover rate.

There are several important limitations to this study. First and foremost is the small sample size of alcohol-dependent patients who actually completed the study. Despite the high turnover in the treatment wards, many patients have comorbid disorders and take other psychiatric medications which excluded them from participation in the study. The ones who were suitable were recruited but not all were compliant to treatment and stayed for the whole duration of treatment. Of the 20 alcohol-dependent patients screened and recruited into the study, some left the hospital without completing treatment and assessment, some were transferred to other units for clinical or medical reasons and some did not return for the second part of the assessment. This could be due to the non-compliance factor mentioned earlier, or the lack of reward or incentive for the patients and controls in the study (other than the altruistic motives to contribute to science), or the time taken to complete questionnaires repeatedly and the tedious testing needing subjects to fast overnight and sit relatively still for about five hours. Not even all the 14 control subjects screened and recruited for the study returned for the second part. An additional factor for the control subjects' non-compliance could also be the abstinence from smoking for almost 16-17 hours, which is obviously very difficult for smokers. Thus, only 11 alcohol-dependent patients and 12 healthy controls completed the study. This decreased the statistical power of the study.

Only ACTH levels were measured; cortisol, prolactin and adrenalin/noradrenalin levels were not assessed. This was a consideration to reduce the amount of blood taken from alcohol-dependent patients who are generally malnourished and unmotivated. Repeated blood taking in larger amounts may scare off patients and demotivate them from returning for the second examination day. Though patients and controls were gender- and age-matched, their education levels, IQ, social status and family history were not taken into consideration. All these could have contributed in part to the severity and course of their alcohol history and their prognosis. Furthermore, use of the CPT requires a small level of technical familiarity which most of our patients, being less well educated and unemployed, may not have and may be intimidated by. They were trained before the actual testing to familiarize them with the procedures, but as shown in the results, even control subjects had high anxiety levels prior to the examination, and even on days when placebo was applied. Lastly, the tests were conducted in a laboratory setting unfamiliar to most people, including control subjects, and presenting a 'surreal' condition which could also have contributed to their anxiety levels and errors on CPT.

10 Conclusion

SSRIs are emerging as effective treatment for depression and impulsivity, child conduct disorders and antisocial personality disorders in adults. Citalopram application in this study was shown to significantly reduce errors made by alcohol-dependent patients compared to control subjects and to placebo, thus inferring some positive influence on impulsive behavior inhibition in alcohol-dependent patients. Citalopram also significantly raised the ACTH levels in both alcohol-dependent subjects and control group, and this may be a good sign of a compensating mechanism of the serotonin system. However, no significant influence of functional 5HTTLPR alleles (S and L) was detected.

Thus it can be concluded that citalopram application has some significant influence on behavioural traits in alcohol–dependent patients and supports the notion that SSRIs can be used as an adjunct to psychotherapy to improve impulsive behaviour in alcoholics.

11 Abstrakt

Die Alkoholabhängigkeit ist eine häufig chronisch verlaufende und multifaktoriell verursachte Erkrankung. Aus einer Reihe von Untersuchungen ist bekannt, dass ein signifikanter genetischer Einfluss auf das Risiko einer Alkoholabhängigkeit besteht. Außerdem wurden in den vergangenen Jahrzehnten große Anstrengungen unternommen, biologische Marker und so genannte intermediäre Phänotypen (Endophänotypen) zu identifizieren, die mit dieser Erkrankung im Zusammenhang stehen. Ein wichtiger zentraler Neurotransmitter ist Serotonin (5-HT), der u.a. auch die Regulation von endokrinen Funktionen, wie etwa der limbisch-hypothalamisch-hypophysär-adrenergen Hormonachse (LHPA) beeinflusst. Umgekehrt besteht auch ein Einfluss der LHPA auf die zentralnervöse serotonerge Funktion. Bei Alkoholabhängigen wurde durch vorangegangene Studien über Veränderungen dieses Systems berichtet, die von erhöhten Stresshormonwerten bei Intoxikationen und im Entzug bis hin zu Störungen der Stresshormonantwort (Cortisol und ACTH) auf exogene und endogene Stressoren reicht.

