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Nicotine Addiction

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Abstract

This report discusses the current knowledge on nicotine dependence, devoting a special chapter to smoking among youths, given that most smoking careers start in adolescence. The transition period, in which youths go from elementary to high school (ages 13-14), shows to be particularly risky for smoking initiation. The earlier youths start smoking, the more likely they will become dependent, and the more likely they will be heavier cigarette smokers in adult life. Since nicotine seems to be able to cause addiction in a short period of time, the aim should be to prevent children experimenting with cigarettes.

Studies have shown that heavy smokers experience less aversive effects during their first smoke than light smokers. 'Light' cigarettes and flavour-enhancing additives could reduce aversive effects during smoking compared to regular cigarettes without such additives. Therefore, prohibiting the use of 'light' cigarettes and the use of flavour-enhancing additives could prevent adolescents from trying a second cigarette and ultimately contribute to preventing addiction among youths.

Contents

SAMENVATTING.....	4
SUMMARY	6
1 INTRODUCTION.....	9
2 YOUTH SMOKING AND NICOTINE ADDICTION	11
2.1. STARTING A SMOKING CAREER	11
2.2. ARE YOUTH SMOKERS ADDICTED TO NICOTINE?	12
2.2.1. <i>Cotinine levels</i>	12
2.2.2. <i>Withdrawal symptoms</i>	13
2.2.3. <i>Smoking behaviour</i>	14
2.2.4. <i>Conclusion</i>	15
2.3. FIRST TOBACCO USE.....	15
2.4. PSYCHOSOCIAL RISK FACTORS IN THE INITIATION OF TOBACCO USE	17
2.5 GENETICS AND THE RISK OF BECOMING A SMOKER	18
2.5.1 <i>Twin studies</i>	18
2.5.2 <i>Candidate genes affecting smoking initiation and smoking persistence</i>	20
2.6. GENDER DIFFERENCES IN ADOLESCENT SMOKING	20
2.7. ETHNIC DIFFERENCES IN YOUTH SMOKING	21
2.8. ANIMAL DATA ON YOUTH NICOTINE ADDICTION	21
2.9 CONCLUSION.....	22
3. HOW ADDICTIVE IS CIGARETTE SMOKING?	23
3.1 INTRODUCTION	23
3.2. TIME PATH OF NICOTINE ADDICTION	23
3.3 TOBACCO CHIPPERS	24
3.4 RELAPSE AND CONDITIONED REINFORCEMENT.....	25
3.5 CONCLUSION.....	27
4 RECOMMENDATIONS.....	29
4.1 SMOKING INITIATION	29
4.2 NICOTINE ADDICTION.....	29
4.3 CONCLUSIONS	30
APPENDIX I GENERAL ASPECTS OF NICOTINE ADDICTION	31
APPENDIX 2 PHARMACOKINETICS AND METABOLISM	37
APPENDIX 3 RECEPTORS.....	47
APPENDIX 4 THE BIOLOGICAL MECHANISM OF NICOTINE ADDICTION	53
APPENDIX 5 GENETICS	63
REFERENCES.....	71

Samenvatting

De meeste rokers beginnen op jonge leeftijd. De overgangsjaren van basisschool naar middelbare school (13 tot en met 14 jaar oud) blijken een hoog-risico periode te zijn voor het beginnen met roken. Hoe eerder een kind begint met roken hoe waarschijnlijker het is dat hij of zij verslaafd raakt. Daarnaast correleert vroeg starten met veel roken op latere leeftijd.

Het beginnen met roken wordt door verschillende factoren beïnvloed zoals, sociaal-milieu-, gedrags-, persoonlijkheids-, genetische en sociaaldemografische factoren. Tot nu toe is er geen simpele verklaring waarom kinderen starten met roken, alle afzonderlijke factoren hebben een geringe invloed. Wel is het zo dat anti-rook campagnes bijdragen aan een positiever beeld van niet-rokers. Het niet-roken als sociale norm kan bijdragen aan het niet of op latere leeftijd starten met roken.

De effecten die gedurende het roken van de eerste sigaret worden ervaren, bepalen in belangrijke mate of later regelmatig tabak zal worden gebruikt. Retrospectieve studies laten zien dat zware rokers minder aversieve effecten hebben ervaren tijdens het roken van hun eerste sigaret dan lichte rokers. 'Light' sigaretten kunnen dus mogelijk bijdragen aan de snelle ontwikkeling van nicotine verslaving in de jeugd, omdat verwacht kan worden dat minder nicotine en teer correleert met minder aversieve effecten tijdens het roken van de eerste sigaret. Ook smaakverbeterende additieven, kunnen op basis van die eigenschappen ervoor zorgen dat er minder aversieve effecten tijdens het roken van de eerste sigaret zullen optreden.

Het tijdspad waarbinnen jongeren verslaafd raken aan sigaretten is niet zondermeer duidelijk. Studies die jongeren een aantal jaren volgen concluderen vaak dat jongeren zich in 2 tot 3 jaar van niet-roker naar regelmatige roker ontwikkelen maar geven geen duidelijkheid over het moment waarop sprake is van verslaving. Een aantal retrospectieve studies gaan er vanuit dat het roken van slechts een aantal sigaretten (<10) voldoende is om rookverslaving te ontwikkelen. De snelle ontwikkeling van rookverslaving in jongeren is meetbaar als een snelle ontwikkeling van onthoudingsverschijnselen (enkele dagen tot een maand) nadat begonnen is met roken. In dierexperimenteel onderzoek is aangetoond dat nicotinegebruik tijdens de adolescentie sneller en minder omkeerbaar is dan nicotinegebruik in volwassenen, en leidt tot een toename in het aantal nicotine receptoren in hersengebieden die met verslaving en beloningspaden geassocieerd worden.

Niet alle rokers zijn echter verslaafd aan nicotine. Zogenaamde 'Chippers' ervaren geen ontwenningverschijnselen gedurende een periode van onthouding. 'Chippers' verschillen echter niet van verslaafde rokers in hun absorptie en metabolisme van nicotine. Ze verschillen ook niet van verslaafde rokers in de mate van tolerantie en in de directe farmacologische effecten van nicotine.

Er zijn verschillende aanbevelingen te maken met betrekking tot de preventie van nicotineverslaving.

1. Het is waarschijnlijk dat nicotineverslaving zich zeer snel kan ontwikkelen tijdens het experimenteren op jeugdige leeftijd. Daarom zou de nadruk primair moeten liggen op het voorkomen van nicotineverslaving met extra aandacht voor de jeugd van 13 tot

en met 14 jaar oud. In die preventie zou gerefereerd moeten worden aan niet-roken als een sociale norm.

2. 'Light' sigaretten kunnen mogelijk bijdragen aan de snelle ontwikkeling van nicotineverslaving in de jeugd, omdat verwacht kan worden dat er minder aversieve effecten tijdens het roken van de eerste sigaret zullen optreden.

3. Een andere optie is om de sigaretten minder attractief te maken door het gebruik van smaakverbeterende additieven te verbieden.

Summary

The majority of smoking careers start in childhood. The transition period, in which children go from elementary to high school (ages 13-14), proves to be particularly risky for smoking initiation. The earlier children start smoking, the more likely they will become dependent and the more likely they will be heavier cigarette smokers in adult life. Therefore, preventing children from smoking at a young age is important, since the chance of becoming addicted when older is less and fewer cigarettes per day will be smoked in adult life.

Initiation of cigarette smoking is influenced, for example, by socio-environmental, behavioural, personal, genetic and socio-demographic factors. To date, there has been no simple explanation of why children start to smoke; furthermore, factors taken separately have little effect. No-smoking campaigns contribute to a more positive image of the non-smoker in general. Making non-smoking the social norm in the Netherlands, can contribute to deciding not to smoke or starting to smoke at an older age.

The effects produced during a first tobacco episode determine to a great extent if the person will be a regular user of tobacco in adult life. Retrospective studies indicate that heavy smokers experience less aversive effects during their first cigarette than light smokers. 'Light' cigarettes may therefore contribute to the rapid development of nicotine addiction among adolescents. This is because of the expectation that less nicotine and tar will correlate with less aversive effects occurring during the smoking of one's first cigarette. The use of several flavour-enhancing additives, which make the cigarettes more attractive in taste, can also play an important role in masking the negative effects during the first tobacco episode.

The time path in which adolescents become addicted to cigarettes is unclear. Follow-up studies often conclude that adolescents go from non-smoking individuals to regular cigarette consumers in 2-3 years. These studies do not provide clarity on the moment marking the beginning of addiction. Retrospective studies indicate that the smoking of only a few cigarettes (<10) is enough to determine whether someone will develop an addiction to cigarette smoking. The rapid development of cigarette addiction is measurable as a rapid development of withdrawal symptoms (some days to a month) after taking up smoking. This statement is also supported by animal studies in which nicotine given during adolescence was shown to produce a pattern of nicotinic receptor up-regulation in brain regions associated with addiction and reward pathways much more rapidly and less irreversibly than in adults.

Not all smokers are addicted to nicotine. So-called 'Chippers' do not experience withdrawal symptoms following a period of abstinence. However, they do not differ from dependent smokers in their absorption and metabolism of nicotine. 'Chippers' are also similar to dependent smokers in their degree of tolerance and in the direct pharmacological effects of nicotine.

A number of recommendations for nicotine addiction prevention follow:

1. Since it has become clear that nicotine addiction develops very quickly during experimentation in adolescents, the aim of prevention should be primarily to prevent

nicotine addiction in youths between 13 and 14 years of age. Prevention should be directed to making non-smoking the social norm.

2. 'Light' cigarettes may contribute to the rapid development of nicotine addiction among adolescents, because less aversive effects may be expected to occur.

3. Another option is to make cigarettes less attractive by prohibiting the use of flavour-enhancing additives.

1 Introduction

Although several studies are available on nicotine addiction, it is still unclear how a smoking career develops. In the Netherlands 71% of the adolescents have ever tried a cigarette by age 18 (1) and smoking rates are rising in adolescents. Helping young people to avoid starting to smoke is a widely endorsed goal of public health, but there is uncertainty about how to do this. From a public health perspective it is important to know if the currently used prevention strategies, still coincide with scientific views on youth smoking.

This report focuses on how youths become addicted to nicotine. In chapter 2 several aspects of adolescent smoking are discussed. When do adolescents try their first cigarette and how innocent is this experimenting? When adolescents are addicted to nicotine, how can this be measured and do they experience withdrawal symptoms upon cessation? Several risk factors are known for starting to smoke, of these genetic influences are evaluated in more detail. Another question that is often raised is how soon does a person become addicted to nicotine or cigarette smoking?

The addictiveness of cigarettes is an interesting subject and will be discussed in chapter 3. The time path of nicotine addiction is postulated. An extraordinary group of cigarette smokers, called 'chippers', is described. This group of cigarette smokers is considered to be non-dependent. Since cigarette smoking is more than nicotine dependence, relapse and the effect of conditioned reinforcement are described.

Finally, the information on youth smoking will be translated into recommendations for the prevention of smoking in adolescents. The recommendations for smoking prevention are discussed in chapter 4.

In the appendices background information is included on the general aspects of addiction, pharmacokinetics and metabolism of nicotine, nicotine receptors, the biological mechanism of nicotine addiction and genetics. These chapters provide detailed information on the subject nicotine addiction. This information is not necessary to understand this report, but provides additional information on the subject.

2 Youth smoking and nicotine addiction

Before discussing several studies on youth smoking it is good to realise that the most used method to study youth smoking and nicotine addiction is by asking adolescents to recall and report their tobacco use episodes. This method is mentioned often as a critical note. For ethical reasons more invasive research is not performed.

2.1. Starting a smoking career

Most smokers begin smoking during childhood or adolescence: 89% of daily smokers tried their first cigarette by or at age 18, and 71 % of persons who have ever smoked daily began smoking daily before or by age 18 (2). In the Netherlands 71% of the adolescents have ever tried a cigarette by age 18 (1), which is comparable with data from the USA where 68.8% of all persons have tried a cigarette by age 18 (2).

