Vulnerability to Substance Dependence

Joanne Marie Lusher

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Abstract

The purpose of the current studies was to examine associations with genetic variation, impulsive sensation seeking (ImpSS) and responsiveness to substance-related stimuli among four hundred individuals (100 alcoholics, 100 heroin abusers, 100 smokers and 100 controls), who each completed questionnaires measuring demographics; substance history; ImpSS levels; addiction severity and mood status. Participants provided a sample of their DNA, genotyped using PCR, and completed a modified Stroop task. Heroin users were found to be significantly higher impulsive sensation seekers than all other groups and all substance-using groups were found to be significantly higher sensation seekers than controls. The pleasure/reward seeking characteristics of high ImpSS are thought to be governed by the mesolimbic dopamine reward pathway, this system appears unregulated in substance abusers, encouraging them to seek out substances to satisfy their neurochemical urge for pleasure. The dopamine reward pathway is where D4 receptors are most densely populated and having the long variant at the DRD4 gene was found to predispose individuals to a high ImpSS personality type. Moreover, genotype alone predicted ImpSS behaviour amongst the heroin group, shedding light onto the controversy surrounding the influence of DRD4 on ImpSS behaviour and illustrates the importance of other factors (e.g. age, sex and mood status) on this association. ImpSS could mediate the genetic influence on addiction; the DRD4 gene variant predisposed individuals to heroin and nicotine dependence, but not alcohol dependence. Therefore, the dopaminergic polymorphisms contribute to individual differences in addiction and ImpSS behaviour. High impulsive sensation seekers were significantly faster to respond to stimuli on the emotional Stroop task and both heroin and smoking groups were distracted by stimuli that were associated with their drug of choice. Individuals with the long variant at the DRD4 gene spent longer responding to words related to heroin and cigarettes. Therefore, the DRD4 gene polymorphism represents a genetic mechanism that could be associated with substance-specific cue-associated responding to drug-related environmental stimuli, whilst demonstrating individual differences in susceptibility to sensitisation. Ultimately, the thesis demonstrates the importance of genetic variation in substance dependence; it advances our understanding of the personalities of substance abusers and increases our knowledge of neurobehavioural influences on addiction, thus offering a multidisciplinary approach to studying vulnerability factors to substance dependence.

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DEDICATION

For Richard, 1945-2005. Thanks for being my dad. You demonstrated courage, strength, bravery, persistence, incontrovertible endurance and an overwhelming grace right to the very end. You are an inspiration. You are sorely missed.

Contents

Chapter one

Vulnerability to Substance Dependence

A General Introduction

Epidemiological reports show that around one third of people under 30 years old experiment with at least one illicit substance (European Monitoring Centre for Drug and Drug Addiction EMCDDA Annual Report, 2002), not all of these (around 6%) continue their use to form a dependency on the substance. Addiction does not occur simply due to the reinforcing properties of the substance that make people want to continue taking it after initial use. Some people are more prone to become addicted to a substance than others are, due to several factors including, differences in genes, personality types and neurobehavioural adaptions. Therefore, this thesis argues that some people are more vulnerable to the effects of a substance than others and that genes, personality traits and brain reward circuitry influence ones vulnerability to substance dependence. These factors will be reviewed in light of the diathesis-stress model and the incentive sensitisation theory of addiction. However, to illustrate the magnitude of the problem, a description of addiction will be given first, with a brief epidemiological account of some social and health-related problems generated by substance use in the United Kingdom.

A description of addiction

Diagnosis is not a simple step in identifying addictions. The problem with diagnosing substance use disorders is that one needs to distinguish between use, abuse and dependence, however difficulty in maintaining consistency is apparent when the terms are frequently used interchangeably. Abuse and dependence warrant a diagnosis under the DSM-IV criteria (American Psychiatric Association (APA), 1994). Dependence can be seen as the state of needing a drug to operate within normal limits, whereas abuse is the use of a drug, which leads to problems for the user (Altman, Everitt, Glautier, Markou, Nutt, Oretti, Phillips and Robbins, 1996). Diagnosing addiction is fraught with problems. For example, dependence is difficult to diagnose because it is not an all-or-none condition but instead relates to many factors: from frequency and route of use, persistence of use despite damage and disruption to everyday life and withdrawal and tolerance. Therefore, substance users must be defined in terms of their degree of dependence as opposed to whether they are dependent or not. However, by

using the DSM-IV criteria it is possible for clinicians to have a common ground in which to work under so that communication about cases can be made more simple and structured. The DSM-IV criteria are comparable to the ICD-10 criteria developed in the UK (World Health Organisation (WHO), 1992) and these are detailed below:

DSM-IV Criteria for Substance Dependence (APA, 1994)

The DSM-IV criteria for substance dependence follow that a person must have a maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three or more of the following criteria, occurring at any time in the last 12-month period:

1) Tolerance, as defined by either of the following:
   (a) A need for markedly increased amounts of the substance to achieve intoxication or desired effect
   (b) Markedly diminished effect with the continued use of the same amount of the substance
2) Withdrawal, as manifested by either of the following:
   (a) The characteristic withdrawal syndrome for the substance
   (b) The same (or closely related substances) is taken to relieve or avoid symptoms
3) The substance is often taken in larger amounts or over a longer period of time than was intended
4) There is persistent desire or unsuccessful efforts to cut down or control substance use
5) A great deal of time is spent in activities necessary to obtain the substance, use the substance, or recover from it's effects
6) Important social, occupational or recreational activities are given up or reduced because of substance abuse
7) The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been exacerbated by the substance.

ICD-10 F1x.2 Dependence syndrome (WHO, 1992)

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 behaviour in terms of its onset, termination or levels of use, as evidenced by: the substance being taken in larger amounts or over longer period than intended; or by a persistent desire or unsuccessful efforts to reduce or control the 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 or 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 the substance;

(5) Preoccupation with substance use, as manifested by important alternative pleasures or interests 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.

An original description of the alcohol dependence syndrome (Edwards and Gross, 1976) emphasised that the observable phenomena of the syndrome were often clustered together, without implying any particular underlying cause. Although the original description referred specifically to alcohol dependence, this has since been expanded to include other drugs. Seven elements of the syndrome were identified, which are influenced by personality and cultural factors:

The Dependence Syndrome (Edwards & Gross, 1976)

1) *Increased tolerance to the drug:* refers to the observation that with repeated doses of the drug, less effect is produced. Or, increased quantities of the drug are required to produce the same effect.
2) *Repeated withdrawal symptoms:* the onset of withdrawal occurs following a period of abstinence from the drug. The timing, severity and onset are variable depending on the substance used.
3) *Subjective awareness of compulsion to take the drug:* is often associated with withdrawal and refers to the psychological state 'craving' for the drug.
4) *Salience of drug-seeking behaviour:* as dependence develops, obtaining the drug has increasing importance over the individual's life.
5) *Relief or avoidance of withdrawal symptoms:* where a person will continue using a drug to prevent the unpleasant effects of withdrawal. For example, a dependent drinker may leave a drink by the bedside at night so that withdrawal is avoided the following morning.
6) *Narrowing of the repertoire of drug taking:* the pattern of drinking or drug taking becomes more and more stereotypic as an increasingly strict daily routine develops.
7) *Reinstatement following a period of abstinence:* after a period of abstinence, the person will quickly escalate to a pre-abstinence level of consumption.

The concept of dependence includes specific psychological and physiological consequences of substance use, namely the elements of the dependence syndrome (withdrawal and tolerance), whereas substance abuse is a rather more psychological diagnosis. One or more of the following criteria, occurring at any time in the last 12-month period warrants a diagnosis of substance abuse:

DSM- IV Criteria for Substance Abuse (APA, 1994)

A) A maladaptive pattern of substance use leading to clinically significant impairment or distress, as manifested by one or more of the following, occurring within a 12-month period:
   (1) Recurrent substance use resulting in a failure to fulfil major role obligations at work, school or home
   (2) Recurrent substance use in situations in which it is physically hazardous

(3) Recurrent substance-related legal problems

(4) Continued substance use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of the substance

B) The symptoms have never met the criteria for substance dependence for this class of substance.

Epidemiology

Data concerning the situation of substance use, abuse and dependence in the United Kingdom has been selected from the national and regional research centres and official government statistics (General Household Survey, GHS, 2002; EMCDDA, 2002; Home Office, 2004). Both general public and problem substance-use will be reported here to illustrate the extent of use and the nature of its misuse.

Experimentation with illegal substances is still spreading among the UK population, particularly among those under 25 years of age. The homeless, those attending raves and dance clubs and those living in London are most likely to have used an illegal substance. Within the UK adult population, 34% have used an illegal substance at some point in their life. With 12% using within the last year and 8% in the last month (EMCDDA, 2002) and the number using a substance is still increasing in the UK. The under 30 year-old age group have the highest adult rates of substance use in the UK, with half having used an addictive substance in the past year. It is estimated that one quarter of the under thirties are using illicit drugs on a regular basis (within the last month). Male drug users outweigh female users two to one and there are three times as many men than women reporting to drug treatment services. The highest prevalence of substance use is found among the unemployed, with 40% reporting use within the last year.

Problematic substance users are more likely to be long-term unemployed and living with their parents or partners. It is estimated that nine out of ten young people living on the streets are using a substance. For those seeking treatment for substance abuse in the UK, 56% enter treatment for heroin abuse, followed by Methadone use at 11%. Heroin continued to be the drug most commonly associated with drug-related deaths. In London, 3.9% of male and 1.5% of female IV drug users are HIV positive and one

in five IV drug users are infected with Hepatitis C. The social and economic costs of illicit drug use in 2000 were estimated at around £19 billion.

The GHS (2002) statistics show that for legal drugs, around 12 million people aged 16 years and over in the UK are cigarette smokers and a further 1.3 million smoke pipes or cigars. Moreover, about 9.6 million adults in the UK are ex-smokers and an estimated 22% of secondary school children are regular smokers (GHS, 2002). In the UK, 364 thousand smokers are admitted to NHS hospitals each year due to smoking-related diseases and world wide, around five million people die prematurely each year as a result of smoking (WHO, 2003). Additionally in the UK, nearly six and a half million people drink at harmful levels (up to 35 units a week for women and 50 units for men) and a further 1.8 million people drink over this amount, with an estimated 2.9 million people in Britain dependent on alcohol. About 150,000 hospital admissions per year are associated with excess drinking and alcohol-related problems are estimated to cost the NHS up to £1.7 billion per year (Alcohol Concern, 2003).

Substance abuse and dependence are the most prevalent of the psychiatric disorders in the United Kingdom (EMCDDA, 2002). Despite the psychological, social and behavioural problems caused to the individual abusing a substance, drugs and alcohol have a widespread effect not only on the users themselves but also on others, e.g. victims of domestic violence, theft, motor vehicle accidents, and the disruption to everyday family life. Understanding the biological, psychological and social factors that underlie the addiction process is necessary for effective reduction in substance abuse and treatment for abusers.

Summary of literature from a diathesis-stress perspective

The diathesis-stress model is well established and has been applied to the aetiology of many psychiatric disorders including schizophrenia (Read, Perry, Moskowitz & Connolly, 2001) and depression (Conley, Haines, Hilt & Metalsky, 2001). The theory claims that a genetic predisposition towards a disorder creates an over sensitivity to stress. Genetic susceptibility is the diathesis and the stress is psychosocial events, such as responses to stress in the environment.

Diathesis

The concept of diathesis suggests a genetic vulnerability factor that makes some people more susceptible to particular degrees of environmental stresses than others (Meehl, 1962; Gottesman and Shields, 1967). Substance dependence can be provoked in a vulnerable person by relatively minor stresses or difficulties, whereas only catastrophic events may induce a similar reaction in a non-vulnerable person. In a non-vulnerable person, substance abuse is short lasting and does not last beyond the stress situation itself. For example an individual may drink alcohol when under pressure from peers for acceptance into a social circle or because of stress at work. However, when that person is not in that social situation, the individual does not feel the need to drink alcohol. Alternatively, a genetically vulnerable person, who may be more susceptible to particular degrees of stress than others, would turn to alcohol at the first sight of daily life stress and consequently form a dependency upon the substance.

Behaviour genetics and substance dependence

Classic gene studies aim to determine the role of genes, biology and environmental factors to explain variations in human behaviour (Plomin, Owen, & McGuffin, 1994). In animal research, selective breeding, cross breeding and inbreeding methods are used to determine genetic control over behaviour and have proved beneficial to our understanding of vulnerability to addiction. Behavioural genetics has profited from selective breeding methods, whereby individual rodents are mated, based on their physiological and behavioural traits, to produce lines of rodents that differ genetically. The most common trait selected for study is the tendency to drink ethanol solutions (Crabbe, 2003). During the experimental procedure, animals are given the choice over a period of weeks to drink either tap water or water containing ethanol. Findings show that genetically different rodents vary in their preference for ethanol (reviewed in Browman, Crabbe & Li, 2000). This is supported by research that has used inbred strains of mice and rats. Inbred rodents have genetically identical genes so can be compared in an environment where other factors can be controlled. One specific strain of mice (C57BL/6) have been found consistently to prefer alcohol, when compared to DBA/2 strain mice, who abstained from alcohol (e.g. McClearn & Rodgers, 1959 and Tarantino, McClearn, Rodriguez & Plomin, 1998). Ultimately, animal research has demonstrated that genetically different strains differ in their sensitivity to the effects

of various drugs, physiological dependence and metabolism of drugs (Crabbe & Harris, 1991), however, the specific genes responsible for influencing these individual differences in drug response cannot be recognised by this type of research.

One way in which specific genes related to addiction can be identified in animal research, is to use transgenic and knockout mice, whereby foreign DNA are inserted into the genome (transgenic) or the gene of interest is deleted (knockout), to form mutations. This ability to manipulate single genes provides a powerful tool in which to identify specific gene functions and the role of different variants of gene expression. For example, D4 knockout mice have been found to be more sensitive to the stimulant effects of alcohol (Rubenstein, Phillips, Bunzow, Falzone, Dziewczapolski & Zhange, 1997). Furthermore, deleting a specific sequence (exon 6) of the Dopamine D2 receptor gene signifies that the mouse under observation will only have the short form of the receptor (D2 receptors naturally occur in two alternative variants, long and short). This approach allows the function of the variant to be identified. It has been reported that the D2 short form contributes to the release of dopamine during high synaptic concentrations, such as that achieved during drug treatments (Rouge-Pont, Usiello, Benoit-Marand, Gonon, Piazza & Borrelli, 2002). Deregulation of dopamine D2 receptor functioning could play a role in mediating the vulnerability to substance dependence because firstly, animals who are vulnerable to self-administer drugs are characterised by enhanced release of dopamine in response to addictive drugs (Rouge-Pont, Piazza, Kharouby, Le Moal & Simon, 1993). Secondly, these animals show a lower number of D2 receptor binding sites and reduced receptor sensitivity (Marinelli & White, 2000).

Despite the obvious benefits that this animal research has in advancing our understanding of genetic vulnerability to addiction, these studies should be interpreted with caution because like with all research designs, there are limitations attached. These include growing constraints on the number of strains that can be accommodated in laboratories (Knight & Abbott, 2002). Crawley (2000), however, highlights that when animals are available for research there can be problems with interpretation. For example, with transgenic mice, other genes can be disrupted when foreign DNA is inserted at random locations on the gene. Additionally, the number of copies of the gene that have been inserted is unknown and this can influence the level of expression

of the protein encoded by the gene. The very procedure of manipulating genes may induce changes that could be the cause of the behaviour being measured. Moreover, consequences can occur during development because neurotransmitters are involved in determining neuronal connectivity during development as well as neuronal communication in adults. With knockout mice, if one gene is deleted then another gene, which serves a similar function, could take the role of the deleted gene, to compensate. The ultimate criticism, however, is that one cannot directly extrapolate findings from animal to human behaviour. Nevertheless, this animal research has offered insights that have contributed significantly to our understanding of addictive behaviour.

Turning to behaviour genetics research that has used human participants, in the past researchers relied upon experiments of nature to study genetic control over behaviour. These make up classic family, twin and adoption studies. Studies of this nature are beneficial because identical twins share all of their genes, whereas fraternal twins only have half of their genes in common, like normal siblings. As both twins share the same family environment and are the same age, these shared environmental factors can be controlled with differences between identical and fraternal twins being due to heredity. Adoption studies are more beneficial, especially those using twins reared apart (Reviewed in Pickens & Svikis, 1991; and more recently in Ball & Collier, 2002). They are useful in estimating the relative influences of heredity and shared environment. If substance dependence does run in families and shows the expected genetic relationship, depending on the degree of biological relatedness, there is some evidence of a possible genetic diathesis.

Genetic and environmental factors have been examined to a greater extent in alcoholic dependence than in drug dependence. This is for several reasons, including the fact that there is a higher rate of alcohol misuse (compared to drug misuse), due to its availability and accessibility and because large numbers of alcoholics seek treatment (Ball & Collier, 2002), thus the pool of participants is greater. The research has demonstrated that alcoholism tends to run in families. Early work (that included 39 family studies) reviewed by Cotton in 1979 reported that alcoholics are six times more likely than non-alcoholics to report parental alcoholism (Ball & Collier, 2002). In recent times, further studies have reported evidence for a genetic predisposition to

alcohol dependence. Schukitt (1994) found that one half of alcoholics seeking treatment have at least one alcoholic close relative. Another study showed that the risk of becoming alcohol dependent was increased by 167 percent if both a first and second-degree relative were affected (Dawson, Harford & Grant, 1992). Together, these studies provide convincing evidence for a genetic diathesis in alcohol addiction, in relation, family studies examining drug addiction are sparse, however, the evidence that is available suggests that drug dependence also runs in families. Kendler, Davis & Kessler (1997) confirmed early findings (Vaillant, 1966) that having a family history of drug addiction put individuals at risk of addiction themselves.

Adoption studies demonstrate a similar picture, a Danish study found that of their sample, 18% of alcoholics had an alcoholic biological father, compared to 5% of alcoholics whose biological father was not alcoholic (Goodwin, Schulsinger, Molley, Hermansen, Winokur, & Guze, 1974). The adoptees never knew their biological fathers so this large difference represents a purely genetic effect. This Danish adoption study was groundbreaking because it was the first to clearly reveal an increased risk of alcoholism in adopted away sons of alcoholic biological parents. A Swedish study, the Stockholm Adoption Study, supported these findings of increased rates of alcoholism in adopted away offspring of biological parents who were diagnosed as alcohol abusers (Cloninger, Bohman & Sigvardsson, 1981; Cloninger, Bohman, Sigvardsson & Van Knorring, 1985; Sigvardsson, Bohman and Cloninger, 1996). Finally, American studies have shown that if a biological first degree relative was alcoholic then 62% of their male and 33% of their female adopted away children became alcoholic. This was compared to 24% of male and 5% of female children whose biological first-degree relative was not alcoholic (Cadoret, Troughton, O'Gorman and Heywood, 1985; Cadoret, Troughton and O'Gorman, 1987).

Adoption studies that have looked at genetic influences in drug dependence demonstrate that drug addiction is more prominent in adoptees whose biological parents are substance dependent. Evidence for a significant genetic effect has been found in illicit drug dependence (Kendler, Bulik, Silberg, Hettema, Myers & Prescott, 2000) and in smoking and nicotine dependence (Heath & Madden, 1997; True, Xian, Scherrer, Madden, Bucholz & Heath, Eisen, Lyons, Goldberg & Tsuang, 1999). Although environmental factors, and also having a biological parent with antisocial

personality disorder, have been shown to increase this risk of drug dependence in adoptees (Cadoret, Troughton, O'Gorman and Heywood, 1986).

Twin studies offer additional evidence for a genetic risk in substance dependence by estimating heritability. The heritability estimate is the proportion of observed variance in scores that can be attributed to genetic factors. The variance not accounted for by genetic factors is explained by environmental (shared and non-shared) factors and measurement error. Heritability is a method of giving the relative importance of genes in accounting for variation in the behaviour being measured and this population statistic can vary depending on the behaviour being investigated, how it is being measured and other population characteristics such as the age and sex of the cohort.

Early onset of problem drinking before 25 years of age shows a very high heritability of 73 percent, whereas late onset alcoholism has a weak heritability rate of 30%. Shared and non-shared environmental factors are important in late onset and non-dependent alcohol abusers (Pickens, Svikis, McGue, Leykken, Heston, and Clayton, 1991). A twin study using a sample of drug abusers found heritability rates of 63% for identical twins and 44% for fraternal twins. These differences between the two groups of twins only reached significance in men (Pickens & Svikis, 1991).

The role of genetics in drug abuse is also more apparent in men than in women. Generally, drug abuse is influenced more by environmental than genetic factors. To illustrate this, a large twin study showed that drug dependence could be accounted for by 34% genetic influences, 28% shared environmental factors and 38% non-shared environmental factors (Tsuang, Lyons, Eisen, Goldberg, True, Lin, Meyer, Toomey, Faraone, and Eaven, 1996). This study reported part of a population-based twin study that examined genetic influences on drug abuse and dependence using the Vietnam Era Twin (VET) registry, which included 3, 372 twin pairs. Findings have further support from another large-scale research project, which incorporated 3, 132 twin pairs, born between 1940 and 1974, sampled from the Virginia Twin Register (VTR). These studies have shown heritability estimates for drug addiction at between 50% and 80% and a summary and review of these studies advocated strong evidence for a substantial, genetic contribution to the development of substance use, abuse and

dependence, with environmental factors having major importance (Ball & Collier, 2002).

Together, family, twin and adoption studies provide clear affirmation that alcohol dependence and drug dependence are genetically influenced. These studies also provide evidence that environmental factors play a key role in the initiation, development and maintenance of substance dependence because solely genes cannot account for all of the variance in addiction. However, they do not tell us which genes may contribute to this genetic vulnerability.

Molecular genetics and substance dependence

Recently, it has been possible for psychological research to use molecular genetics to determine associations with genes and behaviour. DNA can be used to identify specific genes that contribute to genetic variance in substance dependence. It is now possible to identify genes that influence individual differences in addictive behaviour.

Genes are grouped together on chromosomes, they are the smallest chemical unit of hereditary information located inside the body's billions of tiny cells and are made up of DNA (deoxyribonucleic acid). Each cell contains a complete set of genetic material, which is composed of many genes and each DNA molecule consists of two strands that are held together weakly by pairs of four bases (base pairs): adenine (A), thymine (T), guanine (G) and cytosine (C). Due to their particular properties (shape and structure), A always pairs with T and G with C, which coil around each other to form a helix of DNA, which are positioned in any order along a sugar and phosphate spine. Due to this specific pairing of bases, DNA is able to replicate itself and to synthesise proteins. DNA is packaged into chromosomes. Humans receive 23 chromosomes from each parent (22 autosomes and a sex chromosome). At conception, both parents contribute one complete set of chromosomes to the first whole cell (zygote). Therefore, each person receives two of each of the autosomes, which form a matched pair, along with a pair of sex chromosomes, which will be either XX (female) or XY (male). The DNA contains all the information (in the form of genes) that is required for a zygote to develop into an adult human.

The entire human genome (the sum total of DNA molecules within every cell) consists of about 70, 000 genes distributed within a total DNA sequence of about three billion base pairs of DNA. Of these DNA sequences, 99.9% are the same for all people. Identifying the 0.1% of the DNA sequence that differs is a goal for the human genome project because three million DNA sequences are responsible for the genetic differences among humans (Plomin & Crabbe, 2000; Craig & McClay, 2003). The goal here is not to find a single gene responsible for a particular behaviour, as multiple genetic and environmental factors probably influence addiction behaviour, but rather to detect a gene that may contribute (to varying effects) to the variance within a behaviour or trait. This is because DNA controls the production and regulation of proteins, genes that regulate the proteins involved in the function of the central nervous system must be important in the behavioural and cognitive functions that emerge from it. One strategy is allelic association, which examines the association between a particular allele and behaviour. Allelic association studies are used to detect small amounts of genetic contribution to complex behaviours. This method is appropriate for studying associations between alleles of a DNA marker among unrelated individuals and power can be increased by the size of the sample (Plomin & Caspi, 1998), thus enabling the study of gene-environment interaction using measured genotypes.

When examining genetic differences among humans, specific genes, or potential candidates for a particular disorder, can be investigated. In substance use disorders, several genes could theoretically influence ones susceptibility and an increasing number of genes are now being identified for their associations with addictive behaviours. For example, dopaminergic system genes such as dopamine D2 and D4 receptor genes (DRD2 & DRD4) and the dopamine transporter gene (DAT1) and genes involved in metabolism, including the aldehyde dehydrogenase gene (ALDH2), are among others that have been identified (see Kreek, Nielsen & LaForge, 2004, for an overview).

Dopaminergic system genes are of particular interest in the study of addiction because the system is *"centrally involved in the rewarding effects of drugs of abuse. Alcohol, cocaine, and heroin raise dopamine levels in certain reward areas of the brain, such as the nucleus accumbens but also the caudate putamen."* [Kreek, et al., 2004, pp. 90-

91]. More specifically, dopamine receptor genes are interesting candidates in addiction research because dopamine plays a major role in the mesolimbic system of the brain and this reward pathway is critical in drug reinforcement and pleasure-seeking behaviours (Reviewed in Robinson & Berridge, 2000). The mesolimbic system is the key primary reward system, which connects brain structures including the orbitofrontal cortex with the amygdala and the nucleus accumbens, although the main source of dopaminergic input into these areas is the ventral tegmental area (Grilly, 2002). The mesolimbic reward system was first identified in the 1950s, when it was discovered that rodents would engage in various behaviours when electric currents were passed through these particular areas of the brain (Olds & Milner, 1954). Wise, Bauco, Carlezon & Trojinar (1992) provided further evidence for this pioneering work by demonstrating how addictive drugs, such as opiates and ethanol can enhance brain stimulation reward in the mesolimbic system of rodents. Findings like these have been reviewed (Wise, 1998) and provide convincing evidence that the mesolimbic system is associated with appetitive behaviours, such as sex, eating and drug taking.

As dopamine is involved in pleasure and reward, differences in the expression of this process may be due to genes. The function of dopamine in the brain is mediated by receptors that transfer a signal across the cell membrane to alter neuronal function. Therefore, differences between the expression, structure and allelic composition of dopamine receptors can affect neurotransmitter functioning and potentially be implicated in disorders for which the system plays a role (Oak, Oldenhof & Van Tol, 2000). Identifying genes that code for dopamine receptors, reveals potential genetic differences in receptor proteins, linked to individual differences in behaviour and variations in neurotransmitter activity levels, allowing for varying degrees of diathesis.

One gene implicated in addictive behaviour is the ALDH2 gene (Kreek, et al., 2004). Absence of a protective mechanism, as well as the presence of genetic traits could make alcohol more or less attractive to some people. For example, those who become immediately ill after only one or two alcoholic drinks are unlikely to drink very often, or to become addicted because of the aversive conditioning that they have experienced, making the alcohol unattractive to that person. Alcohol causes a flushing

reaction in individuals who carry a particular genetic variant, by elevating blood aldehyde concentrations after ingestion of alcohol, causing alcohol avoidance due to the negative response it produces. The gene that causes this response is the alcohol metabolising liver enzyme aldehyde dehydrogenase (ALDH2) gene. The allelic variant (ALDH2*2) results in deficient ALDH activity, producing sickness when the effected individual takes a drink, thus acting as a protective factor against alcohol dependence (Wall, Peterson, Peterson, Johnson, Thomasson, Cole & Ehlers, 1997).

Another gene that may be associated with substance dependence is the DRD4 gene because of its expression primarily in the limbic system of the brain, an area responsible for pleasure and reward seeking. Genetic processes related to dopamine are shared across behaviours involved in attention, pleasure and reward. As dopamine is involved in central reward processes, differences in the expression of these processes may be due to genes. Many genes are probably involved in substance abuse, but association studies have attempted to find major genes that may be associated with the disorder (McGue, 1993). Association studies have been used to compare gene marker frequencies in unrelated individuals with a disorder (substance use) to those in control individuals, without a disorder. Adamson, Kennedy, Petronis Virkkunen, Linnoila & Goldman (1995) argue the necessity to examine the DRD4 gene in association with substance abuse due to its unique structure among neurotransmitter receptors. Although the DRD4 gene is similar to other dopamine receptor genes, in that they all belong to a family of G-coupled receptors, the DRD4 gene is highly polymorphic, which can produce altered receptor functioning (Oak, et al., 2000). The coding region (exon 3) causes allelic variants and the number of repeats alters the structure, length and efficiency of the receptor (Asghari, Sanyal, Buchwaldt, Paterson, Jovanovic & Van Tol, 1995).

The DRD4 gene is located on the short-arm of chromosome 11 and contains a number of polymorphisms (differences in DNA between individuals) in it's coding sequence (Asghari, et al., 1995). The most extensive polymorphism is located in the third coding region (exon 3) a region that encodes the putative third cytoplasmic loop of the receptor (Van Tol, Wu, Guan, O'Hara, Bunzow, Civelli, Kennedy, Seeman, Niznik & Jovanovic, 1992). This polymorphism has a variable number of tandem repeats (VNTR), in which a 48 base pair (bp) sequence exists. The DRD4 marker consists of

alleles involving 2-10 repeats and the number of repeats changes the length of the third cytoplasmic loop of the receptor. Variants located at this site can be grouped into long and short alleles on the gene and in the Caucasian population, about 70% of alleles are short and 30% long (Plomin & Caspi, 1998).

There is evidence that differences between the long and short forms of this polymorphism have a moderate functional significance, for example, the short alleles (2-5 repeats) code for the receptor that is more efficient in binding dopamine (Asghari, et al., 1995). Alleles are given out two at a time as genotypes. Inheriting one allele from the mother and one from the father forms the individual's genotype. Individuals can therefore have three genotype combinations if alleles are grouped into long (L) and short (S) forms. Individuals who are homozygous could have either a LL or SS genotype, whereas heterozygotes have the LS genotype. In the Caucasian population, the most common DRD4 allele is 4, and the most common genotype is 4-4. The long alleles (6-10 repeats) have been associated with a range of behavioural dimensions, which include both substance dependence and the human personality trait sensation seeking (SS) (see Lusher, Chandler & Ball, 2001, for a review). Hypothetically, individuals who have the long variant at the DRD4 gene are dopamine deficient so therefore seek sensations (e.g. drug seeking behaviour) to increase dopamine release in the brain. As the DRD4 gene has been associated with sensation seeking and addiction, it seems that a genetic process related to dopamine is shared across diverse behaviours that are involved in attention, pleasure and reward.

One example of how dopaminergic system genes can influence addictive behaviour is illustrated with the examination of the A1 form of the dopamine D2 receptor gene (DRD2), which has been linked to alcoholism in some studies, but not in others (Noble, 1996; Uhl, Perisco, and Smith, 1992). Individuals with the A1 allele have fewer D2 receptors. Noble suggested that those with fewer D2 receptors attempt to compensate for this deficiency by stimulating their dopamine release in the reward circuit of the mesolimbic dopamine system of the brain by using alcohol and other drugs (Noble, 1996).

DRD4 and Substance dependence

The DRD4 gene has been found to occur significantly more in substance abusers than in controls (Reviewed in Ebstein and Belmaker, 1997 and Lusher, et al., 2001). In one study the effect of the DRD4 allele was found only in alcoholics with the ALDH2 gene variant, suggesting a polygenetic effect of genes on alcoholism (Muramatsu, Higuchi, Muraymama & Matsushita, 1996). Since the DRD4 gene is also found to be associated with other types of substance abuse this could represent a broad vulnerability factor for addiction (Smith, O'Hara, Persico, Gorelick, Newlin, Vlahov, Solomon, Pickens & Uhl, 1992). A further association was found between the DRD4 gene and alcohol abuse (George, Cheng, Nguyen, Isreal & O'Dowd, 1993), however, these authors borrowed their control group from published genotype frequencies, rather than properly matching participants in their sample.

Studies that have found significant associations between the long alleles at DRD4 and substance abuse have used samples of opiate abusers. Li, Xu, Deng, Cai, Liu, Wang, Xiang, Zhao, Murray, Sham & Collier (1997) found a higher frequency of long alleles at DRD4 amongst a sample of 121, Chinese, heroin abusers, when compared to 154 matched controls. These findings were supported in two similar studies with Israeli opiate abusers (Kotler, Cohen, Segman, Gritsenko, Nemanov, Lerer, Kramer, Zer Zion, Kletz & Ebstein 1997; Mel, Kramer, Gritensko, Kotler & Ebstein 1997). These findings suggest that possession of particular variants at DRD4 receptor gene predispose individuals to opiate abuse, but not alcohol abuse.

A recent study which included severity of dependence in the association analysis found that having long alleles at DRD4 did not increase an individual's susceptibility to heroin dependence per se, but having this genotype partially determined severity of dependence (Lusher, Ebersole & Ball, 2000). This finding has not previously been examined, but it is possible that severity of dependence is a variable pertinent to this association. Comings, Gonzalez, Wu, Gade, Muhleman, Saucier, Johnson, Verde, Rosenthal, Lesieur, Rugle, Miller & MacMurray (1999) provide support for this view because they found higher drug use severity scores reported by individuals with the DRD4 long variant and the lowest drug use severity score was observed for the SS genotype. Future work would therefore benefit by examining associations with the DRD4 gene across different substances of abuse, clearly defining severity of

dependence in an attempt to comprehend the way in which the DRD4 gene predisposes individuals to addictive behaviour.

To summarise, data from both behavioural and molecular genetics research suggests that substance dependence is in part heritable. However, it is possible that there are no specific genes that predispose people to substance dependence, but only genes that predispose personality traits, such as impulsive and sensation seeking types, which intercede drug taking. Behaviour genetics research has been less successful in addressing factors that may act as mediators between genes and behaviour, but could ultimately answer the question about how these genetic effects arise (Heath, Madden, Bucholz, Nelson, Todorov, Price, Whitfield & Martin, 2003). A prime mediator could be a personality factor such as Sensation Seeking (SS), because this trait, as with substance dependence, is linked to brain reward mechanisms where dopamine D4 receptors are most dense.

Personality

Personality is the organisation of traits that characterise the individual. Traits are dispositions for individuals to act in relatively consistent ways in certain kinds of situations. Traits are rather stable overtime, however much of personality emerges in person-situation interactions. The diathesis-stress model is the embodiment of person-situation interactions. Personality develops from interactions of genetics and experiences during the early years of life. It stabilises and becomes quite reliable by early adulthood (Zuckerman, 1999). Personality functions as a moderator of response to stress and may explain why two people, who are exposed to the same amount of stress, may behave in two totally different ways. Personality cannot be explained as the diathesis, as it is a function itself, and is made up of its own genotypes and life experiences. However, personality could act as a mediator for substance abuse, for example a person may have a genetic predisposition to addiction, which is mediated by their sensation seeking personality type.

High sensation seekers have a general need for thrill and excitement, they like taking risks and become bored easily (Zuckerman, 1979). Sensation seeking (SS) is measured using four sub-scales: thrill and adventure seeking; experience seeking;

disinhibition and boredom susceptibility. However, more recently, a new form was developed based on factor analyses of several scales and items within those scales (Zuckerman, 1993) and a major factor that emerged from this analyses combined impulsivity and sensation seeking. For this reason, Zuckerman and colleagues developed a new scale to measure Impulsive Sensation Seeking (ImpSS) (Zuckerman, Kuhlman, Joireman, Teta & Kraft, 1993). Impulsive sensation seekers have a drive to seek out stimuli that are novel or exciting in attempts to maintain an optimal level of cortical arousal and a tendency to act impulsively without thinking or planning ahead (Zuckerman, et al., 1993). ImpSS correlates significantly with Eysenck's psychoticism factor and the openness to experience factor of the five-factor model (Zuckerman, et al., 1993). ImpSS is also considered as a close equivalent to Cloninger's Novelty Seeking (NS) (Zuckerman & Cloninger, 1996) and are both related to the same behaviours, which are characterised by the need for complex and novel sensations, which are obtained by taking physical and social risks, such as drug taking behaviour. For instance, opiate abusers have been found to demonstrate high levels of boredom, therefore, may require novel sensations to relieve excess amounts of boredom (O'Connor, Berry, Morrison & Brown, 1995). Moreover, similar biological and genetic bases have been postulated for both ImpSS and NS (Zuckerman, & Cloninger, 1996).

Behaviour genetics and personality

Animal studies have demonstrated that a genetic basis for NS/SS exists. For example, early work revealed that the C57BL strain of mice (compared to the BALB strain) are most active in the open-field test, in that they demonstrate exploratory behaviour and also show reactivity to novelty in other types of situations (McClearn & Rodgers, 1959). More recently, Bardo, Donohew & Harrington (1996), demonstrated that the tendency for rats to enter a novel section of a maze, rather than remaining in a familiar section, was related to the rats willingness to consume addictive substances. These findings from selective breeding studies convey a genetic basis for personality.

Evidence from human behaviour genetics has also established that a strong genetic contribution to personality exists. Family, twin and adoption studies have demonstrated a genetic basis for all major personality factors (Bouchard, Lykken, McGue, Segal & Tellegan, 1990; Markon, Krueger, Bouchard & Gottesman, 2002).

An estimate of the overall heritability of personality is about 40-50%, although heritabilities vary depending on the different personality traits being measured (Riemann, Angleitner & Strelau, 1997). Since the early work of Shields (1962), the largest and the most prominent study of twins reared apart (TRA) has been the Minnesota study of twins reared apart (MISTRA), pioneered by Bouchard and colleagues (1990). Findings from the MISTRA studies have been published in numerous books and articles and have been summarised most recently by Bouchard & Loehlin (2001), showing a strong genetic diathesis for personality. Extensive research on the genetics of personality has been conducted using Eysenck's super factors (extraversion, neuroticism and psychoticism) and taken together, results have consistently shown that these factors are indeed heritable (reviewed in Zuckerman, 1991). Using the big five factors (neuroticism, extraversion, openness to experience, agreeableness and conscientiousness), extraversion and openness to experience have been found to be the most heritable and these factors, as with Eysenck's psychoticism factor, correlate strongly with sensation seeking (Zuckerman, 1994).

The first, human behaviour genetics study to demonstrate a genetic basis for sensation seeking was conducted in London and used information from 422 pairs of twins. Findings revealed that 58% of the variance in SS could be attributed to genes (Fulkner, Eysenck & Zuckerman, 1980). This finding was supported by a further, more recent, examination of the genetic and environmental variance in sensation seeking (Hur & Bouchard, 1997), in which 106 twins reared apart were compared on all four sub-scales of the SS scale. Results showed the thrill and adventure seeking and experience seeking sub-scales to be the most heritable (54% and 57% respectively). Despite convincing evidence for a genetic influence in personality, behaviour genetics research is unable to recognise the extent to which specific genes contribute to variation in personality, however this point is less notable since the advent of molecular genetics research.

Molecular genetics and personality

Tentatively, it can be argued that various specific genes influence different personality characteristics and that chiefly, dopamine receptor genes (DRD1-5) are related to impulsive and compulsive personality types. This can be illustrated by evidence that has shown associations with the DRD2 gene and pathological gambling, substance

abuse and attention deficit hyperactivity disorder (ADHD) (Comings, et al., 1999). In view of the findings, logical speculations can be made that dopaminergic genes will be associated with impulsive, aggressive and sensation seeking personality traits because these traits are characteristic of such disorders. This speculation has been supported by research that has examined narrower traits, such as impulsivity, NS and SS, as well as lending support for associations with broader personality factors, such as psychoticism.

Comings and his colleagues have conducted a large number of ongoing studies, to examine the role of a number of genes in substance dependence and other psychiatric disorders. These studies also looked at genes in relation to several personality traits and were based on a population of 204, non-Hispanic Caucasian university students and participants from an addiction treatment unit in the United States. Conclusions that have been drawn from these studies are that each gene accounts for only a small percentage of the variance in personality and that different traits have several genes in common. To illustrate this, the dopaminergic genes (DRD1-5 and DAT1) made significant contributions to both neuroticism and extraversion factors of the big five. However, the DRD1, DRD2, DRD4 and DAT1 (dopamine transporter gene) genes also contributed to 5.25% of the variance of the NS factor. These dopamine genes seem to play a greater role in NS, than in Cloninger's other traits (reward dependence and harm avoidance) (Comings, et al., 1999; Comings, Gade-Andavolu, Gonzalez, Wu, Muhleman, Blake, Mann, Dietz, Saucier & MacMurray, 2001 and Burt, McGue, Iacono, Comings, & MacMurray, 2002).