Serotonin wird mit einer Reihe von psychischen Störungen, wie Abhängigkeitserkrankungen, Impulskontrollstörungen, Angststörungen und Depression, ursächlich in Verbindung gebracht. Insbesondere impulsive Verhaltensweisen beinhalten ein erhöhtes Risiko für das Entstehen von Abhängigkeitserkrankungen. So tragen Impulsivität als Verhaltensdisposition möglicherweise zu einem früheren Beginn, höherer Trinkmenge und vermehrter Rückfälligkeit bei.

Wichtiger Bestandteil des serotonergen Systems ist der Serotonintransporter 5-HTT, der, präsynaptisch lokalisiert, durch den Rücktransport von Serotonin aus dem synaptischen Spalt die Konzentration und Wirkdauer dieses Neurotranmitters erheblich beeinflusst. Dieser Transporter ist der Wirkort von Serotoninwiederaufnahmehemmern (SSRI), zu denen auch Citalopram zählt. Für das Gen des 5-HTT wurde in der Promoterregion eine funktionell relevante genetische Variante (5-HTTLPR) mit 2 Allelen (S und L) berichtet, der sowohl die exprimierte Anzahl als auch die Wiederaufnahmekapazität des Transporter signifikant beeinflusst (S < L).

Neuropharmakologische Untersuchungen, so genannte „Challenge - Studien“ mit serotoninagonistisch wirkenden Substanzen, wie etwa Fenfluramin oder p-Chloroamphetamin, berichteten über einen Zusammenhang zwischen der Funktion des serotonergen Systems, einer verminderten endokrinen Responsibilität mit vermehrt impulsivem Verhalten und Craving (Suchtdruck, Trinkdruck) bei Alkoholabhängigen im Vergleich zu gesunden Kontrollen. Der selektive Serotoninaufnahmehemmer Citalopram, der auch in einer intravenösen Applikationsform zur Verfügung steht, ist seit vielen Jahren als Medikament zur Behandlung von Depressionen und Angststörungen in Verwendung. Dieser

SSRI hat wahrscheinlich im Vergleich zu bisher verwendeten serotonergen Substanzen den Vorteil der spezifischeren Wirksamkeit am 5-HTT und der besseren Verträglichkeit.

Ziel dieser doppelblinden, randomisierten und kontrollierten pharmakologischen Challenge-Studie an Alkoholabhängigen und Kontrollpersonen mit Citalopram 0.4mg/kg Körpergewicht vs. Placebo ist es, die Wirkung dieses SSRI auf impulsives Verhalten und endokrine Responsibilität in Abhängigkeit vom Genotyp des 5-HTTLPR Polymorphismus zu messen. Dabei wurde als primäre Hypothese angenommen, dass Citalopram impulsives Verhalten, erfasst mit dem Continuous Performance Test (CPT), signifikant beeinflusst. Darüber hinaus wurde angenommen, dass die endokrine Responsibilität, erfasst über periphere ACTH Spiegel, durch den Genotyp des 5-HTTLPR Polymorphismus signifikant beeinflusst wird. Ebenfalls wird ein signifikanter Effekt des SSRI auf Craving, Befindlichkeit und Intoxikation vermutet. Die Messungen von Verhalten und ACTH Spiegel fanden jeweils zu 2 Zeitpunkten vor und bis zu 6 Zeitpunkten nach der Gabe von Citalopram (CIT) oder Placebo statt.

Eingeschlossen wurden 11 männliche Patienten mit der DSM-IV- und ICD-10- Diagnose einer Alkoholabhängigkeit (Durchschnittsalter 36,5 ± 7,7 Jahre), abgeschlossenem Entzug und ohne aktuelle psychopharmakologische Behandlung, psychiatrische oder somatische Komorbidität sowie 12 geschlechts- sowie altersparallelisierte gesunde Kontrollpersonen (Alter: 32,5 ± 6,4 Jahre). Die Patienten wiesen eine durchschnittliche Dauer der Alkoholabhängigkeit von 8.9 ± 3.4 Jahren auf und konsumierten durchschnittlich 326,4 ± 220,8 g/Tag Alkohol in der Woche vor der Entzugsbehandlung. Die durchschnittliche Dosis von Citalopram betrug bei den Patienten 31,96 ± 4,45 mg und den Kontrollen 34,22 ± 7,65mg.