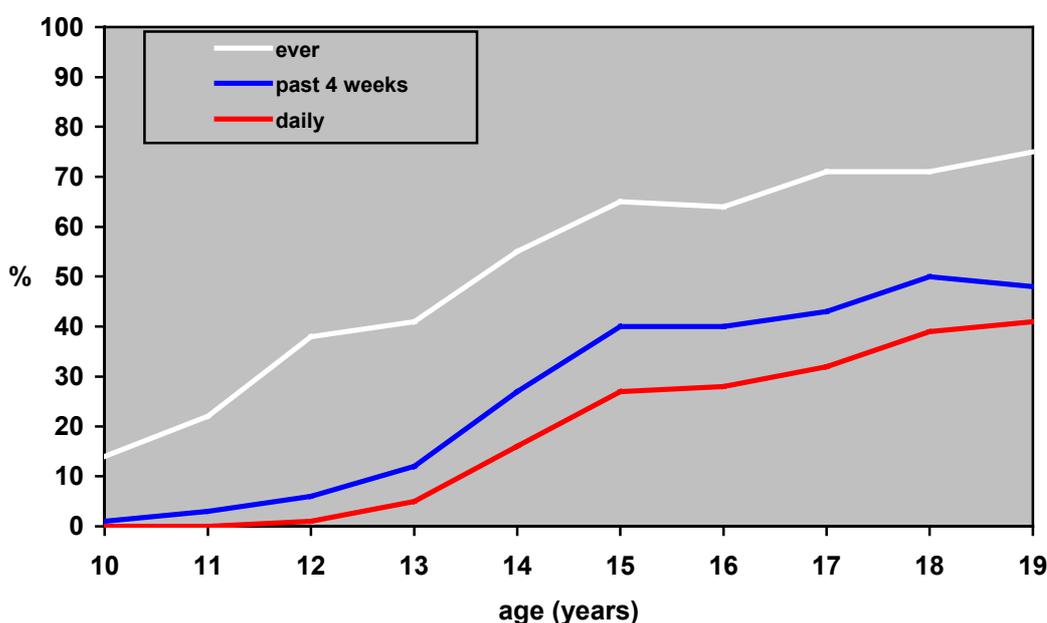


Figure 1 Percentage youth smokers (ever, past 4 weeks, daily) by age in 2000 (in %) (1).

It can be concluded that most smoking careers start in adolescents, but at what age do children actually start to experiment with smoking? In the Netherlands there is an escalation of experimental smoking between the age of 13-15 years old. Figure 1 shows a rapid increase in children who had smoked in the past 4 weeks and children who smoke daily beginning at age 13 (1).

Not only does the smoking career start early in adolescents, numerous studies have found that smoking in adolescence is a strong predictor of smoking in adulthood (3) (4) (5). The earlier in life a child tries a cigarette the more likely he or she is to become a regular smoker (that is, to smoke monthly or more frequently) or a daily smoker. For example, 67% of the children who start smoking between the age of 11 and 12 years old become regular adult smokers, and 46% of teenagers who initiate smoking between the age of 15 and 16 years old become regular adult smokers (6).

Chassin *et al.*(4) demonstrated that even very light, experimental use (i.e. smoking only one or two cigarettes 'just to try') in adolescence raises the risk for adult smoking. Regular (at least monthly) adolescent smoking raises the risk for adult smoking by a factor of 16 compared to non-smoking adolescents.

Furthermore, the earlier a youth begins smoking, the more cigarettes he or she will smoke as an adult (Figure 2) (5), and the more severe the tobacco-related health consequences one is likely to experience in adult life (3).

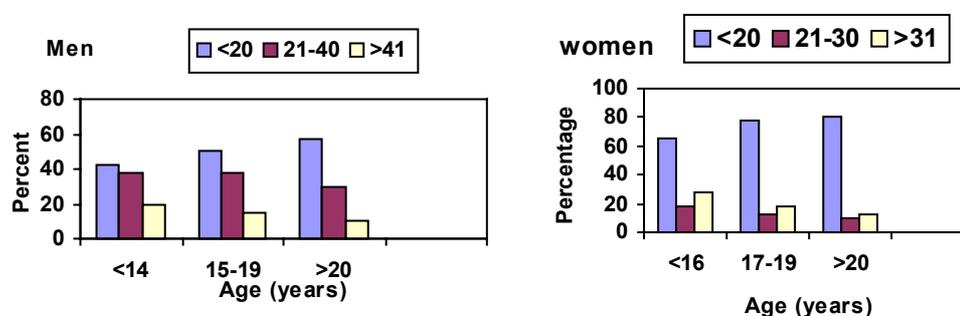


Figure 2 Relation between the age at the start of smoking and the number of cigarettes smoked per day in adulthood, according to sex, adapted from (5).

In summary, most smoking careers start in childhood. The transition years from elementary to high school, ages 13-14 appear to be a particularly high-risk time for initiation. The earlier a youth starts smoking the more likely he or she will become dependent in adult life and the more likely he or she will smoke more cigarettes.

2.2. Are youth smokers addicted to nicotine?

Some scientists argue that youth smokers can not be nicotine dependent due to their relatively short smoking careers. However, many youths describe themselves as being dependent on tobacco, and there is evidence that nicotine dependence does become established in youthful smokers. The evidence reveals that (1) youths consume substantial levels of nicotine, (2) youths report subjective effects and subjective reasons for smoking, (3) youths experience withdrawal symptoms when they are not able to smoke, and (4) youths have difficulty in quitting tobacco use (6).

2.2.1. Cotinine levels

That youths consume substantial amounts of nicotine was shown in a three year study of 197 London schoolgirls who entered the study between ages of 11 and 14. Saliva cotinine concentrations in girls who were smokers throughout the three years were higher at each year's evaluation. Average salivary cotinine levels were 103, 158, and 208 ng/ml. The level of 208 ng/ml is similar to that of many adult daily smokers. The ratio of salivary cotinine per cigarette per day, an index of the amount of nicotine taken in per cigarette, was similar for girls with various levels of cigarette consumption, and similar to that for adults. Thus, there seems to be the same intake of nicotine per cigarette among adolescent girls as among adults. It is remarkable that smokers who smoked at the time of all three surveys, as well as smokers who were

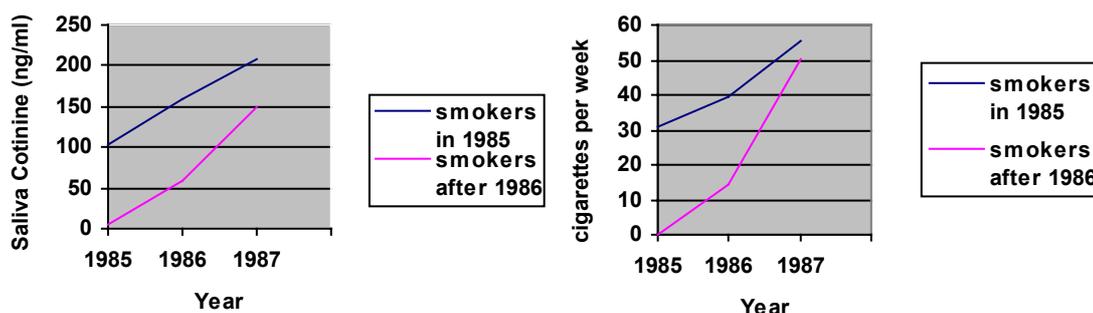


Figure 3 Cotinine concentrations and cigarette consumption by adolescent girls. Adapted from (6), original data (7). The group of smokers in 1985 were smokers in 1985; the group of smoker after 1986 were non-smokers in 1985, but became smokers in 1986-1987.

occasional smokers or non-smokers at the time of the first survey but who subsequently became daily smokers, showed escalation of cigarette consumption and saliva cotinine levels each year (Figure 3) (6) (7). If young smokers can inhale and absorb as much nicotine and carbon monoxide per cigarette as adults do, tolerance is induced with the first dose of nicotine. Since tolerance can begin immediately, it may not be long before other symptoms of dependence follow (8) (for tolerance see appendix 3).

2.2.2. Withdrawal symptoms

If youths are addicted to nicotine, withdrawal symptoms after quit attempts can be expected. Colby *et al.* (3) reviewed several studies on adolescent nicotine dependence. Daily smoking adolescents reported experiencing the following symptoms: persistent desire to quit or cut down (85.4%); smoking more or longer than intended (78.5%); marked tolerance (78.5%, defined as smoking more cigarettes per day); experience of any withdrawal symptom (75.1%); smoking to relieve or avoid withdrawal symptoms (42.1%); and continued smoking despite knowledge of a physical health problem due to smoking (29.9%) (9). The prevalence of withdrawal symptoms of several studies are presented in Figure 4. It can be concluded that adolescents report withdrawal symptoms, but between studies there is a large variation in results.

In the reviewed studies craving was most commonly reported (61%), followed by restlessness (46%), appetite increase or weight gain (45%), and irritability or anger (42.7%). It was also concluded that adolescents can experience withdrawal symptoms in the absence of attempts to quit smoking, e.g. when smoking is restricted, following overnight sleep, or as a result of significantly reducing nicotine intake (3).

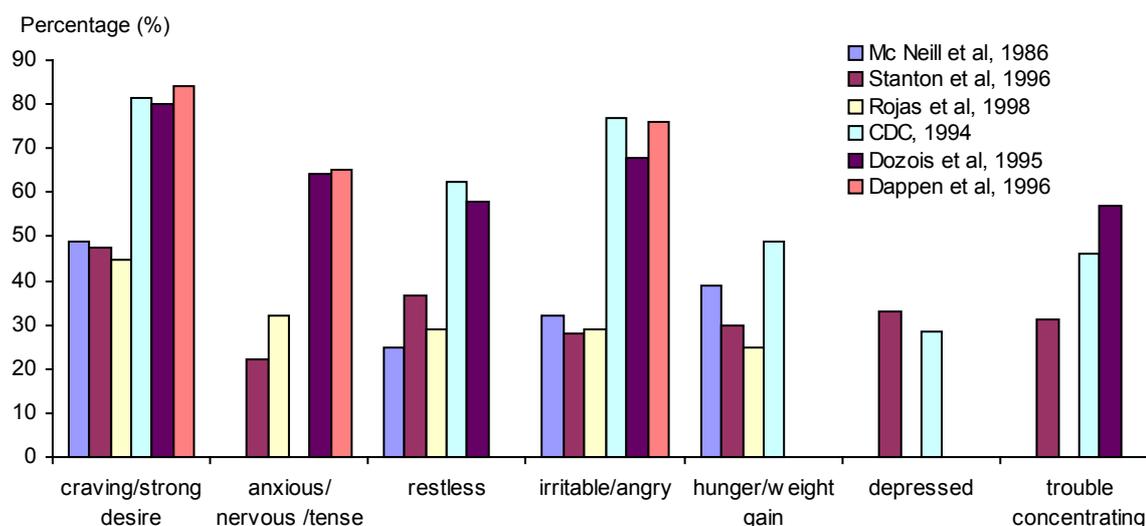


Figure 4 Prevalence of withdrawal symptoms reported by adolescent smokers in reference to a previous unsuccessful quit attempt adapted from (3). Mc Neill et al, 1986: 11-17 year old female smokers in a school setting (n=116); Stanton et al, 1996 14-16 year old smokers in a school setting (n=744); Rojas et al, 1998 10th grade smokers in a school setting (n=249); CDC, 1994 10-18 year old daily smokers in the TAPS-II National Survey Household; Dozios et al, 1995 12-17 year old smokers in a youth detention center (n=41); Dappen et al, 1996 13-19 year old smokers, vocational students (n=72). Adapted from (3).

2.2.3. Smoking behaviour

Do adolescents differ from adults in smoking behaviour? The study of daily smoking vocational students conducted by Prokhorov *et al.* (10) provided an item-by-item comparison between adolescent smokers and adult smokers. Differential endorsement rates were present for several items, with the overall pattern suggesting a less intense and pervasive smoking pattern among adolescents. Specifically, adolescents were much more likely to endorse the lightest smoking category (70% smoked 15 or fewer cigarettes per day) while few adult smokers were in this category (9.8%). Adolescents were less likely to smoke their first cigarette within the first 30 minutes of the day (50%) compared with adults (82.7%), and were also less likely to report smoking when ill (38%) than adults (57.2%). Adolescents were also less likely to report always inhaling when they smoke (81.7%) compared with adults (94.8%) (see Table 1). Adolescent and adult smokers responded to the remaining items (i.e. smoking more in the morning than during the rest of the day, hating most to give up their first cigarette and difficulty refraining from smoking in places where it is forbidden) at comparable rates. It is difficult to interpret the similarity in response rates across these items. It may be that these items reflect features that emerge early in the course of acquiring dependence. On the other hand, it may be that they are not strongly related to dependence, and thus are simply poor discriminators between dependent and non-dependent smokers (3).

Colby *et al* (3) concluded that the data on prevalence of nicotine dependence and related features lead to several conclusions. At face value, these studies suggest that adolescents experience dependence and withdrawal at substantial rates, with 20-68% of adolescent smokers classified as dependent, and two-third or more of adolescent smokers reporting some form of withdrawal upon cutting back or quitting smoking.

Regardless of the type of measure used to assess dependence, adolescents are typically classified as dependent at about half the rate that adults are. Moreover, patterns found across and within studies vary consistently with factors that seem most clearly related to dependence and withdrawal. For example, daily smokers have higher dependence and withdrawal prevalence than non-daily smokers; among daily smokers, those who smoke more cigarettes per day are more dependent than lighter smokers. Still, the high rates of dependence, and particularly the high rate of withdrawal among non-daily adolescent smokers and those who smoke fewer than five cigarettes per day are somewhat surprising (3).