DRD4 and Sensation Seeking

The dopamine receptor gene that has gained the most attention for its influence on the trait novelty seeking is the DRD4 gene. Like with substance abuse, variants at the DRD4 gene have been associated with novelty seeking. As NS scores correlate very highly with ImpSS scores ($r = 0.68$, Zuckerman, et al., 1993), it is likely that a similar association with DRD4 and sensation seeking would exist. Associations have been examined amongst healthy individuals who do not abuse drugs or alcohol. Roughly half of the studies have found a significant association with high SS/NS levels and long alleles at DRD4. However, results have been controversial due to

methodological differences making findings difficult to compare (Ebstein & Belmaker, 1997 and Lusher, et al., 2001).

The first studies to examine associations with NS and DRD4 gene variants, in the early to mid 1990s, did not yield any significant results (George, et al., 1993 and Adamson, et al., 1995). However, soon after there were two reports of associations between significantly high NS scores and long DRD4 alleles amongst healthy populations in both Israel and the United States (Ebstein, Novick, Umansky, Priel, Osher, Blaine, Bennett, Nemanov, Katz, & Belmaker 1996 and Benjamin, Li, Patterson, Greenberg, Murphy, & Hamer, 1996). More reports followed and by the turn of the century, the number of studies to examine the long variant at the DRD4 gene in association with the personality trait novelty seeking, almost reached thirty (reviewed in Prolo & Licinio, 2002), and this figure is continuously growing.

However, the controversy that surrounds these studies has produced heightened debate in the literature because of the many design variations between studies and the mixed results they have produced. For instance, significant results have accumulated among studies that have recruited younger participants (e.g. Ono, Manki, Yoshimura, Muramatsu, Misushimi, Higuchi, Yagi, & Kanba-Asai, 1997; Noble, Ozkaragoz, Ritchie, Zhang, Belin & Sparkes, 1998; Strobel, Wehr, Michel & Brocke, 1999 and Okuyama, Ishiguro, Nankai, Shibuya, Watanabe & Arinami, 2000). This is likely to be a result of the fact that NS diminishes with age. Alternatively, disparity between findings could be a result of the various ethnic groups that have been used in the studies. Amongst others, studies have included, Caucasian (Noble, et al., 1998); Japanese (Okuyama, et al., 2000); Israeli (Benjamin, Osher, Kotler, Gritsenko, Nemanov, Belmaker & Ebstein, 2000) and German (Strobel, et al., 1999) populations. However, genetic variants vary across different ethnic groups, so the association between the long alleles at the DRD4 gene and elevated NS scores may be genuine in some, but not all populations considered. Additionally, the gender of participants can influence the results obtained from these studies. For example, Ono, et al., (1997) only included women in their study and yielded a significant finding, whereas Malhotra, Virkkunen, Rooney, Eggert, Linnoila & Goldman (1996) restricted their

sample to men and failed to obtain any significant association between the long alleles at DRD4 and NS. Several other methodological factors can complicate the interpretation of association studies, including the sample size and the NS measure employed in the study, factors that have been devoted to chapter four.

As associations between DRD4 variants and novelty seeking and DRD4 variants and substance abuse, have been reported, there may be a biological link between NS or ImpSS and drug abuse. This link could be due to individual differences in the sensitivity of the mesolimbic dopamine reward system because sensation seekers are characterised by their investigatory behaviours, which activate the dopamine reward pathway, thereby producing reinforcement of the behaviour (Bardo, et al., 1996). More specifically, drugs have the ability to reduce control that receptors have over dopaminergic neurons in the ventral tegmental area. This in turn will increase the release of dopamine in the nucleus accumbens, a structure within the dopamine reward pathway that controls feelings of pleasure, thus increasing the feeling of pleasure (Grilly, 2002). As possession of long alleles at DRD4 has been associated with behavioural dimensions, including sensation seeking, it would appear that individuals with the long genotype have fewer dopamine receptors, so therefore seek novel sensations (drug-taking behaviour) to increase dopamine in the nucleus accumbens (Plomin, 1998). However, to date, there has been a lack of research that has looked at DRD4 in association with both substance abuse and sensation seeking together, especially in the UK. However, early assessment of sensation seeking scores amongst individuals genetically susceptible to drug taking behaviour could provide a tool for directing therapeutic strategies (O'Connor et al., 1995).

Stress

Returning to the diathesis-stress model, the stress element here refers to environmental factors in addiction. Stress can be social, psychological or physical pressures that can influence the substance-using individual and is a term usually used to describe a situation affecting the person, their internal reactions to the situation in terms of physiological arousal and subjective emotional responses and their behavioural reactions. It is questionable as to whether stress alone can produce a substance dependence in the absence of a diathesis. However only a minority of

people exposed to catastrophic events, such as war or rape, will develop a substance dependency (Zuckerman, 1999). The fact that stress alone is usually not sufficient to explain substance dependence argues the necessity to include other factors such as personality into the equation. However, alcoholics tend to drink to reduce stress, but they also drink when they are not stressed. The question is whether trauma and stress provoke severe drinking, although it is difficult to determine the cause-effect relationship because much of the stress can be produced by consequences of the individual's alcohol use. Alcoholism is co-morbid with some anxiety and depressive disorders; such as Anti-Social Personality Disorder (ASPD), however it could be argued that ASPD could also be a result of alcoholism. Moreover, alcohol represents one way of coping with chronic stress like post-traumatic stress or loss of a job or death of a spouse. Unless there has been some sign of previous history of substance dependence, a life stress, such as death of a loved one, is unlikely to trigger alcohol dependence in the majority of people. Family and social risk factors of alcohol dependence include a family history of substance abuse, family conflict, rejection and criminal behaviour, and parental antagonism and having an alcoholic father in the family are high predictors of alcoholism in boys (Zuckerman, 1999).

Like alcohol abuse, stress could be a contributing cause of drug abuse (including cigarette smoking), a result of abuse, or both. Differences between treatment seeking and non-treatment seeking drug abusers include consequences and problems in life, being married and having a job. Therefore, if drug abusers have work and family ties and commitments, they are more likely to seek help for their drug abuse (Zuckerman, 1999). Availability of drugs, price and prevention of use are strong determinants of use (Hesselbrock, Hesselbrock & Epstein, 1999), so to a degree these factors can determine the likelihood of drug use in a person who is vulnerable due to genetic and personality factors. Concordance in drug use amongst friends is high, however this may be due to choosing like-minded friends rather than peer pressure (Wills, Vaccaro & McNamara, 1992). Drug abuse can lead to decreased school performance, emotional distress and less conformity, which is a vicious circle leading to social alienation, reduced self-esteem and more drug abuse (O'Connell, 1989; Segal & Stewart, 1996). Employment possibilities may therefore become limited to low-paid and unskilled jobs (Ogden, 2004), which can be rather frustrating and unsatisfying for high sensation seeking drug abusers.

It is viewed here that the probability of substance dependence increases as both stress level and diathesis strength increase beyond a minimal level. Certain kinds of stress such as the death of a loved one may be unrelated to the diathesis. However, other types of stress, such as consequences of substance dependence may have a direct relationship with the diathesis. For example, anti-social tendencies in an alcoholic may cause the family and society generally to reject them because of the socially deviant behaviours attached to the disorder. This may produce stress in the individual that interacts with the diathesis itself. Another way in which the diathesis can interact with stress relates to biological stress rather than to social stress. That is to say, genetic predispositions not only motivate the initiation of drug use, during experimentation with the drug, but also can influence the addiction process at both the maintenance and relapse stages. The extent to which an individual finds a drug physiologically reinforcing and the extent to which an individual becomes conditioned to the drug and the environmental context in which the drug is taken can be predisposed by genetic factors. Individual differences in the susceptibility to sensitisation of the drugs effects and drug-related cues in the environment can influence these neurobehavioural elements of addiction. A theory that supports this view is the incentive sensitisation theory of addiction (Robinson & Berridge, 1993).

The incentive sensitisation theory

The theory states that addictive drugs produce long lasting neuroadaptions in the brain system that is involved in incentive motivation and reward. The brain system under debate is the mesolimbic dopamine pathway, where dopamine is projected to the nucleus accumbens. The critical neuroadaptions for addiction leave this brain reward system sensitised to drugs and drug-related stimuli. Sensitisation occurs when the magnitude of a response grows when a drug is repeatedly administered at the same dose, or when a response from a drug remains the same when the dose is decreased. This can be seen as the opposite to tolerance or an increase in the drug effect. According to the theory, the sensitised brain system does not mediate the pleasurable effects of drugs ("liking" the drug), but mediates the incentive salience of drugs ("wanting" the drug), an element of reward. When sensitised, the incentive salience procedure generates compulsive drug-seeking behaviour (Robinson & Berridge, 1993). The drug user becomes sensitised to the drug effect and related stimuli through

associative learning. That is, when the user is repeatedly exposed to the drug, the interaction of neural sensitisation with associative learning makes objects and stimuli associated with drug taking powerful incentives (Robinson & Berridge, 2000).

Evidence for this theory can be seen in animal studies, which have shown that when an addictive drug is repeatedly administered to rats, an increase in the drug effect (sensitisation) can be seen. This is measured by displays of the drugs psychomotor effects seen in rats and mice, for example via the assessment of locomotor activity and rotational behaviour (Stewart & Badiani, 1993; Anagnostraras & Robinson, 1996). Psychomotor sensitisation has also been shown to occur in studies that have exposed rats to repeated alcohol administration (e.g. Hunt & Lands, 1992; Lessov & Phillips, 1998) and consistently, these studies point towards a relationship between sensitisation and dopamine-related brain circuitry (reviewed in Robinson & Berridge, 2000).

Research examining sensitisation in humans was sparse until quite recently when several reports were published by one research group (Strakowski, Sax, Setters & Keck, 1996; Strakowski & Sax, 1998; Strakowski, Rosenberg, Del-bello & Sax, 1999). These reports illustrated how repeated amphetamine administration elicited a greater increase than initial doses, in activity, mood, eye blink rate and other behavioural measures. As well as providing evidence of sensitisation in humans, Strakowski & Sax (1998) also substantiated the hypothesis that sensitisation applies to the wanting and not the liking of drugs, made by Robinson & Berridge (1993) because the participants' reports of drug liking did not increase with successive drug doses. Further evidence using human participants has been assembled from imaging studies. Childress, Mozley, McElgin, Fitzgerald, Reivich & O'Brien (1999) showed substance abusers video tapes of drug-taking scenarios and found that whilst watching these videos, drug abusers experienced changes in their cerebral blood flow in limbic regions of the brain (amygdala and caudate putamen). In the same year, heroin and heroin-related stimuli were found to activate those brain structures as well as the ventral tegmental area, the region that projects dopamine to the nucleus accumbens (Sell, Morris, Bearn, Frackowiak, Friston & Dolan, 1999).

Those dopamine systems found to be associated with sensitisation are, according to Robinson & Berridge, crucial for the *wanting* of incentive motivation rather than the *liking* of the drugs pleasurable effects (Robinson & Berridge, 1993; Berridge, 1996; Berridge & Robinson, 1998). Therefore, the incentive sensitisation theory opposes the popular view, stated initially by Wise in 1982, that enhanced dopamine levels leads to enhanced pleasure and Berridge & Robinson (1998) have supplied convincing support for their opposing claim. To illustrate, dopamine systems have been found to become activated not only by positive (pleasurable) stimuli, but also negative stimuli and stressful events (Salamone, Cousins & Snyder, 1997). Observations that substance abusers can dislike the smell and taste of the drug, but still want to use it, provides further support and Robinson & Berridge themselves describe: *"The neural systems that mediate the subjective pleasurable (hedonic) effects of drugs do not appear to sensitize. This may be why addiction is characterized by an increasing dissociation between the incentive value of drugs (how much they are wanted) and their subjective pleasurable effects (how much they are liked). With the development of an addiction drugs become pathologically wanted ("craved") and this can occur even if drugs are liked less and less.* [Robinson & Berridge, 2000, pp. 105].

Another issue, central to the incentive sensitisation theory, if drug induced neuroadaptions underlying sensitisation do play a role in the development of addiction, then there should be individual differences between how sensitised people become to stimuli associated with the drug. For the reason that it is through associative learning that enhanced incentive value becomes focused specifically on drug-related stimuli. The fact that individual differences in susceptibility to sensitisation are related to the disposition to addiction is a source of debate that has previously received some attention (Robinson, 1988; Robinson & Berridge, 2000), however, there is a shortage of evidence to support individual variability in sensitisation among humans. Of the evidence that is available, the dopamine D4 receptor gene emerges as a prime candidate for individual differences in sensitisation.

DRD4 and sensitisation

The DRD4 gene is of interest because it is expressed in specific areas (limbic regions), which strongly suggests that this receptor has privileged functioning (Oak, et al., 2000). This gene is thought to be critical to sensitisation because D4 antagonists

block the sensitisation of the limbic pathways (Van Tol, Bunzow, Guan, Sunahara, Seeman, Niznik & Civelli, 1991; Feldpausch, Needham, Stone, Althaus, Yamamoto, Svensson & Merchant, 1998). In addition, activation of dopamine receptors has been associated with craving (Robinson & Berridge, 2000) and so a plausible gene involved in craving would be the DRD4 gene because it expresses functional differences in dopamine receptors (Hutchison, LaChance, Niaura, Bryan & Smolen, 2002).

Hutchison, et al., (2002) examined whether the DRD4 gene moderated the effects of smoking cues on craving by examining associations with the polymorphism and cue-elicited craving for nicotine. They hypothesised that participants carrying the DRD4 long alleles would show an increased craving response after exposure to smoking cues when compared to participants carrying the short alleles. This hypothesis is based on a report that, as opposed to the short variants, long variants at the DRD4 gene dampen the intracellular response to dopamine agonists (receptor activators), making dopamine less effective at inhibiting adenylyl cyclase, cyclic AMP (cAMP) formation and the cAMP-dependent protein kinase (PKA). So it is likely that individuals with the long allele at the DRD4 gene have chronically elevated cAMP and PKA levels and elevated cAMP levels enhance dopaminergic signalling (Asghari, et al., 1995). Therefore, individuals with the DRD4 long allele could be more sensitive to the effects of dopamine receptor stimulation, which is triggered by the exposure of drug-related stimuli (Hutchison, et al., 2002).

Despite using a relatively small sample size of 68 participants (88% Caucasian) in their study, Hutchison, et al., (2002) showed that participants with the long alleles showed significantly greater craving, more arousal, more negative affect and more attention to smoking cues than did participants with only short alleles. This is consistent with the incentive sensitisation theory because possession of the DRD4 long allele enhances dopaminergic signals, so individuals with this variant may be more sensitised to the effects of dopamine stimulation that is triggered by exposure to drug-related stimuli. Furthermore, reduced baseline levels of stimulation among individuals with the long alleles would be more likely to take drugs to stimulate dopamine release (Lerman, Caporaso, Audrain, Boyd, Bowman & Shields, 1998). This is in line with the theory because the incentive sensitisation theory asserts that

mesolimbic dopamine activation influences the motivational and appetitive properties of drugs by controlling the incentive salience of drug-related stimuli (Wise, 1988; Robinson & Berridge, 1993; Berridge & Robinson, 1998; Robinson & Berridge, 2000). Therefore, repeated drug use and the associated release of dopamine produces craving (incentive salience) in the sensitised neural system. After the mesolimbic pathway has become sensitised, expression of incentive salience for drugs can be activated in response to drug-related cues (de Wit, 1996). Empirical support for the role that the DRD4 gene plays in reactivity to drug-related cues and the connection this has with incentive sensitisation is presently limited and more work is needed to further elucidate the connection, although cue reactivity studies in addiction research have recently incorporated the incentive motivation view.

Essentially, cue exposure is a general process of classical conditioning, although it is not restricted to these basic principles. Fundamentally, a stimulus or a cue is presented and the response is dependent on the participants' previous exposure with the stimulus. For example, heroin produces feelings of pleasure and euphoria, which become paired with a neutral stimulus such as a syringe. After repeated pairings, the syringe becomes associated with the pleasurable effects of the drug so what results is that the syringe alone can produce a conditioned response of pleasure. Cues can be of any kind, for example, those related to the drug before it is taken (e.g. alcohol advertisements, sight of the drug), or stimuli related to drug ingestion (i.e., drugs effect on neuroreceptors). Also, stimuli related to mood, emotion, cognition (e.g. beliefs about the drug) and withdrawal-related stimuli, such as the unpleasant physiological withdrawal syndrome (Drummond, Tiffany, Glautier and Remington, 1995). The potential for exposure to various different cues is highly variable and the extent to which an individual becomes conditioned to different cues must also be partly due to individual differences.

Identifying individual differences in cue reactivity could benefit research by explaining some of the discrepancies in previous research. For example, as discussed earlier, substance dependence has been shown to be influenced by genetic and personality factors. Although there is a lack of evidence to suggest that genes and personality are associated with enhanced drug cue association, these factors could

play a role and this argument has received several sources of support (see Rees & Heather, 1995, for an overview).

Firstly, Gray (1987) proposed that the personality trait impulsivity is associated with variation in sensitivity to conditioned cues. He argued that when conditioned cues are encountered, impulsive people are more likely to show an increase in positive mood, an increase in reward-seeking and increased sensitivity to other environmental cues, which in 1991, Gray explained as a result of an overactive Behavioural Activating System (BAS). Various studies have supported Gray's BAS theory, including extensive studies carried out by Powell and her colleagues. Powell, Dawkins & Davis (2002) used the Card Arranging Reward Responsivity Objective Test (CARROT), a simple card sorting task, to measure cue reactivity in 26 smokers after smoking and also after abstaining from nicotine and compared these findings with 26 non-smokers. The authors found that abstinent smokers showed lower responsiveness to financial incentive than non-smokers. Therefore, these results provide indirect evidence for a link between reward responsiveness, positive affect and dopaminergic activity, postulated by Gray (1987).

Kambouropoulos & Staiger (2001) provide additional support that Gray's BAS theory of personality assists in explaining individual differences in cue reactivity. They used the CARROT to assess the role of sensitisation to reward in mediating drinkers' reactivity to alcohol-related cues. During an experimental session, two groups of heavy and light drinkers were administered the CARROT after exposure to the sight, smell and taste of a neutral stimulus and an alcohol-related stimulus. Findings revealed that drinkers displayed a significant increase in responsiveness to reward (measured using the BAS scales), positive affect and urge to drink after exposure to the alcohol-related stimulus. These findings are consistent with other cue reactivity studies that have demonstrated increased craving for alcohol after exposure to alcohol-related cues (e.g. Greeley, Swift, Prescott & Heather, 1993), but they also advocate that a BAS type mechanism may mediate responses to alcohol-related cues (Kambouropoulus & Staiger, 2002).

A second source of support that individual differences in personality can influence cue reactivity refers to Eysenck's (1967) theory that introverts are more easily

conditioned to cues than extraverts because they have a higher level of cortical arousal that results in enhanced formation of arousal-related connections. McCusker & Brown (1991) lend support to this theory as they found that introverted alcoholics experienced significantly greater cue-elicited reactivity than did extraverts.

Another potentially important factor that could influence the extent to which an individual can become conditioned to substance-related cues in the environment is mood status. Stewart, de Wit & Eikelboom (1984) used an appetitive motivational model of substance dependence to explain how negative mood states increased the incentive salience of drugs and drug-related stimuli. Based on their findings, vulnerability to cue reactivity was apparent among individuals with a negative mood state at the time of exposure, with the highest level of attentional bias to drug-related cues found among those with a depressed mood. This demonstrates that negative mood can elicit craving during withdrawal because of an increased incentive for the drug.

Finally, individual differences in severity of dependence could influence cue reactivity because severity of dependence is closely related to physiological dependence and physiological dependence is closely associated with cue reactivity (Heather & Greely, 1990; McCusker & Brown, 1991). Research in this area is currently somewhat limited and the evidence available is contradictory and therefore inconclusive. McCusker & Brown (1991) found that the number of years of drinking alcohol was not associated with the desire to drink or salivation responses to alcohol-related cues. However, Monti, Rohsenow, Rubonis, Niaura, Sirota, Colby & Abrams (1993) found that more severely dependent drinkers showed a greater urge to drink and showed an attentional bias to alcohol-related cues than they did to neutral cues.

Other factors to be borne in mind when investigating cue exposure, is the way in which cue reactivity is measured because this may influence the degree of reactivity observed. Laboratory controlled, experimental methods rely on the participants' past drug use histories, whereby cues associated with the drug are used to elicit conditioned responses, which are measured and differences in responses between cases and controls are assessed (Rees & Heather, 1995). Studies of this nature have shown that exposure to smoking related cues elicits greater reactivity than does

exposure to neutral cues. Sayette & Hufford (1994) found this to be the case using a cigarette as a smoking-related cue, and Suraway, Stepney & Cox (1985) used a video tape and a live viewing of others smoking to elicit smoking urges in participants. With heroin users, studies have used drug-related video presentations and real life scenarios whereby heroin paraphernalia (e.g. syringes, powder) are used as cues to measure induced withdrawal and craving among heroin users (Powell, Bradley & Gray, 1992). Another method of studying cue reactivity is to use a modification of the Stroop paradigm (Stroop, 1935). This method is especially useful in measuring cue reactivity to test whether stimuli capture participant's attention. Stroop tasks can be used to determine the level of activation of a word component of the stimulus, whereby its increased activation makes the suppression of its meaning more difficult.

The Stroop effect is becoming more commonly used in addiction research, although smoking behaviour was the biggest focus of this research until quite recently. Gross, Jarvis & Rosenblatt (1993) measured the reaction times of smokers to smoking-related and neutral words. Successful performance of the Stroop task requires suppression of the meaning of the stimulus word in favour of activation of the colour name. Results were as predicted, Gross, et al., found that abstinent smokers were slower at colour naming smoking-related than non-smoking-related words. This processing bias makes the suppression of meaning of smoking-related words more difficult and leads to greater interference during the task. Successful colour naming requires the attention of participants, so Stroop interference arises when the meaning of the words to be colour named captures the attention of the participant at the expense of the task.

However, findings have not been consistently replicated and contradictory findings could be a result of methodological differences between studies (Powell, Tait & Lessiter, 2002). For example, some studies have used a computerised Stroop task (e.g. Gross et al., 1993), whereas others have used a standard card format (e.g. Powell, et al., 2002). There are several other design issues that need to be addressed when interpreting findings from Stroop tasks and these include time pressure (Sharma & McKenna, 2001). Whether a blocked or unblocked format is used and on what basis the drug-related stimuli are decided upon and how these words are matched to the

neutral stimuli (Cox, Pothos, Johnsen & Laberg, 2001). These issues are addressed in chapter six and have also been summarised in Lusher, Chandler & Ball (2004).

Despite these methodological issues attached to the Stroop task, it can be argued that from the view of the incentive sensitisation theory, exposure to smoking-related words in smokers mediates the maintenance of their addiction by producing craving. That is, it makes the smoker want to smoke by being shown stimuli that capture their attention and remind the smoker of smoking. This, being an important factor in understanding the basis of relapse as craving or wanting, has a triggering effect on relapse and demonstrates a powerful environmental factor that can influence substance use behaviour (Robinson & Berridge, 1993).

To summarise, the interactive model of diathesis-stress (Monroe & Simons, 1991) illustrates how substance dependence can be a result of both genetic and neurobehavioural vulnerability, personality and stress. This model allows for substance dependence to emerge in some cases in which there is no diathesis, but also in some cases, the diathesis may play a causal role in the symptoms. The diathesis for substance dependence is defined as the genetic predisposition and the biological traits produced by the genetic programming, biological stressors or both. The diathesis produces the vulnerability to stress. Personality predispositions may be a function of the diathesis or may have their own genetic and environmental origins. The interactive diathesis-stress model postulates that the diathesis is a necessary but not sufficient condition of substance dependence. Stress may produce addiction in a person with a weak or no diathesis, but it takes much more stress than it would in a vulnerable person with a strong diathesis. In light of the diathesis-stress model and the incentive sensitisation theory of addiction, genes, personality and neurobehavioural factors that influence vulnerability to addiction behaviour are explored.

This research demonstrates an original contribution to the field of psychology of addictive behaviour. It offers a multidisciplinary approach to a multifaceted behaviour, by incorporating neuroscience, personality theory and molecular genetic techniques. There has been a lack of research looking at the associations with the DRD4 gene and substance abuse and personality. No single, published study in the United Kingdom had examined the DRD4 gene across different substances of abuse

or severities of dependence and personality within the same population. In addition, previous research has used different ethnic groups and employed varied scales to measure personality, thus making research findings difficult to compare. The Stroop task has not been used extensively in addiction research, nor has previous research of this kind accounted for differing levels of dependence severity, anxiety and depression, which could affect Stroop performance. Finally, there is a lack of research examining the incentive sensitisation theory using human participants. This research attempts to overcome previous shortfalls and to fill some of the gaps in research within this field. This thesis attempts to explain factors that influence vulnerability to substance dependence.

The following chapter describes the methodology employed throughout the thesis. The overall design used for this research remains the same, as all participants completed all levels of each study. Chapter three introduces study one and explores addiction and personality trait influences. Chapter four examines genetic influences on personality in study two and study three is detailed in chapter five investigating genetic influences on addiction. Chapter six discusses susceptibility to sensitisation and explores how substance abusers respond to substance-related cues in study four. This thesis will be brought together in study 5 (chapter seven), which investigates causal pathways to substance dependence, with a concluding discussion, reintroducing the diathesis-stress model, with an overview of the findings and implications for substance abuse treatment and future work.

Chapter two

Methodology

A series of standardised questionnaires were administered to participants using a semi-structured interview technique. At the same time, cheek swab kits were given to participants to collect DNA samples. Finally, participants completed a reaction time task on a laptop computer.

Participants

A total of four hundred, unrelated individuals consented to participate in the study (100 opiate dependent, 100 alcohol dependent, 100 cigarette smoking comparison group and 100 non-dependent comparison group). For the purposes of multiple regression analyses and for a minimum R^2 of 0.4 that can be found statistically significant with a power of 0.80 for up to nine independent variables a sample size of 400 would be sufficient (Hair, Anderson, Tatham & Black, 1998). For the purposes of ANOVA a power analysis using Gpower (Faul & Erdfelder, 1992) was carried out to determine the appropriate sample size that would enable any effects to be identified. A minimum effect of 0.25 and a power of 0.80 was decided and the power analysis revealed that a combined sample size of 200 (40 per group) or more would be large enough to detect such effects. Therefore, a sample size of 400 was concluded as being large enough to confidently detect any significant effects.

National Health Service (NHS) clients (n=200) were selected from treatment centres in east and south London, on the basis that they were attending an outpatient programme at the treatment centre during the period of the study. Inclusion criteria were met if firstly participants were dependent on opiates or alcohol. Dependence was measured using the Severity of Dependence Scale (SDS) and the Severity of Alcohol Dependence Scale (SADQ) for the alcohol group and the SDS alone for the heroin group. Cigarette smoking urges were measured using the Smoking Urges Questionnaire (QSU). Secondly the inclusion criteria were met if participants were aged between eighteen and fifty-five years. This criteria was used to satisfy ethical requirements of using consenting adults and the cut-off point of fifty-five years was necessary to ensure a relatively young sample for an accurate recording of Impulsive Sensation Seeking as this personality trait diminishes with age in normal, healthy individuals, Zuckerman (1979).

Ethnicity was an important factor in this study for genetic reasons. Ethnic differences in genes for complex disorders are to be expected since the allele frequencies vary widely (Van Tol, et al., 1992). Due to these differences between ethnic groups, the final inclusion criteria were met if participants' were western European. This criterion was used to minimise confounding of genetic test results.

The comparison group (n=200) consisted of two random samples of the general population from General Practitioner surgeries in south and east London. The comparison group consisted of 100 smokers and 100 non-smokers who did not abuse illicit drugs or alcohol.

The entire sample was approached individually and the purpose of the study was explained. Participants were invited to read an information sheet. Written consent was obtained before participation in the study. All participants completed a demographic questionnaire, a personality scale, a mood scale and were administered a cheek-cell sample kit. Finally, participants completed a computer-based Stroop task.

Summary of descriptive data on study sample

Of the total 400 individuals who participated in the study, 100% completed the questionnaire and provided a sample of their DNA (31.25% female, mean age 35.69, 98.25% Caucasian)[1]. Finally, 88.25% of the entire sample completed the Stroop task. The sample comprised 100 opiate abusers (17% female, mean age 35.40) 100 alcohol abusers (16% female, mean age 40.57) 100 smokers (45% female, mean age 31.60) and 100 controls (47% female, mean age 35.21). Genetic data from 91.5% of the entire sample could be used for analysis. The 2-, 3-, 4-, 5-, 7- and 8-repeat alleles accounted for 10.1%, 2.7%, 76.2%, 0.1%, 10.8% and 0.1% of alleles, respectively. The stimulant group (identification numbers 201 to 300) was dropped from the study because this drug using population did not access the services used in the study (full details of participants' demographic characteristics are given in Table 2.1).

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[1] Despite one of the inclusion criteria being that participants should be Caucasian, this criteria could not be fully met as on one occasion during the data collection period participants were invited to participate in the study during an outpatient group meeting, which was made up of various ethnic groups.

For group, there was no significant effect of ethnicity ($\chi^2 = 7.397$, df = 3, $p > 0.05$), but there was a significant effect of sex, with more women in the control groups than in the experiment groups ($\chi^2 = 40.521$, df = 3, $p < 0.001$). In addition, one-way ANOVA revealed significant differences between groups for age. Smokers were younger than all other groups and alcoholics were older than all other groups, F (3, 396) = 17.540, $p < 0.001$. Finally, the experiment groups left school at a younger age than the control groups, F, (3, 396) = 19.165, $p < 0.001$ and they reported lower academic achievement ($\chi^2 = 97.270$, df = 3, $p < 0.001$). Therefore, age, sex and educational level were factors to be considered as possible confounding variables in subsequent analyses.

Table 2.1. Demographic characteristics of the study sample

Characteristic	Heroin group		Alcohol group		Smoking group		Control group		Total Sample	
N	100		100		100		100		400	
	Mean	(S. D.)	Mean	(S. D.)	Mean	(S. D.)	Mean	(S. D.)	Mean	(S. D.)
Age	35.40	(7.31)	40.57	(8.60)	31.60	(8.73)	35.21	(10.35)	35.69	(9.34)
School leaving age	15.52	(1.82)	15.80	(1.16)	16.56	(1.63)	16.88	(1.08)	16.19	(1.55)
No. of children	1.17	(1.30)	1.61	(1.51)	0.67	(1.06)	0.88	(1.23)	1.08	(1.33)
Sensation Seeking	12.45	(4.02)	10.26	(3.98)	10.11	(4.65)	7.09	(4.44)	9.98	(4.67)
Mood Disturbance	51.09	(20.83)	51.27	(20.43)	39.35	(18.37)	33.65	(13.90)	43.84	(20.02)
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Sex										
Male	83	(83)	84	(84)	55	(55)	53	(53)	275	(68.75)
Female	17	(17)	16	(16)	45	(45)	47	(47)	125	(31.25)
Ethnicity										
Caucasian	100	(100)	96	(96)	97	(97)	100	(100)	393	(98.25)
White/Mixed race			4	(4)	3	(3)			7	(1.75)

Table 2.1. Cont.	Heroin		Alcohol		Smoke		Control		Total	
Educational level	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
None	50	(50)	37	(37)	17	(17)	3	(3)	107	(26.75)
GCSE	32	(32)	32	(32)	21	(21)	22	(22)	107	(26.75)
A Level	11	(11)	10	(10)	28	(28)	23	(23)	72	(18)
Higher education	7	(7)	21	(21)	34	(34)	52	(52)	114	(28.5)
Employed	18	(18)	22	(22)	78	(78)	90	(90)	208	(52)
Occupation										
Professional	2	(2)	12	(12)	5	(5)	11	(11)	30	(7.5)
Supervisory	2	(2)	7	(7)	14	(14)	14	(14)	37	(9.25)
Employees	13	(13)	13	(13)	42	(42)	45	(45)	113	(28.25)
Skilled manual	48	(48)	39	(39)	19	(19)	18	(18)	124	(31)
Unskilled	35	(35)	29	(29)	20	(20)	12	(12)	96	(24)
Marital status										
Married	7	(7)	21	(21)	30	(30)	48	(48)	106	(26.5)
Single	51	(51)	37	(37)	38	(38)	32	(32)	158	(39.5)
Divorced/separated	14	(14)	31	(31)	11	(11)	6	(6)	62	(15.5)
Widowed	1	(1)	1	(1)	1	(1)	2	(2)	5	(1.25)
Cohabiting	27	(27)	10	(10)	20	(20)	12	(12)	69	(17.25)

Table 2.1. Cont.	**Heroin**		**Alcohol**		**Smoke**		**Control**		**Total**	
Accommodation	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
Private	16	(16)	30	(30)	50	(50)	68	(68)	164	(41)
Rented	56	(56)	41	(41)	37	(37)	19	(19)	153	(38.25)
Sheltered housing	9	(9)	13	(13)	2	(2)	0		24	(6)
No fixed abode	5	(5)	2	(2)	1	(1)	0		8	(2)
Living with parents	14	(14)	14	(14)	10	(10)	13	(13)	51	(12.75)
Mental illness diagnosis	26	(26)	39	(39)	5	(5)	3	(3)	73	(18.25)
Medication for mental illness	25	(25)	28	(28)	3	(3)	0		56	(14)
Family history of substance abuse	49	(49)	70	(70)	37	(37)	27	(27)	183	(45.75)
Completed Stroop	97	(97)	96	(96)	80	(80)	80	(80)	353	(88.25)
Genotype										
Long/Long	4	(4.3)	1	(1.1)	6	(6.5)	4	(4.4)	15	(4.1)
Long/Short	17	(18)	16	(18)	13	(14.1)	4	(4.4)	50	(13.6)
Short/Short	73	(77.7)	72	(80.9)	73	(79.4)	83	(91.2)	301	(82.3)

Treatment seeking group characteristics are given in Table 2.2. The heroin group was older than the alcohol group when drug of choice was first tried (22 years compared to 15 years). However, the time it took for substance use to become a problem for the individual was much lower for the heroin group than the alcohol group (3 years compared to 13 years). Peer pressure and social reasons, followed by coping with life stresses were the most common reasons for both groups to first begin using. The same pattern of responses was found to explain reasons for relapse. Over two-thirds of the experimental group had previous abstinence attempts prior to the study.

Cigarette smokers' characteristics are detailed in Table 2.3. Smokers in the control group smoked on average 13.52 cigarettes per day. They had smoked cigarettes for a mean of 15.45 years and reported lower urges to smoke (mean 75.45) than smokers in the heroin and alcohol groups. The SDS and SADQ were both used to measure dependence severity among the alcohol group, these two scales showed a significant positive correlation ($r = 0.596$, $n = 100$, $p < 0.01$, two-tailed) so the SDS alone was used in the data analyses for the alcohol group. Finally, the depression sub-scale of the POMS-SF correlated significantly with total mood disturbance ($r = 0.837$, $n = 400$, $p < 0.01$, two-tailed), therefore a total mood disturbance score alone was used for analyses.

Table 2.2. Characteristics of treatment seeking groups

Characteristic	**Heroin group**		**Alcohol group**	
	Mean	**(S. D.)**	**Mean**	**(S. D.)**
Age first used substance	22.06	(7.52)	15.00	(4.39)
Age substance use first became problem	25.22	(8.00)	28.15	(9.59)
Subjective craving level	2.73	(2.94)	2.85	(3.02)
Severity of dependence	10.34	(2.74)	10.02	(3.12)
	N	**(%)**	**N**	**(%)**
Reasons for first using				
Availability	15	(15)	5	(5)
Social/Peer pressure	35	(35)	55	(55)
Curiosity	10	(10)	12	(12)
Seek sensation (experience the buzz)	5	(5)	9	(9)
Coping strategy (deal with stress)	19	(19)	12	(12)
Boredom	7	(7)	4	(4)
No reason offered	9	(9)	3	(3)
Had previous abstinence attempt	68	(68)	67	(67)
Reasons for relapse				
No relapse	32	(32)	33	(33)

Table 2.2. Cont.	Heroin		Alcohol	
	N	(%)	N	(%)
Availability	5	(5)	2	(2)
Social/Peer pressure	11	(11)	7	(7)
Seek sensation (for the buzz)	1	(1)	0	
Coping strategy to avoid stress/withdrawal	29	(29)	43	(43)
Boredom	10	(10)	6	(6)
No reason offered	12	(12)	9	(9)
Medication for substance use	62	(62)	11	(11)
Last used substance				
Within an hour	8	(8)	8	(8)
Within a day	39	(39)	17	(17)
Yesterday	23	(23)	19	(19)
Within the last week	17	(17)	22	(22)
Within the last month	4	(4)	27	(27)
Within the last year	7	(7)	7	(7)
Over a year ago	2	(2)	0	

Table 2.3. Characteristics of cigarette smokers

Characteristic	Heroin group		Alcohol group		Smoking group	
Smoking status	**N**	**(%)**	**N**	**(%)**	**N**	**(%)**
Current smoker	85	(85)	77	(77)	100	(100)
Ex-smoker	6	(6)	9	(9)	0	
Non-smoker	9	(9)	14	(14)	0	
	Mean	**(S. D.)**	**Mean**	**(S. D.)**	**Mean**	**(S. D.)**
No. of cigarettes	17.76	(8.10)	26.05	(12.14)	13.52	(8.98)
No. of years smoked	19.93	(7.60)	24.34	(9.00)	15.48	(8.66)
Smoking urges	99.88	(32.32)	100.90	(36.10)	75.45	(36.20)
Subjective craving level					3.23	(2.88)
Smokers last cigarette					**N**	**(%)**
Within an hour					36	(36)
Within a day					25	(25)
Yesterday					18	(18)
Within the last week					9	(9)
Within last month					5	(5)
Within the last year					1	(1)
Over a year ago					1	(1)

Materials

Self-Report Instruments:

Personal details sheet (see appendix 1): Personal details were recorded on a sheet containing purely factual data regarding age; sex; ethnicity; employment, marital and accommodation status; psychiatric history, treatment status and drug of choice. All participants completed this sheet to allow the researcher to allocate each participant to the appropriate group, experiment or control.

Substance Use History (see appendix 2): The experiment group were asked a series of forced-choice questions including questions concerning drug of choice; age first used; age substance use problems began; circumstances surrounding relapse and family history of substance abuse.

Severity of Dependence Scale (SDS) (Gossop, Darke, Griffiths, Hardo, Powis, Hall & Strang, 1995) (see appendix 3): A short, five-item instrument that measures severity of dependence to a substance. This scale was administered to the experiment group. Completion time was less than one minute and the SDS was designed as a research tool. It provided a short, easily administered scale to measure the degree of psychological dependence experienced by substance users. Severity was scored on a four-point scale from 0-mild dependence to 3-severe dependence. A total SDS score was obtained by adding the scores for each of the five items, with higher scores indicating higher levels of dependence (range 0-15). A typical mean SDS score amongst a London population of heroin users is 8.7 (SD 4.0) (Gossop et al., 1995). The SDS was an appropriate tool for this study because degree of dependence may be a contributing factor to a genetic vulnerability, which was hypothesized in the study. The SDS has been validated using various substance using populations including 100 Spanish benzodiazepine users aged 18-75 years (de las Cuevas, Sanz-Emilio, de la Feunte, Padilla and Bereguer, 2000), 408 English heroin users, 150 English cocaine users, 222 Australian methadone clinic attendees and 532 Australian amphetamine users (Gossop, et al, 1995). The SDS correlates significantly with heroin dose ($r = 0.24$) and frequency of heroin use ($r = 0.43$) and Severity of Opiate Dependence Questionnaire (SODQ, Sutherland, Edwards, Taylor, Phillips, Gossop and Brady, 1986) scores ($r = 0.57$).

Severity of Alcohol Dependence Questionnaire (SADQ) (Stockwell, Hodgson, Taylor & Rankin, 1979) (see appendix 4): A twenty-item scale that was administered to the alcohol group. This standardised questionnaire measures dependence severity experienced by alcohol users. It measures severity on a four-point scale (0-3) and like the SDS, scores are obtained by adding up the scores from each item to get a total SADQ score. Therefore, the higher the total score (range 0-60), the more severe the dependence syndrome will be, severely dependent individuals typically score 30 or above. This is a pure measure of the degree to which help-seeking problem drinkers experience a syndrome of alcohol dependence. The SADQ has been shown to have good reliability and validity when compared to the Drinking Pattern Interview (DPI), using a sample of treatment seeking alcoholics in London (Stockwell, Murphy and Hodgson, 1983) and 40 alcoholic patients (mean age 37.5 years) in America (Cooney, Meyer, Kaplan and Baker, 1983).