Als erstes Ergebnis konnte festgestellt werden, dass Patienten und Kontrollpersonen eine nahezu gleich Anzahl an Fehlern im CPT machten. Allerdings war die Leistung bei beiden Gruppen unter CIT nach 180 Minuten signifikant gegenüber Placebo verbessert. Diese Veränderung war bei Alkoholabhängigen signifikant deutlicher. Somit konnte die erste Hypothese teilweise bestätigt werden.

Während sich die endokrine Responsibilität von ACTH unter Placebobedingungen bei Alkoholabhängigen niedriger als bei Kontrollen zeigte, war sie bei beiden Gruppen unter CIT signifikant größer als unter Placebo (bis 90 minuten nach Gabe, $p < 0.01$). Allerdings konnte kein signifikante Unterschied zwischen Alkoholkranken und Kontrollen für die ACTH Spiegel unter CIT gefunden werden. Genetische Varianten des 5-HTT wiesen keinen signifikanten Zusammenhang mit ACTH Spiegeln unter CIT oder Placebo auf. Die Gruppe der Alkoholabhängigen wies aber unter CIT im Vergleich zu Placebo nicht signifikant mehr Craving auf. Demgegenüber berichteten die Patienten und die Kontrollen unter CIT mehr über Angst (für beide Gruppen, $p< 0.05$) im Vergleich zu Placebo. Abschließend berichteten die

Kontrollpersonen unter Verum über mehr subjektive Zeichen einer alkoholartigen Intoxikation als die Alkoholkranken ($p < 0.05$).

Somit konnte in dieser placebokontrollierten und doppelblinden Studie die Hypothese bestätigt werden, dass der SSRI CIT einen eher günstigen Einfluss auf impulsives Verhalten hat. Dies ergibt möglicherweise Hinweise auf den sinnvollen therapeutischen Einsatz dieser Gruppe von Antidepressiva bei Alkoholabhängigen. Demgegenüber konnten kein Einfluß auf Craving bei der Patientengruppe gefunden werden. Ebenfalls konnte die Hypothese des Zusammenhanges von genetischen Varianten des Serotonintransporters, der auch Wirkort von CIT ist, mit der endokrinen Responsibilität (ACTH) nicht bestätigt werden. Limitation der Studie ist sicherlich die relativ kleine Fallzahl (11 Patienten und 12 Kontrollpersonen).

Abstract in English

Alcoholism is a multifactorial disease and often a chronic addiction. A series of research have shown that genetics exert a significant influence on the risk of alcohol dependence. Furthermore, in the last decades much effort has been undertaken to identify biologic and genetic markers and endophenotypes associated with alcoholism. Serotonin (5-HT) is a neurotransmitter involved in regulating the limbic-hypothalamic-pituitary-adrenal (LHPA) axis, and the LHPA axis in turn governs 5-HTergic activity. Alcoholism has been associated with long-lasting alterations in LHPA axis function, from an increase in cortisol secretion during intoxication and withdrawal, to disturbances in stress hormone responses (cortisol and ACTH) to exogenous and endogenous stressors.

Disturbances of the serotonergic neurotransmission have been associated with a number of psychiatric disorders, including alcoholism, disorders of impulse control, panic and depression. In particular, impulsive patients have a higher risk for substance abuse. Impulsivity may be a predisposing behaviour contributing to an earlier begin, higher consumption and increased relapses.