Table 1 A comparison between reported smoking behaviour in adolescent and adult smokers adapted from (3).

Smoking behaviour	Adolescents (%)	Adults (%)
Lightest smoking category (15 or less cigarettes per day)	70	9.8
Smoking the first cigarette within 30 minutes of the day	50	82.7
Smoking when ill	38%	57.2%
Always inhaling	81.7%	94.8

2.2.4. Conclusion

In the study with the English school girls it was seen that adolescents are capable of consuming substantial amounts of cotinine, comparable to that of adults. Also adolescents are capable of experiencing several withdrawal symptoms after a quit attempt. The smoking behaviour, however, differs for adolescents compared to adults. Compared with adult smokers, adolescent smokers tend to smoke less regular; they are less likely to smoke daily, and when they do, they tend to smoke fewer cigarettes per day. This can be due to less opportunity to smoke and less money available to purchase cigarettes compared to adults.

2.3. First tobacco use

No data are available on the use of nicotine alone in adolescents, therefore the use of tobacco will be described in this paragraph. One has to realise that tobacco dependence is a much wider term than nicotine dependence. Not only nicotine is thought of as addictive substance, also other tobacco smoke constituents are thought to have addictive properties. Moreover, environmental cues such as smoking with friends, at a party, the package, and many more cues are considered to play a role in tobacco addiction (see appendix 4).

It has been suggested that the effects of the first tobacco use episode may play a role in initiating or preventing a regular pattern of tobacco use by producing positive effects in the eventual regular user, but producing aversive effects in the eventual non-regular user. A low incidence and/or severity of aversive effect of tobacco use may be important in determining which adolescents become regular users of tobacco. Interestingly, many of the effects of tobacco use reported retrospectively are consistent with the delivery of pharmacological doses of nicotine. Bewley *et al.* (1974) (11) identified heavy smokers (≥ 1 cigarette per day), light smokers (≤ 1 cigarette per day), and experimental smokers (ever puffed or smoked a cigarette) in a survey of 7115 English children aged 10-12. The data suggest that fewer heavy smokers were made sick by their first cigarette: 20.7% of heavy, 39.6% of light, and 35.5% of experimental smokers reported they felt sick after their first cigarette. More regular smokers (heavy and light) reported positive effects and that they enjoyed their

first cigarette (27.6% of heavy, 22.9% of light) as compared to experimental smokers (13.2%). In each group about 31% reported that they felt nothing from their first cigarette (see Table 2).

Table 2 Retrospective data on the effects of smoking the first cigarette for heavy smokers, light smokers and experimental smokers (11).

	Made sick (%)	Positive effects (%)	Felt nothing (%)
Heavy smokers (≥1 cigarette per day)	20.7	27.6	±31
Light smokers (≤1 cigarette per day)	39.6	22.9	±31
Experimental smokers (ever puffed or smoked a cigarette)	35.5	13.2	±31

In another survey study of 1431 US high school students, similar results were observed. Persistent experimentation with tobacco products (i.e. greater than ten uses; 37% of the 1431 high school students) was associated with more reports of 'feeling high' and fewer reports of 'feeling sick' after the cigarette, relative to minimal experimentation, especially amongst girls. Even amongst the persistent users, however, only 40% of the boys and 41% of the girls reported 'feeling high'. These retrospective data are consistent with the idea that the effects produced during a first tobacco episode may help to predict later regular use of tobacco (12).

Aversive nicotine-like effects are experienced by some first-time tobacco users, but these effects become less intense with repeated tobacco exposure. For example, 157 children aged 11-16 years old who were persistent smokers were interviewed regarding their first three smoking episodes. As a whole, few subjects who had tried at least three cigarettes reported pleasant effects (e.g. high, relaxed), but reports of unpleasant effects (dizziness, sickness) were more frequent. The unpleasant effects decreased in frequency across the first three smoking episodes, suggesting some form of tolerance. Reports of negative effects decreased from first to most recent use, but positive effects did not increase. Reports of pleasant effects of smoking were more likely in persistent users relative to minimal users. Thus the results of these studies indicate that tobacco produces both aversive and positive effects in initial users, and that both of these effects may be important in determining the likelihood of continued use (12).

Dizziness is a major aversive effect in smoking initiation. Reports of dizziness after the first cigarette were associated in a stepwise regression with a quick progression to a second cigarette within a week in a cohort of 386 urban public school children aged 7-15 years old (12). Dizziness is often described as 'rush' or 'buzz' and therefore contributes to rapid addiction.

In addition to the aversive effects social factors are important. An important factor that may influence how the effects of tobacco are perceived among first-time users is the presence or absence of more experienced users during the first use episode. Experienced users may minimize the importance of negative effects, such as nausea while identifying other effects, such as dizziness, with positive descriptors, such as 'rush' or 'buzz' (12).

It can be stated that effects during a first tobacco episode help predict later regular tobacco use. The more negative effects occur during this first episode the less likely it is that a second cigarette is smoked. The introduction of 'low nicotine' cigarettes may have contributed to the increase in adolescent smoking by decreasing the likelihood of

nicotine intoxication in novice smokers (12). Also the use of several flavour enhancing additives, which make the cigarettes more attractive in taste, can play an important role in masking the negative effects during first tobacco use.

2.4. Psychosocial risk factors in the initiation of tobacco use

Initiation of cigarette smoking is influenced by several kinds of factors: environmental, behavioural, personal, and sociodemographic (Table 3). Also genetic factors are often suggested to play a role in smoking initiation, and will be discussed in the next paragraph.

In a study on smoking initiation, the main risk factors for the 47% of the children who had tried at least one cigarette were grade level in school (that is the higher the grade the older the child, the higher the likelihood of trying a cigarette), having a best friend who was a smoker, and risk-taking behaviour. Progression to a second cigarette (32% of those who smoked one cigarette) was predicted by life stress, friends who smoked, lack of negative attitudes towards smoking, and experience of dizziness when smoking the first cigarette. Progression toward a third cigarette (in 77% of those who had smoked two cigarettes) was predicted by best friend smoking, feelings of helplessness and rapid progression to the second cigarette. These analyses support the idea that initiation of cigarette smoking is primarily a consequence of environmental factors, whereas progression appears to be more influenced by personal and pharmacological effects (see for more details appendix 5) (6).

Behavioural analysis indicates that cigarette smoking is often an early manifestation of problem behaviour. A number of personal characteristics of adolescents have been linked to cigarette smoking: low self-esteem, poor self-image, sensation-seeking, rebelliousness, low knowledge of the adverse effects of smoking, depression and/or anxiety, pharmacological response. Smokers are more likely than non-smokers to have a history of major depression, even preceding initiation of smoking and smokers with a history of depression have been found to have lower smoking cessation rates than smokers without depression (13) (14).

Other environmental risk factors, which are considered important in smoking initiation, are:

- being a girl
- having brothers or sisters who smoke
- having parents who smoke
- living with a lone parent
- having relatively less negative views about smoking
- not intending to stay on in full-time education after 16
- thinking that they might be a smoker in the future

Analysis showed that apart from parental smoking, which only has an effect if siblings do not smoke, all these characteristics were associated independently with starting to smoke, and that their independent effects were all fairly small and of similar magnitude. This can be interpreted that there is no simple explanation of why children start to smoke since many factors are involved (15).

Table 3 Psychosocial risk factors in the initiation of tobacco use among adolescents adapted from (6).

Risk factors for smoking initiation	
Sociodemographic factors	Behavioural factors
Socioeconomic status	Academic achievement
Developmental stage	Other problem behaviours
	Constructive behaviours
	Behavioural skills
	Intentions
	Experimentation
Environmental factors	Personal factors
Accessibility	Knowledge of consequences
Advertising	Subjective expected utility
Parental use	Self-esteem/self-image
Sibling use	Self-efficacy
Peer use	Personality factors
Normative expectations	Psychological well-being
Social support	

Socio-demographic factors that predispose youths to cigarette smoking include low-socio-economic status, low level of parental education, and the individual's developmental state of adolescence. With respect to the latter, the transition years from elementary to high school, grades 7-10 (ages 11-16) appear to be a particular high-risk time for initiation (6).

2.5 Genetics and the risk of becoming a smoker

Genes that are polymorphic, that is, in different individuals the same gene has slight variations called alleles, could cause a part of the variation in smoking behaviour as seen in the general population.

There are several ways to investigate genetic influences on smoking behaviour and nicotine addiction. First of all there are numerous twin studies available on this subject. If genetic factors are involved, identical twins, who share the same genes, will be more similar in their use of tobacco products than fraternal twins, who on average only share half of their genes.

Another possibility to study genetic influences on nicotine addiction or smoking behaviour is by screening for candidate genes in groups of smokers compared to non-smokers. These candidate genes do not only involve the dopamine pathway (see appendix 4), but also differences in metabolism (see appendix 2 and 5) or other related genes (see appendix 5).

However, several factors exist that contribute to the difficulty in finding reproducible associations between candidate genes and nicotine dependence. One is the multiple comparison problem: in searching for associations between candidate genes, there will be false positive and false negative associations. A second factor is that it is difficult to detect multiple genes of modest effect because association and linkage studies have low statistical power to detect these types of associations. A third factor is differences between studies in the definition of a smoker (16).

2.5.1 Twin studies

In twin studies, researchers compare patterns of tobacco use in fraternal and identical twin pairs, who typically are exposed to common environmental influences. If genes play a role in determining tobacco use, identical twins, who share the same genes, will be more similar in their use of tobacco than fraternal twins, who share on average roughly half of their genes (17).

Initial support for a genetic influence on the use of tobacco came from cross-sectional studies in twins and showed a mean heritability of cigarette smoking of 0.53 with a range of 0.28-0.84 (18) (19). This means that between 28% and 84% of the observed variation in current smoking behaviour in the population from which the data were drawn, can be accounted for by genetic factors. These studies have also indicated that these genetic factors relate to two distinct aspects of smoking behaviour: initiation and persistence.

Hall *et al.* (16) re-analysed data from original twin studies of cigarette smoking around the world (see Table 4). Despite the wide range of cultures, ages, and birth cohorts represented in these papers, estimates of heritability of smoking initiation were substantial for both men and women; they ranged between 37% and 84% in women and between 28% and 84% in men. By contrast there was little consistency between studies for the importance of family environment. In some studies shared family environment was estimated to account for 50% of the variance in smoking initiation among women and 49% of the variance among men. Yet other studies reported no significant shared environmental influences on smoking initiation (16).

Table 4 Estimates from twin studies of genetic (G), shared environmental (SE), and non-shared environmental (NSE) influences on smoking initiation and persistence in women and men (16).

	Women			Men		
	G	SE	NSE	G	SE	NSE
Initiation						
Sweden	44	42	14	51	39	10
Denmark	79	-	21	84	-	16
Finland	37	50	13	50	33	17
Australia	77	4	19	28	43	29
Australia;b	60	26	14	80	-	20
USA Second war veterans	-	-	-	59	21	20
USA Virginia	84	-	16	84	-	16
USA Vietnam veterans	-	-	-	39	49	12
Persistence						
Sweden	59	-	41	52	-	48
Finland	71	-	29	68	-	32
Australia	53	-	47	53	-	47
Australia;b	62	-	38	62	-	38
USA	58	-	42	58	-	42
USA Vietnam veterans	-	-	-	69	-	31

Taken together, this evidence suggests that there is consistent evidence for a genetic influence on smoking initiation, and substantial evidence for a shared environmental influence on initiation, but the relative importance of genetic and environmental factors is highly variable across populations. The risk of smoking persistence is primarily a function of genetic factors, and less of environmental factors. It is important to realise that the same genetic factors that influence smoking initiation could also influence persistence, and thus cause problems when interpreting results (19) (20).

But is this also the case in adolescents? In a study with 1676 Dutch adolescent twin pairs, it was shown that there is not one underlying continuum of liability to smoking initiation and the number of cigarettes consumed. For smoking initiation there is an important influence of shared environmental factors, while for quantity smoked only genetic factors are important. Some of the genetic factors that influence smoking

initiation might also be involved with quantity smoked. Other studies have shown that both smoking persistence and nicotine dependence in adults are highly heritable. The study with adolescent twins shows that even in adolescents, given that they are smokers, genetic factors determine to a large extent the number of cigarettes consumed. There were no differences between males and females in the magnitude of the genetic and environmental influences on individual differences in smoking initiation and quantity smoked. Smoking initiation was influenced by genetic factors (39%) and shared environmental influences (54%). Once smoking is initiated genetic factors determine to a large extent (86%) the quantity that is smoked (21).