Smoking Urges Questionnaire (QSU) (Tiffany & Drobes, 1991) (see appendix 5): This was administered to all participants who smoked cigarettes. It measures desire and intention to smoke along with both anticipation of positive outcomes from smoking and relief from nicotine withdrawal using thirty-two statements which respondents rate their agreement by using a seven-point Likert-type scale (1-strongly disagree to 7-strongly agree). For example, statement number 30:

"I would do almost anything for a cigarette right now".

Scores are obtained by adding up the scores from each item to get a total QSU score, with higher numbers showing more agreement with the statement and more urges to smoke (range 32-224). Of the total 32 items, 14 were reversed to detect participants who responded to the items without regard to the truth. For example, item number 4:

"I am not missing smoking right now".

Smoking urges were reported in the study to determine level of psychological dependence to cigarette smoking. After scoring the scale, a sum of 32 was taken from each score to give a range of 0-192. QSU scores increase as abstinence increases so an individual who smoked immediately before completing the questionnaire would typically score lower than a person who had not smoked for several hours. The QSU was initially validated by Tiffany and Drobes (1991) who found the two subscales (intention and desire to smoke and anticipation of relief from negative effect) to be highly reliable ($r = 0.95$ and $r = 0.93$) and significantly correlated ($r = 0.71$) using a

sample of 230 cigarette smokers (141 men, mean age 21.4 years) who smoked on average 22.3 cigarettes per day. Davies, Willner and Morgan (2000) further validated the scale using a sample of 271 Welsh regular smokers (aged 17-59 years) and found a two-factor structure almost identical to that published by Tiffany and Drobes (1991), indicating that the QSU is a psychometrically sound instrument for assessing smoking urges.

Zuckerman-Kuhlman Personality Questionnaire (ZKPQ) Impulsive-Sensation Seeking Scale (ImpSSS) Form V (Zuckerman, et al., 1993) (see appendix 6): The total questionnaire consists of five scales (99 items). Only the ImpSSS was used for this study, as Impulsive Sensation Seeking was the only variable from the questionnaire to be analysed in the study. The ImpSSS measures individual drive to seek out stimuli that are novel or exciting in attempts to maintain an optimal level of cortical arousal and a tendency to act impulsively without thinking or planning ahead. The ImpSSS measures the personality trait using 19 items and correlates about 0.7 with the SSS-V Total score (Zuckerman, 1996). Participants were required to read a series of statements and decide for each one, whether the statement applied to them. They responded by either placing a tick (true) or a cross (false) in a box beside the corresponding item. For example, statement number 23:

"I like wild, uninhibited parties".

An additional four items, from a sequence of ten lie detection items, were included in the scale to eliminate subjects who carelessly responded to items without regard to the truth. For example, statement number 4:

"I have always told the truth".

These are exaggerated statements that are unlikely to apply to anyone (item numbers 2, 4, 10 and 13). These four items are 'infrequency items' and do not constitute a scale, but may be used to eliminate respondents who have carelessly completed the scale. The items are true scored so respondents score one point for every item that they answered truthfully. They are exaggerated or socially desirable statements but are unlikely to be true about anyone. Any score above three suggests either inattention to the content of the items or very strong social desirability.

Scores were obtained by giving each participant one point for every item that they responded true or false, as indicated to produce a high level of SS. Therefore, the

highest obtainable score being 19. On average, men score 11 and women score about 10 on the ImpSSS (Zuckerman, et al., 1993). These typical scores have been confirmed in a more recent study that found a mean score of 9.6 (Aluja, Garcia & Garcia, 2004), although it is predicted that substance abusers will score higher than the general population. Previous related studies have used the Tridimensional Personality Questionnaire-Novelty Seeking Scale (TPQ-NS) to measure SS, however, both instruments measure this personality trait as a heritable component of human temperament and the two scales are highly correlated (McCourt, Gurrera & Cutter, 1993). Therefore, generalisability and replication of findings with comparable research will be possible by using the ImpSSS alone. The ImpSSS has been tested for its reliability and validity using several different populations. Stephenson, Hoyle, Palmgreen and Slater (2003) found high correlations with the ImpSSS and the Brief SSS ($r = 0.83$) using a sample of 5187 American school children (85% Caucasian). Additionally, the ImpSSS has been found to have good internal consistency using a sample of 450 cocaine abusers (Ball, 1995).

Profile of Mood States-Short Form (POMS-SF) (McNair, Lorr & Droppleman, 1981) (see appendix 7): The POMS is a test of personal adjustment that is used widely for the assessment of mood. It consists of 65 adjectives. Respondents indicate the degree to which each adjective describes how they have been feeling over the past week using a 4-point Likert type scale. Standard scoring yields a global distress score referred to as Total Mood Disturbance as well as a score from each of the six sub-scales: Fatigue-Inertia, Vigour-Activity, Tension-Anxiety, Depression-Dejection, Anger-Hostility and Confusion-Bewilderment. In the POMS-SF (short form), several items from each of the sub-scales were removed, leaving a total of 30 items, which did not lose the face validity or internal consistency of the scale. The short scales were formed using the reliability program from statistical packages for social sciences (Hull, 1981). This program assesses the contribution of items to the internal consistency of the scale. A total score of 120 can be yielded from the POMS-SF, with participants scoring a minimum of zero and a maximum of 20 for each of the six sub-scales. Therefore, a higher score indicated a higher level of mood disturbance. An average POMS-SF subscale score of 4.61 was reported amongst a healthy population (Shachman, 1985), scores higher than this are expected in the substance using population.

The POMS-SF was employed rather than a more commonly used tool such as the BDI because it is a shorter and validated measure of depressed mood, which correlates highly with the BDI (Malouff, Schutte & Ramerth, 1985). Malouff et al. (1985) tested the reliability and validity of this scale, in comparison with the POMS, using 131 outpatients with divorce related depression (r = 0.98). This high correlation was consistent with that in another sample of 83 cancer patients (r = 0.97, Shachman, 1985). In addition, a short form of the scale was appropriate for this study to promote ease of administration and to prevent boredom or fatigue as multiple scales were used to measure different factors in the study. The POMS-SF takes three minutes to complete. Mood was a variable measured in this study for the purposes of the computer-based Stroop task. Although depressed patients show a general performance deficit across a range of tasks, little work has been done on how depression affects attentional performance amongst different clinical and non-clinical samples. Ellis and Ashbrook (1987) demonstrated how depressed mood has effects similar to state anxiety, as in the mood disturbance diverts attentional capacity and cognitive effort to task-irrelevant processing. Therefore, as high proportions of substance abusers tend to suffer with depression and anxiety, these variables need to be measured and accounted for in the data analyses. Although there is little solid evidence for this assumption, it is important to be aware of and take into consideration these assumptions when interpreting emotional Stroop effects.

All scales have been previously validated and shown to be standardised, reliable instruments. An information sheet (appendix 8) was given to each participant to briefly communicate the purpose of the study. A consent form (appendix 9) was used to comply with ethical considerations by obtaining a signature from each participant who agreed to participate in the study. Completion of all questionnaires did not exceed fifteen minutes.

Genetic Test Materials:

A DNA cheek swab kit was used to collect the data for genetic testing. The kit provides a non-invasive and in-expensive method for collecting DNA samples (Freeman, Ball, Powell, Craig & Plomin, 1997). It comprises ten cotton wool swabs and a 15ml tube, containing 2.5mls of Proteinase K storage solution (for full

procedure for making storage solution see appendix 10). The kit is used by rubbing the inside of the cheek with cotton wool swabs on ten occasions, then placing the swabs head down into the tube (full procedure for collecting cheek cell samples see appendix 11). This method was used as an alternative to using blood, as it is a cheaper, painless and an easily administered method for collecting DNA samples. The current method for DNA extraction from cheek cells was used in this study (detailed in procedure below).

Modified emotional Stroop task:

The Stroop task was designed based on previous literature and pilot work conducted prior to the study (detail given in chapter 6). This task was carried out on a Laptop computer with a control pad displaying four colour buttons (red, blue, green, and yellow) and a rest bar (space bar key). Each participant received 154 trials during the task. A practice trial preceded the main task. This comprised ten words (red, blue, green, yellow, heroin, alcohol, cocaine, cigarette, impulsive and door) that were chosen during the piloting procedures (see chapter six). Stimulant- (cocaine, amphetamine) related words were included in the task originally as a group of stimulant users were going to be recruited for the study. However, the recruitment of stimulant users proved too difficult, as this drug using population did not access the treatment services approached for the study.

The main task consisted of 144 trials grouped into blocks of 48 trials for each of the three conditions. Condition one consisted of 16 opiate-related, 16 alcohol-related and 16 neutral words. Condition two contained 16 stimulant-related, 16 smoking-related and 16 neutral words and Condition three comprised 16 sensation seeking words, 16 colour words and 16 neutral words.

Condition 1. Word list

Heroin, chasing, foil, gear, bag, inject, methadone, smack, heroin, chasing, foil, gear, bag, inject, methadone, smack, alcohol, booze, drunk, drink, hangover, pub, DTs, shakes, alcohol, booze, drunk, drink, hangover, pub, DTs, shakes, door, table, picture, sofa, chair, cabinet, rug, desk, door, table, picture, sofa, chair, cabinet, rug, desk.

Condition 2. Word list

Cocaine, charlie, snort, powder, pipe, coke, crack, line, cocaine, charlie, snort, powder, pipe, coke, crack, line, cigarette, smoking, lungs, fags, lighter, nicotine, tobacco, ashtray, cigarette, smoking, lungs, fags, lighter, nicotine, tobacco, ashtray, door, table, picture, sofa, chair, cabinet, rug, desk, door, table, picture, sofa, chair, cabinet, rug, desk.

Condition 3. Word list

Impulsive, novelty, sensation, chance, wild, fun, glide, surf, impulsive, novelty, sensation, chance, wild, fun, glide, surf, red, yellow, red, green, blue, green, yellow, red, blue, yellow, green, yellow, blue, green, red, blue, door, table, picture, sofa, chair, cabinet, rug, desk, door, table, picture, sofa, chair, cabinet, rug, desk.

The words appeared on the screen in one of four different colours (red, blue, green, and yellow) on a black background. All colours were randomized and linked to the storage system. The conditions were counterbalanced to control for order effects. Words always appeared at the same place, in the centre of the screen in arial regular font size 14 with a screen resolution 1024 * 768, which followed a white fixation point (+). The reaction time, error rate and total duration of task were recorded.

Procedure

Ethical approval was obtained from the Maudsley and Bethlem Royal Hospitals NHS Trust, Croydon Local Research Ethics Committee, East London and City Health Authority and London Guildhall University prior to commencing this procedure. As substance abusers attended their treatment centre and controls attended their GP surgery, they were informed that a study was taking place. Those who communicated that they were interested in participating in the study were offered an information sheet (see appendix 8) to explain the purpose of the study. After potential volunteers had read and understood the information, and the researcher had answered any questions, they were asked whether they wished to take part. Each volunteer was reminded of the anonymity of the study and reassured that no information regarding individual genetic results would be communicated to anyone. Each participant, stating that they had read and understood the information provided and were willing to participate, signed a consent form (see appendix 9).

A personal details sheet (see appendix 1) was then administered to participants for self-completion. Participants were allocated to their corresponding group (experiment or control). This procedure determined what set of instruments would be applicable to each respondent.

Participants in the experiment group were administered a substance history questionnaire (appendix 2), SDS (appendix 3), SADQ (appendix 4) (if in the alcohol group), QSU (appendix 5) (if a smoker), ZKPQ-SSS Form V (appendix 6) and POMS-SF (appendix 7). The control groups completed the QSU (if a smoker), ZKPQ-SSS Form V and POMS-SF. All groups self-completed the instruments, with the researcher present throughout the procedure, for assistance in case any difficulties arose.

On completion of all questionnaires, participants were given a cheek swab kit to obtain a sample of DNA. The tube was labelled with an identification number and group name (001-100 opiate; 101-200 alcohol; 301-400 smoker and 401-500 control). This label corresponded with a matching label attached to each questionnaire so that they could be married up at a later date for analyses. The corresponding identification number was typed into the laptop computer programme so that computer data could be matched up with all other data.

Participants were informed of the contents and instructed how to collect a cheek cell sample. The participant rubbing the inside of their own cheek, mouth and gums with the cotton wool swabs provided in the kit obtained the DNA sample. Each swab was carried out for about fifteen seconds using a different part of the mouth each time. The swabs were taken together and placed in their own tube, head down, and returned to the researcher during the time of the study (for full instructions on collecting mouth cells see appendix 11).

Prior to completing the computer task, participants were asked to complete a series of questions on a computer task information sheet (see appendix 12). To determine the ease in which participants could complete the task and the extent to which reaction time could be compared. Handedness, native language, whether the participant had normal vision and whether the participant was colour blind were questions asked.

Participants were also asked to tick from a list, what substances they were using at the time of the study and to state when they had last used each one (cigarettes, alcohol, heroin/other opiate, cocaine/amphetamine or other to specify). This was important to record so that levels of intoxication and physical and mental state could be considered in the data analyses.

Participants were asked to rate on a visual analogue scale, with 0 meaning 'not at all' and 10 meaning 'extremely', how much they were craving for their drug of choice immediately prior to the Stroop task. Subjective craving levels were recorded firstly to determine level of craving in case the results were affected by this variable and secondly to ensure that the task itself did not induce any unwanted craving in participants. On completion of the task, participants were asked to rate how much they were craving for their drug of choice on a scale from 0 (not at all) -10 (extremely).

Finally, the emotional Stroop task was administered to participants using a laptop computer. A practice trial preceded the main task, which consisted of ten trials, four colour words and one of each word from each of the other word types (opiate, alcohol, stimulant, smoking, sensation seeking and neutral). At the end of this trial the participants score appeared on the screen to determine error rate and to decide whether the participant could successfully go on to complete the main task.

The program began with the participant reading the on-screen instructions (see appendix 13). Blocks of coloured words or words typed in different colours were presented on the screen. Participants were instructed to name the colour of the words, which appeared on the screen, while ignoring the content of the word. They were told to press the appropriate coloured key on the keypad that corresponded with the colour in which the word was printed.

When the participant had read and understood the instructions they were asked to press the space bar to begin the practice task. A fixation point appeared in the centre of the screen for one second followed by the first word. When the participant responded to the stimuli the second word appeared on the screen, which was preceded by a fixation point. This procedure was repeated until responses for all trials in the block had been given. The final response initiated a break whereby the participant was

instructed by the on screen instructions to press the space bar to continue onto the next stage of the task. When the participant pressed the space bar, this initiated the first word in the second block to be presented. This procedure continued until the end of the task, at which point the phrase

"You have completed the task, thank you for your time"

appeared on the screen to inform the respondent that they had finished the task. Participants were thanked for their time and participation in the study. No participant received any payment. Before leaving the experiment room, participants were asked again to rate their level of craving, for their drug of choice, on a scale from 0-10. If craving levels were higher after completion of the Stroop task, than they were prior to completing the task, participants were briefed to reduce their subjective craving levels. Then participants were asked for a third time what their level of craving was on a scale from 0-10 to ensure that craving levels had been reduced and that the researcher was confident that participants craving levels were stable before leaving the treatment centre.

The DNA was extracted from the swabs within a month of collection, to prevent degradation. On receiving the samples, they were stored at room temperature. To activate the Proteinase K and release the DNA, samples were incubated at 65°C. The DNA was purified and compared to a standard to confirm the results.

Current Method for DNA Extraction

On the first day of the extraction procedure, when loaded with cells, swabs were stored in 2.5mls of Protinase K at 20mg/ml. The tubes were scanned then spun for five minutes at 3000rpm, to bring all of the liquid to the base of the tubes. They were then placed in a water bath at 65°C for two hours to activate the Protinase K. The tubes were then spun for a further three minutes at 3000rpm to recover any condensation. Caps were removed from the original tubes and then tipped upside down into 50ml falcon tubes and spun down at 3500rpm for five minutes, allowing all of the liquid to collect at the bottom of the 50ml tube. The now dry swabs were removed from the falcon tubes, leaving the liquid in the falcon tubes. 1200µl of liquid from the falcon tubes was put into the microtubes (two microtubes per sample) with 200µl of phenol chloroform mixture (a laboratory solution called *magic mix,* a mix of

Yeast Reagen 3 supplied by Autogen and 100% Ethanol). The tubes were then capped and shaken vigorously for one minute.

The microtubes were spun at 13000rpm for 10 minutes in a microfuge to bring the debris to the base of the tubes. With one swift movement the supernatent from each tube was tipped into a fresh, appropriately labelled microtube and the tube containing the debris was discarded. 800µl of 100% isopropanol at room temperature was added and the tubes were shaken gently then spun at 13000rpm for a further 10 minutes. The 100% isopropanol was tipped out leaving the pelleted DNA in the bottom of the tube to dry for 30 minutes. The DNA was washed with 1ml of cold 70% ethanol, then spun at 10000rpm for three minutes. The ethanol wash was discarded, the tubes inverted and the DNA pellets left to dry at room temperature for 30 minutes to one hour. The DNA was re-suspended in 600µl of TE buffer (H_20, Tris-Hc1 & EDTA) by being shaken overnight in a hybridisation oven set at 45°C.

DNA quantification

On the second day of the extraction procedure, the samples were analysed to determine the purity and concentration of the DNA. Samples were removed from the oven and spun down until they reached 10000rpm to recover all of the liquid to the bottom of the tubes. The DNA from each sample was then put into the second microtube so that again there was one tube per sample. For each sample, 20µl of DNA with 40µl of TE buffer was transferred into wells in a quartz plate supplied by Spectramax. The plate was then placed into a spectrophotometer to analyse the samples. DNA concentration was estimated using spectrophotometery (GeneQuant, supplied by Amersham International). In this technique the absorption, measured as optical density (OD), of different wavelengths of ultra violet (UV) electromagnetic radiation are used to provide an estimation of concentration and purity. The reading gives an indication of the amount of protein in the sample. With OD at a wavelength of 260nm the absorption can be used to calculate DNA concentrations of 50ng/µl. However, the reading at 280nm gives an indication of the amount of protein in the sample. For example, pure preparations of DNA have OD ratios at 260/280 of 1.8. However, if this ratio is significantly less than the value given above, contamination prevents an accurate estimation of the nuclei acid concentration. Therefore, DNA

concentration and purity readings were highlighted and three readings were used to obtain a median figure to obtain a standard concentration of DNA (see DNA dilutions procedure below). The now quantified DNA samples were transferred into 2ml microtubes then scanned and placed in a freezer until Polymerase Chain Reaction (PCR) was performed (see below).

DNA Dilutions

Prior to genotyping the DNA samples, each sample was diluted and 50µl of stock was placed into microtiter storage plates as a preparation procedure for the PCR. First, the samples were mixed using a Vortex gene machine to retrieve all DNA that could have settled in the bottom of the tubes whilst frozen. The samples were then spun down to remove any DNA from inside the caps using a Sorvall biofuge. Calculations were performed to determine the amount of DNA and distilled water to add to the storage plates to make a total volume of 50µl of each sample. This was calculated based on DNA concentration readings. If the DNA concentration reading was 25ng/µl or less then 50µl of stock was used. However, if DNA concentrations were above 25ng/µl, then a concentration of 25 was multiplied by 50 and divided by the actual DNA concentration to determine the amount of water to be added to dilute the DNA. Plates were labelled, sealed and placed in a refrigerator until PCR was performed.

Polymerase Chain Reaction (PCR) procedure

PCR is a widely used technique that amplifies a specific segment of DNA of interest. For genomic DNA, following an initial prolonged denaturation step, the standard PCR employs three basic steps determined by temperature: Firstly, denaturation of double stranded DNA template, followed by annealing of two oligonucleotide primers, then extension of primers to produce the template copy.

The initial denaturation, that typically takes place at 93-95°C, is prolonged to ensure that the maximum amount of genomic DNA is denatured and therefore accessible to the binding of the complementary oligonucleotide primers. Subsequent denaturation steps take place at the lower limit of this range to preserve enzymatic activity. Following denaturation the reaction is cooled to allow annealing of the primers to the target DNA template. The primers are oligonucleotides, usually exactly

complementary to the margins of the target template, typically 20 bases long. The primer pairs should be selected to be specific, balanced regarding annealing properties and non-complementary to each other and themselves. A typical annealing temperature is 57 °C. After the annealing period the temperature is altered to the optimum for the DNA polymerase enzyme (which copies the DNA sequence) used, typically 72 °C. The DNA polymerase extends the primers in a 5' to 3' direction using four deoxynucleoside triphosphates (dATP, dCTP, dGTP and dTTP, in combination known as dNTPs) to form a complementary copy of the template DNA. The direction of polymerisation, combined with the specificity of the primers, enables the production of a specific PCR product. As such, the primers flank the template of interest and the polymerase extends one primer towards the other. In theory, the number of copies doubles each cycle, however in practice, a plateau is reached at around a million-fold amplification, which is determined by various factors including target strands competing with primer annealing and enzyme activity decline. The activity takes place in a magnesium-containing buffer, designed to provide the maximum specific product to which enhancers of the reaction may be added, for example, dimethyl sulfoxide (DMSO), glycerol and 7 deaza-dGTP. Consequently, once a reaction has been established, it then undergoes a process of fine-tuning during which the cycling parameters and other variables are adjusted to maximise specific product yield. Several types of thermal cyclers can be used, including those made by Hybaid, Applied Biosystems/Perkin Elmer and MJ Research.

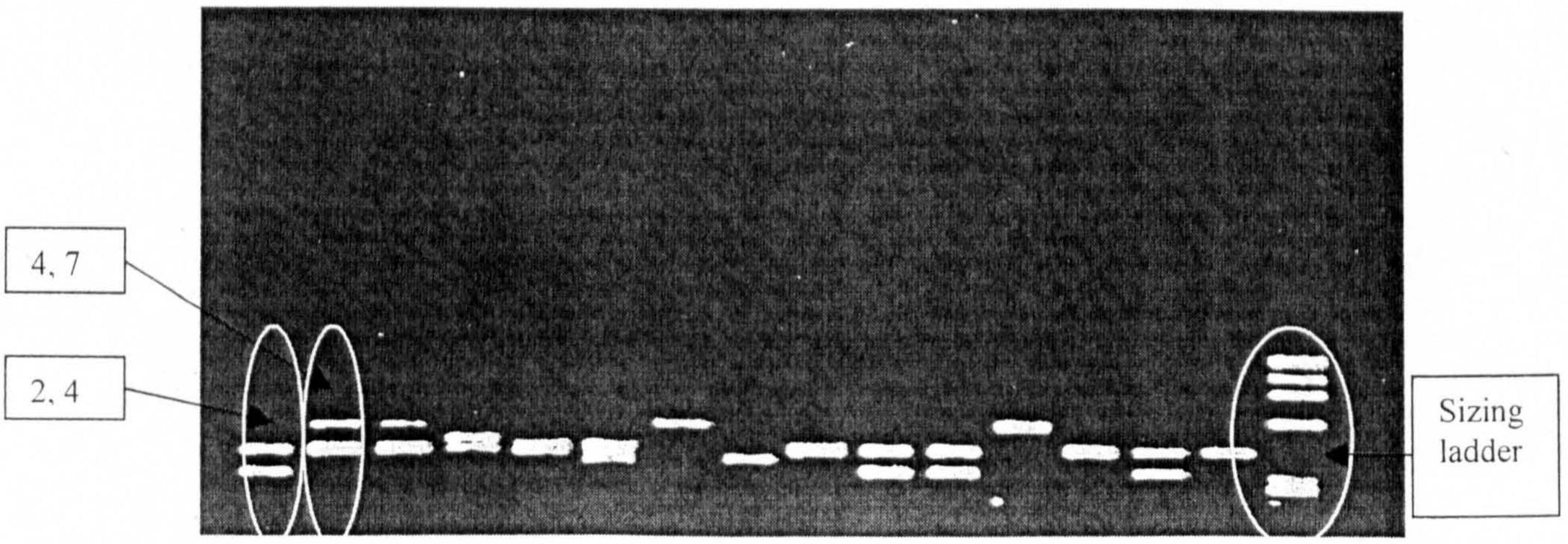
In the present study, DNA samples were removed from storage and variants located in exon 3 VNTR of the dopamine D4 receptor gene (DRD4) were genotyped simultaneously using PCR. The region containing the 48 base pairs (bp) repeat in exon 3 was of interest and therefore was amplified using primers D4-1 (5`- GGT CTG CGG TGG AGT CTG - 3') and D4-2 (5' - GCG ACT ACG TGG TCT ACT - 3'). From the DNA sequence, 20bp on both sides of the polymorphism (DNA differences between individuals) were synthesised. The PCR reaction was performed in a 20µl volume containing 100ng genomic template, 10pmol of each primer (D4-1 and D4-2) and 200 µmol/l of each dNTP (ATP, CTP, TTP, 50% DEAZA GTD, 50% GTP) (the building blocks). In addition, a Perkin Elmer buffer was used to enhance enzymatic activity, plus 1.5mls MgC12, 10% DMSO (detergent) and Taq (polymerase). After an

initial denaturation of 10 minutes at 95°C, the reaction was set at one minute at 55°C, one minute at 72°C, then 33 cycles of amplification at 55°C and a final extension step of 10 minutes at 72°C was performed in a PTC 200 MJ Research DNA engine. The 10 minutes of denaturation at 95°C provides a hot start to the reaction, which reduces a non-specific product and activates a specific product in the G-C rich region. These PCR conditions were determined by following the standard method used in the laboratory that previously had been tried and tested.

Agarose gel electrophoresis

Electrophoresis through agarose gels was used to separate DNA fragments by size, for the purposes of checking PCR products and analysis of restriction fragment length polymorphisms (RFLPs). 2% agarose gels were used to separate fragment length differences and fragments were visualised by ethidium bromide staining and UV transillumination. The results being recorded using a Polaroid camera (see figure 2.1). Allelic and genotypic frequencies were calculated by direct counting of genotypes and alleles then independently verified by a specialist. The genotype for each individual was categorised into short (S) (2-5 repeat) or long (L) (6-9 repeat) alleles on the gene. Genetic data was married up with the questionnaire and computer-task data and stored on a computer spreadsheet then analysed using SPSS statistical package and AMOS 4 graphics.

Figure 2.1. DRD4 alleles on a gel



Note to figure 2.1: The most common allele is 4. Shorter alleles are below the 4's as they run more quickly down the gel. Longer alleles are situated above the 4 markers. For example, from left to right, the first lane contains a 2, 4 genotype, the second and third 4, 7 genotypes and the forth 4, 5. The final lane on the far right side is the sizing ladder.

Statistical analyses summary

For study 1, a one-way between-participants ANOVA was used. The between-participants factor, group, had four levels: heroin, alcohol, smoker and control. The dependent variable was mean impulsive sensation seeking (ImpSS) scores. Hierarchical regression was employed with ImpSS as the criterion variable and predictor variables were group, age and mood status.

An unrelated design was employed in study 2, using independent samples t-tests with genotype, which had two levels (long or short alleles) as the independent variable and ImpSS as the dependent variable. A correlational design was used to determine associations with the dependent variable and other factors followed by standard multiple regression, with ImpSS scores as the criterion variable and genotype, mood, sex and age as potential predictors.

In study 3, a 2*4 Chi-square design was used to explore the association with genotype (long or short) and group (heroin, alcohol, smoker or control).

Study 4 employed a series of repeated measures ANOVA with stimulus type as the within-participants factor, which had two levels (heroin/neutral; alcohol/neutral; smoking/neutral words) and group as the between-participants factor, which had two levels (heroin/control; alcohol/control; smoking/control groups). Regression analyses were used with Reaction Time (RT) difference scores as the criterion variable. A mixed design was adopted using a 2*2 ANOVA where stimulus type (substance related/neutral) was the within-participants factor and ImpSS type (high or low) and genotype (long or short) were between-participant factors.

Finally, study 5 used structural equation modelling to identify causal pathways to addiction, with eight causal variables (alleles, ImpSS, Stroop RT difference scores, age, age first used substance, dependency age and mood status) included in the prediction of nicotine, heroin and alcohol dependence severity.

Chapter three

Study 1: Addiction and personality trait influences

Introduction

This study does not attempt to resurrect the theory that drug abusers can have an *addictive personality*. The addictive personality theory has been extensively studied in psychology and there appears to be no single underlying personality trait that on its own can make an individual became addicted to a substance. Other factors that are pertinent to the aetiology of substance abuse, including genes and environmental factors must also be considered. Therefore, this study focuses on a certain personality trait that may influence ones susceptibility to substance dependence, a factor that may influence ones vulnerability to addiction, rather than attempting to find a personality trait that *causes* addictive behaviour.

Personality is defined as an organisation of traits that characterise individuals (Zuckerman, 1999). Traits are relatively stable overtime although this does not imply that traits are consistent over responses and across all situations from one time to the next. For example, Sensation Seeking (SS) is a personality trait that diminishes with age, from around forty-five years a decline in individual sensation seeking levels can be seen. SS is particularly relevant to addiction research because it is a personality trait characterised by the individual having a general need for thrill and excitement, a need for novelty and change and a preference for unpredictable situations and friends (Zuckerman, Neary & Brutsman, 1970). These are characteristics that could bring about drug-seeking behaviour. Moreover, sensation seekers are impulsive people that act on the spur of the moment, without thinking or planning ahead. Sensation seeking correlates positively with Impulsivity (Zuckerman, 1974) and both traits appear to be mediated by shared genetic factors (Hur & Bouchard, 1997). Moreover, in a recent study of 400 alcohol dependent inpatients, smokers were found to be significantly more impulsive than non-smokers and ex-smokers (Skinner, Aubin & Berlin, 2004). High sensation seekers tend to become easily bored and require plenty of stimulation to keep their exploratory and novelty-seeking behaviours satisfied (O'Connor, et al., 1995). These people tend to have risky occupations such as fire fighting and are more likely to take up dangerous leisure pursuits such as bunjee jumping (Zuckerman, 1979).

Sensation seeking was introduced by Zuckerman, Kolin, Price & Koob (1964) and is measured using the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ), which has undergone several revisions. However, due to the link between sensation seeking and impulsivity, Zuckerman (1994) developed a scale that measured impulsivity and sensation seeking in combination, which he called Impulsive Sensation Seeking (ImpSS, Zuckerman, et al., 1993; Zuckerman, 1994). Impulsive sensation seekers look for novel and exciting experiences, acting impulsively, without thinking ahead (Zuckerman, et al., 1993).

ImpSS correlates significantly with Cloninger's Novelty Seeking (NS) (Zuckerman & Cloninger, 1996) and both traits are related to behaviours that are represented by a need for novel sensations and similar genetic bases have been put forward for both ImpSS and NS (Zuckerman, & Cloninger, 1996). Additionally, McCourt, et al. (1993) examined the relationship between the two measures of personality using a sample of alcohol and drug dependent patients seeking treatment for their addiction. Total sensation seeking scores were significantly correlated with total novelty seeking scores in the sample. As ImpSS is comparable to Cloninger's NS (Cloninger, Pryzbeck & Svrakic, 1987), the terms novelty seeking and sensation seeking are often used interchangeably.

Cloninger, et al. (1987) classified three personality traits that distinguish type 1 and type 2 alcoholics. Type 1 alcoholics show low novelty-seeking/sensation-seeking levels, high anxiety (harm-avoidance) and high dependence (on reward). Type 2 alcoholics are alcohol seekers with high sensation-seeking levels, low harm avoidance (fearless) and low reward-dependence (autonomous), drinking to reduce stress or anxiety. Type 2 alcoholics become more aggressive and show more features of anti-social personality than type 1 alcoholics (see table 3.1).

Table 3.1. Alcoholic subtypes and genetic risk of alcoholism (Cloninger, et al. 1987)

Characteristic Features	Type 1	Type 2
Usual age of onset	After 25 years	Before 25 years
Inability to abstain (alcohol seeking)	Infrequent	Frequent
Arrests when drunk (fighting/aggressive)	Infrequent	Frequent
Loss of control (psychological dependence)	Frequent	Infrequent
Guilt and fear about dependence	Frequent	Infrequent
Personality		
Novelty/Sensation seeking	Low	High
Harm avoidance	High	Low
Reward dependence	High	Low

Sensation seeking has been related to heavy drinking and early onset of drinking in adolescent and young adult drinkers (Zuckerman, 1994). Type 2 alcoholics score significantly higher on sensation seeking than type 1 alcoholics (Oreland, Hallman, Von Knorring & Edman, 1988). High SS/NS alcoholics tend to be sociable, self-confident and engage in risky behaviours, for example, drink drivers score higher on the sensation seeking scale than non-alcoholic drivers (Mookerjee, 1986). Chronic alcoholics score higher on sensation seeking than acute alcoholics (Malatesta, Sutker & Treiber, 1981) and Zuckerman (1999) reported that sensation seeking is associated with more aggressive, psychopathic type alcoholics, as predicted by Cloninger's typology.

Galen, Henderson & Whitman (1997) tested whether novelty seeking was related to alcohol dependence. They found that novelty seeking was significantly related to frequency of drinking and problem drinking with 140 adolescent psychiatric inpatients. In addition, Cloninger, Sigvardsson, Pryzbeck & Svvakic (1995) found that novelty seeking increases the likelihood that young people will start drinking and go on to develop drinking problems, in a study of 1019 American adults. Finally, Galizio, Gerstenhaber & Friedensen (1985) found an association with sensation seeking and alcohol use, that is, alcoholics were significantly higher sensation seekers than controls.

Stronger evidence for the association with the personality trait sensation seeking and substance use can be found amongst studies of drug users. High sensation seekers engage in risky and uninhibited behaviours. They are characterised by their need for novel sensations, which are obtained by taking risks such as drug taking. To illustrate this, Scourfield, Stevens & Merikangas (1996) examined substance use, sensation seeking and gender differences in 262 drug users and 261 relatives using a semi-structured interview technique and standardised personality scales. They found that substance abusers, fulfilling the criteria for a diagnosis of substance abuse under DSM criteria were significantly higher sensation seekers than a control group of participants with anxiety disorder. This finding was supported by a further study that found 80 opiate abusers demonstrated significantly higher sensation seeking levels than controls (Vukov, Baba-Milkic, Lecic, Mijalkovic & Marinkovic, 1995).

Masse & Tremblay (1997) examined the usefulness of measures of personality dimensions for predicting onset of smoking, alcohol consumption and drug use among adolescents. They found that high sensation seeking could predict early onset of substance use and therefore concluded that understanding vulnerable personality types is important for prevalence measures of substance use in schools. Opiate users have been shown to demonstrate higher levels of boredom when compared to other substance users. O'Connor, et al., (1995) divided drug using participants into categories according to their drug of choice. When compared to users of other drugs, opiate users scored significantly higher in their susceptibility to boredom. It could therefore be argued that opiate users require novelty to relieve excess boredom.

Luthar, Anton, Merikangus, & Rounsaville (1992) examined vulnerability factors to drug abuse in 132 siblings of opioid abusers. They found that sensation seeking significantly correlated with drug use and peers use of drugs. Kosten, Ball & Rounsaville (1994) assessed this relationship between sensation seeking and drug use in 201 opiate abusers and 133 siblings. Drug using siblings showed greater sensation seeking levels than non-drug using siblings did, which accounted for most of the variance in the regression analysis. More recently, a twin study on sensation seeking and marijuana use demonstrated a strong association with drug use and high sensation

seeking levels. High sensation seekers were more likely to use marijuana (Miles, Van den Bree, Gupman, Newlin, Glantz & Pickens, 2000).

Vukov, et al. (1995) compared personality dimensions of opiate abusers to a control sample of Yugoslav undergraduate students. They found that the opiate group demonstrated a specific personality profile, being impulsive, quick-tempered, disorderly, quickly distracted and easily bored. These traits demonstrate classic features of the sensation seeking personality type. A high prevalence of anti-social personality can also be found amongst drug abusers. Drug abusers tend to show poor sociability, impulsiveness, aggressive and sensation seeking personality types (Zuckerman, 1999). Anti-social personality traits precede and predict drug abuse. This can be illustrated by following cohort studies of Swedish and Canadian children from birth to 28 years of age. It has been found that high novelty seeking, low harm avoidance and low reward dependence ratings in childhood can predict substance use in adulthood (Cloninger, Sigvardsson & Bohman, 1988). Masse & Tremblay (1997) looked at teachers' ratings of novelty seeking and harm avoidance. They found those children who scored high on these measures, demonstrating a type 2 personality, at six and ten years of age could predict drug and alcohol use at 11-15 years.

Trull & Sher (1994) conducted a concurrent study of psychopathology and personality using Costa & McCrae (1992) five-factor model of personality and DSM criteria for substance dependence to build profiles of alcohol and drug dependence. They concluded that substance abusers could be characterised by high scores on openness and low scores on conscientiousness. Openness to experience was related to at least one type of sensation seeking (experience seeking). Conscientiousness had a negative, moderate correlation with impulsive sensation seeking (Zuckerman, et al., 1970).

This evident link between impulsive sensation seeking personality and substance dependence could be due to neurobehavioural factors. The mesolimbic dopamine pathway of the brain could be responsible for these high sensation-seeking levels found amongst drug abusers. This system of the brain, which includes structures such as the nucleus accumbens and amygdala, is involved in intrinsic reward properties that are produced by substances and exciting experiences (e.g. drugs and sex, Wise, 1998). Reward seeking may be one of the basic mechanisms involved in substance

dependence and could also be a function of an unregulated dopaminergic system (Stewart, et al., 1984), which will be discussed in more detail in chapter six. However, in support of the claim, a study was conducted with opiate abusers and their siblings. Participants completed the SS scale of the ZKPQ (Zuckerman, et al., 1993). Results yielded a strong association between high SS scores and opiate abuse. This study concluded that the early assessment of SS scores amongst individuals genetically susceptible to drug taking behaviour provides a tool for drug abuse prevention (Kosten, et al., 1994). Another family study, examining drug use vulnerability among opiate abusers, revealed a variety of risk variables for opioid abusers. Among these, high sensation seeking and familial history had a strong association with participants' drug taking behaviour (Luthar, et al, 1992).

In summary, impulsive sensation-seeking/novelty-seeking is a personality trait characterised by the individual having a need for thrill and excitement, a need for novelty and change and a preference for unpredictable situations. Substance abusers tend to be high sensation seekers so it could therefore be argued that substance abusers require novel sensations to relieve excess amounts of boredom. Sensation seeking behaviour may be a useful variable to predict individual differences in addiction behaviour. This relationship may be due to differences in the sensitivity of the mesolimbic dopamine system that makes sensation seekers more vulnerable to substance dependence.

The first study aims to examine personality trait influences on addiction. It is hypothesised that there will be an overall significant association with impulsive sensation seeking levels and addictive behaviour. This association will be examined within heroin abusers, alcohol abusers and cigarette smokers.

Method

One hundred heroin abusers and 100 alcohol abusers from outpatient treatment services and 100 smokers and 100 non-substance abusing controls from GP surgeries in London consented to participate in the study. All participants completed a demographic information sheet (descriptive data on the study sample are provided in tables 2.1 page 43, 2.2 page 47 and 2.3 page 49), the ImpSSS (Zuckerman, et al., 1993) and the POMS-SF (McNair, et al., 1981). Additionally, smokers completed the

QSU (Tiffany and Drobes, 1991), heroin abusers completed the SDS (Gossop, et al., 1995) and alcohol abusers completed the SDS and the SADQ (Stockwell, et al., 1979). For the purposes of subsequent studies (detailed in chapters 4-6) participants provided a sample of their DNA using a cheek swab kit and completed an emotional Stroop task on a laptop computer. Full details of the methodology used to test the hypothesis are detailed in chapter two (p 40-64).

Results

As predicted, high impulsive sensation seeking (ImpSS) levels were apparent amongst all substance using groups when compared to a control group who do not use any substances (see Table 3.2). One way ANOVA revealed significant differences between groups on their ImpSS scores $F(3, 396) = 26.473$, $p < 0.001$. Employing the Tukey HSD post-hoc test, significant differences were found between the heroin group and alcohol ($p = 0.002$), smoking ($p = 0.001$) and control ($p < 0.001$) groups. The control group were significantly lower impulsive sensation seekers than all other groups ($p < 0.001$). There were no significant differences in ImpSS scores between the smoking and alcohol groups ($P = 0.995$).