An important part of the serotonin system is the serotonin transporter, 5-HTT, which is located presynaptically and exerts a significant influence on the neurotransmitter's concentration and duration of action through synaptic reuptake. This reuptake is the site of action of the SSRIs (selective serotonin reuptake inhibitors), to which citolopram belongs.The 5-HTT gene in the promoter region has been reported to have functionally relevant genetic variants (5-HTTLPR) with 2 alleles (S and L) which significantly influence the transporter's reuptake capacity (S<L) und presynaptic number.

Neuropharmacologic investigations, so-called 'Challenge studies' with serotonergic substances like fenfluramin or p-chloroamphetamin, showed a relationship between the function of serotonergic systems and increased impulsivity with reduced endocrinologic responsivity, increased impulsive behavior and craving in alcohol-dependent patients compared to healthy controls. Recently, the Selective Serotonin Reuptake Inhibitor, Citalopram, is available for i.v. application. This medication is clinically administered for the treatment of anxiety and affective disorders, and due to its specificity on 5-HTT and better tolerability, has more advantage than previously administered substances.

In this double-blind, placebo-controlled and randomised pharmacological challenge study, it was the aim to investigate whether the administration of Citalopram 0.4mg/kg would influence the impulsive behavior (measured with Continous Performance Test, CPT) in male alcohol-dependent patients, and their endocrinologic responsivity (ACTH), depending on their genotype of serotonin transporters (5HTTLPR), compared to age-matched controls. A significant effect of the SSRI on subjective feelings of craving for alcohol, anxiety and

intoxication was also hypothesized. Measurements of behaviour and ACTH levels were made at 2 time points before and up to 6 time points after citalopram (CIT) or placebo application.

11 adult male patients meeting the DSM-IV and ICH-10 diagnosis of alcohol dependence aged 36.5 ± 7.7 years, detoxified, without comorbid psychiatric or medical diseases, or concurrent medications completed the study with 12 age-matched controls aged 32.5 ± 6.4 years ($p=0.19$). Patients had a mean duration of illness of 8.9 ± 3.4 years, consumed a mean of 326.4 ± 220.8 g alcohol/day in the week prior to detoxification. Citalopram dose administered to patients was 31.96 ± 4.45 mg, to controls was 34.22 ± 7.65 mg ($p = 0.408$).

The results showed that both alcohol-dependent patients and healthy controls made equal number of errors prior to any application, but both performed better 180 minutes after CIT application than after placebo. This difference was more significant in alcohol-dependent patients. Thus the first hypothesis could be confirmed.

ACTH levels were lower with placebo application in alcohol-dependent patients compared to controls, and significantly raised in both groups under CIT application (up to 90 minutes post-application, $p < 0.01$). However, there was no difference in ACTH levels between patients and controls under CIT application. Genetic variants of 5-HTT did not have any significant influence on ACTH levels in either group in either application. Alcohol-dependent patients did not show significantly higher levels of craving for alcohol during both runs compared to controls. Citalopram application did not reduce craving in alcohol-dependent patients. Instead, CIT run induced more anxiety in patients and controls (in both groups $p < 0.05$) compared to placebo. Lastly, healthy controls felt more intoxicated after citalopram application ($p < 0.05$) than alcohol-dependent patients.

Thus, it was confirmed in this placebo-controlled and double-blind study that citalopram application exerts a favourable influence on impulsivity. This may support the therapeutic use of SSRI in alcohol dependence. By contrast, no influence on craving could be detected in the patient group. The hypotheses of an associaton begween the genetic variants of serotonin transporters with the endocrine resposivity (ACTH) cannot be confirmed. Limitation to the study was the small sample size (11 patients and 12 controls).

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13 Lists of Tables and Figures

14 Acknowledgement / Danksagung

To my husband
You are the wind beneath my wings. Without you, I could not have flown so far.

An Prof. Dr. med Michael Soyka
Für Ihre Betreuung, Unterstutzung und Beratschlagung meiner Promotionsarbeit.

An Chefarzt Dr. med. Bernd Langer
Für Ihre Unterstutzung und die Chance eines Neuanfangs.

至：我的家人
谢谢你们这些年来的支持与爱心

Sehati sejiwa, sehidup semati.

15 Lebenslauf

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