It is important to realise what the limitations of twin studies are. A critical assumption of the twin study method is that monozygotic and dizygotic twins have equal exposures to environmental influences that affect the trait under study. If this assumption is not met, then twin studies provide inflated estimates of genetic influences on behaviour. The traditional twin method also assumes that the environments of twins and singleton siblings are comparable. This assumption may not hold because twins have higher rates of obstetric complications and low birth weight than singleton births and there are different patterns of family and sibling interactions in families with twins than in those without. A final limitation of the twin study is that it has low statistical power to test for gene-environment interactions and gene-environment correlation effects in the aetiology of smoking and other behaviours.

Despite the limitations in experimental design, there is strong support for genetic factors playing a role (along with the environment) in tobacco smoking (16).

2.5.2 Candidate genes affecting smoking initiation and smoking persistence

It is generally thought that variations in genetic properties between persons can contribute to differences in smoking behaviour, or being a smoker or a non-smoker. Genes that are polymorphic, that is, in different individuals the same gene has slight variations called alleles, could cause a part of the variation in smoking behaviour as seen in the general population.

Studies comparing ever smokers versus never smokers have shown that positive associations with smoking initiation are polymorphisms in the serotonin transporter gene, polymorphisms in the cytochrome P450 enzyme 2A6 gene, and polymorphisms in the dopamine D2 receptor (DRD2) and the dopamine D4 receptor (DRD4) genes. Candidate genes that may influence persistent smoking include the dopamine transporter (DAT1), DRD2 and DRD4 genes (19). These genes and their polymorphisms are described in more detail in appendix 5.

2.6. Gender differences in adolescent smoking

Colby *et al.* (3) reviewing adolescent nicotine dependence, described gender differences. So far, no conclusive data are available on this subject. Some studies found no differences in nicotine dependence between males and females (9)(22) (23). Other studies found only a difference in smoking and dependence prevalence in an adult group of 18-49 years old. Smoking and dependence prevalence were higher among females compared with males. Further analyses showed that the gender differences in dependence were only found among whites and not in other ethnic groups (24). In adolescents one study found no gender differences, another study found that males smoked significantly more than females, and scored significantly higher on a measure of nicotine dependence (3) (25).

Gender differences in withdrawal experiences have been shown in several studies. Both Stanton (9) and Dappen *et al.* (26) found that adolescent females were more

likely than males to report weight gain and appetite increase during nicotine withdrawal. Females also reported more often to smoke to relieve withdrawal symptoms than males. In a subsequent study, Stanton *et al.* (27) found additional gender differences, with adolescent females reporting more stress and depression after quitting or cutting down smoking compared with males. Interestingly, females also reported more positive effects of quitting than males (e.g. having more energy, feeling better about oneself) (3).

Taken as a whole these data suggest that males and females may differ in the factors that maintain smoking, but more research is clearly needed to fully elucidate potential differences and their implications (3).

2.7. Ethnic differences in youth smoking

In a Dutch study the four-week prevalence of tobacco, alcohol and cannabis use were studied among secondary high school students of Dutch, Caribbean, Surinamese, Turkish and Moroccan descent using questionnaires. Compared to Dutch students, four-week prevalence of tobacco, alcohol and cannabis amongst Moroccan students was significantly lower. After controlling for confounders students of Surinamese and Turkish descent were also found to have significant lower prevalence of tobacco, as well as alcohol compared to Dutch students. Although prevalence differed, there were minor and non-significant differences between ethnic groups with regard to amounts used (28).

Ethnic differences in nicotine metabolism in adults have recently been demonstrated. Afro-Americans show in several studies (29) (30) to have higher levels of cotinine, normalised for cigarettes smoked per day. Recently, Perez-Stable *et al* administered deuterium-labelled nicotine and cotinine to Afro-American and Caucasian smokers. Afro-American metabolise cotinine more slowly than Caucasians due to slower oxidation to trans-3'-hydroxycotinine and slower N-glucuronidation (31) (32). For more information on nicotine metabolism see appendix 2.

Instead of slower cotinine metabolism there are also ethnic groups with slower nicotine metabolism. Benowitz *et al.* (33) administered deuterium-labelled nicotine and cotinine to Chinese-Americans, Latinos and whites. Total and non-renal clearance of nicotine via the cotinine pathway were similar in Latinos and whites and significantly lower in Chinese-Americans (35% slower). The fractional conversion of nicotine to cotinine and the clearance of nicotine were also significantly lower in Chinese-American smokers than in Latinos and whites. The half-life of nicotine was significantly longer in Chinese-Americans than in members of the other ethnic groups. In other words in this ethnic group metabolism of nicotine appears to be slower than in Caucasian smokers. In contrast to the situation in African-Americans, the intake of nicotine per cigarette by Chinese-Americans (0.73mg) was significantly lower than by Latinos (1.05 mg) or whites (1.10 mg).

These results are discussed in more detail in appendix 5 genetics.

2.8. Animal data on youth nicotine addiction

The ability of nicotine to produce unique effects in adolescents likely stems from the fact that brain development continues in this period. Apoptosis, synapse formation and the functional programming of behavioural responses are all consolidated during adolescence, refuting the now-outdated view that brain development is essentially complete in early childhood (34).

Nicotine given during adolescence produces a pattern of nicotinic receptor up-regulation in brain regions associated with addiction and reward pathways. With adolescent nicotine treatment, male rats show prolonged nicotinic receptor up-

regulation that remains evident even a month after the termination of drug exposure, a much longer span than in foetal or adult rats exposed to comparable or even higher levels of nicotine (35). Furthermore, adolescent nicotine exposure produces long-term alterations in cell number, and gene expression, commensurate with brain cell damage. Ultimately, animals exposed to nicotine during adolescence display long-term changes in the functioning of the reward pathway (34). For more information see appendix 4.

2.9 Conclusion

The majority of smoking careers start in childhood. The earlier a youth starts smoking the more likely he or she will become dependent in adult life and the more likely he or she will smoke more cigarettes. The transition years from elementary to high school, ages 13-14 appear to be a particular high-risk time for initiation. Youths consume substantial levels of nicotine, report subjective effects and subjective reasons for smoking, experience withdrawal symptoms when they are not able to smoke, and they have difficulty in quitting tobacco use. This indicates that youths can be addicted to nicotine.

The effects produced during a first tobacco episode may help to predict later regular use of tobacco. Retrospective studies indicate that heavy smokers experience less aversive effects than light smokers during their first cigarette. The more negative effects occur during this first episode the less likely it is that a second cigarette is smoked. The introduction of 'low nicotine' cigarettes may have contributed to the increase in adolescent smoking by decreasing the likelihood of nicotine intoxication in novice smokers. Also the use of several flavour enhancing additives, which make the cigarettes more attractive in taste, can play an important role in masking the negative effects during first tobacco use.

Initiation of cigarette smoking is influenced by various factors like: environmental, behavioural, personal, and socio-demographic factors. Twin studies suggests that there is consistent evidence for a genetic influence on smoking initiation, and substantial evidence for a shared environmental influence on initiation, but the relative importance of genetic and environmental factors is highly variable across populations. There are some indications that there exists some gender differences in smoking and dependence prevalence, but more research is needed to elucidate the potential differences and their implications. Ethnic differences have been reported on tobacco prevalence. Studies comparing ever smokers versus never smokers have shown that there exist positive associations with several genetic polymorphisms and smoking initiation and smoking persistence.

It is now evident from animal studies that nicotine given during adolescence produces a pattern of nicotinic receptor up-regulation in brain regions associated with addiction and reward pathways. With adolescent nicotine treatment, male rats show prolonged nicotinic receptor up-regulation, which remains evident even a month after the termination of drug exposure, a much longer span than in foetal or adult rats exposed to comparable or even higher levels of nicotine. Furthermore, adolescent nicotine exposure produces long-term alterations in cell number, macromolecular characteristics, and gene expression, commensurate with brain cell damage. Ultimately, animals exposed to nicotine during adolescence display long-term changes in the functioning of the reward pathway.

3. How addictive is cigarette smoking?

3.1 Introduction

The first cigarette smoked is often perceived as aversive, producing coughing, dizziness, and/or nausea. With repeated smoking, tolerance develops to the noxious effects of cigarette smoking, and smokers tend to report positive effects of smoking. As the daily intake of nicotine increases, the development of physical dependence, that is, experiencing withdrawal symptoms between cigarettes or when cigarettes are not available, becomes established. Thus, there appears to be a progression over time from smoking initially for social reasons to smoking for pharmacological reasons. The latter includes both smoking for positive effects of nicotine and smoking to avoid withdrawal symptoms (6) (36). Often the question raised is: how addictive is cigarette smoking? How many cigarettes are needed to become addicted to cigarette smoking? The time path of nicotine addiction is described in paragraph 3.2. Tobacco ‘chippers’ form an extraordinary group and are considered as not addicted, this group will be discussed in paragraph 3.3. Since cigarette smoking is considered to be more than nicotine addiction alone, relapse and conditioned reinforcers are described in paragraph 3.4.

3.2. Time path of nicotine addiction

Some scientists state that smoking a few cigarettes may result in the development of tobacco dependence. Rusell calculated from self-report studies on past and current smoking behaviour that 94% of those who smoke more than 2-3 cigarettes in their life, go on to become regular smokers as adults (37). Chassin *et al.* (4) concluded that even very light, experimental use (i.e. smoking only one or two cigarettes ‘just to try’) in adolescence significantly raises the risk for adult smoking. Regular (at least monthly) adolescent smoking raises the risk of adult smoking by a factor 16 compared to non-smoking adolescents. It is possible that this figure is inflated by adult non-smokers who forget that they had tried a cigarette in adolescence or perhaps felt that smoking the odd cigarette many years previously does not really count as having smoked (38).

In contrast to the hypothesis that smoking even a few cigarettes may result in addiction, there exists the assumption that heavy daily use (one half pack per day) is necessary for dependence. This hypothesis is derived from observations in ‘chippers’, adult smokers who have not developed dependence despite smoking up to five cigarettes per day over many years (see also paragraph 3.3.). Also many smokers who are instructed to quit, report cutting down to about 10 cigarettes per day and cannot reduce their consumption to fewer than 10. At 10 cigarettes per day smokers can still absorb adequate nicotine to maintain nicotine addiction (6).

Parallel to the discussion if only a few cigarettes in life are needed to become addicted or that smoking more than 5 cigarettes a day can cause addiction there is the discussion if the onset on addiction is rapid or develops slowly in 2 till 3 years. It has been suggested that unlike adults, in whom intermittent or light smoking may be a stable and relatively non-addictive pattern of smoking (‘chippers’), children who are light smokers are often in a phase of escalation, with a typical interval from initiation to addiction in 2-3 years. The interval between initiation and addiction is based on a comparison of the cumulative prevalence curves for trying a first cigarette and smoking daily) and the interval between initiation of smoking and the rise of salivary cotinine concentrations to adult levels (Figure 3) (6). In this scenario fits the development of nicotine addiction in youths characterised as a series of five stages:

1. Preparatory
2. Initial trying
3. Experimentation
4. Regular use
5. Nicotine addiction

The 'preparatory' stage includes formation of knowledge, beliefs, and expectations about smoking. 'Initial trying' refers to trials with the first 2 or 3 cigarettes. 'Experimentation' refers to repeated, irregular use over an extended period of time; such smoking may be situation-specific (for example, smoking at parties). 'Regular smoking' by youths may mean smoking every weekend or in certain parts of each day (such as after school with friends). 'Nicotine addiction' refers to regular smoking, usually every day, with an internally regulated need for nicotine. Thus, for individual youths, there is a progression of smoking over time from initiation to experimentation with light smoking to regular and heavy smoking (6).

Goddard (15) states, however, that the behaviour of some children does fall into this pattern, but that of the majority does not. Her survey included a sample of secondary school children who were interviewed three times in 1986, 1987 and 1988, when they were at the beginning of the second, third and fourth year (age 11-15 years old). In this longitudinal study among school children, over a 2-year period, approximately 48% of those pupils who had tried smoking once did not smoke again. This suggests that trying a single cigarette is not inevitably followed by rapid escalation to regular smoking in 2–3 years (38).

Withdrawal symptoms, however, develop rapidly after smoking initiation. In a cohort of young adolescents 22% of the 95 subjects who had initiated occasional smoking reported a symptom of nicotine dependence within four weeks of initiating monthly smoking (8). One or more symptoms were reported by 60 (63%) of these 95 subjects. Of the 60 symptomatic subjects, 62% had experienced their first symptom before smoking daily or began smoking daily only upon experiencing their first symptom. The first symptoms of nicotine dependence can appear within days to weeks of the onset of occasional use, often before the onset of daily smoking. These results were obtained in a longitudinal study of 681 students age 12-13 years old. Subjects were interviewed individually in school three times for one year, no measures of cotinine or nicotine were taken. Some have postulated that youths' experience of withdrawal symptoms may be influenced by their expectations. This raises the question as to whether repeated inquiries regarding symptoms of dependence may have prompted youths to over report symptoms in subsequent interviews. There was no difference in the rapidity of onset of symptoms among those who had reported symptoms during the first interview and those who reported symptoms only after repeated interview (8). There are also studies which concluded that adolescents can experience withdrawal symptoms in the absence of attempts to quit smoking, e.g. when smoking is restricted, following overnight sleep, or as a result of significantly reducing nicotine intake (3).