Table 3.2. Group differences between mean ImpSS levels.

Group	Total (n)	SS	S. D.	Range
Heroin	100	12.45	4.02	2-19
Alcohol	100	10.26	3.98	1-18
Smokers	100	10.11	4.65	1-19
Controls	100	7.09	4.44	0-17
Total	400	9.98	4.67	0-19

However, independent-samples t-tests revealed significant differences between groups with respect to their age and levels of mood (see chapter two). These possible confounding variables were entered into a hierarchical regression model. The results of the regression analysis with ImpSS score as the dependent variable are shown in table 3.3. At step one, group was entered into the model $F(1, 398) = 62{,}475$, $p < 0.001$, adjusted R square = 0.134, followed by age at step two $F(1, 397) = 58.282$, $p < 0.001$, adjusted R square = 0.223 and mood status in the final step $F(1, 396) =$

44.763, $p < 0.001$, adjusted R square = 0.248. Each predictor variable made significant independent contributions to the prediction of ImpSS levels, and together accounted for 25% of the variance. However model one, with group entered alone as a predictor, best predicted impulsive sensation seeking levels, above and beyond age and mood.

Table 3.3. Regression coefficient (beta values) with ImpSS scores as the dependent variable.

	Beta	P
Group	-0.352	0.0001
Age	-0.303	0.0001
Mood	0.174	0.0001

Discussion

In support of the hypothesis, an association between the personality trait impulsive sensation seeking and addiction behaviour was apparent in this study. It was found that heroin users were significantly higher impulsive sensation seekers than alcoholic, smoking and non-substance using groups, and the control group of non-substance users were significantly lower impulsive sensation seekers than all substance using groups. Despite the fact that possible confounding factors such as age and mood disturbance could not be controlled in this study, regression analysis revealed that group membership predicted impulsive sensation seeking levels, independent of these factors.

These findings are consistent with previous research that has compared sensation-seeking levels of substance users, with that of controls (e.g. Galizio, et al, 1985; Oreland, et al, 1986; Vukov, et al, 1995; Scourfield, et al, 1996 and Low & Genaszek, 2002) and could be explained from a neurobehavioural standpoint insofar as demonstrating the importance dopamine plays with the personality trait sensation seeking. Impulsive sensation seekers have a general need for thrill and excitement, enjoying change and preferring unpredictable situations. Impulsive people tend to act on the spur of the moment, without thinking or planning ahead, becoming easily bored and requiring plenty of stimulation to keep them satisfied. These sensation-

seeking characteristics can be likened to the pleasure and reward seeking properties that are governed by the mesolimbic dopamine pathway of the brain, which may be unregulated in substance users, which encourages them to seek out substances to satisfy their neurochemical drive for pleasure. For example, animal studies have consistently shown that novel experiences activate the mesolimbic pathway in the same way that substances of abuse can (Bardo, et al., 1996). Therefore, impulsive sensation seekers could be more likely to abuse addictive substances than low sensation seekers because they are tapping into the same chemical reward mechanisms. To illustrate this, Bardo and colleagues (1993) in a series of studies found that initially, rats preferred a novel environment, but by blocking dopamine receptors, this preference could be removed. They also found that when rats were placed in unfamiliar, novel environments, a marked increase in dopamine levels in the mesolimbic pathway was present (Bardo, Bowling, Robinet, Rowlett, Lacy & Mattingly, 1993).

Although these results cannot be directly extrapolated to human behaviour, they do give some insight into individual differences in sensation seeking behaviour and help to explain why impulsive sensation seekers are more prone to substance abuse than low sensation seekers. Indeed evidence from human studies can be used to support the findings from animal research. According to Gray (1987), a key indicator of impulsivity is a heightened sensitivity to rewards, and Powell, Al-Adwawi, Morgan, & Greenwood (1996) found significant increases in reward responsivity after bromocriptine (dopamine agonist) was administered to a group of brain injured participants. Reward sensitivity is measured using the BAS scale (Carver & White, 1994), which consists of three sub-scales that measure behavioural activity in terms of fun seeking, reward responsivity and drive variation. Using the BAS scale, evidence suggests, consistent with Gray's (1987) original theory, that sensitivity to reward, and therefore impulsivity, is associated with dopaminergic transmission, heightened motivation and positive mood (Carver & White, 1994; Powell, et al., 1996; Zelenski & Larsen, 1999; Kambouropoulos & Staiger, 2001).

An alternative explanation by Marvel & Hartmann (1986), pose an economic theory of addiction, arguing that people who abuse substances like alcohol or illicit drugs do so because they do not space out their intoxication's far enough apart to let their

moods return to baseline. The difference between substance abusers and people who do not abuse substances is the inability for substance abusers to tolerate discomfort such as depression, anxiety or boredom for a long enough period. That is, substance abusers require drugs to enhance dopamine release in order to produce pleasure and to avoid discomfort (withdrawal). Marvel & Hartmann (1986) conducted studies to test this theory and a strong positive relationship was found with sensation seeking and substance abuse.

Teichman, Barnes & Rahav (1989) conducted a prospective study in Israel and demonstrated using multiple regression analyses, that the only trait adding significance to the prediction of drug abuse was sensation seeking. Zuckerman, et al (1970) found that 74% of high and 23% of low sensation seekers reported ever having used an illegal drug, this finding was later supported by Kumar, Pekala & Cummings (1993) who reported 69% and 23% respectively. Ball, Carroll & Rounsaville (1994) added that the highest sensation seekers were polydrug users who could be characterised by the number and variety of drugs they used concurrently or over their life history, rather than by the particular type of drugs that were abused.

Findings do have implications for substance abuse treatments as it is suggested that novel experiences could be used as a substitute therapy for impulsive and high sensation seeking substance abusers. If a stimulating and rewarding alternative to harmful substance could be provided then this could reduce substance use among this sub-group of individuals. It has been found that high sensation seekers keep fewer treatment appointments and are less likely to stay in treatment than low sensation seekers (Zuckerman, 1999). This is of vital importance for treatment outcome studies because it demonstrates that it may not be the intervention that is failing treatment seeking substance abusers, but that the intervention is not appropriate for a sub-group of participants (i.e. high sensation seekers). Thus helping to explain why large proportions of substance abusers relapse during or shortly after treatment.

Helmus, Downey, Arfken, Henderson & Schuster (2001) also highlight the importance of the current findings to treatment factors in addiction. They recruited 68 cocaine users for a 29-week treatment programme. By measuring personality types of those in treatment, the authors found that high sensation seeking individuals were

more likely to drop out of treatment. High sensation seekers had a better retention rate for the first eight weeks of treatment but this effect reversed from the eighth week onwards, demonstrating high sensation seekers susceptibility to boredom. It was thought the reason for this was that a novel pharmacological treatment was used in the treatment programme, facilitating retention in the early part of the study. Likewise, Kravitz, Fawcett, McGuire, Kravitz & Whitney (1999) in their clinical trial found that high SS scores increased the odds of dropping out of alcohol treatment by 7%. Finally, Meszaros, Lezinger, Hornik, Fureder, Willinger, Fischer, Schonbeck & Aschauer (1999) found that high SS scores predicted relapse in detoxified alcoholics.

It has been shown consistently that a risk factor for poor treatment retention and relapse after an intervention is personality typology (reviewed in Zuckerman, 1999). By making treatment interventions more interesting and by introducing novel protocols to treatment for high sensation seeking substance abusers, it is possible that treatment retention could be improved for susceptible drug users. One example of this could be to identify the high sensation seeking population, then place them on a highly adventurous treatment programme, such as adventure therapy (a treatment used in American rehabilitation centres). Instead of receiving standard treatments such as relapse prevention techniques, one-to-one counselling and pharmacological substitution therapies, participants would encounter intensive activity such as pole jumping (as a confidence/self-esteem builder); active goal-directed teamwork and assort course activity (for problem solving). Trials of this kind of treatment would need to be carefully thought out and would therefore be expensive, requiring highly trained professionals to design and manage a protocol like the one described. Impulsive sensation seeking, treatment seeking substance abusers tend to drop out of treatment prematurely (Helmus, et al, 2001) and high sensation seeking levels predict relapse (Meszaros, et al, 1999). Therefore, a treatment programme tailored towards impulsive sensation seekers, like the one described could benefit substance abusers and treatment outcome statistics. Future research could focus on this issue by screening participants for their substance use and ImpSS levels, then placing them on individually tailored treatment protocols that could satisfy their sensation seeking needs. Therefore, it would firstly be important to recruit appropriate participants for the trial that are accurately diagnosed as high sensation seekers and secondly to include behaviour modification training to assist participants with maintaining their

high level of novel and stimulating activities when they graduate from the treatment programme. This would be beneficial in helping to prevent relapse, to prevent participants going from an intensive, stimulating activity, to a laborious, monotonous lifestyle, which they may have had prior to treatment.

These suggestions for improving relapse prevention rates in impulsive, high sensation seeking substance abusers should be viewed with caution. It could be argued that these implications have been overstated because high sensation seekers tend to be impulsive risk takers who become easily bored, so it could be the case that high sensation seekers will never be rewarded adequately and could switch from one risk-taking activity (e.g. drug abuse) to another (e.g. bungee jumping), continuing to seek out sensations, without ever becoming fully satisfied. The evidence that "cross-addiction" can occur (Gossop, et al., 2001; reviewed most recently in Haylett, Stephenson and Lefever, 2004) can support this argument. That is, as an addictive behaviour decreases, a compensatory increase in another arises. Therefore, adventure therapy and other treatment interventions may only satisfy the high sensation seeking drug abuser's need for pleasure momentarily (Stephenson, Maggi, Lefever and Morojele, 1995).

The issue concerning appropriate screening for high sensation seekers relates to a potential limitation of this present study and that is the use of a self-report instrument to measure impulsive sensation seeking. The disadvantages of using this type of design are that firstly there is no universally accepted measure of impulsive sensation seeking, although the one used for the study has been shown to have high reliability and validity, and correlates with related instruments (e.g. TPQ NS scale). In addition, participants may lack insight into their own behaviour patterns and so be unable to accurately report their behaviour. Moreover, because these instruments tend to ask direct questions about the behaviour in question this could lead to a response bias. Therefore, it may be beneficial for future research to consider the use of both self-report and laboratory based tools to tap into individual sensation seeking and risk taking behaviours witnessed among substance using individuals to supply a more stringent assessment of behaviour. Future directions could also differentiate types of drug use (e.g. whether marijuana users differ from amphetamine users), and examine

impulsive sensation seeking among non-substance addictions (e.g. whether pathological gamblers and compulsive shoppers are comparable to substance abusers).

To overcome the shortfalls of using self-report instruments to measure risk taking and impulsive behaviour among substance abusers, researchers have begun validating alternative tools, including a laboratory-based behavioural measure of risk taking, called the Balloon Analogue Risk Task (BART) (Lejuez, Read, Kahler, Richards, Ramsey, Stuart, Strong & Brown, 2002). This task allows participants to make decisions about taking risks that are similar to real world situations. Risk-taking behaviour is rewarded up until a point to which further risk taking would lead to a poorer outcome on the task. Lejuez, Aklin, Richards, Strong, Kahler & Read (2003) employed the BART to examine risk-taking behaviour among smokers. They found that the sensation seeking scale and the BART contributed to the differentiation of smokers and non-smokers. Another task that has recently been developed, validated and applied to measure a key indicator of impulsivity, is the Card Arranging Reward Responsivity Objective Test (CARROT, Powell, et al., 1996). This is a behavioural task that is used to measure participant's responsiveness to financial rewards. As quickly as possible, participants are instructed to sort 100 cards into three numbered trays that correspond with the numbers printed on the cards. Of the four trials, during the third, participants are told that they will be offered a monetary reward for every five cards sorted. Reward responsivity is measured by any increase in the number of cards sorted during that rewarded trial. These tools are implicit measures of impulsiveness and risk taking. The present study found that heroin abusers scored significantly higher than alcoholics, smokers and controls on a self-report, explicit measure of impulsive sensation seeking. Controls scored significantly lower on the ImpSSS than all of the other groups, regardless of the group differences in age and mood. This chapter has argued that individual differences in dopaminergic brain activity may be able to explain this trend.

Chapter four

Study 2: Personality and genetic influences

Introduction

The view that personality is partially determined by genes is now well established (see Heath, Cloninger & Martin, 1994 for a review). This can be illustrated by reviewing a huge body of literature on classic gene studies. These studies have concluded that around half of the variance on personality tests conducted with twins reared apart is due to genetic factors (Bouchard & McGue, 1990). Individuals may pick and choose their environment and events depending on their genotype. That is, people may help to create their own environment and how they respond to stresses and stimuli within it.

Traditionally, behavioural genetics research employed three methods to investigate genetic influences involved in human personality differences. The first, family studies have been used to compare relatives of probands with a disorder, with control participants without the disorder. If the percentage of disordered relatives of the disordered proband is higher than that of control relatives, without the disorder, it can be concluded that the disorder is familial. This method does not however manage to separate environmental factors that may confound results. Therefore, adoption studies are used to disentangle genetic factors from environmental influences, as one parent provides the genes and the other provides the environment. The final classic method used in genetic studies is the twin study method. Monozygotic (MZ) twins are genetically identical whereas Dizygotic (DZ) twins only share about half of their DNA. If MZ twins are reared apart, then they only share the same genes so it could be argued that environmental influences could be separated from the analysis. If a relationship with genes and personality is identified in twin studies, then the relative contribution of genes and environment can be calculated.

However, Joseph (2001) reviewed classic research on genes and personality and provided a harsh critique formed on the basis of the methodology employed in classic studies, with an emphasis on MISTRA (Minnesota study of twins reared apart, Bouchard & McGue, 1990). He argued that such studies did not support the existence of a genetic component in personality differences. Overall, it was argued that volunteer-based studies are biased toward greater twin similarity and that evidence

from twins reared apart does not support the role for genes in personality and behavioural dimensions generally.

The major methodological flaws of classic studies are that, twins are not separated straight from birth so, in fact, share the same environment, are raised by other family members or they are placed with families of similar socioeconomic status (SES) as their biological parents. In addition, the twins are often aware of each other and have had previous contact. In addition, raters who assess twins are often not blind to study variables, which could cause bias to the study. It has been found that 90% of MZ twins reared apart are actually reared in the same or similar environment, so it is often not the case that twins environments are in fact different, as is often assumed (Faber, 1981).

In support of these claims, twins that have been used in classic gene studies are discovered on the basis of similarity and therefore have knowledge of each others existence so therefore are not representative of separated twins forming a similarity bias in the studies. Furthermore, previous studies have failed to provide case note data or share information of the twins. In the MISTRA study, there were problems with reported frequencies of contact between twin pairs. For example, with twenty weeks contact being reported, huge differences can be seen on personality influences. That is, if the twin pair spent those twenty weeks together in the first twenty weeks of their lives, compared to if they had spent one week of the year together, every year, over twenty years of their lives (Joseph, 2001).

Joseph (2001) continues to argue that twins reared apart and together have a lot more in common than their genes. Important environmental factors that should be considered are that twins used in these studies are of the same age, sex and ethnicity. They are of similar appearance and attractiveness. They share the same pre-natal environment and typically spend time together so are probably treated quite similarly. It could be that it is the environment adapting their personalities as opposed to their genes, and it is essential to consider these factors when conducting such research.

According to Joseph (2003), an appropriate control group for twins would have to be biologically unrelated pairs of strangers, matched to the twins for age, sex, ethnicity,

appearance and attractiveness. Controls would also have to be from the same culture and social background. There would have to be no differences between the two groups other than their genetic make-up and pre-natal environment. Joseph concludes that twin studies are sufficiently flawed that no conclusions about the role of genetic influences on human personality and behaviour can be drawn from them.

However, in defence, Plomin & Colledge (2001) highlight that when classic gene studies are conducted, they are not simply attempting to show the degree to which genes influence personality, but these studies also shed light onto the equal importance that environmental factors play in personality. That is, when behaviours are found to be 50% genetically influenced, the fact that the other 50% of the variance must be non-heritable is discovered. Thus leaving psychologists the task of unravelling the complex role that shared and non-shared environmental factors play in personality, and how these non-genetic factors interact with genetic factors.

The extent to which genetics influence the variation in personality is no longer limited to time consuming and expensive classic studies because psychologists can now use new tools to identify specific genes that are responsible for personality. Molecular genetic studies have been employed and associations with genes and personality have been identified. These methods are advantageous, as they not only show the extent of genetic variation, but also:

> *"...In the case of correlations between DNA variants and behaviour, the behaviour of individuals does not change their genome. Expression of genes can be altered but the DNA sequence itself does not change. For this reason, correlation's between DNA differences and behavioral differences can be interpreted causally."* [Plomin & Colledge, 2001, p236.]

Sensation seeking and DRD4

The DRD4 gene has received the most attention in the literature for its association with personality and particularly the sensation-seeking (SS) trait. This is due to information that dopamine is a major neuromodulator of individual differences in personality traits that encompass novelty-related behavioural tendencies and sensitivity to signals of reward (Le Moal & Simon, 1991; Depue & Collins, 1999). Variants at the D4 dopamine receptor gene have been associated with the personality

trait novelty seeking (NS), which is equivalent to Zuckerman's impulsive sensation seeking trait (ImpSS, Zuckerman & Cloninger, 1996) so the term sensation seeking alone is predominantly used here in order to minimise textual rambling.

High sensation seekers seek thrills, which they do by taking risks. The association between the DRD4 gene variants and sensation seeking levels has been examined in a normal, healthy sample of non-drug users. An association of high sensation seeking levels and the 7-repeat (long) allele on the DRD4 gene was found in a group of 124 unrelated Israeli subjects, regardless of their age, sex, or ethnicity. Although sensation seeking decreases with age, age did not effect the results in this study as the mean age of subjects was young (29 years) (Ebstein, et al., 1996). Benjamin, et al. (1996) also reported this association in a population of 315 family members. They confirmed the result that there is an association between long alleles on DRD4 and high levels of sensation seeking. From these studies it can be concluded that the association is the result of a direct relationship between the gene and the personality trait, rather than population stratification (e.g. having unmatched controls). Paired-sample t-tests revealed that the siblings with long-repeat alleles had significantly higher sensation seeking scores than did siblings from the same family with short-repeat alleles.

The number of studies that have examined the association between DRD4 gene variants and SS/NS is growing rapidly, but to the author's knowledge, the number of studies to date exceeds 40 studies (reviewed most recently in Prolo & Licinio, 2002). These studies have examined the DRD4 gene and its association with sensation seeking, however mixed ethnic groups (Caucasian, Asian, African) and various instruments to measure the personality trait have been used, thus providing controversial results that are difficult to compare. Half of these studies to date have found significant associations with the long-repeat genotype at DRD4 and high SS levels. Ebstein et al (1996) used the Tridimensional Personality Questionnaire (TPQ), Novelty Seeking scale (NS) (which is comparable with Zuckerman's ImpSS scale) to measure personality among staff and students, which was devised by Cloninger, et al. (1987) to measure NS as a heritable component of human temperament. Noble, et al. (1998) also used the TPQ with a sample of Caucasian high school members. These significant results have been found mainly using opportunity samples of staff and students at the research institution where the studies have been conducted, although a

fair number of studies have employed substance abusing volunteers (see Table 4.1. for a summary of these studies).

Associations with sensation seeking and DRD4

Benjamin, et al (1996) found a significant association with the DRD4 long variant (6-8 repeats) and NS using the NEO-PIR. Three hundred and fifteen, healthy volunteers, participated in the study (92% Caucasian; 95% male) and data was statistically corrected for age, sex, ethnicity and educational level. The following year, Ono, et al. (1997) also found an association with the DRD4 long alleles (5-8 repeats) and sensation seeking (measured using the TCI, NS scale; Cloninger, Svrakic & Pryzbeck, 1993). The sample consisted of 157 Japanese, female students (mean age 18.7 years). Ebstein, Nemanov, Klotz, Gritsenko & Belmaker (1997) later found additional evidence for the DRD4 and its association with personality. They used 94 healthy, Israeli staff and student volunteers, commenting that failure to replicate these association studies may be a result of noise from methodological differences between studies obscuring a weak effect of this gene on personality.

Noble, et al (1998) measured NS using the TPQ in association with the DRD4 7-repeat variant amongst a sample of 119 healthy Caucasian boys (mean age 12 years). They found a significant association and concluded that it was more apparent in the boys who also had a DRD2 variant. Ekelund, Lichtermann, Jarvelin & Peltonen (1999) administered the TCI to 4,773 Finnish volunteers and took 100 people (50% male) with the highest scores and 100 (50% male) with the lowest scores to compare their genotypes. The authors found a significant difference between groups. Those who scored high on NS were significantly more likely to carry the variant (6-8 repeats) at DRD4. Finally, this large cohort study offered a more robust method of examining the association and it also controlled for variables such as psychiatric history, substance history and sex.

Table 4.1. Summary of association studies examining DRD4 polymorphisms and sensation seeking traits.

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
George, et al. 1993	72 male & female Caucasian alcoholics with a mean age of 40.5 years	Clinical interview	DRD4 exon 3 repeats	Unclear significance due to problems with the control group
Adamson, et al. 1995	226 Finnish male alcoholics & controls	Clinical interview	DRD4 exon 3 repeats	No association
Benjamin, et al. 1996	315 male (n=300) & female normal volunteers (290 Caucasian) with a mean age of 32.4 years	NEO-PI-R	Short versus long DRD4 exon 3	Significant association Long allele ($p = 0.002$)
Ebstein, et al. 1996	114 male & female Jewish normal volunteers with a mean age of 29.8 years	TPQ (NS)	Presence/absence of the 7-repeat allele at DRD4	Significant association Presence ($p = 0.013$)
Muramatsu, et al. 1996	655 alcoholics & 114 controls, Japanese men & women with a mean age of 50 years	Clinical interview	DRD4 exon 3 repeats	Significant association Alcoholics only ($p < 0.001$)

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Malhotra, et al. 1996	331 male Finnish alcoholics & controls with a mean age of 32 years	TPQ (NS)	7 repeat allele at DRD4	No association
Ebstein, et al. 1997	120 Israeli male & female healthy volunteers with a mean age of 30 years	TPQ (NS)	Long repeat at DRD4 & 5-HTTLPR variant	Sample size too small to detect such effects (n = 6)
Chang, et al. 1997	127 Chinese alcoholics & controls	Clinical interview	DRD4 exon 3 repeats	No association
Garpenstrand, et al. 1997	49 male & female Caucasian psychiatric Pts.	SSS-Total score	DRD4 exon 3 repeats	No association
Geijer, et al. 1997	139 Swedish alcoholics & controls	Clinical interview (NS)	DRD4 exon 3 repeats	No association
Kotler, et al. 1997	251 male Israeli heroin abusers & controls with a mean age of 39 years	TPQ (NS)	7 repeat allele at DRD4	Significant association Heroin abusers only (p = 0.02)
Jonsson, et al. 1997	126 male & female Swedish controls (41 yrs)	Karolinska Scales of Personality (KSP) (NS)	DRD4 exon 3 repeats	No association

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Sander, et al. 1997	252 male Caucasian alcoholics with a mean age of 41.9 years	Clinical interview	DRD4 exon 3 repeats	No association
Vandenbergh, et al. 1997	200 male & female elderly (mean age 61 years) Caucasian controls	NEO-PI-R	7 repeat allele at DRD4	No association
Li, et al. 1997	121 male & female Chinese heroin abusers mean age 27 years	Clinical interview (NS)	Long versus short allele at DRD4	Significant association $P < 0.002$.
Ebstein, et al. 1997	94 healthy Jewish volunteers	TPQ (NS)	7 repeat allele at DRD4	Significant association $P < 0.001$.
Ono, et al. 1997	153 female Japanese healthy volunteers with a mean age of 18.7 years	Tridimensional Personality Inventory (TPI) (NS scale)	DRD4 exon 3 repeats	Significant association $P = 0.045$.
Gelernter, et al. 1997	341 American healthy controls aged 35 years	TPQ	DRD4 exon 3 repeats	No association

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Ebstein, et al. 1998	81 male & female 2-week old Jewish babies	Neonatal Behavioural Assessment Scale (NBAS)	7 repeat allele at DRD4	$P = 0.02$.
Noble, et al. 1998	119 healthy Caucasian boys (aged 12 years)	TPQ (NS)	7 repeat allele at DRD4	Significant association
Sullivan, et al. 1998	267 male & female Caucasian alcoholic & depressed participants mean age 36 years	Temperament & Character Inventory (TCI) (NS scale)	7 repeat allele at DRD4	No association
Benjamin, et al. 1998	124 male & female Jewish healthy volunteers with a mean age of 29.7 years	TPQ (NS)	DRD4 exon 1 repeats	No association
Pogue-Geile, et al. 1998	92 MZ & 61 DZ male & female American twins aged 18-27 years	SSS-Total score TPQ (NS) NEO	Presence/absence of the 7-repeat at DRD4	No association
Jonsson, et al. 1998	167 Israeli men with a mean age of 56.7 years	KSP	DRD4 exon 3 repeats	No association

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Ricketts, et al. 1998	95 male & female Caucasian Parkinson's disease patients & 47 controls (mean age 68 yrs)	TPQ (NS)	7 repeat allele at DRD4	No association
Mel, et al, 1998	143 male Israeli controls & 57 heroin abusers with a mean age of 32 years	TPQ (NS)	7 repeat allele at DRD4	Significant association $P = 0.001$.
Auerbach, et al. 1999	76 Israeli 2-month old male & female babies	Infant Behaviour Questionnaire (IBQ)	7 repeat allele at DRD4	Significant association $P = 0.005$.
Strobel, et al. 1999	136 German male & female healthy volunteers mean age 23.6 years	TPQ (NS)	7 repeat allele at DRD4	Significant association $P = 0.001$.
Ekelund, et al. 1999	200 Finnish controls (50% female, 33 years)	TCI	2-, 3-, 4-, 5-, 6-, 7-, 8-repeat alleles at DRD4	Significant association $P = 0.03$.
Kuhn, et al. 1999	120 healthy male German students (20-30 years)	TCI	7 repeat allele at DRD4	Significant association

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Bau, et al. 1999	110 Caucasian male alcoholics with a mean age of 41 years	NS	DRD4 exon 3 repeats	No association
Tomitaka, et al. 1999	69 Japanese healthy students (mean age 25 yrs)	TCI	Long alleles at DRD4	Significant association
Gebhardt, et al. 2000	109 male & female Austrian volunteers with a mean age of 32.5 years	TCI	2-, 3-, 4-, 5-, 6-, 7-, 8-repeat alleles at DRD4	No association
Benjamin, et al. 2000	455 healthy Israeli volunteers	NS	7 repeat allele at DRD4 & 5HTTLPR short allele	Significant association
Persson, et al. 2000	330 male & female Swedish controls	NEO-PI-R (extraversion)	DRD4 exon 3 repeats	No association
Swift, et al. 2000	47 healthy volunteers	NS	Presence/absence of 6-8 repeats at DRD4	No association
Okuyama, et al. 2000	86 healthy Japanese, male students with a mean age of 22.8 years	TCI	CC genotype at DRD4 5' promoter region	Significant association

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Bau, et al. 2001	226 Brazilian male alcoholics	NS	7 repeat allele at DRD4 & DAT 1 10-repeat	Significant association $P < 0.0005$.
Mitsuyasu, et al. 2001	173 Japanese healthy volunteers	TCI (NS)	DRD4 exon 3 repeats	No association
Ronai, et al. 2001	109 Hungarian female healthy volunteers	NS	CC genotype at DRD4 5' promoter region	Significant association $P < 0.01$.
Strobel, et al. 2002	276 (205 female) German healthy volunteers aged 18-41 years	TPQ (NS)	DRD4 exon 3 repeats & -521C/T polymorphism in promotor region of DRD4	No association
Burt, et al. 2002	137 families (n = 348) 97% Caucasian	Multidimensional Personality Questionnaire (MPQ-NS)	DRD4 exon 3 repeats & presence/absence of A1 allele at DRD2	No association
Kluger, et al. 2002	Meta-analysis (n = 3907)	Various	DRD4 or polymorphism	No association on average
Soyka, et al. 2002	181 German alcoholics (43 female, mean age 40.6 years)	TCI (NS) NEO-FFI Sensation Seeking Scale	DRD4 exon 3 repeats	No association
Jonsson, et al. 2002	Swedish healthy controls	TCI (NS) & NEO-PI	DRD4 exon 3 repeats	No association

Table 4.1. Continued...

Authors of the study	Participants	Personality measure used	Polymorphism	Significance
Lakatos, et al. 2003	90 Hungarian children aged 12 months	IBQ & observed response to novelty	7 repeat allele at DRD4 5-HTTLPR short allele	Significant interaction
Strobel, et al. 2003	115 healthy German twins 86 female aged 18-67 yrs	NEO-FFI NS and Extraversion	DRD4 exon 3 repeats & DRD4 -521 T allele	No association
Keltikangus-Jarvinen, et al. 2003	154 (50% female) Finnish controls aged 20-35 years	TCI (NS)	DRD4 exon 3 2- or 5- repeat allele	Significant association $P = 0.026$.

Strobel, Wehr, Michel & Brocke (1999) conducted an association study with 136 healthy German, Caucasian volunteers (48 men), using younger participants to overcome shortfalls from previous comparable research (mean age 23 years). They found an association with the dopamine D4 receptor gene and sensation seeking using the TPQ. Looking at presence versus absence of the 7-repeat allele, they found that high novelty seekers were significantly more likely to have the long variant, accounting for 8% of the variance in personality. Thus indicating that other genes are involved in the genetic transmission of temperament as classic gene studies have commonly found around 50% of the variance accounted for by genes (Heath et al, 1994). Plomin, et al (1994) explain heritability as a statistical estimate that describes the proportion of phenotypic variance in a population that can be attributed to genetic influences. Heritability ranges from 40-50% for personality (and about 30% for addiction, see chapter five for detail).

Bau, Almeida, Costa, Garcia, Elias, Ponso, Spode & Hutz (2001) examined the dopamine receptor gene (DRD4) 7-repeat allele and the DAT1 10-repeat, a dopamine transporter gene, as modifying genes in alcoholism. They also examined how sensation seeking and level of alcohol consumption may interact with these genes. Both genes were examined simultaneously as they are implicated with SS behaviour (Strobel, et al, 1999). Among 114 alcoholics and 112 controls from Brazil, the authors found a significant effect with the DRD4 7-repeat allele, the DAT1 10-repeat and sensation seeking on level of alcohol consumption. The DRD4 gene accounted for 6.6% of the variance ($p = 0.0005$) and the DAT1 gene accounted for 4.9% of the variance ($p = 0.002$).

Multiple genes appear to be responsible in influencing human personality, for example, the -521 C/T polymorphism in the promoter region of DRD4, COMT (Cathecol-O-Methyltransferase) gene and the promoter region of the serotonin transporter gene 5-HT-TLPR have all been implicated (Benjamin, Osher, Belmaker & Ebstein, 1998). Bau et al (2001) investigated the DAT1 10-repeat in the 3' region of the transporter gene. They claimed that genes such as this might influence the association with the DRD4 long variant and SS. This association was also found amongst a sample of 190 healthy male students (aged 20-30 years) in Germany (Kuhn, Meyer, Nothen, Gansicke, Papassotiroponlos & Maier, 1999).

Benjamin, Osher, Kotler, Gritsenko, Nemanov, Belmaker & Ebstein (2000) examined the DRD4 association with 455 normal, healthy volunteers recruited from a staff and student population in Israel. They found an association with the DRD4 7-repeat allele and sensation seeking and concluded that SS is strongly expressed in those participants who lack the short allele of the 5-HTTLPR gene and have the D4 long allele variant (6-8 repeats). Benjamin et al (2000) argue that COMT is another gene that may play a further role in modulating the effects of both the DRD4 and 5-HT-TLPR genes on personality and that both dopamine and serotonin dually mediate SS behaviour.

Evidence from a Japanese population of 69 healthy medical students (mean age 25 years) revealed a significant association with SS and DRD4 long repeat using the TCI (Tomitaka, Tomitaka, Otuka, Kim, Matuki, Sakamoto & Tanaka, 1999). Moreover, Okuyama, Ishiguro, Nankai, Shibuya, Watanabe & Arinami (2000) examined the DRD4 association with 86 normal, healthy Japanese, male students (mean age 22.8 years) and found a significant association with the personality trait SS and DRD4 in the 5' promoter region. Ronai, Szekely, Nemoda, Lakatos, Gervai, Staubt & Sasvian-Szekely (2001) replicated this study using 109 Hungarian participants. They also found a significant association with high SS and the CC genotype in the 5` promoter region of the DRD4 gene, especially among women ($p < 0.01$), thus helping to explain discrepancies found with these studies, that could be explained by gender differences (Ebstein, et al, 1997). Ronai, et al (2001) claim that further work is in progress, using a larger sample to investigate the genetic interaction within the DRD4 gene long variant and other genes that have been shown to influence sensation seeking behaviour.

Lack of association with sensation seeking and DRD4

In contrast to these significant findings, as illustrated in Table 4.1, the other half of these association studies have failed to support the association with the DRD4 gene and SS. For example, Gelernter, Kranzler, Coccaro, Siever, New & Mulgrew (1997) examined the DRD4 gene and sensation seeking in 341 American participants. Failure to find an association may have been due to ethnic differences among the sample as 65.3% were African and 34.7% were of European decent. In addition, the mean age of

participants was 35.4 years, thus being older than participants in studies that have found significant associations (e.g. Okuyama, et al, 2000). Gelernter et al (1997) from their study, concluded that failure to replicate previous associations with DRD4 and SS could not be due to sample size (large sample of 341), or the use of different personality measures (TPQ adopted, as in Ebstein et al (1996) who did detect an association). Therefore, results may have been due to linkage disequilibrium. That is, another gene close to DRD4 may be responsible for significant associations in previous studies (COMT- Benjamin et al, 2000, 5' promoter region- Okuyama, et al, 2000), rather than the DRD4 long variant having a direct influence on personality.

Sander, Harms, Dufeu, Kuhn, Rommelspacher & Schmidt (1997) also failed to detect an association with DRD4 and SS. They used 197 German blood donors and compared their SS scores to 252 alcohol dependent volunteers. In the same year, Ebstein, Gritsenko, Nemanov, Frisch, Osher & Belmaker (1997) examined TPQ scores in association with DRD4 amongst 120 Israeli staff and students members. Jonsson, Nothen, Gustavsson, Neidt, Brene, Tylec, Propping & Sedvall (1997) used 126 Swedish, healthy unrelated volunteers (mean age 41 years) and also failed to observe an association. This result could have been due to the older age of participants, compared to significant association studies that used younger participants. As sensation seeking diminishes with age, failure to detect an association may be due to the sample having lower sensation seeking scores as they were older, rather than due to genetic factors. In addition, Vandenbergh, Zonderman, Wang, Uhl & Costa (1997) failed to find an association with DRD4 and SS but their sample was also made up of older participants.

The association with SS and the DRD4 gene variant has been examined with different populations. For example Sullivan, Fifield, Kennedy, Mulder, Sellman & Joyce (1998) used patients in treatment for alcohol dependence and depression. The lack of association could have been due to the mood of participants, altering their personality scores. Jonsson, Nothen, Gustavsson, Neidt, Forslund, Mattila-Evenden, Rylander, Propping & Asberg (1998) used a different measure, the Karolinska Scales of Personality (KSP), to measure sensation seeking. The KSP was used with 167 Israeli men (mean age 56.7 years). Furthermore, this study may have failed to find an

association not simply due to the measure of personality but also participants were older and only men participated in the study.

Pogue-Geile, Ferrell, Deka, Debski & Manuck (1998) conducted a twin study with 306 same sex twins (mean age 21.6 years, 56% female, 97% white). They failed to find associations with the DRD4 7-repeat allele and SS. This could have been due to their method of analysing DRD4 as previous studies have compared short (2-5) versus long (6-8) alleles on the gene, rather than limiting the association to presence or absence of the 7-repeat allele.

Malhotra, Virkkunen, Rooney, Eggert, Linnoila & Goldman (1996) found an association with high SS and the D4 gene amongst a sample of 138 alcoholics, but the association was in the opposite direction. Finally, Bau, Roman, Almeida & Hutz (1999) failed to detect the association amongst a sample of 110 Caucasian male alcoholics. However, it could be argued that this non-significant result was due to several methodological factors. Firstly, all participants were men and this association may be more prominent in female participants. Secondly, the mean age of these men was 41 years, which is around the age that SS levels start to decline. Finally, this association has not been demonstrated with alcohol dependent populations, so it could be that the genetic association with personality is in fact substance specific or predominant in healthy populations.

Although mixed gender and ethnicity groups have been examined and similar instruments have been employed to measure SS, it appears that the differences between studies may be due to demographic factors such as age and gender. These are important factors in allelic association studies. For example, Vandeburgh et al (1997) failed to detect an association with the long-repeat genotype and SS, compared to Benjamin et al (1996) who did find a significant association. Both studies administered the same personality scale to white Americans, but the significant association was only found in the Benjamin et al (1996) study that consisted of young men. One explanation for this inconsistency in findings is that participants were younger in the Benjamin et al study (mean age 32 years) compared to participants in the Vandeburgh et al study (mean age 61 years) as SS declines with age. Alternatively, inconsistency in findings could be due to the assertion that DRD4 and

SS associations are more prominent in men than in women (95% men in the Benjamin et al study compared to 56% men in the Vandeburgh et al study).

Paterson, Sunohara & Kennedy (1999) offer several reasons as to why some studies have failed to find a significant association with sensation seeking and the DRD4 gene variant. Firstly, it could be due to the presence of another nearby association (i.e. linkage disequilibrium, whereby another stretch of DNA is responsible for the effect). Furthermore, lack of replication could be due to population stratification (i.e. having a mixture of different ethnic groups in the sample or unmatched controls), or because a small effect is present, but there is not enough power to detect the effect (due to small sample sizes used in this research). Finally, failure to replicate could be explained as due to inconsistent measures of the personality trait being employed in these studies. It is therefore crucial in this research when examining associations with genes and personality to use mixed gender groups with younger participants and comparable tools to measure the personality trait.

To summarise, despite advances in modern molecular genetics techniques, classic methods are still important, as the problem with complex traits is that they are probably due to a combined action of many different gene variants and a variety of environmental factors, all interacting to varying degrees. The challenge is to detect the genes that are responsible for small effects. Classic studies can aid this search by being a valuable supplement to modern techniques. They can steer association studies toward disorders that are most heritable and when genes have been identified, twin studies can provide descriptive models to trace the pathway from the molecular level to behavioural level.

Classic studies can help to identify the likelihood of an individual with a genetic vulnerability developing a disorder, depending on environmental risk factors. Family studies are also important in determining these environmental factors and twin studies can demonstrate the degree that shared and non-shared environmental factors can influence treatment outcome and intervention planning. Twin studies would be useful in assessing differences in reactions to environmental stimuli and exposure to environmental factors. For example, if a twin has a genetic vulnerability to a particular personality type, non-shared environmental factors could be examined that

may lead only one twin with the vulnerability to a particular behaviour, despite both twins sharing the same genes and shared environment.

Modern association studies, despite research carrying some methodological pitfalls, are of benefit to our understanding of personality as they provide the means for psychologists to detect genes responsible for complex behavioural mechanisms. By understanding the genetic basis of normal personality, research can lead to the discovery of some of the genetic and environmental reasons why individuals develop disorders and respond in different ways to environmental factors. Finding how much genetic factors effect personality is important in understanding the origin of individual differences and the influences this has on shared and non-shared environmental factors that interact with personality. Furthermore, identifying the etiological links between normal and abnormal personality and to discover the nature-nurture interplay will increase the objectivity of personality research. This study aims to overcome some methodological shortfalls, that have previously undermined research examining the genetic basis of personality, by using large samples of younger participants, controlling for age, sex and ethnicity, ensuring that appropriate measures of personality that are comparable across studies are employed.

Therefore, the second study examined the association with the dopamine D4 receptor gene (DRD4) and the personality trait sensation seeking. It is hypothesised that there will be a significant association with the DRD4 gene and impulsive sensation seeking levels.

Method

One hundred heroin abusers and 100 alcohol abusers from outpatient treatment services and 100 smokers and 100 non-substance abusing controls from GP surgeries in London consented to participate in the study. All participants completed a demographic information sheet (descriptive data on the study sample are provided in tables 2.1 page 43, 2.2 page 47 and 2.3 page 49), the ImpSSS (Zuckerman, et al., 1993) and the POMS-SF (McNair, et al., 1981). Additionally, smokers completed the QSU (Tiffany and Drobes, 1991), heroin abusers completed the SDS (Gossop, et al., 1995) and alcohol abusers completed the SDS and the SADQ (Stockwell, et al., 1979). DNA was obtained from cheek cell samples and the DRD4 gene

polymorphism was genotyped following standard laboratory procedures. DRD4 gene alleles vary in size (2-10 repeats of a 48bp fragment) and these were grouped into short (2-5 repeats) and long (6-10 repeats) alleles. Fragments were resolved by agarose gel electrophoresis and visualised by ethidium bromide staining. Participants also completed an emotional Stroop task on a laptop computer for the purposes of succeeding studies (detailed in chapter 6). Full details of the methodology used to test the hypothesis are detailed in chapter two (p 40-64).

Results

Independent-samples t-test revealed significant differences between groups with the DRD4 gene variant and ImpSS levels ($t = -2.308$, $df = 364$, $p = 0.022$). Participants with the long variant at DRD4 were significantly higher sensation seekers than those with the short variant (see table 4.2).

Table 4.2. Associations with DRD4 gene variants and ImpSS levels.

Gene Variant	Total (n)	SS (mean)	SD
Long	65	11.14	5.25
Short	301	9.66	4.56
Total	366	9.92	4.72

This result remained significant when firstly only heroin users were included in the analysis ($t = -2.909$, $df = 92$, $p = 0.005$). Secondly, the result remained significant when only cigarette smokers were included in the analysis ($t = -2.294$, $df = 90$, $p = 0.024$). However, this result did not remain significant when alcoholics ($t = 1.775$, $df = 87$, $p = 0.079$)[1] or control participants ($t = 1.422$, $df = 89$, $p = 0.159$) were included alone in the analyses (see table 4.3. for mean ImpSS scores for each group).[2]

[1] Although this effect did not quite reach significance ($p = 0.079$) it is close enough to warrant discussion rather than outright rejection. It is also interesting to note that this relationship is reversed for alcoholics. It appears that alcoholics with the short variant, as opposed to the long variant, have increased ImpSS levels.

[2] Note that the association with the DRD4 gene variant and ImpSS only reached significance in the heroin and smoking groups.

Table 4.3. Associations with DRD4 gene variants and ImpSS levels among the different groups of participants.

Gene Variant	Total (n)	ImpSS Mean	SD
Heroin Group			
Long	21	14.62	3.29
Short	73	11.79	4.08
Alcohol Group			
Long	17	8.65	4.03
Short	72	10.56	3.98
Smoking Group			
Long	19	12.21	4.69
Short	73	9.56	4.43
Control Group			
Long	8	4.75	5.09
Short	83	7.08	4.37

Therefore, associations with ImpSS levels and other factors were further explored. There was a significant correlation between ImpSS levels and genotype (rho = 0.128, N = 366, p = 0.014, two-tailed), sex (rho = -0.194, N = 400, p < 0.0001, two-tailed), age (r = -0.240, N = 400, p < 0.0001, two-tailed) and mood (r = 0.291, N = 400, p < 0.0001, two-tailed). These possible confounding variables were entered into three regression models using the enter method and significant models emerged. For the whole group, age, sex and mood were all significant predictors of impulsive sensation seeking, but genotype did not add significance to this model, F (4, 361) = 26.486, p < 0.0001, adjusted R square = 0.218 (see table 4.4).

Table 4.4. Regression coefficient (beta values) using the whole sample with ImpSS levels as the dependent variable.

	Beta	P
DRD4 variant	0.070	0.139
Mood	0.281	0.0001
Sex	-0.240	0.0001
Age	-0.298	0.0001

However, the second regression that only included the heroin group in the analysis revealed that genotype was the only significant independent predictor of ImpSS levels, F (4, 89) = 3.957, p = 0.005, adjusted R square = 0.113 (see table 4.5.)

Table 4.5. Regression coefficient (beta values) amongst heroin users with ImpSS levels as the dependent variable.

	Beta	P
DRD4 variant	0.293	0.006
Mood	0.055	0.606
Sex	-0.151	0.133
Age	-0.196	0.052

Furthermore, together with age and sex, genotype could significantly predict ImpSS levels amongst cigarette smokers, F = (4, 87) 7.369, p < 0.0001, adjusted R square = 0.219 (see table 4.6).

Table 4.6. Regression coefficient (beta values) amongst cigarette smokers with ImpSS levels as the dependent variable.

	Beta	P
DRD4 variant	0.188	0.050
Mood	0.145	0.131
Sex	-0.290	0.003
Age	-0.327	0.001

Discussion

The purpose of the present study was to examine associations with the dopamine D4 receptor gene and impulsive sensation seeking. Initially the hypothesis that there would be an association between DRD4 and ImpSS was supported. Individuals carrying the long variant at DRD4 were significantly higher impulsive sensation seekers than individuals with the short variant at DRD4. This indicates that having the long variant at DRD4 predisposes individuals to an impulsive sensation seeking

personality type, thus supporting early research (e.g. Benjamin et al, 1996; Ebstein et al 1996; Ebstein et al, 1997).

However, controversy between study findings has led to criticism of the methodology and data analyses that has been employed in reports that have found a positive association (Paterson et al, 1999). For this reason, the association with DRD4 and ImpSS was examined more closely in the current investigation in an attempt to detect any spurious results that may be due to other factors that are known to influence sensation seeking behaviour, including age, sex and mood (see introduction).

Firstly, the association was examined separately with each group of participants (heroin, alcohol, smoking and control) within the entire sample because sample type can alter the effect of DRD4 association results. Significant associations have been found more often among drug users (Li et al, 1997; Kotler et al, 1997; Mel et al, 1998) than alcoholics (Adamson et al, 1995; Malhotra et al, 1996; Chang, Ko, Lu, Paktis & Kidd, 1997). When groups were analysed separately in the present study, the original association detected using the whole sample only remained significant for the heroin and cigarette smoking groups. Therefore, these groups appeared to be responsible for the effect of DRD4 in the original analysis. When these results were corrected for multiple testing, the effect only remained significant for heroin users ($p < 0.01$). This sheds some light onto the controversy between previous study findings because it demonstrates that this effect may not be apparent in all populations. This being the case, significant associations found in studies with healthy volunteers could be due to spurious results with other factors causing the association rather than the genotype itself.

Alternatively, studies that have failed to find the association may have been due to the amount of genetic variance that DRD4 accounts for in sensation seeking levels. It could be that this genetic effect is so small that it has gone unnoticed (false negative result) in previous studies that have used smaller sample sizes. In addition, as the association only remained significant for heroin users, this points to the consideration that the DRD4 gene contributes to other behaviours than merely a sensation seeking personality (examined further in chapter five).

An interesting observation was that (although statistically non-significant) ImpSS levels were higher among individuals who carried the short variant compared to those with the long variant at DRD4 amongst alcoholics and controls. This result is consistent with the findings of Malhotra, et al (1996) who also found the association in the opposite direction in their sample of alcoholics. This finding is consistent with more recent literature that has found associations with the short alleles (2-5 repeats) and novelty seeking (Elovainio, Puttonen, Heponiemi, Reuter, Kivimaki, Vikari & Keltikangas-Jarvinen, 2005), demonstrating that the DRD4 gene variants may not affect behaviour in the same way for every individual. It is likely that other factors are modifying the association with DRD4 and ImpSS. Low-density lipoprotein (LDL) cholesterol is one factor that has been shown to modify this association (Elovainio, Kivimaki, Puttonen, Heponiemi, Keltikangas-Jarvinen & Viikari, 2005) as fatty acid supplementation can influence dopamine metabolism and modify impulsive behaviours related to dopamine (Hibbeln, Linnoila, Umhau, Rawlings, George & Salem, 1998).

Secondly, the significant association with DRD4 and ImpSS was examined further with respect to other factors beyond participant selection. In the present study, significant correlations were found with ImpSS and age, sex and mood, supporting previous reports (reviewed in Prolo & Licinio, 2002). These factors were entered into a regression model to determine whether the effect of DRD4 was large enough to predict impulsive sensation seeking independent of the effects that age, sex and mood have on the dependent variable. Results showed that for the whole sample, age, sex and mood were all significant predictors of ImpSS, but DRD4 did not add significance to this model. This result supports earlier claims that previous research may have yielded false positive results because they did not account for other factors that are pertinent to this association. However, this disappointing result could be interpreted in a number of ways.

From an optimistic viewpoint, it could be argued that having the long variant at DRD4 does predispose individuals to impulsive, exploratory and novelty seeking behaviour. However, the effect of this gene alone is so small that its effect is masked by other demographic and behavioural measures that have a greater influence on

personality than DRD4 does. That is, the variance accounted for by DRD4 is small and so its effect may be confounded by noise from other variables.

In addition, it could be argued that the significant result does show a true association and type I error is not likely because corrections were made for multiple comparisons and significance was attained for the heroin group. Standard regression with the heroin and cigarette smoking groups separately revealed this. It was found that among heroin users, genotype was the only significant predictor of impulsive sensation seeking and DRD4 accounted for 11% of the genetic variance of impulsive sensation seeking behaviour.

From a more conservative perspective, these results, along with the results from other association studies (see introduction for a review), could be interpreted as false positive results that have emerged as a result of various factors. Balaban (2002) offers a harsh criticism of human association studies that discusses some pitfalls of this kind of research.

"*One reason I am critical of human correlative behavior genetics is that many investigators make simplifying assumptions about heredity, genetics, development and behavior that I find biologically questionable*" [Balaban, 2002, pp. 295].

Taken as a whole, these criticisms are connected with differences between studies and problems with data collection and interpretation.

"*Particular alleles could affect behavior in a particular way in a very small number of lineages-how can we tell a real but rare effect from a false positive correlation? What kind of population sampling methods should one use to test the consistency of an allele's behavioral effects? Alleles that have effects on behavior in only some lineages can either be missed entirely or over-interpreted depending on the design of any particular study*" [Balaban, 2002, pp. 299-300].

In defence of this statement, the present study has acknowledged the problems with association studies by matching the sample for ethnicity and controlling for age and sex in the analyses to prevent population stratification. In addition, a reliable and validated, comparable personality measure was used. The present study employed the ImpSS scale, which correlates about 0.70 with SS (Zuckerman, 1996) and is

equivalent to Cloninger's NS scale (Zuckerman & Cloninger, 1996). Furthermore, corrections were made for multiple testing to limit false positive results and the effect of DRD4 on ImpSS remained significant for heroin users. Therefore, it could be argued that DRD4 does influence impulsive sensation seeking in heroin users. Thus confirming previous association studies and providing an explanation that previous negative findings have missed this small effect of DRD4 due to a combination of noise from other variables and lack of power to detect such effects.

This viewpoint does not rule out the possibility that another nearby polymorphism is in linkage disequilibrium with the DRD4 polymorphism, dismissing that other genes could be working with DRD4 to produce this association, but this is an issue for future investigation. Identifying genes for complex traits is a difficult task that is packed with design considerations. However, detection of such genes that are responsible for these small effects should not be postponed simply because the search is far from clear cut. Identifying such genetic effects will enhance our understanding of gene/environment interactions and as with any study, there are flaws, but these should be scrutinised to aid interpretation of findings. For example, the present study aimed to recruit young participants with mixed genders, but this was not completely successful due to the characteristics of the population examined. That is, the sample was random and consisted of drug and alcohol users in treatment, cigarette smokers and controls. Alcoholics in treatment have a tendency to be older and more men present to treatment for substance dependence than do women. Although groups were not matched for age and sex, these factors were considered in the data analyses and this helped with interpretation of the regression analyses. Moreover, other factors including educational level were normally distributed and so environmental features such as this were not skewed as to cause spurious effects. Furthermore, the sample size was large enough to detect any association with DRD4 with adequate power. Finally, ImpSS was measured using a tool that correlates with other personality scales used in other studies, so results can be compared across studies that have used the same ethnic group.

In summary, this study found that heroin users who carry the long variant at DRD4 are significantly higher impulsive sensation seekers than those who carry the short variant, indicating a genetic predisposition to an impulsive sensation seeking

personality type, being impulsive, exploratory and easily bored. This finding highlights the importance of other factors, as variables including age, sex and mood have an effect on the association with the DRD4 gene and ImpSS. Thus leading to a conclusion that multiple genes are responsible (11% genetic variance accounted for here) and are interacting with multiple environmental factors. Due to the effect only remaining significant when heroin users were selected, it also draws attention to the fact that DRD4 may be responsible for predisposing individuals towards addiction behaviour as well. Impulsive sensation seeking is closely linked to brain reward mechanisms where D4 receptors are most dense, which is the brain area also implicated with addiction behaviour. To conclude, dopaminergic polymorphisms contribute to individual differences in the personality trait impulsive sensation seeking, and could also be implicated in addictive behaviour, which is examined in the next chapter.

Chapter five

Study 3: Addiction and genetic influences

Introduction

Classic gene studies have generally found that about 30% of the variance in addiction can be accounted for by genetic influences (Plomin, et al., 1994). A great deal of research has looked specifically at alcohol addiction (reviewed in Ball & Collier, 2002). Twin studies show moderate heritability for early onset alcoholism in men at 50% but modestly heritable in women (25%), although estimates of heritability range widely (Ball & Collier, 2002). Environmental factors seem to play a greater role in late onset and non-dependent drinking behaviour (Pickens, et al., 1991). Studies of adopted away twins have shown similar findings (Cadoret & Gath, 1978; Bohman, Sigvardsson & Cloninger, 1981; Cadoret, et al., 1985; Sigvardsson, et al., 1996). For example, Sigvardsson, et al (1996) conducted an adoption study in Sweden and found increased rates of alcoholism in adoptees whose biological parents were drug or alcohol dependent. In addition, 62% of men and 33% of women adopted away became alcohol dependent, compared to 24% of men and 5% of women whose biological parents were not alcohol dependent (Cadoret, et al., 1985).

The pattern that has emerged with alcohol dependent populations can to a degree be seen amongst drug abusers. For example, a family study found that with 350 drug dependent individuals and 1478 of their first-degree relatives, male first-degree relatives reported two times more drug use than female relatives (Merikangas, Stolar, Stevens, Goulet, Preisig, & Fenton, 1998). Rounsaville, Kosten, Weissman, Pauls, Anton & Merikangas (1991) conducted the first such family study to include a control group. They recruited 201 opiate users, 877 relatives and 82 controls with 360 of their relatives. It was found that relatives of opiate users displayed more addiction-related problems than did controls. In addition, an eight-fold increased risk of drug disorders has been reported amongst relatives of probands. Family studies however, fail to separate shared environmental influences such as shared home and socioeconomic status from genetic factors.

Therefore, twin studies have been used in the attempt to measure the effect of genes on addiction. One such study found a 78% concordance rate of drug disorders (Pickens, et al, 1991). Moreover, a twin study of drug dependent individuals found a

concordance rate of 63% in men and 22% in women (Pickens & Svikis, 1991). Jang, Livesley & Vernon (1995) conducted a twin study of drug and alcohol users. They used 438 twin pairs and found that shared environmental influences had a small effect (0-20%) but non-shared environmental factors (e.g. divorce, job loss) accounted for most of the variance (53-64%). Genetic factors accounted for 21-41% of the total variance. A much larger study (3,810 twin pairs) found heritability estimates of 42-75% (Heath, Meyer, Jardine & Martin, 1991). Moreover, True, et al. (1999) examined alcohol and nicotine co-use amongst 3356 male-male twin pairs and they found heritability rates of 60.3% and 55.1% for nicotine and alcohol dependence respectively. For dual dependence, a heritability rate of 68% was obtained. Overall, twin studies suggest that male drug use has some genetic influence with a shared environmental influence (Ball & Collier, 2002).

Furthermore, adoption studies have revealed significant association with drug disorders in the adoptee and alcohol problems in the biological parent (e.g. Cadoret, et al, 1986). In addition, Cadoret, Yates, Troughton, Woodworth & Stewert (1995) recruited 95 male adoptees. From this study it was concluded that there are two pathways to addiction, a direct genetic effect and anti-social personality characteristics in adoptive parents. They highlighted the importance of including severity of dependence as a factor in such research. That is, the larger number of relatives affected the more symptoms of substance dependence found among probands.

Although classic studies provide supportive evidence for the role of genes in addiction behaviour, more modern approaches are able to detect specific genes that may be involved. Research using modern molecular genetic studies has revealed that as with the search for a true association with DRD4 and impulsive sensation seeking research (chapter four), the search for genes associated with substance abuse is taking the same theme. That is, conflicting findings are reported in substance abuse with half of these studies, which are exploratory in nature, reporting a significant association with the DRD4 gene variant whilst the other half have failed to replicate this association, demonstrating the need for replication in an attempt to elucidate this association. The pattern that is emerging is that significant associations between DRD4 and substance abuse are found amongst samples of drug abusers (e.g. heroin and nicotine

dependence) and non-significant results are yielded from studies of alcohol abusers. Therefore, the association with DRD4 and substance abuse could be substance specific.

The number of association studies that have compared the DRD4 gene variant in unrelated, substance dependent individuals to those in control individuals, who are not substance dependent, is growing, like with the DRD4 and personality studies. Due to differences between studies and methodological problems, research findings have been difficult to compare as they have examined different ethnic groups (e.g. Chinese, Israelis and Finnish) and different substances of abuse (e.g. alcohol and heroin). These studies have been summarised in Table 5.1.

Associations with substance dependence and DRD4

Half of the studies have found a significant association between the long-repeat allele at DRD4 and substance abuse. Two significant associations were found between DRD4 and alcohol abuse. However, one study that found this association borrowed their control group from published genotype frequencies (George, et al, 1993). The second study found a significant association with DRD4 and alcohol abuse but this was only observed in alcohol abusers who were distinguished by the ALDH2-2 polymorphism. ALDH2-2 (as opposed to ALDH2-1) is an allele whose presence causes a flushing reaction following alcohol ingestion, which therefore acts protectively to lower the incidence of alcoholism in people with this genotype (Muramatsu, et al, 1996).

Of the remaining studies, which have found significant associations between the long-repeat allele at DRD4 and substance abuse, have used samples of drug abusers. Li, et al (1997) found a higher frequency of long-repeat alleles at DRD4 amongst a sample of 121, Chinese, heroin abusers, when compared to matched controls (n=154). These findings were supported in two similar studies with Israeli, heroin abusers (Kotler, et al, 1997, Mel, et al, 1997). From these findings it could be argued that having the variant at DRD4 predisposes an individual to heroin abuse, but not alcohol abuse.

Table 5.1. Summary of association studies examining DRD4 polymorphisms and addiction.

Authors of the study	Participants	Polymorphism	Significance
George, et al. 1993	72 male & female Caucasian alcoholics with a mean age of 40.5 years	DRD4 exon 3 repeats	Unclear significance due to problems with the control group
Adamson, et al. 1995	226 Finnish male alcoholics & controls	DRD4 exon 3 repeats	No association
Muramatsu, et al. 1996	655 alcoholics & 114 controls, Japanese men & women with a mean age of 50 years	DRD4 exon 3 repeats	Significant association $p < 0.001$.
Malhotra, et al. 1996	331 male Finnish alcoholics & controls with a mean age of 32 years	7-repeat allele at DRD4	No association
Chang, et al. 1997	127 Chinese alcoholics & controls	DRD4 exon 3 repeats	No association
Garpenstrand, et al. 1997	49 male & female Caucasian psychiatric Pts.	DRD4 exon 3 repeats	No association
Geijer, et al. 1997	139 alcoholics & controls	DRD4 exon 3 repeats	No association
Kotler, et al. 1997	251 male Israeli heroin abusers & controls, mean age 39 years	7-repeat allele at DRD4	Significant association $p = 0.02$.

Table 5.1. Continued...

Authors of the study	Participants	Polymorphism	Significance
Sander, et al. 1997	252 male Caucasian alcoholics with a mean age of 41.9 years	DRD4 exon 3 repeats	No association
Mel, et al. 1997	Israeli heroin abusers	7-repeat allele at DRD4	Significant association
Parsian, et al. 1997	162 alcoholics & 89 controls	DRD4 exon 3 repeats	No association
Parez de Castro, et al. 1997	68 Caucasian gamblers (21 female) & 68 controls	DRD4 7-repeat allele	Significant association $P = 0.033$.
Li, et al. 1997	121 male & female Chinese heroin abusers & 154 controls mean age 27 years	Long versus short allele at DRD4	Significant association $P < 0.002$.
Sullivan, et al. 1998	267 male & female Caucasian alcoholic & depressed participants mean age 36 years	7-repeat allele at DRD4	No association
Shields, et al. 1998	283 smokers & 192 non-smokers	Long versus short alleles at DRD4	Significant association
Lerman, et al. 1998	289 smokers & 233 controls	DRD4 transporter gene (SLC6A3) variant 9	Significant association
Mel, et al, 1998	143 male Israeli controls & 57 heroin abusers, mean age 32 years	7-repeat allele at DRD4	Significant association $P = 0.001$.

Table 5.1. Continued...

Authors of the study	Participants	Polymorphism	Significance
Ovchinnikov, et al. 1999	42 alcoholics	7-repeat allele at DRD4	Significant association among alcoholics with family history
Sabol, et al. 1999	1, 107 smokers, non-smokers & ex-smokers	SLC6A3-9	Significant association
Roman, et al. 1999	215 Caucasian alcoholics/controls	7-repeat allele at DRD4	No association
Comings, et al. 1999	707 gamblers & 737 controls	Long versus short allele at DRD4	Significant association $P = 0.0001$.
Bau, et al. 1999	110 Caucasian male alcoholics with a mean age of 41 years	DRD4 exon 3 repeats	No association
Franke, et al. 2000	285 German heroin abusers & 197 controls	Presence/absence of 7-repeat allele at DRD4	No association
Li, et al. 2000	Chinese heroin abusers	DRD4 exon 3 repeats	Significant association
Ishiguro, et al. 2000	185 Japanese alcoholics & 286 controls	DRD4 exon 3 repeats	No association
Lusher, et al. 2000	Caucasian heroin abusers (n = 60) 51 alcoholics & 64 controls with a mean age of 35 years	Long versus short allele at DRD4	Significant association with severity of dependence ($P < 0.01$)

Table 5.1. Continued...

Authors of the study	Participants	Polymorphism	Significance
Bau, et al. 2001	226 Brazilian male alcoholics	7-repeat allele at DRD4 & DAT 1 10-repeat	Significant association $P < 0.0005$.
Hutchison, et al, 2002	74 (23 female) American students (21-35 years)	Long versus short allele at DRD4	Significant association with alcohol craving ($p < 0.01$).
Tsai, et al. 2002	103 Chinese methamphetamine abusers & 112 controls with a mean age of 28 years	DRD2 Taq1 & DRD4 exon 3 polymorphisms	No association

In addition, Parez de Castro, Ibanez, Torres, Saiz-Ruiz & Fernandez-Piqueras (1997) found the DRD4 7-repeat allele exclusively in gamblers. The authors recruited 68 Caucasian gamblers (47 male, 21 with a family history of substance abuse) and compared their genotypes with 68 blood donors who were not gamblers. However, this finding only remained significant when women were included in the analysis (p = 0.033). This study maintained that gambling was produced by a critical reduction in chemical release in the dopaminergic reward pathways. The dopamine reward pathway being crucial in the study of addiction as this part of the brain is thought to mediate the motivating effects of drugs (detailed in the following chapter).

The DRD4 gene has also been associated with cigarette smoking. In a study of 283 smokers and 192 non-smokers (African/American and Caucasian), the DRD4 long genotype was associated with a significant increased risk of smoking and reported shorter time to first cigarette of the day. Also, none of the participants who carried the long genotype were abstinent at two months, compared to 35% of the short genotype group, and if replicated this has important implications for treatment planning (Shields, Lerman, Audrain, Bowman, Main, Boyd & Caporaso, 1998).

Lerman, Caporaso, Audrain, Main, Bowman, Lockshin, Boyd & Shields (1998) also examined the association with the dopamine transporter gene SLC6A3 variant 9 with cigarette smoking among a sample of 289 smokers and 233 controls. They found that having the DRD4-long and SLC6A3-9 genetic variants put individuals at a significantly lower risk of smoking and made people significantly less likely to smoke before the age of sixteen years. Therefore, together these genes may influence smoking initiation and dependence. Sabol, Nelson, Fisher, Gunzerath, Brody, Hu, Sirota, Marcus, Greenberg, Lucas, Benjamin, Murphy & Hamer (1999) extended this study further by looking at smoking behaviour and personality types in non-smokers, current smokers and ex-smokers (n=1,107). These authors found significant associations with SLC6A3-9, the dopamine transporter gene variant, and smoking status and low sensation seeking levels. They argued that those carrying the gene variant have altered dopamine transmission, which reduces their need for sensation and reward by external stimuli, such as cigarettes.

Finally, Comings, Gonzalez, Wu, Gade, Muhleman, Saucier, Johnson, Verde, Rosenthal, Lesieur, Rugle, Millar & MacMurray (1999) examined the genes of 737 controls and 707 experimental participants. Results showed that pathological gamblers were significantly more likely to carry the long genotype at DRD4 than controls ($P = 0.0001$). They concluded that their study demonstrated a role for DRD4 in impulsive and addictive behaviours, but it is more complex than merely focusing on presence or absence of the 7-repeat allele. Benjamin et al (1996) recommend a division of alleles into short (2-4) and long (5-8) as it is having the long variant and not just the 7-repeat allele that should be examined.

Lack of association with substance dependence and DRD4

Despite the growing number of association studies detecting a relationship with the DRD4 gene and substance abuse, there are a growing number of similar studies that are failing to replicate. For example, Franke, Nothen, Wang, Knapp, Lichtermann, Neidt, Sander, Propping & Maier (2000) conducted a large study that looked at the DRD4 gene variant and heroin abuse. They genotyped 285 German heroin users and 197 German controls for the 7-repeat allele but no significant association with the gene and substance use was found. This result could be population specific, as the association was found in Chinese and Israeli samples. However, it may be more likely that the DRD4 gene variant is only acting in conjunction with other specific genetic or environmental factors, like with the DRD4 gene and personality trait association studies. For example, Ozkaragoz & Noble (2000) did not find any main effects of genetic or environmental factors on personality, however they did find a significant interaction between DRD2 alleles and environmental variables on extraversion.

However, an alternative perspective is this association could be substance specific. To illustrate this theoretical implication, Sander and colleagues (1997) examined the DRD4 gene and variations in novelty seeking in a sample of alcoholics. Although the DRD4 gene variant was reported to predispose an individual to high levels of novelty seeking, an excess of long-repeat alleles was not present in this group of alcoholics (Sander, et al, 1997). This finding has been supported in a sample of 162 alcohol dependent subjects and 89 unrelated individuals with no diagnosis of substance dependence. The authors of this study found no association with DRD4 and alcoholism, despite the use of a well characterised and ethnically matched control

group (Parsian, Chakraverty, Fisher & Cloninger, 1997). Likewise, Geijer, Jonsson, Neiman, Persson, Brene, Gyllander, Sedvall, Rydberg, Wasserman & Terenius (1997) did not find an association using a sample of 72 Scandinavian alcoholics. Furthermore, researchers have failed to find the association in a study of 185 alcoholic and 286 control participants in Japan (Ishiguro, Saito, Shibuya & Arinami, 2000). Finally, Roman, Bau, Almeida & Hutz (1999) compared genotypic frequencies of 100 Caucasian controls with 115 alcoholics. They failed to find an association with the 7-repeat variant at DRD4 and alcoholism and argued that George, et al (1993) originally detected an association with this genotype and alcoholism in their sample due to their control group being inadequate. George et al (1993) compared 72 severe alcoholics to a database of individuals whose genotype was made available in the literature.

This lack of association between the DRD4 gene variant and alcohol dependence has also been reported amongst a sample of Finnish males (Adamson, et al, 1995) and a Taiwanese population (Chang, et al, 1997). Adamson et al (1995) argued the necessity to examine the DRD4 gene in association with alcoholism due to its unique structure among neurotransmitter receptors makes this gene an interesting candidate for variations in dopamine related behaviours, such as drug seeking, eating and sexual behaviour. As dopamine is involved in central reward processes, differences in the expression of these processes may be due to genes.

A study that analysed severity of dependence reported that the long-repeat variant at DRD4 did not increase an individual's susceptibility to heroin dependence, but that having the long-repeat genotype was associated with severity of dependence (Lusher, et al, 2000). If severity of dependence is not clearly defined, then an association may not be obtained, simply because the sample is not severely dependent. Therefore, severity of dependence is an important variable to be included in such studies, as well as a large sample size because the long-repeat allele at DRD4 is rare in the Caucasian population, so large samples are needed to detect these associations.

The aim of the third study is to examine the association with the DRD4 gene and addiction in a sample of substance abusers and healthy volunteers. It is hypothesised that there will be a significant association with the DRD4 gene and substance dependence.

Method

One hundred heroin abusers and 100 alcohol abusers from outpatient treatment services and 100 smokers and 100 non-substance abusing controls from GP surgeries in London consented to participate in the study. All participants completed a demographic information sheet (descriptive data on the study sample are provided in tables 2.1 page 43, 2.2 page 47 and 2.3 page 49). To determine severity of dependence among experimental groups, the SDS (Gossop, et al., 1995), SADQ (Stockwell, et al., 1979) and QSU (Tiffany and Drobes, 1991) were administered to participants for self-completion. DNA was obtained from cheek cell samples and the DRD4 gene polymorphism was genotyped following standard laboratory procedures. DRD4 gene alleles vary in size (2-10 repeats of a 48bp fragment) and these were grouped into short (2-5 repeats) and long (6-10 repeats) alleles. Fragments were resolved by agarose gel electrophoresis and visualised by ethidium bromide staining. During the procedure, participants also completed the ImpSSS (Zuckerman, et al., 1993), the POMS-SF (McNair, et al., 1981) and an emotional Stroop task on a laptop computer for the purposes of additional studies contained within this thesis (detailed in chapters 3, 4 and 6). Full details of the methodology used to test the hypothesis are detailed in chapter two (p 40-64).

Results

There was a significant association with heroin abuse and presence of the long allele at DRD4 ($\chi^2 = 6.422$, df = 1, p = 0.011). There was also a significant association with the long allele at DRD4 and alcohol abuse ($\chi^2 = 3.999$, df = 1, p = 0.046), and cigarette smoking ($\chi^2 = 5.763$, df = 1, p = 0.016). However, Bonferroni corrections meant that the *p* value be adjusted for multiple testing to $p < 0.016$, therefore, the association with the long allele at DRD4 and addiction did not remain significant for alcoholics (allele frequencies are given in table 5.2).

Table 5.2. Amount (%) within groups with long or short genotypes at DRD4

	Group			
Genotype	**Heroin**	**Alcohol**	**Smoker**	**Control**
Long	21 (22.3%)	17 (19.1%)	20 (20.7%)	8 (8.8%)
Short	73 (77.7%)	72 (80.9%)	73 (79.3%)	83 (91.2%)

Discussion

Study three found that the long variant at the dopamine D4 receptor gene polymorphism is associated with addiction behaviour. Significantly more substance abusers carried the long variant at DRD4 than did controls. It was concluded in study two (chapter four) that the DRD4 long variant may predispose individuals to addictive behaviour rather than impulsive sensation seeking personality per se, which is supported by these results.

However, the association with DRD4 gene variants and substance dependence does not appear to generalise across different substances of abuse, as previous studies of alcoholics have failed to find a similar association (Geijer, et al, 1997; Sander, et al, 1997; Vandenburgh, et al, 1997; Chang, et al, 1997). In addition, the present finding revealed that when Bonferroni corrections were made to the data, the association with DRD4 and addiction only remained significant for heroin and smoking groups. Different populations and varied means of clinical assessment have been employed in such studies making comparison difficult. This controversy suggests that the association is a complex one and multiple genetic and environmental interactions are undoubtedly working together to produce inconsistencies in the findings, therefore highlighting the value of considering a number of factors in this research.

The first factor that needs to be considered is the ethnicity of the population examined in the research. Some discrepancies between study findings could be explained by ethnic differences. To illustrate, allelic frequencies vary among different populations and positive associations have generally been found among Israeli (Kotler et al, 1997; Ebstein et al, 1997), Japanese (Muramatsu et al, 1996) and Chinese (Li et al, 1997) samples. Compared to negative findings that have been gathered among Finnish (Adamson et al, 1995) and Swedish (Geijer et al, 1997) populations. So the importance of using the same ethnic group within the sample population should not be underestimated. The present study did match groups for ethnicity and a significant association emerged among this Caucasian population.

A second consideration for this research is the diagnosis and accurate assessment of the groups. The diagnosis and assessment of substance abuse may be another important factor that could influence the results obtained from these association

studies. Diagnosing substance dependence is not a straightforward process (discussed in depth in chapter one), due to the fact that it is not an all-or-nothing event, but instead a range of interacting symptoms that vary depending on the actual substance being taken and the person taking it. If substance abuse or dependence status is not reliably defined amongst the entire sample, then associations between variables may not be detected due to the substance using groups being too similar to the control group. In addition, severity of dependence may be a factor pertinent to this association. To highlight the importance of dependence severity in association studies a study reported an association between pathological gambling and the Monoamine Oxidase gene (MAOA) polymorphism (Ibanez, Perez de Castro, Fernandez-Piqueras, Blanco & Saiz-ruiz, 2000). The study found no significant differences between pathological gamblers and healthy controls overall in allele distribution at the MAOA gene but when severity of gambling was considered they did find a significant association with allele distribution in a sub-group of severe gamblers. A significant association with DRD4 and heroin and nicotine dependence was found in the present study, regardless of dependence severity. Heroin and alcohol users had a mean severity of dependence score of 10.34 and 10.02 respectively. A typical mean score is 8.7 (Gossop, et al., 1995), so the population used in the present study demonstrated above average severity scores (see chapter two for details). It would seem plausible to argue that this was due to the selection criteria employed for this study, whereby substance users were dependent on their drug of choice during the time of the study.

Finally, social factors may play a part in this association as DRD4 appears to have a small role, likewise with sensation seeking studies, such a small effect may be masked by environmental factors such as educational level or substance use factors such as age of onset. Alternatively, the effect of DRD4 on substance abuse could be working in combination with these environmental variables and other genetic influences. This can be illustrated by the study conducted by Lerman et al (1998) who found that carrying both the DRD2 A2 gene variant and the SLC6A3-9 transporter gene variant acted as a genetic protective factor by putting individuals at a lower risk of smoking cigarettes. Therefore, future work should aim to establish whether and how many other genes might interact with the DRD4 gene as vulnerability factors to addiction behaviour.

The role of dopamine in mediating drug reinforcement and reward mechanisms was what originally prompted researchers to examine the prevalence of the DRD4 gene variant among substance abusers and this line of question can provide a theoretical explanation for the present findings. It has been suggested that some individuals who are drug dependent may be dopamine deficient, therefore seek out psychoactive drugs to increase the release of dopamine, as they are unable to achieve satisfaction or adequate pleasure from normal life events (Zuckerman, 1999). This explanation is strengthened by findings in the previous chapter that impulsive sensation seeking is associated with the DRD4 gene variant among heroin users. In relation to this idea, the DRD4 polymorphism has been found to moderate craving in participants carrying the DRD4 long variant, who demonstrated an increase in craving after alcohol consumption when compared to participants with the short variant (Hutchison, McGeary, Smolen, Swift & Bryan, 2002).

This finding is consistent with appetitive models of addiction that suggest mesolimbic dopamine activation is crucial to the psychological process of drug craving, in that craving is associated with the physiological sensitisation of mesolimbic dopamine pathways in the brain (Berridge & Robinson, 1998). However, currently the state of understanding the true functional significance of DRD4 is limited and the link between DRD4 and substance abuse remains unclear. Nevertheless, the DRD4 gene is localised in the limbic structures that underlie incentive sensitisation (Van Tol, et al., 1991) and DRD4 receptors localised in the nucleus accumbens have been suggested to modulate excitatory transmission (Svingos, Periasamy & Pickel, 2000). Therefore, the DRD4 gene may be involved in both the acquisition and expression of incentive salience (craving) for a variety of substances and appetitive stimuli (Hutchison, et al., 2002).

To summarise, this study found that heroin abusers and cigarette smokers were significantly more likely to carry the long variant at DRD4 than healthy volunteers who do not use any addictive substances. These findings support the view that the DRD4 gene variant predisposes susceptible individuals to substance dependence by arbitrating neurobehavioural changes in the dopamine reward pathway of the brain, an issue that is dealt with in the following chapter.

Chapter six

Study 4: Addiction and neurobehavioural influences: Exposure to substance-related stimuli

Introduction

Substances abused by men and women, including heroin, alcohol and nicotine have the common action of increasing dopamine in the nucleus accumbens (NAcc) (reviewed in Everitt, Dickinson & Robbins, 2001). Additionally, neuroadaptions that occur in the NAcc are induced by drug administration (Koob & Le Moal, 1997 and 2001). Consistent with this line of debate are current neurobehavioural models of addiction, such as the incentive sensitisation theory, which point to the mesolimbic dopamine pathway as the primary brain region involved in addictive behaviour (reviewed most recently in Robinson & Berridge, 2003). This pathway is also thought to play a common role in the rewarding effects of drugs (Wise & Rompre, 1989). This brain region comprises projections from the ventral tegmental area (VTA) to the amygdala and the prefrontal cortex and functionally, activation of this pathway is associated with appetitive and rewarding behaviours such as sex, eating and drug-taking (Wise, 1998).

The incentive sensitisation theory of addiction states that activation of these brain structures is linked to the craving or the wanting of the drug (e.g. motivation) (Robinson & Berridge, 1993; 1998; 2003). The central idea is that drugs alter NAcc-related circuitry (Volkow, Fowler & Wang, 2002; Lingford-Hughes, 2002) that mediate a basic incentive motivational function called incentive salience. This psychological process transforms perceptions of drugs and related stimuli, making them attractive and wanted, so they become especially salient, capturing the attention of the user (Robinson & Berridge, 2003). According to this theory, a consequence of repeated drug use is that these reward pathways become sensitised (Robinson & Berridge, 2003), whereby, repeated drug-use initiates neuroadaptions in the system, making the person hypersensitive to the drug and related stimuli. Sensitisation, as a pharmacological effect, of the dopamine system is controlled, via conditioning, by associative learning, which will turn the wanting of the drug to an intense craving for the drug. Sensitisation is said not to be an inevitable consequence of exposure to addictive drugs. It can be dependent upon psychological and environmental factors

associated with drug administration (i.e. the situation and place where the drug is usually taken, Robinson & Berridge, 1993).

Therefore, this theory is different to earlier models of addiction that have concentrated on the pleasure seeking principle of addiction. The incentive sensitisation theory explains how the drug can be craved intensely, without being liked. It is true for many drug dependent individuals that they do not like the taste, smell or effects of the drug, but they still crave it. According to the incentive sensitisation theory, this sensitised neural system is responsible for excessive drug craving, which can be dissociated from the neural system that mediates the hedonic effects of drugs (i.e., the liking or pleasure) and is governed by a different process with its own neural substrates (Berridge, 2002). The theory also explains the process of chronic relapse, which is fundamental to the majority of substance abusers. As sensitisation is persistent, drug-related stimuli promote relapse in susceptible users, due to an increase in activity in dopamine neurotransmission when substance-related stimuli are present (Robinson & Berridge, 1993). For example, in animals sensitisation has been shown to persist for months and even years after drug treatment ceases (Castner & Goldman-Rakic, 1999).

Earlier neurobehavioral models of drug addiction should be considered here, in light of the incentive sensitisation theory. Negative reinforcement theories ultimately claim that drugs are administered as an escape from distress, to avoid the negative effects of the withdrawal syndrome. This holds true for substances such as opiates and alcohol because clear symptoms of tolerance and physiological withdrawal are present when they are abused. Negative reinforcement theories also claim that addiction is maintained because of the aversive symptoms associated with withdrawal, concentrating on the state in which they alleviate, rather than the state that they produce. Robinson & Berridge (1993) build on this by pointing to the disadvantages of negative reinforcement theories. Firstly, they do not take into account substances like cocaine and ecstasy, which do not produce marked withdrawal symptoms, but nevertheless, are abused. The incentive sensitisation theory accounts for all drugs. Also, drugs such as opiates are self-administered in the absence of negative withdrawal syndromes. Negative reinforcement theories do not take into account relapse after long periods of abstinence when the withdrawal syndrome has passed,

however, the incentive sensitisation theory accounts for this by the persistence of sensitisation (Robinson & Berridge, 2003).