3.3 Tobacco chippers

In 1976, Zinberg and Jacobsen (31) used the term 'chippers' to refer to opiate users who were capable of controlling and limiting their use of opiates, as opposed to the common pattern of escalating and compulsive opiate use which many had come to associate with heroin users. Their paper is one of many in the drug addiction field which recognises that drugs which have strong dependence-producing qualities in many people do not necessarily produce dependence in all users. Since those early studies of non-dependent heroin users, it has been recognised that not all tobacco smokers progress to become highly dependent chain smokers. Shiffman, in the United

States, was one of the first to study systematically the phenomenon of non-dependent smokers, and he also used the term 'chippers' (31).

The question of what proportion of smokers is dependent is not as simple as it might at first seem to be. Degree of dependence is best conceptualised as existing on a continuum, rather than as a dichotomous variable (dependent vs. non-dependent).

There appears to be a consensus in the literature that adults who consistently smoke five or fewer cigarettes per day (but who smoke on at least four days per week) over a long period (e.g. more than a year) are non-dependent. Although cross-sectional surveys find that up to 20% of smokers report smoking fewer than five cigarettes per day, it is unclear what proportion of these people are in a transitional phase of increasing or decreasing consumption. One Australian study found that only 8% of 700 adult smokers smoked five or fewer cigarettes per day. However, most of them were preparing to quit smoking, and so may have been reducing their consumption. It has been estimated that only about 5% of smokers are able to smoke without becoming addicted (31).

The first studies of tobacco 'chippers' compared them to samples of heavy smokers (20-40 cigarettes per day). The light smokers reported no signs of nicotine withdrawal after overnight abstinence and, in contrast to heavy smokers, also reported that they could regularly and easily abstain from tobacco for periods of a few days or longer. This confirms that they were at the low end of the dependence continuum. However, it was also found that the 'chippers' nicotine absorption per cigarette and nicotine elimination rates were similar to those of heavy smokers. The 'chippers' were less likely both to smoke to relieve stress and to report an aversive response to their first ever cigarette. The light smokers also reported having fewer smoking relatives.

It has been suggested that vulnerability to nicotine dependence is related to genetically based high initial sensitivity to nicotine. Consistent with this, people who become highly dependent cigarette smokers, have been found to have more pleasurable sensations at their initial exposure to tobacco. It has also been reported that regular smokers recalled more pleasant reactions to their first cigarette than 'chippers' (31).

Because 'chippers' do not differ from other smokers in their absorption and metabolism of nicotine, some investigators suggest that this level of consumption may be too low to cause nicotine dependence (8). 'Chippers' are similar to dependent smokers in their degree of tolerance and in the direct pharmacological effects of nicotine, and yet withdrawal symptoms do not appear to accompany abstinence (39). Among adults, light or occasional smokers are relatively uncommon (less than 10% of adult smokers); they have higher success in smoking cessation than do heavier smokers, although not all light smokers are able to quit. In contrast, many more children than adults are light or occasional smokers; however, light smoking by children is often not a stable pattern, but rather represents a stage in escalation to become daily smokers (6).

3.4 Relapse and conditioned reinforcement

In the United States, less than 10% of the people who quit smoking for a day remain abstinent 1 year later. Because only 2% to 3% of smokers succeed in quitting smoking, nicotine is considered among the most addictive drugs. High rates of relapse are common for cigarette smokers (40).

Tobacco smoking is more than the pharmacological working of nicotine in the brain. It has also been hypothesised that reinforcing properties¹ of tobacco smoke are important for nicotine addiction. If this is the case, conditioned reinforcers² contribute to maintain the addiction.

In the beginning of the addiction, nicotine and the conditioned reinforcers are connected. This means that when smoking a cigarette, the smell of cigarette smoke or the view of a cigarette package is coupled to the pharmacological effect of nicotine in the brain. When a smoker smokes several cigarettes during the day, desensitisation of the nicotinic acetylcholine receptors occurs. This means that nicotine cannot exert a pharmacological effect in the brain. The conditioned reinforcers may be enough in this case to maintain the addiction. For cigarette smokers, the conditioned reinforcers paired with the nicotine in cigarette smoke are likely to be re-established each day on occasions when they smoke following a period of abstinence which permits reactivation of the receptors responsible for the dopamine response. One such time will be the first cigarette in the morning following the abstinence associated with sleep. Interestingly, many smokers report that this cigarette is the most pleasant of the day. Thus, even for people who smoke in a way, that sustains desensitising nicotine concentrations (i.e. high concentrations) through much of the day, the hypothesis predicts that the primary reinforcing properties of nicotine continue to play a central role in maintaining the addiction by repetitively re-establishing the salience of the conditioned reinforcers present in the smoke (41).

The reinforcers can be divided in positive and negative reinforcers. Negative reinforcement refers to the above-described relief of nicotine withdrawal symptoms in the context of physical dependence (see Table 5). Positive reinforcing effects that are reported include relaxation, reduced stress, enhanced vigilance, improved cognitive function, mood modulation, and lower body weight (42).

Table 5 Nicotine withdrawal symptoms (6).

Nicotine withdrawal syndrome	
Restlessness	Loss of energy/ fatigue
Eating more than usual	Dizziness
Excessive hunger	Stomach or bowel problems
Anxiety / tension	Headaches
Impatience	Sweating
Irritability/anger	Insomnia
Difficulty concentrating	Heart palpitations
Depression	Tremors
Disorientation	Craving for cigarettes

The coupling of other stimuli than nicotine with cigarette smoking is also important in relapse. When a smoker encounters stressors or situational reminders of smoking, these stimuli revivify the pleasurable or other reinforcing aspects of smoking, which then generate the urge to smoke. Such recurrent anticipatory responses may persist

¹ **Reinforcement** A reinforcer increases the likelihood of the act that produced it being repeated. The resultant strengthening of behaviour can be seen as a form of memory, by which voluntary actions with certain outcomes are consolidated into long-term memory (87).

² **Conditioning** Forms of learning and association. They may be either instrumental (in which voluntary actions are controlled by their outcomes; see reinforcers below) or Pavlovian (in which a temporal correlation between events is detected and learned by an animal, even in the absence of voluntary control) (87).

6 months or longer after physical dependence has been overcome, accounting for the relapses that occur beyond the first week or two after cessation of tobacco use. There are various conceptualisations of the nature of the anticipatory response system. One is the conditioning model, in which learned associations between the effects of cigarette smoking and specific cues in the environment motivate smoking. Another model is self-regulation, in which high-risk situations activate cognitive processes in a form of pleasurable expectations and a reduced sense of personal control, which then increases the likelihood of smoking (6).

3.5 Conclusion

Not all smokers are addicted to nicotine. 'Chippers' do not differ from other smokers in their absorption and metabolism of nicotine. They are similar to dependent smokers in their degree of tolerance and in the direct pharmacological effects of nicotine, and yet withdrawal symptoms do not appear to accompany abstinence.

No consensus exists on the time path of nicotine addiction. From retrospective data it has been calculated that smoking only a few cigarettes determines whether someone becomes a smoker or not. Follow up studies conclude often that adolescents develop in 2-3 years to regular cigarette consumption. Withdrawal symptoms appear to develop rapidly after taking up smoking. That nicotine is a very addictive substance is demonstrated by the high relapse rates. In the maintaining of the addiction conditioned reinforcers such as cigarette smoke are thought to play an important role.

4 Recommendations

4.1 Smoking initiation

It can be concluded that most youths start their smoking during adolescence. Especially children in the age of 13-14 years old, i.e. the transition years from preliminary school to high school, are a high-risk group for initiating smoking. The prevalence of 'ever tried to smoke' increase rapidly at this age. When children actually start at such a young age with smoking, the risk of becoming a regular smoker in adult life is much greater than when they start at a later age. Also the amount of cigarettes smoked in adult life is correlated with the age of smoking initiation. The younger a person starts to smoke, the more cigarettes a day will be smoked in adult life. Therefore the aim in smoking prevention should be to prevent smoking at a young age with special care for the high risk period during the transition years from preliminary school to high school.

Smoking initiation is a complex process, which is influenced by several different factors. For instance, having a best friend who smokes, risk taking behaviour, lack of negative attitudes towards smoking, experiencing dizziness when smoking the first cigarette, and age are all factors associated with starting to smoke. Until now there is no simple explanation of why children start to smoke. The aim of prevention should be to create a non-cool image on smoking behaviour. In the Netherlands DEFACTO launched the campaign 'but I do not smoke' to improve the image of non-smoking youths. After the campaign non-smokers were considered to be cooler, tougher and nicer persons. Reporting the percentage of non-smokers instead of the percentage of smokers may contribute to smoking prevention, since it refers to non-smoking as the social norm in the Netherlands (43).

Twin studies suggest that there is consistent evidence for a genetic influence on smoking initiation, and substantial evidence for a shared environmental influence on initiation, but the relative importance of genetic and environmental factors is highly variable across populations. Studies comparing ever smokers versus never smokers have shown that there exist positive associations with several genetic polymorphisms and smoking initiation and smoking persistence. It can be concluded that genetic influences play a role in smoking initiation and persistence, but several other factors are also important.

The effects during a first tobacco episode help predict later regular tobacco use. The more negative effects occur during this first episode the less likely it is that a second cigarette is smoked. The introduction of 'low nicotine' cigarettes may have contributed to the increase in adolescent smoking by decreasing the likelihood of nicotine intoxication in novice smokers. Also the use of several flavour enhancing additives, which make the cigarettes more attractive in taste, can play an important role in masking the negative effects during first tobacco use.

4.2 Nicotine addiction

The question how rapid nicotine causes addiction in adolescents remains. No consensus exists on the time path of nicotine addiction. From retrospective data it has been calculated that smoking only a few cigarettes determines whether someone becomes a smoker or not. Follow up studies conclude often that adolescents develop in 2-3 years to regular cigarette consumption. Withdrawal symptoms appear to develop rapidly after taking up smoking. That nicotine is a very addictive substance is demonstrated by the high relapse rates.

Data from experimental animal studies support the hypothesis that nicotine is able to induce addiction in a short period of time. In adolescence brain development continues and moreover nicotine given during adolescence produces a pattern of nicotinic receptor up-regulation in brain regions associated with addiction and reward pathways. With adolescent nicotine treatment, male rats show prolonged nicotinic receptor up-regulation that remains evident even a month after the termination of drug exposure, a much longer span than in foetal or adult rats exposed to comparable or even higher levels of nicotine. Ultimately, animals exposed to nicotine during adolescence display long-term changes in the functioning of the reward pathway.

Taken all the evidence into consideration it seems justified to state that there is a great likelihood that nicotine is able to cause addiction very quickly. Reducing the number of smokers in the future should start with prevention of smoking initiation. The aim should be to prevent children experimenting with cigarettes. This experimenting is not as innocent as is sometimes thought.

4.3 Conclusions

It seems likely that nicotine addiction develops quickly after experimentation in adolescents. Experimentation with cigarettes by adolescents is not innocent. Therefore, the aim should be primarily to prevent nicotine addiction in youth between 13-14 years of age. Prevention should refer to non-smoking as the social norm.

The introduction of 'low nicotine' cigarettes and the use of flavouring enhancing additives may have contributed to the increase in adolescent smoking by decreasing the likelihood of nicotine intoxication in novice smokers.

Appendix I General aspects of nicotine addiction

1.1 THE HISTORY OF NICOTINE ADDICTION	32
1.2 WHAT IS ADDICTION?.....	32
1.3 ASSESSMENT OF NICOTINE ADDICTION	33

1.1 The history of nicotine addiction

Cigarette smoking and other forms of tobacco use are nowadays regarded as addictive. Until the late seventies tobacco smoking was only considered to be a habit, not an addiction. The literature of the next ten years yielded many studies that supported the view that one of the main reasons for cigarette smoking was to obtain the effects of nicotine. This changed the view of tobacco use as a habit into tobacco use as an addiction (44).

Nowadays it is generally accepted that nicotine can serve as a positive reinforcer in several animal species, including humans, and is considered addictive but less addictive than cocaine and heroin (40).

1.2 What is addiction?

Addiction can be defined by the compulsive use of a drug that develops after repeated drug exposure despite severe, adverse consequences (40). The World Health Organization (WHO) describes drug dependence as ‘a behavioural pattern in which the use of a given psychoactive drug is given a sharply higher priority over other behaviours that once had a significantly higher value’. In other words, the drug comes to control behaviour to an extent considered detrimental to the individual or to society (6). Specific criteria for a drug that produces dependence or addiction have been presented by the U.S. Surgeon General (Table 6), and specific criteria for diagnosing drug dependence or addiction in individuals have been presented by the American Psychiatric Association (Table 7). It is apparent that addiction is associated with euphoria and other psychoactive effects, the development of tolerance and the experience of withdrawal symptoms when the product is no longer used.

Table 6 Criteria for drug dependence (6)

Criteria for drug dependence
Primary criteria
Highly controlled or compulsive use
Psychoactive effects
Drug-reinforced behaviour
Additional criteria
Addictive behaviour often involves the following:
Stereotypic pattern of use
Use despite harmful effects
Relapse following abstinence
Recurrent drug cravings
Dependence-producing drugs often manifest the following:
Tolerance
Physical dependence
Pleasant (euphoric) effects

Table 7 Criteria for substance dependence (6).

Criteria for substance dependence
A maladaptive pattern of substance use, leading to clinically significant impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:
1. Tolerance, as defined by of the following: <ol style="list-style-type: none"> a. A need for markedly increased amounts of the substance to achieve intoxication or desired effect. b. Markedly diminished effects with continued use of the same amount of the substance.
2. Withdrawal, as manifested by either of the following: <ol style="list-style-type: none"> a. The characteristic withdrawal syndrome for the substance (refer to Criteria A and B of the criteria sets for withdrawal from the specific substance). b. The same (or a closely related) substance is taken to relieve or avoid withdrawal symptoms.
3. The substance is often taken in larger amounts or over a longer period than was intended.
4. There is a 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 (e.g., visiting multiple doctors or driving long distances), use the substance (e.g., chain smoking), or recover from its 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 caused or exacerbated by the substance (e.g., current cocaine use despite recognition of cocaine-induced depression, or continued drinking despite recognition that an ulcer was made worse by alcohol consumption).

1.3 Assessment of nicotine addiction

Various measures and methods have been developed to measure dependence on nicotine and its abuse or addiction potential.

*Measures of dependence or severity of dependence include (45):

- Daily or regular smoking (cotinine levels)
- DSM criteria (Diagnostic and Statistical Manual of Mental Disorders)
- International Diagnostic Code
- Surgeon General's report, 1988
- Fagerström Tolerance Questionnaire
- Fagerström Nicotine Dependence Test.

*Methods to assess addictive properties or abuse liability of a drug include:

- Daily use or dependence among the general population
- Daily use or dependence among those exposed to the drug
- Escalation of drug use
- Relapse rate

*Also several laboratory models exist such as:

- Psychoactive or subjective effects
- Drug discrimination
- Drug self-administration
- Withdrawal

Of all these models, a practical model to assess nicotine dependence is the Fagerström Test for Nicotine Dependence (Table 8). The Fagerström test is often used in research

studies on nicotine dependence (46). Although the scale is continuous, a cut-off score of 6-7 or higher has been used to separate low and high level of dependence (45). To see if anyone is addicted to nicotine Heatherton *et al.* (1989) designed a quick questionnaire based on the Fagerström test (Figure 5). If the total score is two or less a person is not addicted to nicotine (47) (48) (46).

Table 8 The Fagerström Test for Nicotine Dependence (46)

Questions	Answers	Points
1. How soon after you wake up do you smoke your first cigarette?	Within 5 minutes	3
	6-30 minutes	2
	31-60 minutes	1
	After 60 minutes	0
2. Do you find it difficult to refrain from smoking in places where it is forbidden e.g. in church, at the library, in cinema, etc.?	Yes	1
	No	0
3. Which cigarette would you hate most to give up?	The first one in the morning	1
	All others	0
4. How many cigarettes/day do you smoke?	10 or less	0
	11-20	1
	21-30	2
	31 or more	3
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?	Yes	1
	No	0
6. Do you smoke if you are so ill that you are in bed the most of the day?	Yes	1
	No	0

* How soon after you wake up do you smoke your first cigarette ?

Within 5 minutes 3 points

6-30 minutes 2 points

31-60 minutes 1 point

After 60 minutes 0 points

* How many cigarettes per day do you smoke ?

10 or less 0 points

11-20 1 point

21-30 2 points

31 or more 3 points

Figure 5. Assessing nicotine addiction (47)

The two parameters used in this quick questionnaire, Time To First cigarette (TTF) and Cigarettes Smoked per Day (CSD) are considered to be valuable indices of heaviness of smoking. These two parameters are also originally used in the Fagerström Test for Nicotine Dependence (FTND). It has been established that smokers who have their first cigarette early in the morning have higher levels of carbon monoxide and salivary or plasma cotinine and nicotine than smokers who postpone having that first cigarette. Those smokers who smoke the most cigarettes per day, also display higher levels of carbon monoxide and salivary or plasma cotinine and nicotine and have greater self-reports of difficulty refraining from tobacco (48). Nicotine addiction is usually assessed by combining questionnaires, such as the FTND, with biomarkers such as expired air carbon monoxide levels. By these means a

more reliable measure of nicotine dependence can be assessed, than by the use of questionnaires alone.

Appendix 2 Pharmacokinetics and metabolism

2.1. NICOTINE IN TOBACCO SMOKE.....	38
2.2. BIOAVAILABILITY.....	38
2.3. NICOTINE ABSORPTION AND DISTRIBUTION	38
2.4. NICOTINE VERSUS NICOTINE REPLACEMENT THERAPY	39
2.5. NICOTINE ACCUMULATES IN THE BODY	41
2.6. PLASMA NICOTINE LEVELS VARY AMONG INDIVIDUALS	41
2.7. NICOTINE METABOLISM	41
2.7.1 GENERAL	41
2.7.2 ETHNIC DIFFERENCES IN NICOTINE METABOLISM	42
2.8. PARAMETERS OF TOBACCO EXPOSURE	43
2.8.1. COTININE	43
2.8.2. CARBON MONOXIDE	43
2.8.3. HYDROGEN CYANIDE	44
2.8.4. NITRIC OXIDE	44
2.8.5. MARKERS RELATED TO CARCINOGENIC ACTIVITY.....	44
2.8.6. TOBACCO ALKALOIDS	44
2.9 CONCLUSION	44

2.1. Nicotine in tobacco smoke

Nicotine is a tertiary amine consisting of a pyridine and a pyrrolidine ring. There are two stereoisomers of nicotine: (*S*)-nicotine and (*R*)-nicotine. (*S*)-nicotine is the active isomer, which binds to nicotinic cholinergic receptors and is found in tobacco. During smoking, some racemisation takes place, and small quantities of (*R*)-nicotine, which is less active, are also found in cigarette smoke (31).

2.2. Bioavailability

The declaration on cigarette packages is the amount of nicotine in cigarette smoke and not in the cigarette itself. The amount of nicotine in cigarette smoke is commonly reported on the basis of a standardised method of the United States Federal Trade Commission. This protocol specifies that each cigarette is machine smoked to a standard butt length by use of a 35 ml puff volume drawn over 2 seconds at a rate of one puff per minute (49). The daily intake of nicotine, however, is poorly correlated with machine-determined yields. This is because smoking machines smoke cigarettes in a standardised way, whereas people can take more puffs, puff more intensively, and occlude ventilation holes in the filter or on the cigarette in order to obtain the desired dose of nicotine from most cigarettes (50) (49) (51).

Compared with the FTC protocol values, smokers of low- and medium-yield brands take in statistically significantly larger puffs (48.6 and 44.1 mL, respectively) at statistically significantly shorter intervals (21.3 and 18.5 seconds, respectively), and they draw larger total smoke volumes than specified in the FTC parameters. They received, respectively, 2.5 and 2.2 times more nicotine and 2.6 and 1.9 times more tar than FTC-derived amounts. Smokers of medium-yield cigarettes compared with smokers of low-yield cigarettes received higher doses of all components. The FTC protocol underestimates nicotine doses to smokers and overestimates the proportional benefit of low-yield cigarettes (49).

On average, the cigarette delivers about 1 mg of nicotine to the circulation of the smoker (52). The variation in intake per cigarette is considerable, however, ranging from 0.3 to 3.2 mg, depending on how the cigarette is smoked (50).

2.3. Nicotine absorption and distribution

During smoking, nicotine is distilled from burning tobacco, and small droplets of tar containing nicotine are inhaled and deposited in the small airways and alveoli. Nicotine is a weak base so that its absorption across cell membranes depends on the pH.

Tobacco smoke reaches the mouth first. Often the question is raised if a cigar smoker, who does not inhale receives a similar dose of nicotine compared to a cigarette smoker who inhales. It is generally thought that absorption of nicotine in the mouth from smoke differs for cigarette smokers and cigar smokers. The pH of smoke from most American cigarettes (blonde tobacco) is acidic (pH 5.5). At this pH, nicotine is mainly ionised and does not freely cross cell membranes. Consequently, nicotine from the blonde tobacco cigarette smoke is absorbed slowly through the buccal mucosa. However, the pH of smoke from tobacco in pipes and cigars is alkaline (pH 8.5), at which a larger part (approximately 85% (53)) of nicotine is unionised and well absorbed from the mouth (31). These data are based on an experiment with cats on absorption of tobacco smoke in the mouth only (53). No recent experiments on nicotine absorption in the mouth have been performed. The question is if these data can be extrapolated to humans, and if the cigarettes and cigars used are comparable to the ones smoked today.

When cigarette smoke reaches the small airways and alveoli of the lung, the nicotine is buffered to physiological pH and rapidly absorbed into the pulmonary alveolar capillary and venous circulation, and hence directly into systemic arterial blood (54) (31).

In the above-described data the smoke pH is buffered in the lungs, but not in the mouth. Rambali *et al.* (2003) concluded that cigarette smoke will not affect the respiratory fluid lining pH and that the deposited nicotine from cigarette smoke will be dissolved in the respiratory fluid (55). This conclusion was based on the calculated buffer capacity of the respiratory lining fluid. Cigar smoke, however, is alkaline and is not inhaled. It can be speculated that the surface of the mouth with its fluid, which is much smaller than the surface of the lungs, is not enough to buffer the smoke pH of cigars and differences of nicotine absorption, such as in the cat experiment, can therefore occur.

It takes 7-9 seconds for nicotine to reach the brain following passage of the pulmonary alveoli. In the brain, nicotine will be present at high levels for 10-19 seconds. Brain levels decline rapidly thereafter, as the drug is distributed to other body tissues (31;54).

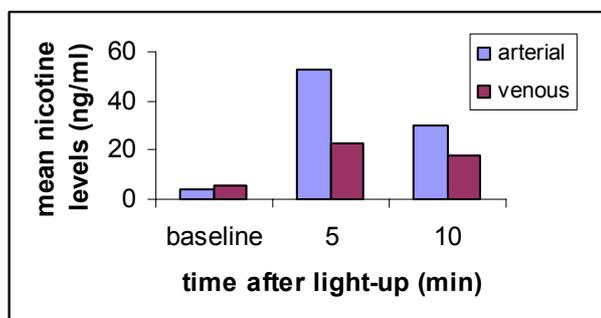


Figure 6 Arterial and venous nicotine concentrations before and after smoking one cigarette. Mean of 8 subjects. Arterial concentrations differ significantly from venous concentrations ($p < 0.05$) adapted from (56).

Arteriovenous differences during cigarette smoking are substantial, with arterial levels exceeding venous levels six to tenfold (42). The difference between arterial and venous blood levels declines rapidly (see Figure 6) (56). The pharmacological relevance of this observation is that during a short time (seconds), there is a high concentration of nicotine in the brain, resulting in an intense pharmacological response. It is generally thought that when nicotine levels in the brain decline between cigarettes, this provides an opportunity for resensitization of receptors so that positive reinforcement can, to some extent, occur with successive cigarettes despite the development of tolerance (for tolerance see appendix 3.2) (42).

Nicotine mainly exists in the particulate phase of cigarette smoke (57)(58). Particle deposition measurements indicate that 50 to 95 % of the particles are deposited in the respiratory tract(59). Measurements on regional deposition showed that 11–23 % of the total particles is deposited in the nasal, oral, pharyngeal, and laryngeal regions, between 45–81 % is deposited in the tracheo-broncheal region and between 26–35% is deposited in the pulmonary region (55).

2.4. Nicotine versus nicotine replacement therapy

Nowadays, there are many nicotine replacement therapy products available. The principles behind nicotine replacements are to (1) provide cigarette smokers a sufficient amount of nicotine to reduce some of the withdrawal symptoms experienced

shortly after tobacco abstinence; (2) to permit progressive reduction of the level of nicotine exposure. Leading to eventual ease of totally withdrawing from nicotine products; and (3) to reduce the abuse potential of nicotine due to the slower rate of nicotine absorption (45).