Positive reinforcement models of addiction posit that drug self-administration is maintained because of the pleasurable state induced by the drug, rather than the alleviation of negative effects produced by the drug. They claim that drugs are taken because they are positively reinforced, that is, pleasure is experienced from the mind-altering effects of the substance and withdrawal symptoms are removed. Problems with positive reinforcement theories are firstly, they assume that the effects are always highly pleasurable, producing enough pleasure to out weigh social, physical and psychological negative effects of repeated drug use. Also, theories have ignored environmental factors involved in craving and relapse. Finally, they do not consider that many drug abusers report relief, rather than pleasure after self-administration of drugs.

The incentive sensitisation theory can explain the features that have been missed by previous theories. Firstly, the incentive sensitisation theory includes an explanation of the dissociation between liking and craving. Why the neural system should activate incentive motivation is also considered. Why relapse persists after long-term abstinence, the extent to which incentive salience is elicited by drugs in the common neural system and an explanation of the progressive development of addictive behaviour through repeated use are all accounted for by the incentive sensitisation theory. The incentive sensitisation theory assumes that repeated use of an addictive drug can cause susceptible individuals to become sensitised to stimuli associated with drug taking. Evidence for sensitisation to the effects of drugs amongst humans is limited. However, Strakowski, et al. (1996) reported results of a double blind, placebo controlled drug study whereby participants were given two treatments, two days apart. They found that the second treatment with amphetamine elicited a significantly greater increase than the first, in energy, mood, speech and eye blink rate. Strakowski and Sax (1998) replicated this study to see if three treatments of amphetamine would induce a progressive increase in the drug effect. Results were as predicted and consistent with the first study and subjective reports of drug liking did not increase with the three drug treatments. This finding is consistent with the theory that

sensitisation applies to the wanting of the drug rather than the liking of the drug (Robinson & Berridge, 2000 & 2003).

Other important features of sensitisation, according to the incentive sensitisation theory, are that individuals vary in their susceptibility to sensitisation. That is, some individuals sensitise more readily, whereas others are more resistant, due to a host of factors including genetic variation (Robinson, 1988). Additionally, there are individual differences in the extent to which stimuli associated with the drug can become wanted by the user (capturing their attention) and the mechanism for this incentive salience is said to be mediated by dopamine (Robinson & Berridge, 1993). Thus, individuals can vary in the extent that the mesolimbic dopamine system is vulnerable to transforming the perception of drug-related stimuli into attractive and wanted stimuli, thus capturing the drug-users attention (Robinson & Berridge, 1993). This has been illustrated by the finding that morphine and amphetamine given to rodents in distinct and novel environments induce a much more robust sensitisation than when given in a home cage (Badiani, Oates & Robinson, 2000). Additionally, dopamine-related systems, such as NAcc circuitry, have been shown to play an important role in conditioning-guided attributions of incentive salience (Dickinson, Smith & Mirenowicz, 2000; Wyvell & Berridge, 2000; 2001, & De Borchgrave, Rawlins & Dickinson, 2002).

Wyvell & Berridge (2000) illustrated that sensitisation, by prior drug administration and direct stimulation of dopamine neurotransmission in the NAcc, increased the incentive salience attributed to a sugar reward cue, causing the cue to elicit an exaggerated craving for the reward. The study implicated that injecting amphetamine into the NAcc of animals did not increase the liking for sugar, however the wanting for sugar was increased. From their study, Wyvell & Berridge (2000) concluded that an increase in dopamine neurotransmission in the NAcc increases the wanting, without increasing the liking for drugs. This finding was supported in a second study by Wyvell & Berridge (2001), who investigated the effect of sensitisation on cue-triggered wanting for a sugar reward by administering rats with several injections of amphetamine, leaving the rats drug-free for a couple of weeks, then after testing, found conditioned incentive salience. Although conducted with rats, these studies seem to point towards human drug addiction being a consequence of drug-induced

neural sensitisation that attributes an incentive salience to particular environmental stimuli. Therefore, it would be of interest to further explore attentional bias to drug-related cues as an indicator of susceptibility to substance dependence. Exposure to substance-related environmental stimuli could mediate the maintenance of addiction by producing craving. That is, making the individual want to use drugs as a result of being shown stimuli that capture their attention and remind them of the drug. This being an important factor in understanding the basis of relapse as craving, or the wanting of drugs has a triggering effect on relapse and demonstrates a powerful environmental factor that can influence substance use behaviour. Exposure to substance-related stimuli in the environment could be an important factor in the maintenance of addiction. Some people may become more sensitised to substance-related environmental cues (such as advertisements) and to the development of that kind of behaviour, than others.

There is, however, currently a shortage of cue reactivity research that has tested the attribution of incentive salience, although animal research suggests that the ability to induce sensitisation is modulated by learning and environmental cues (e.g. Dickinson, et al., 2000; Wyvell & Berridge, 2000; 2001; De Borchgrave, Rawlins, Dickinson & Belleine, 2002). It appears that the interaction of neural sensitisation and associative learning is responsible for the focus that drug abusers have on drug-related stimuli. That is, the behaviours and objects associated with drug taking become powerful incentives themselves and the modulation of the expression of sensitisation via the drug-taking context may contribute to the crucial role that drug-related cues play in precipitating relapse.

Cue reactivity occurs when stimuli, such as the environmental context in which the drug is used or substance-related materials (e.g., cigarette lighters and papers) become associated with the drug and its effects and therefore, due to this pairing with the substance, become conditioned stimuli. This produces a conditioned response whereby stimuli that are repeatedly paired with the substance cause responses to occur, in the absence of the drug. Therefore, drug relevant stimuli become conditioned stimuli that elicit motivational states that can produce physiological and behavioural responses that are similar to that of the drug response (craving) (Niaura, Rousenow, Binkoff, Monti, Pedraza & Abrams, 1988).

Human cue exposure research has indeed shown how attentional bias reflects the activation of the incentive sensitisation system. For example, substance-related paraphernalia (e.g., lighter, ashtray, foil), or a videotape showing a person preparing for a fix of heroin, have been used to demonstrate selective attention whereby participants view the stimulus then are asked to complete a questionnaire rating their craving levels before and after the viewing. Dudish-Poulsen & Hatsukami (1997) used this method when examining subjective and behavioural responses in cocaine abusers, after cocaine-related stimuli were presented. Findings suggested that cocaine abusers reported significantly greater levels of craving after exposure to cocaine-related, as opposed to neutral stimuli. In addition, Childress, McLellan & O'Brien (1986) used the videotape method to examine the role of conditioning factors in drug dependence. They used a neutral tape, which featured a nature story, and a neutral activity that allowed participants to play a computerised game. The drug-related videotape featured a buy, sell, cook-up and shoot-up ritual, and the drug-related activity required participants to go through a mock cook-up and tie-off procedure, with optional self-injection of saline solution. They found that substance abusers showed clear evidence of conditioned craving and withdrawal like responses, with an initial reduction in skin temperature with repeated exposure to drug-related stimuli. Indirectly, this suggests that cues can trigger wanting as a conditioned motivational response.

Other methods, such as a modification of the Stroop paradigm (Stroop, 1935) could be employed to measure attentional bias in substance abusers. This method is especially useful for testing whether stimuli capture the attention of participants. Stroop tasks can be used to determine the level of activation of a word component of the stimulus, whereby its increased activation makes the suppression of its meaning more difficult. In the original Stroop test, participants were presented with lists of colour-words (e.g. RED), which were printed in different colours (e.g. blue). Participants were required to read out the words ("RED") while ignoring the colours in which they were printed (blue). Then, the participant was required to name the colours ("blue") whilst ignoring the words (RED). Stroop (1935) found that to name the colour whilst ignoring the word produced lower performance, so reaction times were longer for this part of the task.

The Stroop effect occurs because the participant's attention is directed partly toward the semantic properties of the stimuli (meaning of the word) and partly toward the perceptual properties (colour in which the word is printed). When the semantic and perceptual properties are inconsistent, interference occurs thus producing longer reaction times. The Stroop task assumes that both types of property of the stimulus are attended to otherwise there would be no interference.

The emotional Stroop task goes further and is able to differentiate between groups of individuals with a psychopathology, such as substance dependence, and control participants, providing a reliable instrument for assessing attentional bias (Williams, Mathews & MacLeod, 1996). The emotional Stroop task uses stimuli that are either neutral in emotional valence or stimuli that are emotionally evocative for participants. For example, studying the cognitive mechanisms underlying substance abuse involves participants' reaction times to emotionally salient substance-related words and unrelated, neutral words. The logic underlying the original Stroop can be extended to interpret results from emotional Stroop tasks. When participants are pre-occupied with, say, alcohol and they encounter a word like "Vodka", they take longer to respond to that word than a neutral word like "Sofa" because extra time is taken to process the semantic properties of the associated stimulus.

Modifications of the Stroop test have been widespread and more recently, the Stroop paradigm has been used to investigate information processing biases underlying clinical disorders. In perceptual and attention tasks, biases in mood have been established in anxious participants. Williams, Watts, MacLeod & Matthews (1988) found that anxious participants are differentially slowed on the Stroop colour naming task when it includes threat-related, rather than non-threat-related words. These effects can also be seen in participants with depressed mood. For example, Pincus, Fraser & Pearce (1998) examined whether chronic pain patients Stroop on pain stimuli. Categories of emotionally salient and neutral words were presented in different colours and response times of patients to name the colour of each word were recorded. It is assumed that the emotive content of salient words will interfere with the colour-naming task, resulting in longer response latencies (Williams et al, 1996). Therefore, chronic pain patients should take longer to colour name pain-related stimuli than neutral stimuli. The Pincus et al study failed to find this result, but argued

that the depressed mood measured in chronic pain patients was likely to be responsible for the slower response in these participants, rather than their pain per se. Emotional Stroop effects have been found across a range of emotional disorders and the size of the effects differ from group to group, being especially low in depressed groups (Williams et al, 1996).

Treisman & Fearnley (1969) suggested that interference with selective attention might occur only when the irrelevant stimulus attribute belongs to the same class as the response. This was tested using a card sorting task whereby participants were asked to sort cards into two piles depending on whether they were the same or different, by various criteria. Two packs of cards were used, each having two items on them. Participants were required to organise the cards depending on the condition they were given. Results demonstrated that the condition whereby participants had to match the colour of the word at the top of the card to the bottom word, ignoring the nature of the word at the top, took the longest. Therefore, the presence of another stimulus value in a different attribute will interfere in proportion to the relative speeds of naming the attributes.

Until recently, the Stroop effect in addiction research had mainly focused on smoking behaviour. Gross, et al (1993) measured the reaction times of smokers to smoking related and neutral words. Successful performance of the Stroop task requires suppression of the meaning of the stimulus word in favour of activation of the colour name. Results were as predicted, Gross, et al found that abstinent smokers were slower to colour name smoking-related than non-smoking-related words. This finding has since been supported by further studies examining attentional bias in smokers, compared to non-smokers (Johnsen, Thayer, Laberg & Asbjornsen, 1997; Waters & Feyerabend, 2000) and among current smokers, ex-smokers and non-smokers (Munafo, Mogg, Roberts, Bradley & Murphy, 2003).

Further support for the Stroop effect witnessed in substance abusers comes from a study conducted with 20 alcoholics and 23 controls. Participants received 16 trials of blocked words that were either alcohol-related or neutral words. Alcoholics took significantly longer to colour name alcohol-related than neutral words when compared to controls (Stormark, Laberg, Nordby & Hugdahl, 2000) and this finding has further

support from Stetter, Ackerman, Bizar, Straube & Mann (1995) and Bauer & Cox (1998). This processing bias makes the suppression of meaning of substance-related words more difficult and leads to greater interference during the task. Successful colour naming requires the attention of participants, so Stroop interference arises when the meaning of the words to be colour named captures the attention of the participant at the expense of the colour-naming task.

From a cognitive perspective, such as the cognitive processing model introduced by Tiffany (1990), cognitive processes that are associated with a substance become automatic through repeated use of the drug. Therefore, cue reactivity results from the activation of non-automatic, conscious processing during abstinence. Non-automatic activation is presented as physiological withdrawal and desire, or craving, to use. This theory is beneficial to our understanding of craving in that it attempts to explain how cognitive processes operate, which is somewhat ignored by the incentive sensitisation theory of addiction. Understanding addiction at the cognitive level is important due to the relevance of cognition in cue-reactivity and attentional bias. To frame the cognitive processing model with attentional bias findings, positive and negative outcome expectancies of substance use are stored in memory and these, in the presence of cues related to the substance, become activated and therefore capture attention, thus producing attentional bias. This assumption is similar to the incentive sensitisation theory, which argues that the mesolimbic dopamine system becomes sensitised to drugs and drug-related stimuli so substance abusers find stimuli particularly salient making the user *want* the drug. Therefore, Robinson and Berridge (1993) offer a similar explanation for the development of addictive behaviour to Tiffany (1990), but argue from a neurobehavioural standpoint, rather than from a cognitive-processing perspective.

From a biopsychosocial perspective, such as the incentive sensitisation theory, exposure to smoking-related words in smokers could mediate the maintenance of their addiction by producing craving. That is, it makes the user want to smoke by being shown stimuli that remind the smoker of smoking. This being an important factor in understanding the basis of relapse as craving, or wanting, has a triggering effect on relapse. The incentive sensitisation theory claims that among individuals prone to substance dependence, the use of a substance sensitises their positive incentive value

of the substance, therefore the substance user becomes highly motivated to seek out drugs and related stimuli. The basis of addiction therefore is not the pleasure of taking the drug per se, but the anticipated pleasure of taking it. The wanting of the drug becomes sensitised and therefore the incentive salience of the drug becomes out of proportion with the pleasure obtained from it. Therefore, the drug is craved regardless of physical and social problems that are incurred from drug use. Cue reactivity is an area where identifying and explaining individual factors that contribute to variability in drug sensitisation is of benefit as it can be used to explain discrepancies in previous research, assisting in understanding why addiction develops in some people who experiment with drugs, but not others. In fact, identifying factors in individual variation and how they influence psychological performance is important in psychological, personality and genetics research.

Firstly, the way in which group factors produce changes to responsivity to substance-related stimuli can be identified. For example, Childress et al (1986) reported that half of their sample of drug users who were on methadone maintenance programmes did not experience craving to heroin-related cues. Therefore, the medication participants are using and when they last used the substance are important factors to consider in cue reactivity research. Certain other factors like dependence severity, personality types (Ball & Zuckerman, 1992) and affect (Pincus, et al., 1998) may also produce individual differences in cue-elicited responses to drug stimuli. For example, dependence severity is positively related to level of reactivity. That is, drug dependent individuals show greater reactivity than non-drug users and greater frequency and history of drug consumption produce a greater cued response (Heather & Greeley, 1990). It is important therefore to use direct measures of dependence severity in cue reactivity research with drug using samples.

Pre-existing affective states might influence cued responses because high levels of depression, anxiety and stress are thought to play a role in initiating and maintaining drug use. Moreover, the appetitive model of addiction claims that negative mood states increase the incentive value of some drugs and make expected effects of drugs more salient (Stewart, et al., 1984). Therefore, it would be expected that cue reactivity would be greatest in those individuals who have salient affective states at time of exposure to drug-related stimuli. Greeley, et al., (1993) provide evidence for this in

two studies that they conducted with alcohol dependent individuals. They found a significant correlation with depression and urge reactivity. That is, a cue-elicited desire for alcohol could be predicted by subjective level of depression and mean daily alcohol consumption. Moreover, negative mood state was found to account for cue-elicited urges in opiate users, whereby feelings of boredom, anxiety and anger strongly correlated to desire to use when heroin-related cues were present (Sherman, Zinser, Sideroff & Baker, 1989). These examples point to the importance of measuring affective states in cue reactivity research, of which is also consistent with theoretical models.

The way in which personality and genes influence substance use behaviour is far from clear, there is currently limited evidence of the risk factors associated with enhanced drug cue associative learning and responding. It may be important to look at how drug cue associative learning relates to vulnerability to substance abuse. Personality can be considered as a mediating variable between genetic vulnerability and actual drug taking behaviour. For example, the physiological basis of drug taking and craving could be said to occur due to distinct neural pathways, making up the mesolimbic dopamine reward system, that differ in response to novel, appetitive and aversive stimuli. These genetically determined neural pathways are thought to allow for enhanced associative learning of drug effects (Cloninger, 1987). If this were true, it would be expected that individuals with high-risk personality types (e.g. sensation seekers) would show signs of enhanced drug-related conditioning. An individuals susceptibility to conditioned responses to drug cues may also develop powerful drug cue associations, therefore, producing greater cue reactivity. In support of this, Gray (1991) argued that impulsive people have increased sensitivity to cues and are more likely to show appetitive responses to cues associated with rewarding outcomes.

Genetic variation has been shown to influence cue reactivity. For example, Hutchison, LaChance, Niaura, Bryan & Smolen (2002) examined the role of the dopamine D4 receptor (DRD4) gene in cue-elicited craving in cigarette smokers. The DRD4 gene is a logical candidate for nicotine craving because of its distribution in the brain. DRD4 receptors are dense in the NAcc and have been shown to be critical to sensitisation of NAcc pathways (Feldpausch, et al., 1998). It has also been reported that the DRD4 gene is involved in incentive salience (Robinson & Berridge, 2000). In support of this,

Hutchison, et al., (2002) found that participants with the long allele at DRD4 showed an increase in craving and attention to cues during exposure to smoking-related stimuli, when compared to participants with the short allele, thus pointing to this polymorphism as a moderator in cue-reactivity. Further work conducted by Hutchison and colleagues (Hutchison, McGeary, Smolen, Swift & Bryan, 2002) has supported this finding in a subsequent study with alcohol dependent participants. During this study, participants consumed three alcoholic drinks or three non-alcoholic drinks and alcohol craving levels were then measured. Results showed that participants with the long alleles at DRD4 demonstrated an increase in craving for alcohol after drinking alcohol, whereas those with the short alleles did not, thus illustrating the moderating effects of the DRD4 gene polymorphism on cue-elicited craving for alcohol. Taken together, these studies support the incentive sensitisation theory of addiction by indicating that the mesolimbic dopamine system is important to the attribution of incentive salience (Robinson & Berridge, 1998).

Family history could also be an important variable in cue reactivity research. Walitzer & Sher (1990), followed by Newlin (1994) suggested how individuals with a family history of drug use have a higher risk of developing drug use problems themselves because they react differently to drug cues than a person with no family history of substance abuse. For example, offspring of alcohol dependent fathers experience greater antagonistic behaviour, show greater subjective intoxication and a faster heart rate, than individuals without a family history, when exposed to alcohol-related cues (Walitzer & Sher, 1990). It seems apparent therefore that individual differences contribute greatly to the degree and type of cue reactivity findings. It is important to consider these variables that may enhance individual susceptibility to acquire conditioned responses to drug cues.

To summarise, the Stroop paradigm would be beneficial in examining the incentive sensitisation theory (Robinson & Berridge, 1993) because it can demonstrate increased salience of drug-related stimuli in substance dependent individuals. If reaction times are slower, and performance is lower for the addicted groups, compared to that of the controls, then it can be concluded that a processing bias makes the suppression of meaning of drug-related words more difficult, leading to greater interference during the task. Therefore, exposure to drug-related words, in substance

abusers, could mediate the maintenance of their addiction by producing craving. Study 4 aims to examine the hypothesis that substance abusers will respond differently to substance-related words than neutral words when compared to control participants. A full description of the methodology employed to test the hypotheses is detailed in chapter two.

Pilot Study 1:

Introduction

The aim of this pilot study was to collect lists of words from substance abusers that are associated with their drug of choice. This procedure was necessary because words were required to build into the emotional Stroop task used in the main study to measure reactions to drug-related words, in the attempt to examine the incentive sensitisation theory of addiction using a human sample.

Method

Substance-related words to be used in the modified Stroop computer task were collected from 60 substance abusers (20 cigarette smokers, 16 alcohol users, 12 stimulant users and 12 opiate users, a representative sample of 15% of the expected entire sample for the main study) in east and south London treatment centres. Participants were asked to list as many words as they could think of, which they would associate with their drug of choice. This procedure was used to ensure that words used for the Stroop programme were actually words that substance users in south and east London would be familiar with. Dialect, language and slang vary from culture to culture, amongst different communities and from time to time. So by collecting words from a representative sample of the population to be recruited for the main study, it could be argued that the words were substance-related according to that particular population at the time the study was conducted.

Results and conclusion

A total of 914 substance-related words were recorded. Frequency distributions determined the 10 most frequently occurring words for each substance group to be included in the programme and were multiplied by two to give a total of 20 words for each substance word list. Word lists compiled for the computer programme are listed below:

Heroin	Alcohol	Stimulant	Cigarettes	ImpSS	Neutral
Heroin	alcohol	cocaine	cigarette	impulsive	cabinet
Brown	shakes	charlie	smoking	wild	door
Chasing	booze	snort	lungs	chance	kitchen
Foil	drunk	powder	fags	magic	desk
Gear	drink	pipe	lighter	novelty	chair
H	hangover	white	nicotine	funfair	table
Inject	pub	coke	tobacco	glide	picture
Methadone	vodka	crack	addictive	adventure	TV
Smack	DT's	line	ashtray	surfing	sofa
Score	beer	sniff	ash	sensation	carpet

Substance-related (heroin, alcohol, stimulant and cigarette) words were matched to the impulsive sensation seeking (ImpSS) words and neutral words by length and syllable frequencies. Stimuli were matched in this way, rather than by frequency, because the time taken to read the words is what is measured during the task. Additionally, it has been argued that *"the frequency with which participants who suffer from a particular disorder (e.g. alcohol abuse) have been exposed to words related to that disorder (e.g. alcohol-related words) is not represented by the frequency with which those words occur in the spoken language, leading researchers to question the relevance of the control"* [Cox et al., 2001, p. 1263]. Nevertheless, it is difficult to choose the correct method of matching stimuli (Sharma, Albery & Cook, 2001a) and choosing how to match words has given rise to lively discussion in the literature, without resolution. Impulsive sensation seeking words were obtained from sensation seeking literature (Zuckerman, 1979), specifically, the ZKPQ-SSS (Zuckerman, 1994) and the neutral words were chosen from a list of random household objects. Household terms were used as they belong to a category and are therefore semantically related to each other and they are not closely associated to colour words, i.e. as sky is to blue (Cox et al., 2001). The colours used were red, blue, yellow and green, which were repeated five times to make a total of 20 colour words in each condition. The word lists were added into a modified Stroop programme and tested in pilot study 2.

Pilot Study 2:

Introduction

The emotional Stroop task was tested using an opportunity sample of undergraduate students at London Guildhall University. This was necessary to ensure that the programme accurately measured reaction times and that the programme did in fact detect a Stroop effect. A minimum of 18 people for each group (smokers and non-smokers) were required to obtain a medium effect (power = 0.29, $\delta = 1.40$, $P<0.05$) (Cohen, 1992).

Method

Participants were invited to take part in a pilot study to test whether a computer-based task that will be used for the main study is accurate in measuring reaction times. Participants were asked to complete a short computer-based task that takes 10 minutes. Participants were informed that the study was entirely voluntary and no pressure would be put on them to take part. Participants were given the opportunity to ask questions before the study began. No participant received payment for his or her participation in the study.

Prior to completing the Stroop task, participants were asked to complete a series of questions on a computer task information sheet. Questions were asked to determine the ease in which participants could complete the task and the extent to which reaction times could be compared, handedness, native language, whether the participant had normal or corrected to normal vision and whether the participant was colour blind were questions asked. Participants were also asked to tick from a list, what substances they were using at the time of the study and to state when they had last used each one (cigarettes, alcohol, heroin/other opiate, cocaine/amphetamine or other to specify). This was important to record so that levels of intoxication and physical and mental state could be considered. Finally, participants were asked to rate on a visual analogue scale, with 0 meaning 'not at all' and 10 meaning 'extremely', how much they were craving for cigarettes immediately prior to the Stroop task. Subjective craving levels were recorded firstly to determine level of craving in case the results were effected by this variable and secondly to ensure that the task itself did not induce any unwanted substance craving amongst individuals. On completion of the task participants were

asked to rate how much they were craving for their drug of choice again on a scale from 0 (not at all)-10 (extremely).

The instructions for the task were displayed on the computer screen. Participants were instructed to press the space bar to continue onto the next stage of the task. Blocks of coloured words, or words typed in different colours, were presented on the screen. Participants were instructed to concentrate on the cross in the centre of the screen preceding each presentation. They were instructed to name the colour of the words while ignoring the word content by pressing the appropriate coloured key on the keyboard that corresponded to the colour the word was printed in.

A practice session preceded the main task. This consisted of 28 words taken from the seven word groups (four of each: heroin, alcohol, stimulant, cigarette, sensation seeking, neutral and colour). At the end of the practice session participants were given the opportunity to ask questions and their correct score was checked to ensure that participants understood how to complete the task.

The main task consisted of five conditions with 28 words in each condition (four words from each word group). The words were presented in random order in blocks of 28, with a total of 140 words presented on a laptop computer. To eliminate order and practice effects, conditions and words in each condition were randomly shuffled by the computer after each participant presentation. The colour in which the word was presented was shuffled randomly after each presentation without allowing the same colour to be presented consecutively.

Results

Incorrect responses were removed from the data prior to computation of the descriptive data. 99.7% (n=6703) of the cases were included in the data analysis and 0.3% (n=17) false responses were excluded. Mean reaction times for each of the three conditions (smoking, neutral and colour words) for each participant (n=40) were computed so that they could be compared to determine presence or absence of a Stroop effect. Only smoking word responses were compared to colour words and neutral words as the sample for this pilot procedure were either smokers or non-smokers and did not abuse any other substance. ImpSS words were not analysed in

this pilot study, as the ImpSS scale was not administered. However, all conditions and all word groups were included in the task for this pilot to determine the length, timing and ease of running the whole task for the main study.

The mean response times and standard deviations were calculated for each condition. Mean response times for smoking words were 723 milliseconds (ms, S.D. 0.143), 737 ms (S.D. 0.147) for neutral words and 805 ms (S.D. 0.251) for colour words. ANOVA demonstrated that participants took significantly longer to respond to colour words than to all other words ($F = (1, 39)\ 7.400$, $p < 0.01$).

Smokers (n=20) mean reaction times for each condition (smoking, neutral, colour words) were compared to that of non-smokers (n=20) using a repeated measures ANOVA. No significant differences between groups were found, therefore, smokers reaction times to smoking-related words were not significantly different to that of non-smokers ($F = (1, 39)\ 1.006$, $p = 0.322$). Non-smokers took on average 697 ms (S.D. 0.135) to respond to smoking-related words, 705 ms (S.D. 0.102) to respond to neutral words and 745 ms (S.D. 0.144) to respond to colour words. Smokers spent on average 750 ms (S.D. 0.149) to respond to smoking words, 770 ms (S.D. 0.179) to respond to neutral words and 865 ms (S.D. 0.318) to respond to colour words.

Due to the non-significant effect, data from smokers were divided into two groups (n=20), those that had smoked within the hour previous to participation in the study (immediate group) and those who had not smoked for at least one hour prior to participation (one hour group). ANOVA was performed on this data but failed to yield a significant effect. Smokers who had not smoked for at least one hour did not respond significantly slower than those who had smoked immediately prior to taking part in the study, $F = (1, 19)\ 0.589$, $p = 0.449$.

Conclusion

This pilot study demonstrated that the modified Stroop task used for the purpose of this study accurately measured reaction times to substance-related words. Evidence for this was obtained by comparing reaction times of participants to words printed in different colours.

However, using the Stroop task, this study did not support previous findings that smokers respond more slowly to smoking-related words than do non-smokers. There may be several reasons as to why this study failed to replicate. Firstly, participants were approached in a common room (sitting area) where smoking is permitted. This was because the common room is a prime location for obtaining an opportunity sample of volunteers to participate in the study. Results may have been affected by this variable due to the fact that smokers were likely to have just smoked a cigarette prior to taking part in the study. Therefore, smokers' would probably have responded more quickly to the smoking-related words having just had a cigarette, compared to if they were craving nicotine. There are two reasons for this; in smokers, memory, learning and cognition are delayed during nicotine withdrawal and craving plays a role in the degree of attentional bias seen in this sample (McCusker, 2001).

In order to test this hypothesis, data from smokers in the sample were divided into two groups, those that had smoked within the hour previous to participation in the study and those who had not smoked for at least one hour prior to participation. However the study failed to yield a significant effect. Smokers who had not smoked for at least one hour prior to the study did not respond significantly slower to smoking related words than smokers who had smoked just before the study. This result could be due to problems with the time limit set, that is, one hour being an insufficient time limit to yield adequate levels of craving for cigarettes. To overcome this problem, the Stroop task could be tested using a sample of substance abusers in treatment, hence abstaining from their drug of choice for a longer period.

Pilot Study 3:

Introduction

In order to test the assumption made in Pilot Study 2, further piloting was carried out.

Method

Alcoholics who were currently in treatment for alcohol dependence and abstaining from alcohol (n=20) mean reaction times for each condition (alcohol, neutral, colour words) were compared to that of controls (n=20) using a repeated-measures ANOVA.

Results and conclusion

No significant differences between groups were found, alcoholics reaction times to alcohol-related words were not significantly different to that of controls, $F = (1, 39)$ 0.056, $p = 0.813$. As this result yielded a non-significant effect it was concluded that the programme fails to measure an emotional Stroop effect. Therefore the programme was to be modified.

Pilot Study 4:

Introduction

Blocking the conditions modified the emotional Stroop task. Originally the Stroop task used in Pilot Studies 1, 2 and 3 consisted of unblocked words from all conditions being randomly presented and shuffled after each participant presentation. However, Waters & Feyerabend (2000) adopted both the blocked and unblocked format and found an attentional bias to smoking-related words in the blocked format, but not in the unblocked format. The reasons for this are not yet clear, but could be due to carryover effects whereby participant's attention is still on the smoking-related word when a neutral word is presented, thus slowing participant's response time to the neutral word. This difference has also been observed with social phobia. Holle, Neely & Heimberg (1997) found in their study that participant's suffering with social phobia were significantly slower to colour name threat-related words than neutral words when the word types were blocked together.

Method

This hypothesis was tested using a sample of 11 heroin dependent participants who were currently in treatment for heroin dependence (heroin users were employed for the piloting procedure this time because of the nature of data collection being that access to the various substance using groups varied from one period to the next during the data collection stage).

Results and conclusion

Results demonstrated that heroin dependent participants took significantly longer to colour name heroin-related words (mean 946 ms, S.D. 0.190) than they did to colour name neutral words (mean 901 ms, S.D. 0.161), $t = (1, 10)$ 2.662, $p < 0.05$. As this result yielded a significant effect it was concluded that the programme does in fact

measure an emotional Stroop effect so the Stroop task was changed to a blocked format for the main study.

Main Study

Introduction

For the main study an emotional Stroop task was designed based on previous literature and pilot work conducted prior to the study (detailed above). This task was carried out on a Laptop computer with a control pad displaying four colour buttons (red, blue, green, and yellow) and a rest bar (space bar key). Each participant received 154 trials during the task. A practice trial preceded the main task. This comprised 10 words (red, blue, green, yellow, heroin, alcohol, cocaine, cigarette, impulsive and door). The main task consisted of 144 trials grouped into blocks of 48 trials for each of the three conditions. Condition one consisted of 16 opiate-related, 16 alcohol-related and 16 neutral words. Condition two had 16 stimulant-related, 16 smoking-related and 16 neutral words and Condition three comprised 16 sensation-related words, 16 colour words and 16 neutral words.

Method

A sample of 400 volunteers (100 heroin abusers, 100 alcohol abusers, 100 cigarette smokers and 100 healthy controls) consented to take part in the study. The heroin and alcohol groups were invited to take part in the study as they attended their outpatient treatment service and cigarette smokers and controls were invited to participate in the study as they attended their GP service for routine appointments. Participant characteristics, including substance use history, are given in tables 2.1-2.3, pages 43-49. Mood status was assessed using the POMS-SF (McNair, et al., 1981). Severity of dependence was measured using the SDS (Gossop, et al., 1995), SADQ (Stockwell, et al., 1979) and the QSU (Tiffany and Drobes, 1991).

The emotional Stroop task was carried out on a laptop computer that featured 144 trials. A practice trial preceded the main task. The program began with participants reading the on-screen instructions stating that blocks of coloured words, or words written in different colours, would be displayed on the screen. Participants were instructed to name the colour of each word, whilst ignoring the word content, as quickly and as accurately as possible (full procedural details are provided in the

methodology chapter, pages 54-59). Participants were also given a visual analogue scale to measure their craving levels (0 = not at all to 10 = extremely) before and after the Stroop task to ensure that subjective craving levels had not been induced by the task itself. Paired samples t-tests revealed no significant differences between subjective craving levels before and after the Stroop task in the heroin group (t [1, 97] = 1.808, p = 0.074, mean difference in craving level 0.19), the alcohol group (t [1, 99] = 0.214, p = 0.831, mean difference in craving level 0.03), or the smoking group (t [1, 93] = 1.264, p = 0.209, mean difference in craving level 0.09).

Participants also provided a sample of their DNA using a cheek swab kit for the purposes of additional studies contained within this thesis (detailed in chapters 4 and 5). Full details of the methodology used to test the hypothesis are given in chapter two (p 40-64).

Results

The heroin group made on average 0.65 errors (S.D. 2.517) compared to the control group who made on average 0.53 errors, (S.D. 0.993). No significant differences between groups were found with regard to the number of errors made, t (1, 174) = 0.404, p = 0.307. Additionally, participants did not delay their responses to ensure accuracy during the task (r = 0.122, n = 176, p = 0.108, two-tailed). Finally, the majority of participants reached ceiling effects that is 73.3% of responses made were correct. Secondly, the alcohol group made on average 0.32 errors (S.D. 1.034) compared to the control group who made on average 0.54 errors, (S.D. 0.993). No significant differences between groups were found with regard to the number of errors made, t (1, 173) = 1.439, p = 0.122. Additionally, participants did not delay their responses to ensure accuracy during the task (r = 0.082, n = 175, p = 0.281, two-tailed). Finally, the majority of participants reached ceiling effects, that is, 77.1% of responses made were correct. Finally, smokers made on average 0.49 errors (S.D. 1.079) compared to the control group who made on average 0.48 errors, (S.D. 0.871). No significant differences between groups were found with regard to the number of errors made, t (1, 158) = 0.081, p = 0.393. Additionally, participants did not delay their responses to ensure accuracy during the task (r = 0.120, n = 160, p = 0.132, two-tailed). Finally, the majority of participants reached ceiling effects, that is, 73.8% of responses made were correct.

Therefore, mean correct reaction time (RT) scores from the computerised Stroop task were analysed using repeated measures ANOVA with stimulus type (heroin-related versus neutral stimuli) as the within participants factors and group (heroin versus control) as the between participant factor. The within participant effect for stimulus type was significant, $F(1, 174) = 20.936$, $p < 0.001$. There was also a significant interaction between stimulus type and group, $F(1, 174) = 5.742$, $p = 0.018$. Planned comparisons t-tests indicated that the heroin group spent significantly longer responding to the heroin-related words than they did to the neutral words when compared to controls ($t[1, 174] = 3.411$, $p < 0.001$. For mean RT see table 6.1).

Table 6.1. Heroin and control groups mean Stroop RT in milliseconds (ms) on stimulus type.

Stimulus	**Group**					
	Heroin		**Control**		**Total**	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Heroin	1025	0.384	866	0.177	953	0.317
Neutral	918	0.244	834	0.178	880	0.220
Interference score	107	32			73	

Possible confounding variables were entered into a hierarchical regression model with reaction time difference score (RT heroin words minus RT neutral words) as the dependent variable (for beta values see table 6.2). At stage one, group status was entered into the model, $F(1, 174) = 5.742$, $p < 0.05$, adjusted R square = 0.026, followed by mood at step two, $F(2, 173) = 3.074$, $p < 0.05$, adjusted R square = 0.023, ImpSS at step three, $F(3, 172) = 2.060$, $p > 0.05$, adjusted R square = 0.018, and sex and age at the final step, $F(5, 170) = 1.882$, $p > 0.05$, adjusted R square = 0.025. Results showed that group status, entered alone, best predicted RT difference score, independent of these possible confounding variables.

Table 6.2. Regression coefficient (beta values) with RT difference scores as the dependent variable.

	Beta	P
Group	0.179	0.018
Mood	0.054	0.515
ImpSS	0.023	0.798
Sex	-0.142	0.080
Age	-0.024	0.764

Mean correct RT scores from the computerised Stroop task were analysed using repeated measures ANOVA for comparisons with alcoholics and controls. Stimulus type (alcohol-related versus neutral stimuli) was the within participants factors and group (alcohol versus control) was the between participant factors. The within participant effect for stimulus type was significant, F (1, 173) = 6.863, p = 0.010. However there was no significant interaction between stimulus type and group, F (1, 173) = 0.891, p = 0.347, indicating that reaction times were significantly slower for alcohol-related words in both alcoholics and controls (see table 6.3).

Table 6.3. Alcohol and control groups mean Stroop RT (ms) on stimulus type.

Stimulus	**Group**					
	Alcohol		**Control**		**Total**	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Alcohol	1053	0.386	861	0.189	966	0.326
Neutral	982	0.350	833	0.178	914	0.294
Interference score	71		28		52	

Mean correct RT scores from the computerised Stroop task were analysed using repeated measures ANOVA with stimulus type (smoking-related versus neutral stimuli) as the within participants factors and group (smoking versus control) as the between participant factors. The within participant effect for stimulus type was significant, F (1, 157) = 5.666, p = 0.019. There was also a significant interaction between stimulus type and group, F (1, 157) = 11.243, p = 0.001. Planned comparisons t-tests indicated that smokers spent significantly longer responding to the

smoking-related words than they did to the neutral words when compared to controls (t [1, 157] = 3.721, p <0.001. For mean RT see table 6.4).

Table 6.4. Smoking and control groups mean Stroop RT (ms) on stimulus type.

Stimulus	Group					
	Smoking		Control		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Smoking	853	0.211	826	0.164	839	0.188
Neutral	837	0.297	833	0.178	835	0.244
Interference score	16		-7		4	

Possible confounding variables were entered into a hierarchical regression model with reaction time difference score (RT smoking words minus RT neutral words) as the dependent variable (for beta values see table 6.5). At stage one, group status was entered into the model, F (1, 157) = 11.243, p < 0.005, adjusted R square = 0.061, followed by mood at step two, F (2, 156) = 5.896, p < 0.005, adjusted R square = 0.058, ImpSS at step three, F (3, 155) = 3.906, p < 0.05, adjusted R square = 0.052, and sex and age at the final step, F (5, 153) = 2.946, p < 0.05, adjusted R square = 0.058. Results showed that group status best predicted RT difference score independent of other factors.

Table 6.5. Regression coefficient (beta values) with RT difference scores as the dependent variable.

	Beta	P
Group	-0.264	0.001
Mood	0.077	0.335
ImpSS	-0.010	0.914
Sex	-0.116	0.153
Age	0.060	0.476

Mean correct reaction time scores were analysed using a 2x2 repeated measures ANOVA with stimulus type (sensation-related versus neutral words) as the within participant factors and impulsive sensation seeking (ImpSS) type (high versus low) as

the between participant factors. ANOVA was performed to determine whether groups high and low on ImpSS differed from one another with respect to their Stroop performance. The within participant effect for stimulus type was not significant, F (1, 78) = 0.145, p = 0.704 and there was no significant interaction with stimulus type and ImpSS level, F (1, 78) = 0.085, p = 0.771. However, there was a significant main effect of ImpSS, F (1, 78) = 5.343, p = 0.023, indicating that impulsive sensation seekers responded significantly faster on both stimulus types than did low sensation seekers (mean RT table 6.6)

Table 6.6. Mean Stroop RT (ms) across levels of ImpSS and stimulus type.

Stimulus	**ImpSS Level**			
	Low		**High**	
	Mean	S.D.	Mean	S.D.
Sensation	850	0.168	767	0.092
Neutral	860	0.196	769	0.099

A 2x2 repeated measures ANOVA was carried out with stimulus type (heroin-related versus neutral words) as the within participant factors and DRD4 allele (long versus short) as the between participant factors. ANOVA was performed to examine whether presence or absence of the long allele at the DRD4 gene differentiated participants with respect to their Stroop performance. The within participant effect for stimulus type was significant, F (1, 159) = 23:301, p <0.001. There was also a significant interaction between stimulus type and group, F (1, 159) = 5.133, p = 0.025. Planned comparisons t-tests indicated that individuals with the long allele at DRD4 spent significantly longer responding to the heroin-related words than they did to the neutral words when compared to participants without the long allele (t [1, 159] = 2.446, p = 0.016. For mean RT see table 6.7).