The nicotine inhaler is puffed upon like a cigarette, but absorption occurs primarily buccally and from the upper airway and not in the lungs because its design does not provide an aerosol that could be substantially inhaled in the lung. Each cartridge contains 10 mg of nicotine, with 13 μg delivered with each puff, which is about one-tenth the nicotine dose from puffing on a cigarette. The use is ad libitum and the recommended number of cartridges is 6-16. Each cartridge is used in three smoking periods. For the nicotine nasal spray, the recommending dosing is 1-2 sprays per hour, with a minimum recommended treatment of 8 doses per day and a maximum of 40 doses per day (45).

For nicotine gum, a fixed schedule of use (e.g. at least one piece every 1-2 hours, with a maximum of 24 pieces a day) is recommended to achieve sufficient levels of nicotine. The recommended duration of treatment for all these products ranges from 3 to 6 months (45).

So far no currently available nicotine replacement therapy (NRT) formulation can mimic either the extremely high and rapidly acquired arterial nicotine concentrations, which occur when tobacco products are inhaled, or the rapid pharmacological effect that this produces (31). The faster the speed of nicotine delivery, rate of absorption, and attainment of peak level, the greater is the likelihood of continued use or abuse (45). Cigarettes and oral snuff rapidly lead to high plasma concentrations of nicotine. Oral snuff is able to maintain these high concentrations during a much longer period than cigarettes are. Nasal spray and nicotine gum are unable to reach high plasma concentrations. The advantage of nasal spray is that it peaks fast, but with a low concentration compared to cigarette smoking (see Figure 7).

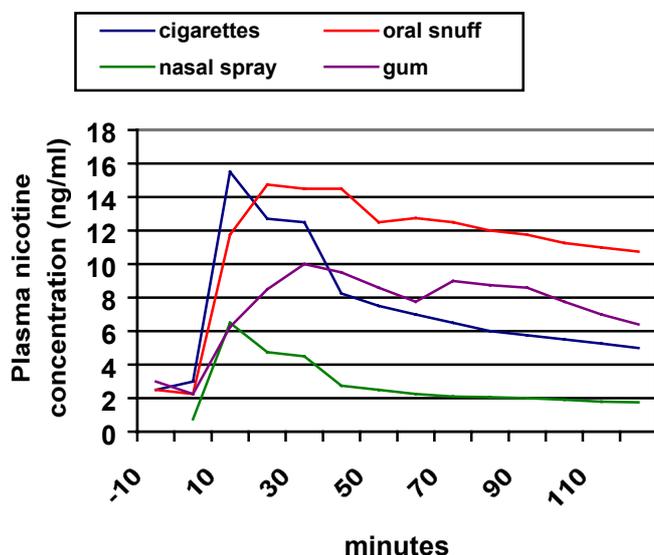


Figure 7 Venous blood concentrations of nicotine as a function of time for various nicotine delivery systems adapted from (60).

There are numerous other nicotine replacement therapies available. These fall, however, outside the scope of this report and will not be discussed any further.

2.5. Nicotine accumulates in the body

As described above, smoking many cigarettes during the day, results in oscillations between peak and trough plasma nicotine levels. Due to its half-life of 2-3 hours, however, nicotine accumulates over 6 to 8 hours (Figure 8)(36) in the body (42). Following its absorption it is widely distributed, at least in rats and rabbits, particularly in liver, lungs, and brain (45).

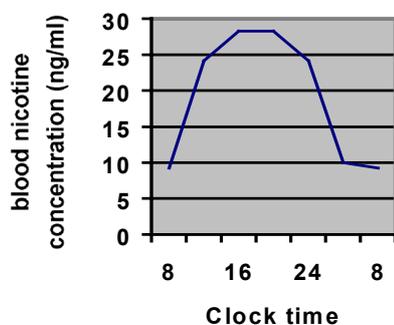


Figure 8 Mean blood nicotine concentration in cigarette smokers. Subjects smoked cigarettes every half four from 08:30 a.m. to 11:00 p.m. for a total of 30 cigarettes per day adapted from (36).

2.6. Plasma nicotine levels vary among individuals

During the day plasma nicotine levels in smokers usually range between 20-40 ng/ml. There is a significant inter-individual variability, however, in plasma nicotine levels and the intake of nicotine from a cigarette. Smokers can manipulate the intake of nicotine from different cigarettes to achieve and maintain the desired level of nicotine. They do this by changing puff volume, the number of puffs per cigarette, the intensity of puffing, the depth of inhalation, and/or by blocking ventilation holes in the filter (36). Because of the complexity of this process the dose of nicotine cannot be predicted from the nicotine content of the tobacco or its absorption characteristics. To determine the dose, one needs to measure blood levels and know how fast the smoker eliminates nicotine. Benowitz and Jacob(22) studied 22 cigarette smokers who smoked an average of 36 cigarettes per day (range 20 to 62) and found an average daily intake of 37 mg, with a wide range of 10 to 79 mg of nicotine. The intake of nicotine per cigarette averaged 1.0 mg but ranged from 0.37 to 1.56 mg. Similar results have been reported in other studies (54) (61) (62). Also other ranges have been reported in the literature for instance 0.3 to 3.2 mg nicotine per cigarette (50) (22) (63).

The pharmacokinetic disposition of nicotine does not seem to be changed to a clinically important extent in elderly subjects (age, 65-76 years) compared with younger adults (age, 22-43) (64).

2.7. Nicotine metabolism

2.7.1 General

Considerable inter-individual variability exists in the rate of metabolism of nicotine (36). Despite of this variability between individuals, the pattern of metabolism is consistent within an individual (65). Also smokers and non-smokers differ in nicotine metabolism. Smokers have on average slower nicotine clearance than non-smokers (36).

Nicotine is metabolised extensively, primarily by the liver, but also to a small extent in the lung (54). Approximately 70 to 80% of nicotine is metabolised to cotinine, and about 4% to nicotine N'-oxide (42), neither of which appears to be pharmacologically

active (54). Although lately it has been suggested that cotinine is pharmacological active. Although the effective concentrations of cotinine at which dopamine release is possible, are not in the concentration range found in plasma of tobacco smokers (66). Cotinine is extensively metabolised to 3'-hydroxycotinine, which is the most abundant metabolite of nicotine found in the urine. Nicotine and cotinine undergo N-glucuronidation, while 3'-hydroxycotinine undergoes O-glucuronidation (42). The possible pathways of nicotine metabolism are presented in Figure 9.

In nicotine metabolism the conversion of nicotine to cotinine is the rate-limiting step. The metabolism of nicotine to cotinine is a two-step process via an intermediary metabolite, the nicotine iminium ion. The first step is metabolism by a Cytochrome P450 (CYP450) enzyme, most likely CYP2A6, while the second step is metabolism by aldehyde oxidase (42).

Cotinine, because of its long half-life (16 to 20 hours), is commonly used in surveys and treatment studies as a marker of nicotine intake (54).

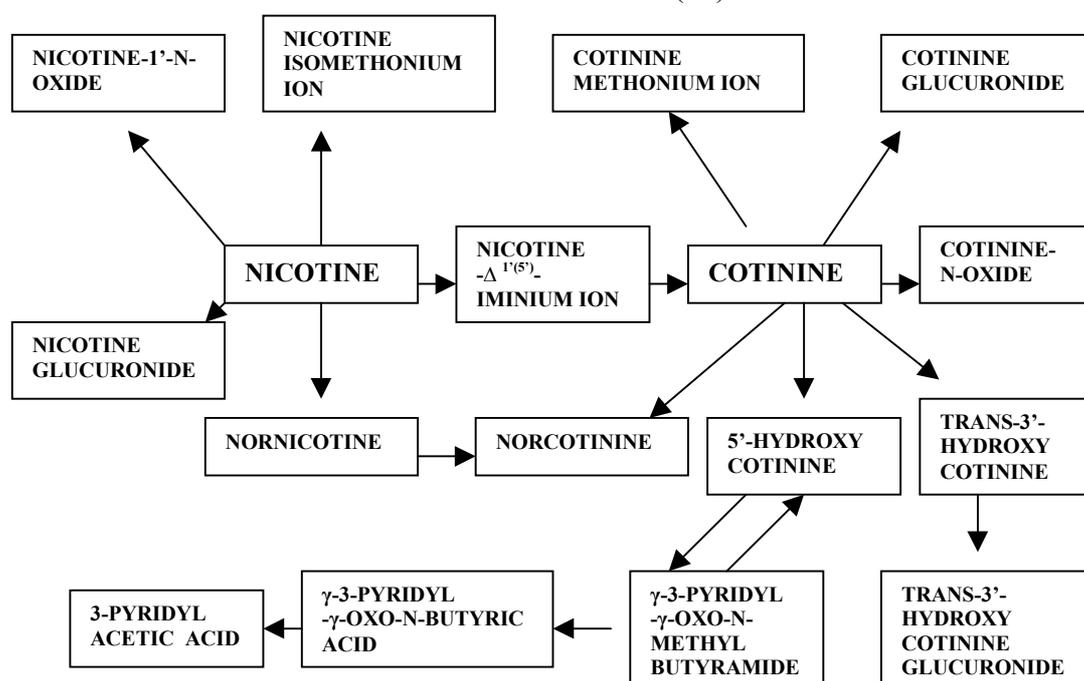


Figure 9 Pathways of nicotine metabolism. Adapted from (65).

2.7.2 Ethnic differences in nicotine metabolism

Ethnic differences in nicotine metabolism have recently been demonstrated. Afro-Americans show in several studies (29) (30) to have higher levels of cotinine, normalised for cigarettes smoked per day. Recently, Perez-Stable *et al.* administered deuterium-labelled nicotine and cotinine to Afro-American and Caucasian smokers. Afro-American metabolise cotinine more slowly than Caucasians due to slower oxidation to trans-3'-hydroxycotinine and slower N-glucuronidation (31) (32).

In stead of slower cotinine metabolism there are also ethnic groups with slower nicotine metabolism. Benowitz *et al.* (33) administered deuterium-labelled nicotine and cotinine to Chinese-Americans, Latinos and whites. Total and non-renal clearance of nicotine via the cotinine pathway were similar in Latinos and whites and significantly lower in Chinese-Americans (35% slower). The fractional conversion of nicotine to cotinine and the clearance of nicotine were also significantly lower in chinese-American smokers than in Latinos and whites. The half-life of nicotine was significantly longer in Chinese-Americans than in members of the other ethnic

groups. In other words in this ethnic group metabolism of nicotine appears to be slower than in Caucasian smokers. In contrast to the situation in African-Americans, the intake of nicotine per cigarette by Chinese-Americans (0.73 mg) was significantly lower than by Latinos (1.05 mg) or whites (1.10 mg).

These results will be discussed in more detail in appendix 5 genetics.

2.8. Parameters of tobacco exposure

2.8.1. Cotinine

The daily intake of nicotine from tobacco can be estimated from the level of cotinine, the principal metabolite of nicotine, in blood, saliva, or urine. Cotinine has a much longer half-life than nicotine. Cotinine plasma concentrations are therefore less dependent on the exact times of blood sampling. The average blood cotinine concentration in addicted smokers is about 300 ng per millilitre. The cotinine plasma level is 14 ng per millilitre per cigarette. Studies involving the infusion of nicotine and cotinine into smokers indicate that the daily intake of nicotine can be estimated as 0.8 times the blood cotinine concentration (52) (67). Other studies suggest that a level of 50 to 70 ng of cotinine per millilitre corresponds to a daily intake of 4 to 6 mg of nicotine (50). Salivary and urinary cotinine concentrations correlate well ($r = 0.8$ to 0.9) with blood cotinine concentrations (67). Therefore, salivary or urine cotinine concentrations should be almost as useful as blood levels in indicating nicotine intake. There are some remarks on using cotinine as an estimate of nicotine intake. When using cotinine as a biomarker of nicotine exposure, one must consider ethnic differences in the relationship between cotinine levels and nicotine intake (33) (see also appendix 2.7.2 and 5.4). Cotinine levels are dependent on both the extent of formation from nicotine by CYP2A6 and the rates of oxidation and glucuronidation of cotinine to 3-hydroxy-cotinine and glucuronide conjugates, respectively, which vary among individuals and among ethnic groups. Therefore, cotinine levels are only approximately correlated with the daily intake of nicotine (45).

Despite the possible variation in metabolism, the use of cotinine is recommended for routine clinical use. It provides the best discrimination (sensitivity 96-97% and specificity of 99-100%) and must be the marker of choice for situations where accuracy is paramount. Non-invasive specimens of saliva or urine give essentially the same information as blood samples (68).

Obviously, the assessment of smoking exposure using nicotine or cotinine cannot be done in smokers who are concomitantly using nicotine replacement products.