Table 6.7. Long and short allele groups mean Stroop RT (ms) on stimulus type.

Stimulus	Group					
	Long		Short		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Heroin	1102	0.426	929	0.298	953	0.317
Neutral	943	0.197	872	0.228	880	0.220
Interference score	159		57		73	

A 2x2 repeated measures ANOVA was carried out with stimulus type (alcohol-related versus neutral words) as the within participant factors and DRD4 allele (long versus short) as the between participant factors. ANOVA was performed to examine whether presence or absence of the long allele at the DRD4 gene differentiated participants with respect to their Stroop performance. The within participant effect for stimulus type was not significant, $F(1, 154) = 2.281$, $p = 0.133$ and there was no significant interaction between stimulus type and group, $F(1, 154) = 0.107$, $p = 0.744$ (see table 6.8.).

Table 6.8. Long and short allele groups mean Stroop RT (ms) on stimulus type.

Stimulus	Group					
	Long		Short		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Alcohol	977	0.258	946	0.321	966	0.326
Neutral	932	0.200	917	0.322	914	0.294
Interference score	45		29		52	

Finally, a 2x2 repeated measures ANOVA was carried out with stimulus type (smoking-related versus neutral words) as the within participant factors and DRD4 allele (long versus short) as the between participant factors. ANOVA was performed to examine whether presence or absence of the long allele at the DRD4 gene differentiated participants with respect to their Stroop performance. The within participant effect for stimulus type was significant, $F(1, 144) = 10.576$, $p = 0.001$. There was also a significant interaction between stimulus type and group, $F(1, 144) = 4.287$, $p = 0.040$. Planned comparisons t-tests indicated that individuals with the long

allele at DRD4 spent significantly longer responding to the smoking-related words than they did to the neutral words when compared to participants without the long allele (t [1, 144] = 3.189, $p = 0.002$. For mean RT see table 6.9).

Table 6.9. Long and short allele groups mean Stroop RT (ms) on stimulus type.

Stimulus	Group					
	Long		Short		Total	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Smoking	965	0.338	823	0.148	839	0.188
Neutral	901	0.267	809	0.147	835	0.244
Interference score	64		14		4	

Discussion

Findings support the hypothesis that heroin users have an attentional bias to stimuli associated with their drug of choice. This attentional bias can be explained as reflecting the activation of the incentive sensitisation system, as heroin-related stimuli are particularly salient cues that are wanted by the user, therefore directing attention toward the cue and away from the task at hand. The degree of activation of incentive sensitisation is said to be the key mechanism that makes heroin attractive and wanted to susceptible individuals, thus directing behaviour towards drug use (Robinson & Berridge, 1993). It appears that repeated administration of heroin leads to sensitisation of the effect that heroin has on dopamine activity in the nucleus accumbens and related circuitry. Robinson & Berridge (2000) argue that the mesolimbic dopamine system becomes enduringly sensitised to specific drug effects and associated stimuli and that this drug-induced brain change psychologically leads to excessive attribution of incentive salience to drug-related cues, causing the user to have a pathological 'want' to take the drug. Although conducted artificially, if the results from this present study were applied to real life, it could be argued that heroin users would be more likely to notice drug-related stimuli in the environment and elicit conditioned responses to those stimuli due to their sensitised brain systems.

The attentional bias, found in this study, occurred independent of other factors that were thought to play a role in this processing bias. Firstly, it was thought that subjective craving levels and mood could influence attentional bias among drug users. For example, Franken, Kroon, Wiers & Jansen (2000) found that reaction times to heroin-related cues were significantly predicted by heroin craving levels. In addition, negative mood state was found to account for cue elicited urges in opiate users, whereby feelings of boredom, anxiety and anger strongly correlated with desire to use when heroin-related cues were present (Sherman, et al, 1989). However, among heroin users, an attentional bias to heroin-related words was apparent in the present study irrespective of other related factors.

Another extraneous factor that could have influenced findings from this study, but was not systematically controlled, relates to priming. During the study procedure, participants were administered a pack that consisted of three parts: a series of questionnaires, a cheek swab kit and a Stroop task. These materials were not systematically counterbalanced, participants were free to complete them in the order in which they chose. If participants completed the questionnaires prior to the Stroop task, then either the substance use history questionnaire or the severity of dependence scale could have potentially influenced their response times on the Stroop task by priming them for the presentation of substance-related stimuli. Priming can automatically activate associated cues and facilitate responses to semantically related stimuli (Fazio, 2001). Therefore, future research should control for this as a possible confounding factor.

Impulsive sensation seeking was also thought to influence attentional bias. Neary & Zuckerman (1976) suggested that high sensation seekers have strong excitatory CNS processes. Zuckerman (1979) proposed high and low sensation seekers process environmental information differently due to differences in their levels of arousal and attention, claiming a relationship between SS and cognitive impulsivity. Sensation seekers search for intrinsic reward or positive arousal that can be gained by novel, complex or unpredictable stimuli to maintain an optimal level of arousal. In support of Zuckerman's theory, Martin (1985) found that high sensation seekers perform well in tasks where selective attention is required. Participants were administered an embedded figures test that required them to attend to a single stimulus whilst ignoring

another. High sensation seekers performed better than low sensation seekers on this task. Ball & Zuckerman (1992) provided further evidence using a dichotic-listening task whereby participants were asked to listen to different stimuli presented to each ear. Focused attention was measured by having the participant repeat each word they heard in the target ear, while ignoring the non-attended ear. They found that with novel sensations (sexual, violent, drug-related words), high sensation seekers had better focused attention than low sensation seekers. To explain this finding, high sensation seekers appear to have increased attention to focal stimuli and are more able to ignore irrelevant information when they are presented with novel stimuli (Martin, 1985). However, despite these previous results, Stroop interference occurred in the present study irrespective of age, sex, mood disturbance and ImpSS levels.

A second finding from this study showed that although high sensation seekers, compared to low sensation seekers, were quicker to respond to stimuli overall, the two groups did not differ in their responses to sensation-related stimuli in the Stroop task. One explanation could be that the sensation seeking stimuli used were not novel or exciting enough to acquire the attention of a high sensation seeker. That is, high sensation seekers may not have experienced the stimuli presented as complex and varied, but as familiar and repetitive, so failure to detect a group difference may indicate that the processing demands of the task were not sufficient. Alternatively, a plausible assumption would be that the effect was not found because participants were recruited from the general population and non-clinical samples do not tend to demonstrate attentional bias to the degree of clinical samples (Williams et al., 1996). Interference is due particularly to threatening stimuli, e.g. the word *spider* presented to a person with arachnophobia. Future research would be necessary to test these assumptions set out.

Another result of the present study was that smokers, like heroin users, showed attentional bias to stimuli related to their drug of choice. This occurred regardless of age, sex, mood disturbance and impulsive sensation seeking levels. A study conducted by Gross et al (1993) was the first of its kind to measure Stroop performance amongst smokers and was followed by Waters & Feyerabend (2000) who recruited 96 smokers, who either abstained for 24 hours or smoked normally and were given blocked and unblocked Stroop task. The authors found that in the blocked format,

abstinence produced an attentional bias to smoking-related stimuli and it was argued that smokers become hypersensitive to smoking-related stimuli in their environments, which could be problematic if they were attempting to quit. The processing of stimuli could lead to conditioned responses such as withdrawal features. From their results, Waters & Feyerabend (2000) concluded that the initial response to smoking-related cues are important determinants of early smoking and attentional bias might be driving smoking behaviour.

Support for the present finding also came from a study by Wertz & Sayette (2001) who examined the emotional Stroop effect using three groups of 30 smokers. The authors found an overall Stroop effect with smoking and neutral words. Smoking opportunity affected the degree of interference, whereby the group who were told that they could smoke during the experiment showed greater interference than the group who were told that they could not smoke throughout the experiment. From this study it was argued that smoking opportunity affects the salience of smoking-related words among nicotine deprived smokers.

The present study also provides additional evidence for the alcohol Stroop paradigm. Alcoholics showed an attentional bias toward alcohol-related stimuli, demonstrating that alcoholic's process information that is emotionally salient to their psychopathology differently to the way that they process neutral information. However, surprisingly, control participants also showed this effect. The first studies to use an alcohol Stroop task were published in 1994. Johnsen, Laberg, Cox, Vaksdal & Hugdahl (1994), in their study, found larger interference scores among problem drinkers than a control group of social drinkers. The following year, interference scores were calculated by subtracting the mean reaction time to neutral words from that of alcohol-related words on a Stroop colour naming task and a larger interference score was found with problem drinkers when compared to controls (Stetter, et al, 1995).

More recently, and in line with the present findings, studies examining Stroop performance in alcoholics and controls have found non-dependent drinkers also show longer response times to alcohol-related words than to neutral words. For example, Bauer & Cox (1998) compared Stroop performance among 20 alcoholics and 20 non-

dependent alcohol drinkers and they found that alcohol-related words were distracting for drinkers in general, regardless of different levels of alcohol consumption. In addition, Sharma, Albery & Cook (2001b) found an attentional bias to alcohol-related stimuli in both problem and non-problem drinkers.

Despite the controversy that surrounds alcohol Stroop effects, it appears that alcohol-related stimuli capture the attention of drinkers in general, and this can also be illustrated by results that have been gathered from research that has measured attentional bias using alternative methods to the Stroop task. Townsend & Duka (2001) examined differences between heavy and occasional social drinkers using a dot probe detection task. Heavy social drinkers showed attentional bias toward alcohol-related stimuli when compared to occasional social drinkers. This study supported cognitive theories of addiction, showing that the ability of alcohol-related stimuli to capture attention plays an important role in substance dependence, craving and relapse. This study demonstrated that although methodological issues should be considered when interpreting results, regardless of the method employed to measure attentional bias in substance dependence, alcohol users respond differently to alcohol-related stimuli than to emotionally neutral stimuli.

The present findings are consistent with Tiffany's cognitive processing model (Tiffany, 1990), which claims that cognitive processes motivate the development of addictive behaviour during the course of substance history, from initiation to maintenance of use. Tiffany claims that automaticity develops through repeated use and experience with substance-related environmental stimuli, which creates a network of substance-related concepts in memory. Therefore, substance abusers find it difficult to ignore substance-related stimuli when compared to control participants.

To frame the incentive sensitisation theory to these present findings, this attentional bias can be explained as reflecting the activation of the incentive sensitisation system. Drug-related stimuli are particularly salient cues that are wanted by the drug user, therefore directing attention toward the cue and away from the task at hand. The degree of activation of incentive salience is said to be the key mechanism that makes drugs attractive and wanted, thus directing behaviour towards substance use (Robinson & Berridge, 1993). Robinson & Berridge (2000) argue that the mesolimbic

dopamine system becomes enduringly sensitised to specific drug effects and associated stimuli and that this drug-induced brain change psychologically leads to excessive attribution of incentive salience to related cues, causing the drug user to have a pathological want to take drugs. Results support the belief that drug users are more likely to notice drug-related stimuli in the environment and elicit conditioned responses to those stimuli due to their sensitised brain systems.

Both the incentive sensitisation theory (Robinson and Berridge, 1993) and the cognitive processing model (Tiffany, 1990) claim that substance-related stimuli have the ability to capture the attention of substance users and that attentional bias plays an important role in substance dependence (Everitt, Dickinson and Robbins, 2001), however, the two models differ in certain respects. For example, the incentive sensitisation theory maintains that dopamine is a key feature for all addictive substances in that drugs are reinforcing because they produce pleasure in the reward system of the brain, therefore negative cues may not produce incentive salience. Alternatively, the cognitive processing model assumes that automaticity develops through repeated use and exposure to substance-related cues and this generates a network of both positive and negative associations in memory. The cognitive processing model states that negative cues can trigger relapse by reducing cognitive resources rather than by directly motivating substance use.

Finally, an interesting finding from this present study was that the DRD4 gene polymorphism influences cue reactivity in heroin users and cigarette smokers. Both groups spent significantly longer to respond to substance-related stimuli if participants carried the long allele at the DRD4 gene, whereas participants without the long allele did not show this difference. This finding is in line with previous research (Lerman, Caporaso, Audrain, Main, Bowman, Lockshin & Shields, 1999; Sabol, et al., 1999) that has found the DRD4 gene to moderate cue-elicited craving in smokers. However, it does not support a previous positive finding conducted with alcohol dependent participants (Hutchison, et al., 2002). Reasons for this discrepancy between findings could be difficult to identify because *"given the current state of knowledge regarding the functional significance of the DRD4 and the biological action of alcohol, the precise nature of the interaction between the DRD4 VNTR polymorphism and alcohol is not clear."* [Hutchison, et al., 2002: p143].

Cautiously, it could be argued that these conflicting findings are a result of the differing populations, measures and methods used in the studies. The present study used a Stroop task to measure attentional bias to alcohol-related cues, whereas previous work has used alcoholic and non-alcoholic beverages as cues to elicit craving for alcohol. Therefore, the two studies are not entirely comparable. Alternatively, failure to identify any influence of the DRD4 gene polymorphism on reactivity to alcohol-related cues in the present study could be due to the relative importance of this particular gene on alcohol dependence. Study three, presented earlier in chapter five of this thesis, reported an association with the DRD4 gene long allele and heroin and nicotine dependence, however the association with this polymorphism did not remain significant in alcohol abusers after Bonferroni corrections had been made for multiple testing. Therefore, the trend that can be seen from the findings reported here appears consistent in that the DRD4 gene has a greater influence on drug addiction than it has on alcohol addiction, with other genes, such as the DRD2 gene, possibly having a greater effect (detailed in chapter 5). Further work, examining the relative importance of other genes in alcohol cue reactivity, is necessary to clarify this issue. Nevertheless, the long allele at the DRD4 gene may represent a genetic mechanism that influences the expression of incentive salience of both heroin and nicotine.

Taken together, these findings can be explained from the view of the incentive sensitisation theory, which was first proposed by Robinson & Berridge (1993). Sensitisation is a permanent process (Robinson & Berridge, 2003) and this might help to explain why substance users go back to compulsive drug use in the absence of any withdrawal effects and after long periods of abstinence. It appears that craving is elicited by cues whereby related stimuli have the ability to direct behaviour toward drug seeking. This being the case, there are implications for the treatment of substance dependence. If an individual were susceptible to sensitisation, then associated cues would be powerful incentives to control in an attempt to remain abstinent weeks, months or years after pharmacological treatment ceases. For example, Naltrexone is used to treat heroin addiction by blocking opiate receptors, so if taken, heroin has no effect. However, the implication for this treatment, and others like it, is that sensitisation is permanent, so further drug use could follow

pharmacological treatment because environmental cues associated with heroin would remain powerful incentives for the user.

In conclusion, it is apparent that most people who experiment with drugs or alcohol do not become addicted to them. Therefore, a current challenge is to discover the neurobehavioral elements that may influence this vulnerability of an individual's transition to drug addiction (Koob & Le Moal, 1997). If neuroadaptions occur when substances are repeatedly administered, then there should be large individual differences in susceptibility to this sensitisation. Results from the present study support the incentive sensitisation theory of addiction and provide evidence from human populations that substance users respond differently to stimuli that are associated with their drug, than they do to neutral stimuli, when compared to controls. Attentional bias measures such as the Stroop task can capture the degree of incentive salience to substance-related words, which in turn may reflect the degree of sensitisation of the neural system. Additionally, individuals with the long variant at the DRD4 gene spent longer responding to words related to heroin and cigarettes, thus demonstrating individual differences in cue reactivity. By investigating individual susceptibility to sensitisation, according to the incentive sensitisation theory, advances could be made into the utilisation of extinction treatment approaches for susceptible individuals. A fuller understanding of individual susceptibility to sensitisation and the mechanisms that underlie attentional bias could lead to some improvement in the effectiveness of treatment by focusing on the long term urge to use drugs and drink that arises from a sensitised brain system that mediates their motivation to use.

Chapter seven

A concluding discussion from a diathesis-stress perspective

Study 5: Vulnerability to substance dependence: A path analysis

Introduction

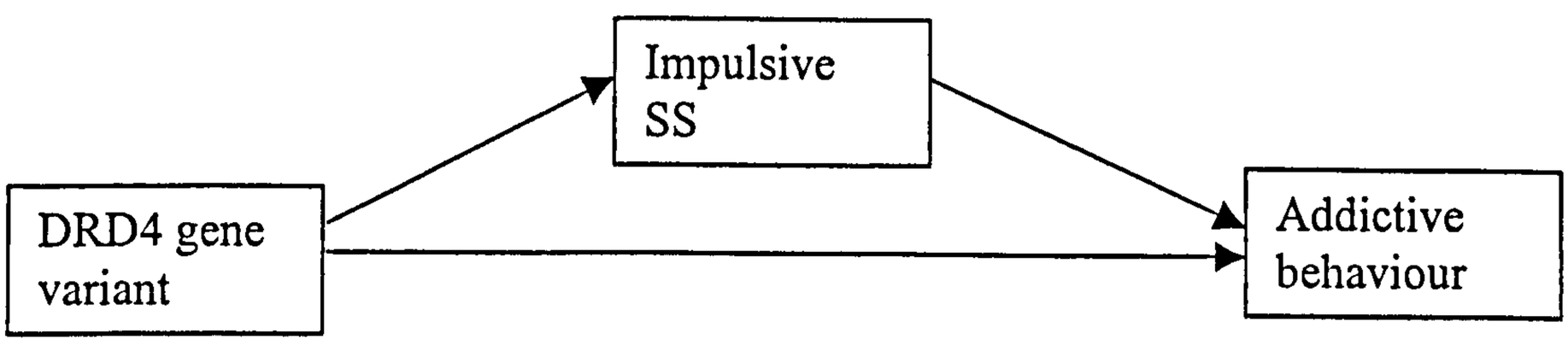
Taken together, findings from studies one to four indicate that vulnerability to substance dependence can be explained within a framework of a diathesis-stress model (detailed in chapter one). The diathesis-stress model emphasises that a number of complex psychological mechanisms and genes are important mediators' in smoking status and sensitivity to nicotine (Gilbert & Gilbert, 1995) and other drugs. The diathesis is the predisposition in terms of biological traits, which create dispositions or vulnerability to the development of substance dependence. Personality acts as a mediator between the diathesis and substance dependence. Biological and environmental stresses may contribute to the development of substance dependence or may also be a trigger for episodes of dependence. Stress must be independently defined as that which happens to a person rather than as a reaction to the stress, reactions to the stress are a function of an individual's vulnerability and genetic factors can influence these reactions to events. Although stress may originate in substance dependence, stress can exacerbate the symptoms. For example, a person may become stressed from work so drink to reduce the stress, lose their job due to drinking and therefore drink more. Stress is relatively non-specific so any kind of stress can interact with specific diathesis producing substance dependence. Finally, from a diathesis-stress perspective, it could be argued that substance dependence is a result of a direct neurochemical pathway to positive mood, which can no longer be satisfied by pleasure from normal life rewards in genetically vulnerable individuals.

To elaborate, ultimately study one (chapter three) found that heroin users were significantly higher Impulsive Sensation Seekers (ImpSS) than all other groups and control participants were significantly lower impulsive sensation seekers than all substance abusing groups. Therefore, in line with the diathesis-stress model, the impulsive sensation seeking personality trait may either demonstrate a direct pathway to substance dependence, or more convincingly, genetic factors influence the impulsive sensation seeking trait, which in turn leads to drug taking behaviour. Impulsive sensation seekers act impulsively and seek out novel and exciting risky

behaviours in an attempt to maintain an optimal level of arousal (Zuckerman, et al., 1993). Therefore, individuals with these characteristics would be more prone to using substances that are a risk to, or compromise, our health and would be more likely to experiment with illegal drugs. Additionally, study two (chapter four) found that impulsive sensation seeking was associated with the long variant at DRD4 among heroin and cigarette smoking groups. This biological link could be due to the fact that investigatory behaviours that characterise sensation seeking, activate the dopamine reward pathway to produce reinforcement of that behaviour (Bardo, et al., 1996).

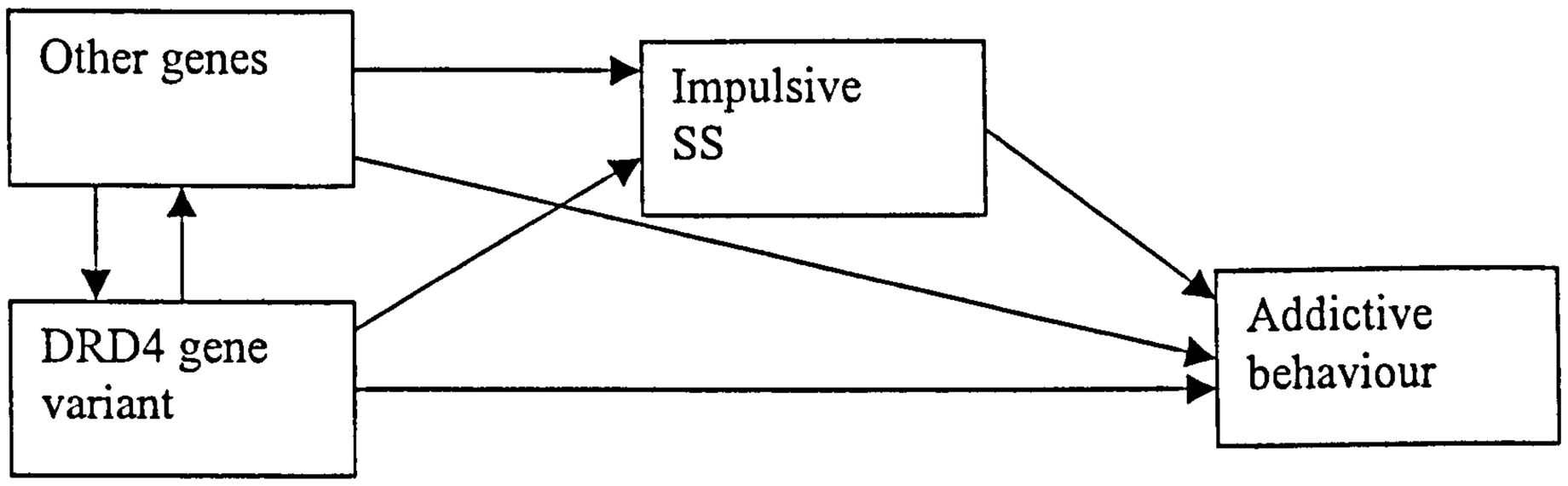
Figure 7.1. illustrates the causal path that has been specified thus far, showing that the DRD4 gene variant can predict addictive behaviour directly, but can also influence addiction via the personality trait impulsive sensation seeking.

Figure 7.1. Proposed causal path to substance dependence: Stage one.



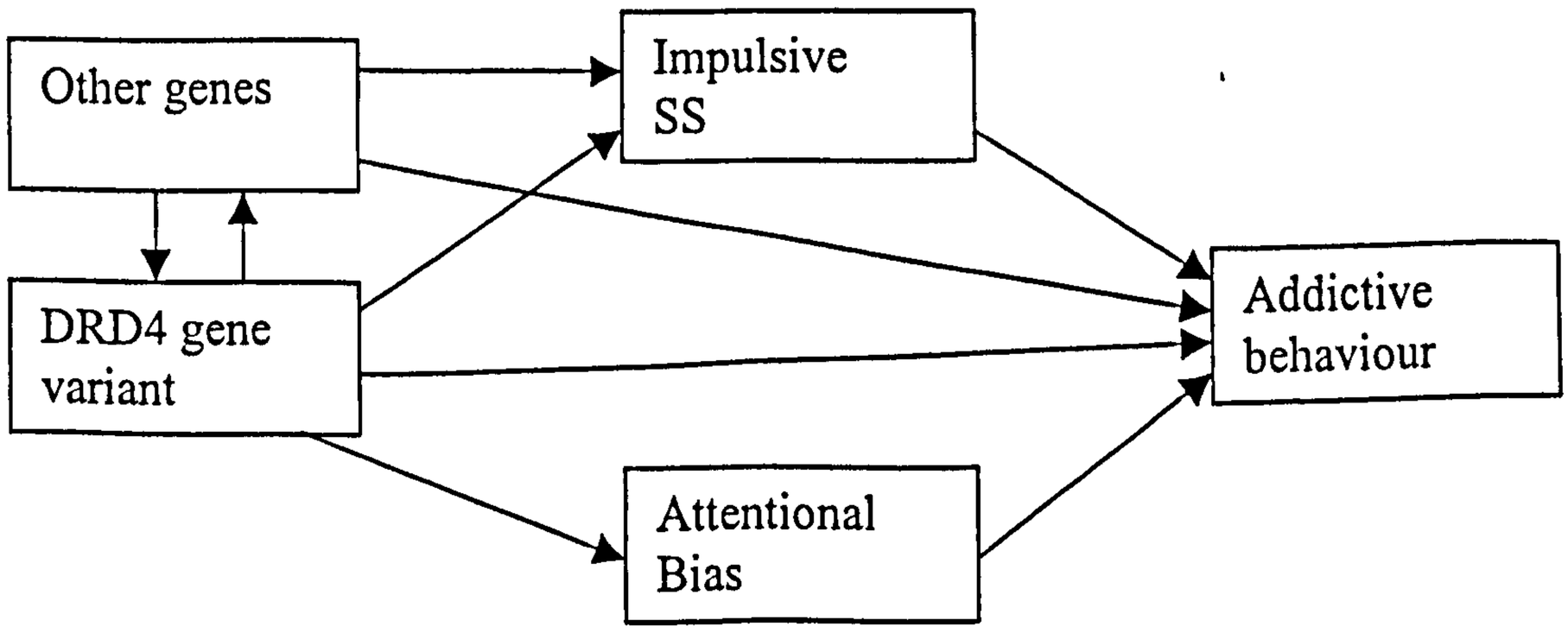
However, study two also reported that the DRD4 gene variant accounted for 11% of the variance in the personality trait ImpSS. Sensation seeking is claimed to be about 50% heritable (Hur & Bouchard, 1997), so it is likely that other genes are interacting with the DRD4 gene and having their own unique effect on ImpSS, and the influence this trait has on predicting substance dependence. This idea is further developed by examining findings from study three (chapter five), which found that heroin abusers and cigarette smokers are significantly more likely than healthy participants to carry the long variant at DRD4. This can be explained from a neurobehavioural standpoint in that substance dependent individuals seek out psychoactive drugs to increase the release of dopamine in the limbic structures of the brain (Hutchison, et al., 2002). A review of the research has also pointed to genes other than the DRD4 gene that contribute to a genetic predisposition to addiction (see Dick & Foroud, 2003). Adding this factor to the model leads to a proposed path illustrated in figure 7.2., which illustrates, theoretically, a direct link between various genes, personality and substance dependence.

Figure 7.2. Proposed causal path to substance dependence: Stage two.



Findings from study four (chapter 6) calls for a further factor to be added to this model, attentional bias. It was found that participants with the long variant at DRD4 showed an attentional bias to drug-related words. Additionally, heroin abusers, cigarette smokers and alcohol drinkers demonstrated an attentional bias to words related to their drug. Therefore, with reference to the diathesis-stress model, these findings posit a model that allows for genetic factors influencing reactions to events and environmental stimuli contributing to addictive behaviour directly, as illustrated in figure 7.3.

Figure 7.3. Proposed causal path to substance dependence: Stage three.



Therefore, taken together data suggest that biological, psychological and social factors influence substance dependence vulnerability either directly or via an interaction. Substance dependent individuals possess a vulnerability to develop a severe dependency to drugs. Often drugs are readily available to all individuals in a particular population, but not all individuals who use drugs go on to form a

dependency on the substance. Therefore, individual differences in personality, genes and neurobehavioural elements interact to play a crucial role in determining the extent of substance dependence. Therefore, based on the research and evidence accumulated in studies one to four, this final study aimed to culminate this thesis by producing three theoretically based causal path models to addiction.

Analytic Method

Structural Equation Modelling (SEM) was used to examine the interaction of Impulsive Sensation Seeking, DRD4 alleles, attentional bias, participant characteristics and dependency factors with the outcome measures heroin, nicotine and alcohol dependence severity. Path analysis, an extension of regression, was used to test the correlation matrix against a theoretically constrained model. The model is depicted in a circle-and-arrow figure, in which arrows indicate causation. A regression is calculated for each variable in the model as a dependent on others, which the model indicates are causes. The regression weights predicted by the model are compared with the observed correlation matrix for the variable and a goodness-of-fit statistic is calculated.

Initially, for each of the three models tested in this study, missing data were removed and only interval level data for all variables were used to satisfy the requirements of the AMOS program used to run the path analysis. A series of path analyses were then conducted using AMOS 4.0 (Arbuckle, 1999). Model selection was based on multiple goodness-of-fit statistics (see Byrne, 2001, for a review). As an absolute fit, the Chi-square value, which tests the overall fit of the model, was examined. A small, non-significant value indicates a good fit of the data, however, because the χ^2 test is sensitive to sample size, tests of relative fit were also used. These included the Tucker-Lewis Index (TLI), the Comparative Fit Index (CFI), the Parsimony adjusted Comparative Fit Index (PCFI) and the Root Mean Square of Approximation (RMSEA). Tucker-Lewis Index (TLI) values of 0.95 or higher indicate a good fit of the data. The CFI should be over 0.9, but 0.95 or above demonstrates a very good fit of the data. The PCFI multiplies the CFI by the percentage of degrees of freedom for testing estimation in a model and values 0.5 or over are considered stable. RMSEA

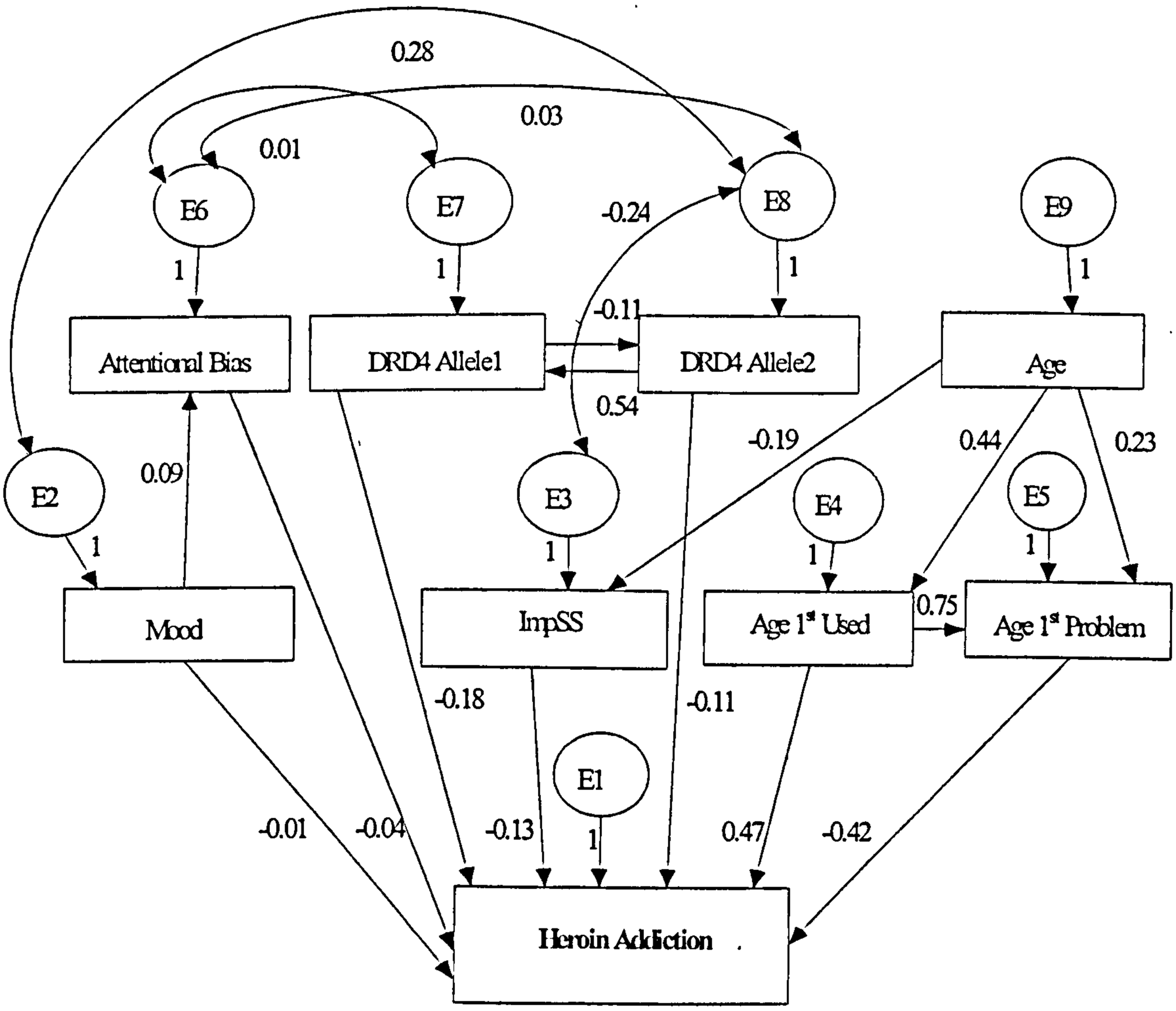
should be less than 0.1, with a value of 0.05 or smaller demonstrating a very good fit (Byrne, 2001).

Three path analyses were fit to explain heroin, smoking and alcohol addiction severity. These were schematically portrayed using a configuration of symbols. Squares or rectangles represent observed variables, circles or ellipses represent unobserved latent variables, single headed arrows represent the impact of one variable on another and double headed arrows represent covariance or correlations between pairs of variables. Associated with each observed variable is a circle with an arrow coming from it, which represents measurement error and unexplained variance.

Heroin path model

As depicted in figure 7.4., the causal path to heroin addiction hypothesised here contains nine observed variables (attentional bias, mood, impulsive sensation seeking, age first used the drug, age first had a problem with the drug, DRD4 allele 1, DRD4 allele 2; and severity of heroin dependence as the outcome measure) and nine unobserved variables (E1-E9), which could account for genetic and/or environmental influences that were not measured directly in the study.

Figure 7.4. A causal path model to heroin dependence (n=90).



From the diagram in figure 7.4, it can be seen that heroin addiction is caused directly by mood, ImpSS, age first problem, DRD4 allele 1, DRD4 allele 2 and attentional bias. However, this model also allows for the hypothesis that other genetic/environmental factors could cause fluctuations in the values of the variables included in the model that would assist in the outcome of heroin addiction. The figure shows that there is shared variance between the unexplained variance of mood and DRD4 allele, attentional bias and DRD4 allele and with DRD4 allele and ImpSS. This model acknowledges that mood can influence the impact that attentional bias has on heroin addiction and that age first used can impact on age first problem, age itself can influence these two variables as well as ImpSS. Finally, the impact that DRD4 allele 1 has on heroin addiction can be influenced by allele 2 and vice versa.

Smoking path model

In line with findings gathered in studies one to four, figure 7.5. shows a similar picture to that of the heroin path model, but instead outlines a causal path to nicotine addiction. Again, the first-order factors (mood, ImpSS, years smoked, allele 1, allele 2 and attentional bias) are all hypothesised to directly effect the outcome variable 'nicotine dependence severity'. Each of these seven observed factors have measurement error and unexplained genetic/environmental variance (E1-E7) attached to them, which can impact on the influence predictor variables have on nicotine addiction.

Figure 7.5. A causal path model to nicotine dependence (n=74).

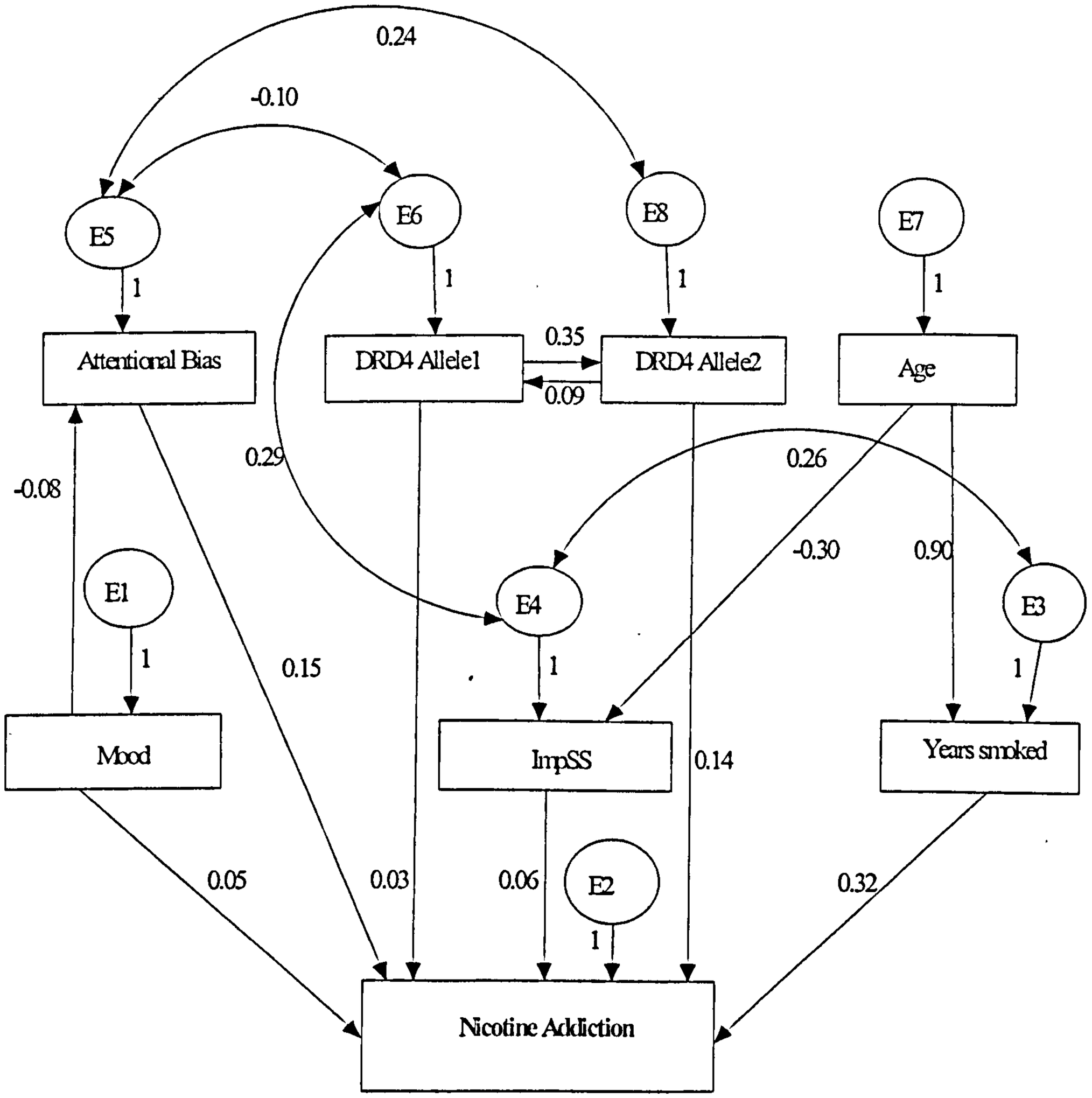
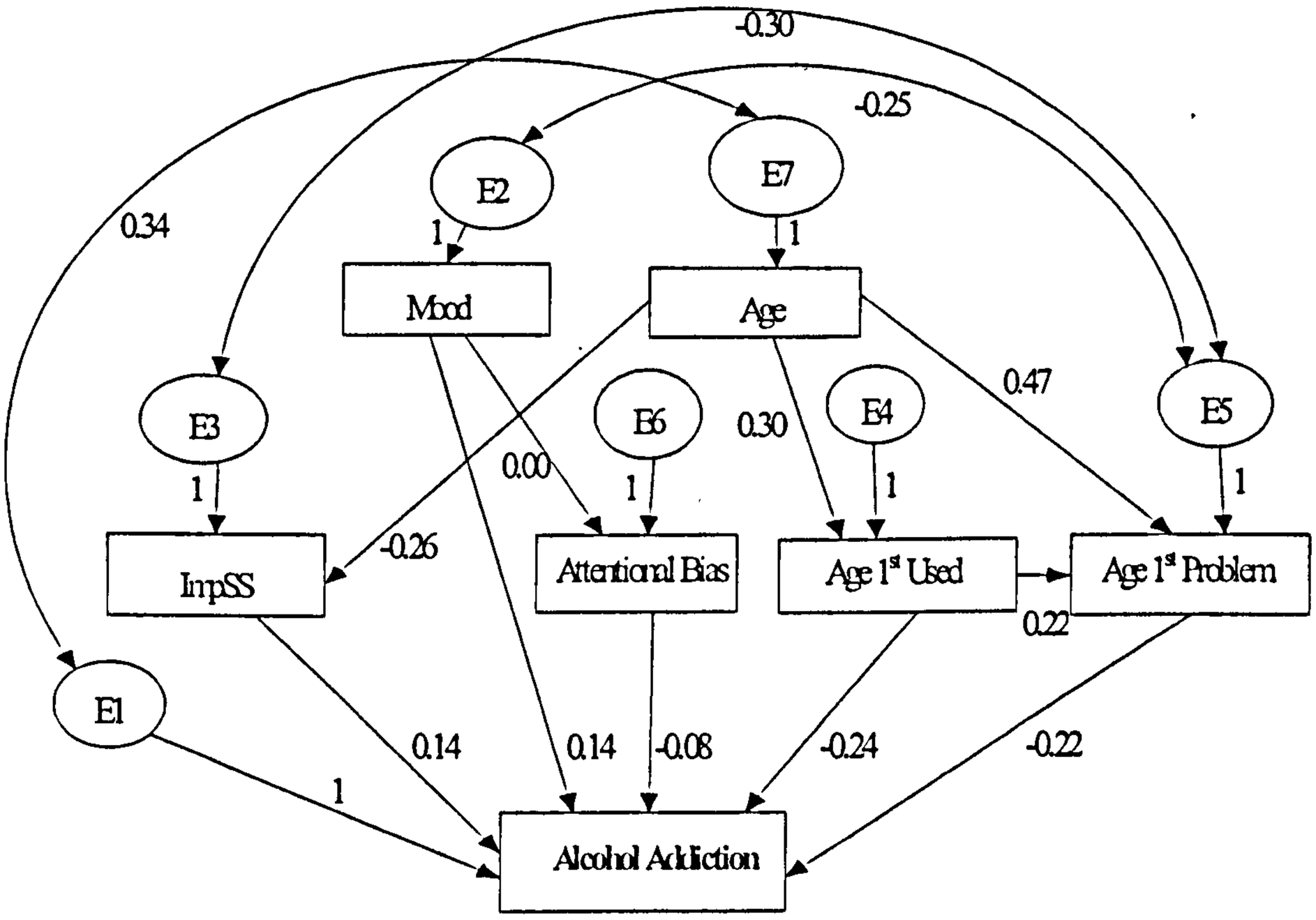


Figure 7.5. illustrates that mood can influence the impact of attentional bias on nicotine addiction. Age, a second-order factor can influence the impact of years smoked and ImpSS and allele 1 and 2 can influence each other. Finally, the unexplained variance attached to ImpSS covaries with that of years smoked and DRD4 allele and the unexplained variance attached to DRD4 allele shares variance with attentional bias.

Alcohol path model

Figure 7.6. shows a theoretically driven path model that is slightly different to those set out for heroin and nicotine addiction. Studies one to four demonstrated that the association with the DRD4 gene variant and substance dependence may be substance specific and this gene did not appear to be as important for alcohol addiction vulnerability as it is for heroin and nicotine addiction. Therefore, the variables included in this model were alcohol dependence severity, as the outcome measure, with ImpSS, attentional bias, mood, age first used and age first problem as the five first-order observed variables and age and mood as the second-order factors. Covariance was indicated for the unexplained variance (E1-E7) related to alcohol addiction and age; ImpSS and age first problem; and age first problem and mood. Again, mood was hypothesised to influence attentional bias and age was hypothesised to influence age first problem, age first used and ImpSS.

Figure 7.6. A causal path model to alcohol dependence (n=85).



Results

The heroin model, illustrated in figure 7.4., demonstrated a very good absolute fit of the model to the data ($\chi^2 = 20.493$, P = 0.428, df = 20). The relative fit statistics also showed the model to fit the data very well in terms of its high TLI (0.995), CFI (0.997), PCFI (0.554) and low RMSEA (0.017).

Figures 7.4., 7.5. and 7.6. show the standardised regression coefficients (beta values) for predictor variables included in the heroin, nicotine and alcohol models respectively. The impact of one variable on another is the square of the path coefficient. Therefore, the predictors included in the heroin model together accounted for around 45% of the variation in heroin addiction severity. Age first used heroin had the greatest impact, explaining over 21% of the variance in heroin addiction severity, with DRD4 alleles explaining less than 5% of the variance.

The nicotine model, illustrated in figure 7.5., demonstrated a very good absolute fit of the model to the data ($\chi^2 = 13.639$, P = 0.400, df = 13). The relative fit statistics also showed the model to fit the data very well in terms of its high TLI (0.991), CFI (0.996), PCFI (0.462) and low RMSEA (0.026).

Over 15% of the variation in nicotine addiction severity was accounted for by the predictor variables included in the model. The number of years smoked had the greatest impact on the dependent variable, followed by attentional bias.

The alcohol model, illustrated in figure 7.6., demonstrated a very good absolute fit of the model to the data ($\chi^2 = 9.263$, P = 0.321, df = 8). The relative fit statistics also showed the model to fit the data very well in terms of its high TLI (0.957), CFI (0.983), PCFI (0.375) and low RMSEA (0.043).

Finally, predictor variables included in the alcohol model explained just below 15% of the variation in alcohol addiction severity. The age that participants first used alcohol was the most predictive factor of severity of alcohol dependence. Attentional bias was shown to have little influence on alcohol addiction severity.

Discussion

Based on study findings discussed in chapters three to six, this final chapter attempted to amalgamate the results by producing a series of three causal pathways to addiction. Findings from the present study demonstrated that heroin, nicotine and alcohol dependence severity can be explained by a combination of the same factors, but the degree of impact that these factors have on dependence severity varies from one substance to the next. For example, the DRD4 gene had a greater influence on heroin addiction than on both nicotine and alcohol addiction. This is in line with previous studies, reviewed in chapter five, which found the long variant at DRD4 to be associated with heroin abuse (e.g., Li, et al., 1997; Mel, et al., 1997: Kotler, et al., 1997), but not alcohol abuse (e.g., Sander, et al., 1997; Geijer, et al., 1997; Roman, et al., 1999). It has been argued that other genes, such as DRD2 and ALDH2, have a greater impact on alcohol addiction than does DRD4 (Dick & Foroud, 2003). Furthermore, the genes thought to contribute to alcohol addiction are likely to vary from one individual to the next, as would the environmental factors that interact with the genes (Dick, Rose, Viken, Kaprio & Koskenvuo, 2001), thus making the task of identifying specific genes involved in addiction even more difficult.

In addition, the impact of the DRD4 gene on heroin addiction was not substantial, accounting for only a small amount of the overall variance in addiction severity. Around 30% of the variance in substance dependence can be accounted for by genetic factors (Plomin, et al., 1994), so it is palpable that future, large-scale studies should include as many genes and environmental factors, thought to be associated with substance dependence as possible into a single path model, in an attempt to explain a significant amount of the variance in addiction behaviour. However, it should be accentuated here that each of the three path models tested demonstrated a very good fit of the data and depicting causal relationships between variables is the primary aim of path analyses, rather than to account for substantial amounts of variance in any given outcome variable.

It is important to acknowledge at this stage, some of the limitations of the present study. One dilemma when constructing the path models was that categorical data could not be included. This was to satisfy the requirements of the AMOS package, which was the only appropriate software available during the time of the study. In

studies one to four, DRD4 gene variants were grouped so the participants with long alleles could be compared with participants with short alleles. Rather than using presence of the long allele at DRD4 as a factor in the model, continuous data indicating the exact length of the allele had to be used here. This was not ideal because it is presence of the long allele, rather than the precise length of the allele, that has been shown to important for an addiction to occur (Asghari, et al., 1995).

Another limitation of this study again relates to the constraints set by the AMOS package used for the analyses. Ideally, the model would have aimed to predict addiction as a discrete category, but the data used in the model had to be continuous, so severity of dependence was used as the outcome measure. This has implications for the findings because it may be the case that the factors included in the model would better predict addiction per se, as opposed to degrees of severity. Replicating this study with an alternative SEM package, such as LISREL, would help to clarify this issue. To illustrate, as shown in the first study and elsewhere (e.g., Scourfield, et al., 1996; Vukov, et al., 1995), heroin abusers are significantly higher impulsive sensation seekers than controls. Once an addiction has been established, the impact that ImpSS behaviour has on characteristics of addiction, such as severity of dependence, may be less focal.

This is an issue that could in fact assist with the interpretation of another key finding from the present study. Impulsive sensation seeking did not have a substantial impact on the prediction of addiction severity in any of the three models tested. In light of the existing evidence, it was hypothesised that ImpSS would influence addiction, which it did, but to a far lesser extent than was anticipated. However, in partial support of the hypothesis, ImpSS did share variance with genetic factors, indicating that there is a relationship between the DRD4 gene and the personality trait ImpSS in explaining addictive behaviour.

A third, key factor built-into each path model was attentional bias. This factor was shown to be less important in the prediction of alcohol addiction than to cigarette smoking and heroin addiction. There is some support for this finding in that study four (detailed in chapter 6) showed that drinkers in general showed an attentional bias to alcohol-related cues. This is possibly due to the distinction between alcoholics and

controls being less clear-cut than the distinction between smokers and non-smokers and heroin abusers and controls. In comparison to heroin abuse and cigarette smoking, a huge proportion of people in our society can drink without forming a dependency on alcohol, so therefore, alcohol-related environmental cues would be more salient to controls than would heroin and nicotine related cues, although this is speculative and was not directly measured.

These findings, in support of the interactive diathesis-personality-stress view of addiction (Monroe & Simons, 1991), do have practical implications for substance abusers and treatment interventions designed for substance abusers. Gaining a better understanding of the vulnerabilities to substance dependence will enable a better understanding of important environmental factors that contribute to substance dependence. Secondly, it will permit a better understanding of the biochemical mechanisms involved in the development of substance dependence. Thirdly, gaining an understanding of the biological mechanisms of substance dependence will better inform treatment approaches targeted to individuals. For example, pharmacotherapy could be used to aid relapse prevention treatments by increasing dopamine release in the brain. Finally, identifying genes that contribute to complex common disorders, such as addictive behaviour, allows for the development of tests used to identify vulnerable individuals prior to the onset of substance dependence. Preventative measures could be adopted by offering genetically predisposed, impulsive, high sensation seeking individuals, alternative methods to satisfy their need for excitement and to avoid boredom. As psychologists can now use DNA in their research to predict genetic risk and biological vulnerability, there is hope for effective intervention. Intervention could be in the form of environmental rather than genetic manipulation. For example, high sensation seeking individuals could be geared to alternative stimuli to satisfy their needs for thrill and excitement rather than through drug taking. In addition, individuals who have already exposed themselves to addictive substances could be given treatment in the form of adventure therapy, whereby they are given alternative behaviours to learn, which would satisfy their sensation seeking appetites. Moreover, there is hope for new treatments in the future, that acknowledge the importance of substance-related stimuli and that these can act as triggers for genetically vulnerable, high sensation seeking substance abusers. With that in mind,

treatments could enhance the use of relaxation training and meditation techniques in the presence of substance-related cues.

Summary and conclusions

Previous studies that have examined DRD4 associations with personality and substance abuse have been varied and difficult to compare. This is due to the differences between demographic and methodological features employed. This was the first study to examine ImpSS, substance abuse and DRD4 using a single, UK population. ImpSS and DRD4 studies have previously concentrated on normal, healthy populations, and substance abuse and DRD4 studies have focused on heroin and alcohol abuse, without considering ImpSS levels and smoking status. There has also been a lack of research examining these associations among Caucasians in the United Kingdom. Previously, ImpSS and DRD4 have been associated, substance abuse and DRD4 have been associated and substance abusers have been found to be high sensation seekers. Therefore, it was appropriate for these phenomena to be examined simultaneously. Twin studies have consistently shown important genetic influences on smoking behaviour but our knowledge regarding specific genes involved is extremely limited. A number of candidate genes for smoking (genes involved in dopaminergic neurotransmission) have been identified but association studies with these genes had not been examined in relation to smoking behaviour previously. Finally, the incentive sensitisation theory of addiction was explored using an emotional Stroop paradigm for the first time, demonstrating that substance abusers respond differently to substance-related cues when compared to matched controls. Interestingly, evidence for individual differences in attentional bias were found in this study, whereby participants with the DRD4 long variant showed an attentional bias to substance-related cues, which opens the door to further research exploring the links between specific genes and responsiveness to environmental stimuli. Previous studies that have incorporated genetic testing have used blood samples. This no longer has to be the case as techniques for collecting DNA have developed. Blood samples can be expensive to collect, painful for the participant and time consuming for both researcher and participant. DNA can now be extracted from cheek swabs. This technique is inexpensive, non-invasive and simple to administer. One study adopted this technique and collected 114 DNA samples from two-year-old children and 116 adults. The samples were sent by mail, then, posted back to the researcher up to a

month later (Freeman, et al, 1997). This method of DNA collection facilitates psychologists in the search for genes and the investigation of the interplay between behaviour and genes (Plomin & Rutter, 1998).

To conclude, the long-repeat allele at the dopamine D4 receptor gene is associated with the personality trait impulsive sensation seeking in substance abusers. It has been argued that individuals with this genotype are dopamine deficient so therefore seek risky and novel sensations such as drug taking to increase the release of dopamine in the brain. Identifying these genetic associations with behaviour aids our understanding as to why some individuals become substance dependent, whilst others do not, thus demonstrating a vulnerability factor for addiction. Findings suggest that substance dependence is partly due to a common neurochemical pathway and gene receptors that are involved in this pathway. Another pathway to substance dependence seems to be through personality traits that are related to disinhibition of behaviour and risk taking to achieve short-term rewards. In support of this claim, heroin users were found to have the highest sensation seeking levels and substance abusers were significantly higher sensation seekers than controls. The effect that the DRD4 gene has on substance dependence may be mediated by the heritable personality trait impulsive sensation seeking. However, data suggest that this gene variant plays a small role in substance dependence so could easily be masked by other factors. Finally, it was demonstrated that regardless of factors including mood status, demographics and substance history, substance abusers responded differently to substance-related cues than neutral cues when compared to controls, implicating that substance users are susceptible to the sensitisation of environmental cues. Future work should investigate the effect of the DRD4 gene in combination with other genes to identify the accumulative and interactive effects that genes have on reactions to substance-related cues and responses to these cues, on sensation seeking and ultimately on addictive behaviour.

Despite the overwhelming complexities apparent in addiction research, it is anticipated that the future will allow researchers to identify all genes that contribute to substance dependence vulnerability and how biological, psychological and social factors interact, accounting for individual variation, to produce substance dependency.

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Appendices

1. Personal details sheet
2. Substance Use History sheet
3. Severity of Dependence Scale (SDS)
4. Severity of Alcohol Dependence Questionnaire (SADQ)
5. Questionnaire of Smoking Urges (QSU)
6. Sensation Seeking Scale (ZKPQ ImpSSS)
7. Profile of Mood States-Short form (POMS-SF)
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13. Computer task instructions
14. List of publications based on material presented within this thesis

Appendix 1

Experiment groups

Personal details sheet

1. Are you male or female? Male ☐ Female ☐
2. How would you describe your ethnic group? ☐
3. What is your age in years? ☐
4. Are you currently employed? Yes ☐ No ☐
5. What is your present or last occupation? ☐
6. How old were you when you left school? ☐
7. What is your highest qualification? None ☐ GCSE ☐ NVQ ☐ Alevel ☐ Higher Education ☐
8. What is your marital status? Married ☐ Single ☐ Divorced ☐ Widowed ☐ Living with partner ☐
9. Do you have any children, if so, how many? ☐
10. What kind of accommodation do you have? Private ☐ Rented ☐ Shelter ☐ NFA ☐ Living with parents ☐
11. Have you ever been diagnosed as having a mental illness? Yes ☐ No ☐
    If yes, are you currently receiving any medication? (Specify) ☐
12. What are you currently in treatment for? Heroin dependence ☐ Alcohol dependence ☐ Cocaine dependence ☐ Amphetamine dependence ☐ Polysubstance dependence ☐ Other (please specify) ☐

Appendix 1

Control groups

Personal details sheet

1. Are you male or female? Male ☐ Female ☐
2. How would you describe your ethnic group? ☐
3. What is your age in years? ☐
4. Are you currently employed? Yes ☐ No ☐
5. What is your present or last occupation? ☐
6. How old were you when you left school? ☐

7. What is your highest qualification? None ☐ GCSE ☐ NVQ ☐ Alevel ☐ Higher Education ☐

8. What is your marital status? Married ☐ Single ☐ Divorced ☐ Widowed ☐ Living with partner ☐

9. Do you have any children, if so, how many? ☐

10. What kind of accommodation do you have? Private ☐ Rented ☐ Shelter ☐ NFA ☐ Living with parents ☐

11. Have you ever been diagnosed as having a mental illness? Yes ☐ No ☐

If yes, are you currently receiving any medication? (Please specify) ☐

12. Are you currently in treatment for substance dependence? Yes ☐ No ☐

Appendix 1

Personal details sheet

Additional Sheet for Control Participants

Does/Did anyone in your family abuse drugs or alcohol?

Mother	Yes	☐	No	☐
Father	Yes	☐	No	☐
Brother	Yes	☐	No	☐
Sister	Yes	☐	No	☐
Uncle	Yes	☐	No	☐
Aunt	Yes	☐	No	☐
Cousin	Yes	☐	No	☐

Other

Appendix 2

Substance Use History

1. What is your main drug of choice?
   Alcohol ☐ Heroin ☐ Other Opiate ☐
   Cocaine ☐ Amphetamine ☐
   Other (please specify) ☐
2. At what age did you first use this substance? ☐
3. What do you think made you first try this substance? ☐
4. At what age did you feel that you first had a problem with your use of this substance? ☐
5. Have you had any periods of abstinence? Yes ☐ No ☐
6. What do you think has caused you to relapse in the past? ☐
7. Does/did anyone in your family abuse drugs or alcohol?
   Mother Yes ☐ No ☐
   Father Yes ☐ No ☐
   Brother Yes ☐ No ☐
   Sister Yes ☐ No ☐
   Uncle Yes ☐ No ☐
   Aunt Yes ☐ No ☐
   Cousin Yes ☐ No ☐
   Other ☐
8. Are you currently taking any medication for substance dependence, if so what? ☐

   E.g.: Acamprosate; Methadone; Naltrexone

Appendix 3

The Severity of Dependence Scale (SDS)

Please rate how each of these four statements applied to you in the past year by putting a number in the spaces provided on a scale from 0-3.

0= never, 1= sometimes, 2= often, 3= always.

1) Did you think your use of your drug was out of control? ☐

2) Did the prospect of missing a fix (or dose) or not Chasing make you anxious or worried? ☐

3) Did you worry about your use of this drug? ☐

4) Did you wish you could stop? ☐

Please rate this statement on a scale from 0 to 3.

0= not difficult, 1= quite difficult, 2= very difficult, 3= impossible.

5) How difficult did you find it to stop, or go without your drug? ☐

-Thank you for your time-

Appendix 4

Severity of Alcohol Dependence Questionnaire (SADQ)

If you are in treatment for Alcohol Dependence then please complete the following questionnaire. If not, please turn over the page.

Please recall a typical period of heavy drinking in the last six months.
Please rate how each of these statements applied to you during this time on a scale from 0 to 3.

0= never, 1= sometimes, 2= often, 3= always.

During that period of heavy drinking:

1. I woke up feeling sweaty
2. My hands shook first thing in the morning
3. My whole body shook violently first thing in the morning if I didn't have a drink
4. I woke up absolutely drenched in sweat
5. I dreaded waking up in the morning
6. I was frightened of meeting people first thing in the morning
7. I felt at the edge of despair when I woke up
8. I felt very frightened when I awoke
9. I liked to have a morning drink
10. I always gulped my first few morning drinks down as quickly as possible
11. I drank in the morning to get rid of the shakes
12. I had a very strong craving for drink when I awoke
13. I drank more than 1/4 bottle of spirits a day (Or 4 pints beer/2 cans strong lager/1 bottle wine)
14. I drank more than 1/2 bottle of spirits a day (or 8 pints beer/ 4 cans strong lager/2 bottles wine)
15. I drank more than 1 bottle of spirits a day (or 15 pints beer/ 8 cans strong lager/ 4 bottles wine)
16. I drank more than 2 bottles of spirits a day (or 30 pints beer/ 15 cans strong lager/8 bottles wine)

Imagine the following situation:
You have been completely off drink for a few weeks, you then drink very heavily for two days. How would you feel the morning after those two days of heavy drinking?

Please rate how you would feel on a scale from 0 to 3.

0= Not at all, 1= Slightly, 2= Moderately, 3= Quite a lot.

The morning after:

17. I would start to sweat
18. My hands would shake
19. My body would shake
20. I would be craving for a drink

Appendix 5

Smoking Urges Questionnaire (SQU)

- I am a Smoker ☐ Non smoker ☐ Ex smoker ☐

If you are currently a smoker please complete the following questionnaire to rate your cigarette smoking. If you are not a smoker then please turn over the page.

- How many cigarettes do you smoke each day? ☐
- How long have you smoked cigarettes? ☐

Below you will find a series of statements that persons might use to describe themselves. Read each statement and decide whether or not it describes you. Please read each statement and decide whether you agree or disagree by circling a number from 1-7 for each question using the scale provided.

1 2 3 4 5 6 7

Strongly Agree — Strongly Disagree

1.	Smoking would make me feel very good right now	1 2 3 4 5 6 7
2.	I would be less irritable now if I could smoke	1 2 3 4 5 6 7
3.	Nothing would be better than smoking a cigarette right now	1 2 3 4 5 6 7
4.	I am not missing smoking right now	1 2 3 4 5 6 7
5.	I will smoke as soon as I get a chance	1 2 3 4 5 6 7
6.	I don't want to smoke now	1 2 3 4 5 6 7
7.	Smoking would make me less depressed	1 2 3 4 5 6 7
8.	Smoking would not help me calm down now	1 2 3 4 5 6 7
9.	If I were offered a cigarette, I would not smoke it immediately	1 2 3 4 5 6 7
10.	Starting now, I could go without smoking for a long time	1 2 3 4 5 6 7
11.	Smoking a cigarette would not be pleasant	1 2 3 4 5 6 7
12.	If I were smoking this minute, I would feel less bored	1 2 3 4 5 6 7
13.	All I want right now is a cigarette	1 2 3 4 5 6 7
14.	Smoking now would make me feel less tired	1 2 3 4 5 6 7
15.	Smoking would make me feel happier now	1 2 3 4 5 6 7
16.	Even if it were possible, I probably wouldn't smoke now	1 2 3 4 5 6 7
17.	I have no desire for a cigarette right now	1 2 3 4 5 6 7
18.	My desire to smoke seems overpowering	1 2 3 4 5 6 7
19.	Smoking now would make things seem just perfect	1 2 3 4 5 6 7
20.	I crave a cigarette right now	1 2 3 4 5 6 7
21.	I would not enjoy a cigarette right now	1 2 3 4 5 6 7
22.	A cigarette would not taste good right now	1 2 3 4 5 6 7
23.	I have an urge for a cigarette	1 2 3 4 5 6 7
24.	I could control things better right now if I could smoke	1 2 3 4 5 6 7
25.	I am going to smoke as soon as possible	1 2 3 4 5 6 7
26.	I would not feel better physically if I were smoking	1 2 3 4 5 6 7
27.	A cigarette would not be very satisfying right now	1 2 3 4 5 6 7
28.	If I had a lit cigarette in my hand I probably wouldn't smoke it	1 2 3 4 5 6 7
29.	If I were smoking now I could think more clearly	1 2 3 4 5 6 7
30.	I would do almost anything for a cigarette now	1 2 3 4 5 6 7
31.	I need to smoke now	1 2 3 4 5 6 7
32.	Right now, I am not making plans to smoke	1 2 3 4 5 6 7

Appendix 6

Zuckerman-Kuhlman Personality Questionnaire (ZKPQ)

Impulsive-Sensation Seeking Scale

Below you will find a series of statements that persons might use to describe themselves. Read each statement and decide whether or not it describes you. If you agree with the statement, answer by ticking the box. If you disagree with the statement, answer by putting a cross (X) in the box beside the statement.

1. I tend to begin a new job without much planning on how I will do it ☐
2. I never met a person that I didn't like ☐
3. I usually think about what I am going to do before I do it ☐
4. I have always told the truth ☐
5. I often do things on impulse ☐
6. I very seldom spend much time on the details of planning ahead ☐
7. I like to have new and exciting experiences and sensations even if they are frightening ☐
8. I often get so carried away by new things, I never think of possible complications ☐
9. Before I begin a complicated job, I make careful plans ☐
10. I have never been bored ☐
11. I'd like to take off on a trip with no pre-planned or definite routes or timetables ☐
12. I enjoy getting into new situations where you can't predict how things will turn out ☐
13. I never get annoyed when people cut ahead of me in line ☐
14. I like doing things just for the thrill of it ☐
15. I tend to change interests frequently ☐
16. I'd like to explore a strange town by myself, even if it means getting lost ☐
17. I sometimes like to do things that are a little frightening ☐
18. I'll try anything once ☐
19. I'd like the kind of life where one is on the move, with lots of change & excitement ☐
20. I sometimes do crazy things just for fun ☐
21. I prefer friends who are excitingly unpredictable ☐
22. I am an impulsive person ☐
23. I like wild, uninhibited parties ☐

Appendix 7

Profile of Mood States-Short Form (POMS-SF)

[redacted] DATE _____

SEX: Male (M) Female (F) Identification No. _____

Below is a list of words that describe feelings people have. Please read each one carefully. Then fill in ONE circle under the answer to the right which best describes HOW YOU HAVE BEEN FEELING DURING THE PAST WEEK INCLUDING TODAY.

The numbers refer to these phrases.

(0) = Not at all
(1) = A little
(2) = Moderately
(3) = Quite a bit
(4) = Extremely

	Not at all	A little	Moderately	Quite a bit	Extremely
1. Tense	0	1	2	3	4
2. Angry	0	1	2	3	4
3. Worn out	0	1	2	3	4
4. Lively	0	1	2	3	4
5. Confused	0	1	2	3	4
6. Shaky	0	1	2	3	4
7. Sad	0	1	2	3	4
8. Active	0	1	2	3	4
9. Grouchy	0	1	2	3	4
10. Energetic	0	1	2	3	4
11. Unworthy	0	1	2	3	4
12. Uneasy	0	1	2	3	4
13. Fatigued	0	1	2	3	4
14. Annoyed	0	1	2	3	4
15. Discouraged	0	1	2	3	4
16. Nervous	0	1	2	3	4
17. Lonely	0	1	2	3	4
18. Muddled	0	1	2	3	4
19. Exhausted	0	1	2	3	4
20. Anxious	0	1	2	3	4
21. Gloomy	0	1	2	3	4
22. Sluggish	0	1	2	3	4
23. Weary	0	1	2	3	4
24. Bewildered	0	1	2	3	4
25. Furious	0	1	2	3	4
26. Efficient	0	1	2	3	4
27. Full of pep	0	1	2	3	4
28. Bad-tempered	0	1	2	3	4
29. Forgetful	0	1	2	3	4
30. Vigorous	0	1	2	3	4

MAKE SURE YOU HAVE ANSWERED EVERY ITEM.

SHORT FORM

A C D F T V

Appendix 8

Information sheets

Experiment groups (South London)

A study to investigate vulnerability to drug taking behaviour.

We are inviting you to take part in a study to investigate factors that make an individual more likely to use drugs. You will be asked to complete a set of brief questionnaires, this will last approximately 10 minutes. One questionnaire will measure the extent of any drug dependence and one questionnaire will measure aspects of your personality thought to relate to drug taking for example, how much you seek new sensations. You will then be asked to complete a short computer-based task (10 minutes) to measure your reactions to words that are related to drug taking.

You will also be asked to provide a sample of DNA, the hereditary material, by rubbing the inside of your mouth with several cotton buds. The DNA will be used to see if we can identify genes that contribute to drug dependence. As such this is new research and it does not represent a routine investigation and no individual test will be generated. By doing this we hope to understand some of the reasons why different types of people use different types of drugs.

If you would like to know about the general progress of this study please do not hesitate to call us at the number below.

We would appreciate your participation in this study. The study is entirely voluntary and no pressure will be put on you to take part. Whether or not you participate will not affect the treatment that you receive from your treatment service. You are free to withdraw from this study at any point. Your name will not be communicated to anyone outside of the research team, as the research performed will be totally confidential. Please do not hesitate to ask if you have any further questions.

If you do have any queries then contact: Jo Lusher at

London Guildhall University, Psychology Department,

Calcutta House, Old Castle Street, London E1 7NT.

Telephone: 020 7320 1282.

Appendix 8

Information sheets

Experiment groups (East London)

INVITATION TO PARTICIPATE

A Study to Investigate Vulnerability to Drug Taking Behaviour

We invite you to take part in a research study that we think may be important. The information that follows tells you about it. It is important that you understand what is in this leaflet. It says what will happen if you take part and what the risks might be. Try to make sure you know what will happen to you if you decide to take part. Whether or not you do take part is entirely your choice. Please ask any questions you want to about the research and we will try our best to answer them.

The aim of this study is to establish factors that make an individual more likely to use drugs. To do this we will be asking a group of drug abusers about their drug use. Personality traits and responses to words that are related to drugs will be measured. A sample of DNA will be taken. We have chosen you to take part in the study as we would like information about 300 people who use drugs or alcohol so that we can then compare results from drug abusers with results from people who do not abuse drugs or alcohol. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time. This will not affect the standard of care you receive. If you decide to participate you will be asked to complete a series of brief questionnaires, this will last about 10 minutes. The questionnaires will measure aspects of your personality thought to relate to drug taking for example, how much you seek new sensations. You will then be asked to complete a short, computer-based task (10 minutes) to measure your reaction to words that are related to drug taking. You will also be asked to donate a sample of your DNA, the hereditary material, by rubbing the inside of your mouth with several cotton buds. The DNA will be used to see if we can identify genes that contribute to drug dependence. Your DNA sample is a gift donation so you will therefore have no ownership after donation. Your DNA sample will be given a code number so that it can be married up with your questionnaire. Your name will not appear on any of this material and all DNA samples will be destroyed when the research is complete. This is new research and it does not represent a routine investigation. No individual test will be generated. By doing this we hope to understand some of the reasons why different types of people use different types of drugs.

There are neither advantages nor disadvantages to you agreeing to participate apart from the satisfaction of having helped psychological research in an important way. Identifying particular vulnerability factors to drug taking will enable a better understanding of this behaviour. It will be of value in making diagnoses and influencing treatment. When the study is finished, results will be published in journals. Details will be put on display at your treatment service. All information that is collected about you during the course of the study will be kept strictly confidential. Any information about you that leaves the treatment service will not contain your name or address, so that you cannot be recognized from it. We would appreciate your participation in this study. The study is entirely voluntary and no pressure will be put on you to take part. You don't have to join the study. You are free to decide not to be in this trial or to drop out at any time. If you decide not to be in the study, or drop out, this will not put at risk your ordinary medical care. Please contact us on the details below if you would like any further information.

We believe that this study is basically safe and do not expect you to suffer any harm or injury because of your participation in it. However, London Guildhall University has agreed that if your health does suffer as a result of your being in the study then you will be compensated. In such a situation, you will not have to prove that the harm or injury that affects you is anyone's fault. If you are not happy with any proposed compensation, you may have to pursue your claim through legal action.

Thank you for your time.

Appendix 8

Information sheets

Control groups

Date: 12th September 2000

If you have any queries then contact:
Jo Lusher
Psychology Department
London Guildhall University
Direct line: 020 7320 1282
Email: lusher@lgu.ac.uk

Information Sheet for Control group Participants (Version II)

A Study to Investigate Vulnerability to Drug Taking Behaviour

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information and do not hesitate to ask if you have any questions.

The aim of this study is to establish factors that make an individual more likely to use drugs. To do this we will be asking a group of drug abusers about their drug use. Personality traits and responses to words that are related to drugs will be measured. A sample of DNA will be taken.

We have chosen you as a control participant as we would like information about 200 people from the general population who are not dependent upon drugs or alcohol. We can then compare results from drug abusers with results from people who do not abuse drugs or alcohol.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time. This will not affect the standard of care you receive. If you decide to participate you will be asked to complete a series of brief questionnaires, this will last about 10 minutes. The questionnaires will measure aspects of your personality thought to relate to drug taking for example, how much you seek new sensations. You will then be asked to complete a short, computer-based task (10 minutes) to measure your reaction to words that are related to drug taking. You will also be asked to donate a sample of your DNA, the hereditary material, by rubbing the inside of your mouth with several cotton buds. The DNA will be used to see if we can identify genes that contribute to drug dependence. Your DNA sample is a gift donation so you will therefore have no ownership after donation. Your DNA sample will be given a code number so that it can be married up with your questionnaire. Your name will not appear on any of this material and all DNA samples will be destroyed when the research is complete. This is new research and it does not represent a routine investigation. No individual test will be generated. By doing this we hope to understand some of the reasons why different types of people use different types of drugs.

There are neither advantages nor disadvantages to you agreeing to participate apart from the satisfaction of having helped psychological research in an important way. Identifying particular vulnerability factors to drug taking will enable a better understanding of this behaviour. It will be of value in making diagnoses and influencing treatment. When the study is finished, results will be published in journals. Details will be put on display at your surgery. All information that is collected about you during the course of the study will be kept strictly confidential. Any information about you that leaves the surgery will not contain your name or address, so that you cannot be recognized from it. We would appreciate your participation in this study. The study is entirely voluntary and no pressure will be put on you to take part. Please contact us on the above details if you would like any further information.

Appendix 9

Consent forms

South London

A study to investigate vulnerability to drug taking behaviour.

I have read the information sheet and understand that participation in this study will involve completing a set of brief questionnaires, completing a short computer-based task and providing cheek cell samples. I understand that participation in this study is entirely voluntary and no pressure will be put on me to take part. I understand that I am free to withdraw from this study at any time and the information gathered will be held as strictly confidential.

I agree to participate in this study.....

(please sign)

Name.....

Date.....

Thank you for agreeing to take part in this study. Enter your responses to the questions in the spaces provided or circle the most appropriate answers, where an alternative is given. Please answer as accurately and as honestly as possible. Please do not hesitate to ask questions if you have any problems.

Appendix 9

Consent forms

Control groups (East London)

Date: 12th September 2000

If you have any queries then contact:
Jo Lusher
Psychology Department
London Guildhall University
Direct line: 020 7320 1282
Email: lusher@lgu.ac.uk

Consent Form for Control group Participants (Version II)
A Study to Investigate Vulnerability to Drug Taking Behaviour

1. I confirm that I have read and understand the information sheet dated..... (version 1) for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, without my medical care or legal rights being affected. ☐

3. I agree to take part in the above study. ☐

Name of participant_____Date_____Signature_____

Name of researcher_____Date_____Signature_____

Thank you for agreeing to take part in this study. Enter your responses to the questions in the spaces provided or circle the most appropriate answers. Please answer as accurately and as honestly as possible. Do not hesitate to ask if you have any questions.

Appendix 9

Consent forms

Control groups (South London)

WRITTEN CONSENT FORM:

A Study to Investigate Vulnerability to Drug Taking Behaviour
London Guildhall University

REC Number:
Name of Patient/Volunteer (Block Capitals):

- The study organisers have invited me to take part in this research.
- I understand what is in the leaflet about the research. I have a copy of the leaflet to keep.
- I have had the chance to talk and ask questions about the study.
- I know what my part will be in the study and I know how long it will take.
- I know how the study may affect me. I have been told if there are possible risks.
- I understand that I should not **actively** take part in more than 1 research study at a time.
- I know that the local East London and The City Health Authority Research Ethics Committee has seen and agreed to this study.
- I understand that personal information is strictly confidential: I know the only people who may see information about my part in the study are the research team or an official representative of the organisation which funded the research.
- I understand that my personal information may be stored on a computer. If this is done then it will not affect the confidentiality of this information. All such storage of information must comply with the 1998 Data Protection Act.
- I freely consent to be a subject in the study. No one has put pressure on me. I know that I can stop taking part in the study at any time.
- I know if I do not take part I will still be able to have my normal treatment.
- I know that if there are any problems, I can contact:

Dr/Mr/Ms.....
Tel. No. ..... Bleep No./Ext. .....

Patient's/Volunteer's: Signature .....
Witness's Name .....
Witness's Signature: .....
Date .....

The following should be signed by the Clinician/Investigator responsible for obtaining consent
As the Clinician/Investigator responsible for this research or a designated deputy, I confirm that I have explained to the patient/volunteer named above the nature and purpose of the research to be undertaken.

Clinician's Name:.....Clinician's Signature:.....Date:.....

Appendix 10

Storage solution

One Litre of Proteinase K Storage Solution

Mix	100ml Sodium Chloride	
	10ml Tris Hc1 ph8	=STE Buffer
	20ml EDTA ph8	
Add	50ml SDS (10%)	
	Make up to 990ml with dh 0	
Add	10ml Proteinase K (@20mg/1ml) just prior to use	

Appendix 11

Instructions for collecting mouth cells

Cheek swab kit pack contains:

1 tube containing storage solution

10 cotton wool buds

How to use the cotton wool buds:

1. Make sure that there are no bits of food in your mouth when you collect the mouth cells.
2. Try to use half of the buds to rub the inside upper part of the mouth and half to rub inside the lower part of the mouth.
3. Rub the cotton wool bud along the inside of the mouth (including cheek, lip and gums), with a little pressure against the mouth as you do so. Do this for about fifteen seconds with each bud. It does not hurt at all.
4. Each time a bud has been used, place it in the tube containing the storage liquid-cotton wool ends downwards, into the liquid.
5. When all 10 buds have been used, screw the lid on tightly and return the tube to the researcher.

Appendix 12

Computer Task Information Sheet

Please answer questions 1-6 below (Please circle)

1. Are you right handed? Yes/No

2. Is English your first language? Yes/No

3. Do you have normal or corrected to normal vision? Yes/No

4. Are you colour blind? Yes/No

5. Please tick which of the following substances you are using now and state when you last used each one

- Cigarettes ☐ _____
- Alcohol ☐ _____
- Heroin ☐ _____
- Other Opiate ☐ _____
- Cocaine ☐ _____
- Amphetamine ☐ _____
- Other (specify) ☐ _____

6. If you are a smoker, on a scale from 0-10, with 0 meaning 'not at all' and 10 meaning 'extremely', how much are you **craving** for cigarettes now?

(Circle a number on the scale)

0 1 2 3 4 5 6 7 8 9 10

Not at all extremely

Appendix 13

Computer Task Instructions

The instructions for this task can be read from the computer screen. Press the rest pad or the key indicated in the on-screen instructions to continue onto the next stage of the task.

Blocks of coloured words or words typed in different colours will be presented on the screen. Concentrate on the cross in the centre of the screen preceding each presentation. You will be instructed to name the colour of the words while ignoring the word content. Press the appropriate coloured key on the keyboard that corresponds to the colour in which the word is printed.

You will be given a practice session before the task. Please ask the experimenter if you have any questions.

-Thank you for your time-

Publications based on material presented within this thesis

Lusher, J., Ebersole, L. & Ball, D. (2000) Dopamine D4 Receptor gene and severity of dependence. *Addiction Biology,* **5**, 469-472.

Lusher, J., Chandler, C. & Ball, D. (2001) Dopamine D4 Receptor gene (DRD4) is associated with Novelty Seeking (NS) and substance abuse: The saga continues... *Molecular Psychiatry,* **6**, 497-499.

Lusher, J., Chandler, C. & Ball, D. (2004) Alcohol dependence and the alcohol Stroop paradigm: Evidence and issues. *Drug and Alcohol Dependence,* **75**, 3, 225-231.

Lusher, J., Chandler, C., & Ball, D., (2004) DRD4 and cue reactivity in substance abusers. Poster presented at the Fourth meeting of the European Foundation of Psychiatry Meeting, Beyond Nature and Nurture Conference, November 8-9th.