2.8.2. Carbon Monoxide

Carbon monoxide (CO) is present in high concentrations in tobacco smoke and is a useful marker of exposure to the gaseous fraction of tobacco smoke. The short half-life of CO excretion makes it a measure that is predominantly influenced by smoking within the most recent several hours. There is no reason to believe that smokers adjust their smoking to regulate CO levels in the body. Levels of CO can be measured in expired air or in the blood, the latter as carboxyhemoglobin (COHb). The CO level can be influenced by environmental exposures and the rate of elimination is markedly influenced by the level of physical activity (69) (45).

In general, carbon monoxide provides an acceptable degree of discrimination (sensitivity and specificity of 90%) and is considerably cheaper and more simple to apply than cotinine measurements (68).

2.8.3. Hydrogen cyanide

Hydrogen cyanide is another component of tobacco smoke. In the body, cyanide is metabolized to thiocyanate, which can be measured in blood or saliva. Thiocyanate has been used as a marker of tobacco smoke exposure in many studies. Its main limitation is that there are many dietary sources of thiocyanate, and thiocyanate levels in non-smokers are substantial. Thus measurements of thiocyanate yields relatively poor sensitivity and specificity for tobacco smoke exposure, particularly at low levels of cigarette smoking (69).

2.8.4. Nitric Oxide

Mean plasma concentration of nitric oxides (NO_x) from smokers were significantly greater compared with non-smokers. Plasma NO_x in smokers was not significantly correlated with the average daily number of cigarettes smoked, but was positively and linearly correlated with plasma cotinine (70). Again a limitation is that there are many dietary sources of NO_x.

Besides the levels of plasma NO_x, a reduction of exhaled NO in smokers compared to non-smokers has been found. This reduction was significantly related with cigarette consumption (71).

2.8.5. Markers related to carcinogenic activity

There are also reports of measuring exposure to tobacco smoke carcinogens. Such carcinogens in tobacco smoke include polycyclic aromatic hydrocarbons (PAHs), various nitrosamines, naphthylamines, polonium-210, and others. The most promising carcinogen biomarker is a measurement of nicotine derived nitrosamines. The nicotine derived nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), is specific for tobacco smoke exposure and is metabolised to a butanol metabolite, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide (NNAL-GLUC). Urine levels of NNAL and NNAL-GLUC are elevated in smokers (different yield of cigarettes not tested). The assay for NNAL is technically demanding (69).

Other potential markers of carcinogen exposure include adducts of 4-aminobiphenyl to hemoglobin in red blood cells; adducts of benzo(a)pyrene and other potential carcinogens to DNA in white blood cells; adducts of PAHs to plasma albumin; and urinary hydroxyproline or N-nitrosoproline excretion. None of these markers has been used to date in studying the nicotine up-take in smokers (69).

2.8.6. Tobacco alkaloids

In addition to the determination of cotinine, nicotine and exhaled CO, the levels of specific tobacco alkaloids, such as anatabine or anabasine in the urine can be determined (45;72). In one study alkaloid levels in commercial tobacco products (in milligrams per gram tobacco), were anabasine 0.008-0.030; and anatabine 0.065-0.27. Measurable concentrations of all alkaloids are excreted in the urine. Correlations between nicotine intake from tobacco and alkaloid concentrations in urine were found to be good to excellent. Due to the long half-lives of anabasine and anatabine, 16 hours and 10 hours respectively, levels should be detectable for 1 to 2 days following smoking cessation in a typical smoker (72).

2.9 Conclusion

A cigarette delivers about 1 mg of nicotine to the circulation. Smoking can be viewed as a unique form of systemic drug administration. Nicotine enters the circulation through the pulmonary rather than the portal or systemic venous circulation. Nicotine enters the brain quickly, but brain levels decline rapidly thereafter, due to distribution

to other body tissues. Due to its long half-life, nicotine accumulates in the body. Smokers can manipulate the intake from cigarettes to achieve the desired level of nicotine, which explains the large inter-individual variability of nicotine levels.

The majority of nicotine is metabolised into cotinine. Several ethnic differences in nicotine and cotinine metabolism exist. Afro-American smokers have higher levels of cotinine than Caucasian smokers due to slower metabolism of cotinine and a higher intake of nicotine per cigarette. Chinese-Americans have a slower metabolism of nicotine resulting in a longer half-life of nicotine. The intake of nicotine per cigarette however, is significantly lower than in Caucasian smokers.

In research on cigarette smoking the combined use of questionnaires and parameters of nicotine intake is regarded as a reliable measure of nicotine intake. The parameters that have been most widely used in the quantification of human exposure to tobacco smoke are cotinine and exhaled carbon monoxide (CO) levels.

Appendix 3 Receptors

3.1. NICOTINIC ACETYLCHOLINE RECEPTORS	48
3.1.1. INTRODUCTION	48
3.1.2. COMPOSITION	48
3.1.3. PERIPHERAL EFFECTS	50
3.2. TOLERANCE AND SENSITISATION	50
3.3 CONCLUSION	52

3.1. Nicotinic acetylcholine receptors

3.1.1. Introduction

Nicotinic acetylcholine receptors (nAChRs) are involved in a wide range of neuronal functions and are present at the neuromuscular junction, autonomic ganglia and in the central nervous system (73). Most relevant to nicotine addiction are the neuronal nAChRs. These receptors are found throughout the brain, with the largest number of binding sites in the cortex, thalamus, and interpeduncular nucleus, and substantial binding in the amygdala, septum, and brain stem motor nuclei and locus coeruleus (42).

Nicotine binding to nAChRs induces allosteric changes resulting in several different functional states. These include the resting state, an activated state (channel open), and two desensitized states (channel closed) (42). When the nAChRs are activated, sodium ions flow into the cell and potassium ions flow out of the cell. The ion flow results in a net exciting depolarization of the cell that can trigger an action potential (see Figure 10). An action potential can be conducted along a nerve cell, trigger contraction in a muscle cell or facilitate transmitter release from a nerve ending.

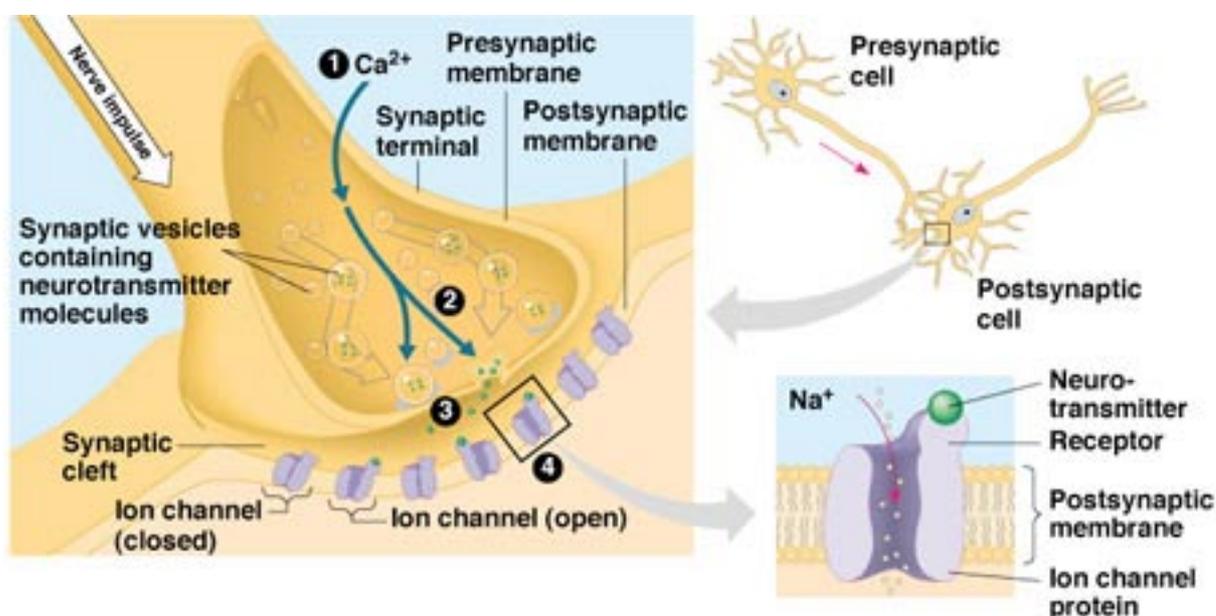


Figure 10 An action potential releases synaptic vesicles containing acetylcholine. In the synaptic cleft the acetylcholine molecule binds to nAChRs. Due to the binding, the ion channel opens and sodium flows into the cell and potassium flows out of the cell. This movement of ions can trigger a new action potential in the postsynaptic cell. In stead of acetylcholine also nicotine can bind to the nAChRs and the same events take place (74).

3.1.2. Composition

All nicotinic acetylcholine receptors (nAChRs) have five subunits organised around the central ion channel. These subunits all contribute to the lining of the ion channel. Neuronal nAChRs are composed of α and β subunits, see Figure 11. The subtype of a nAChR is defined by the combination of subunits that form it. In homomeric acetylcholine receptors (composed of identical subunits), all subunits contribute to the formation of acetylcholine binding sites at specific interfaces between subunits. In heteromeric acetylcholine subunits (composed of different subunits), there are thought to be two acetylcholine binding sites at the interfaces between what is defined as the plus side of an alpha subunit and the minus side of an adjacent subunit (57).

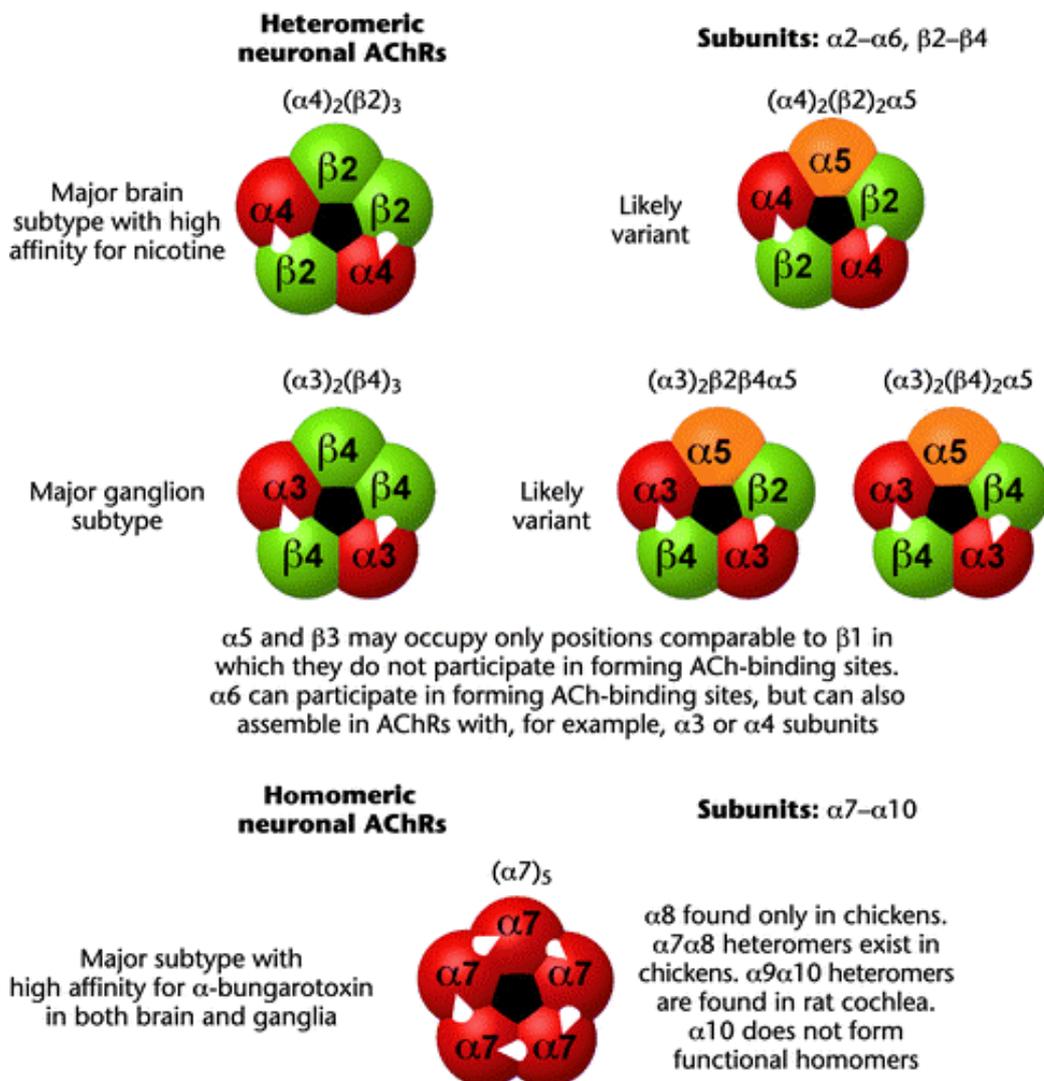


Figure 11 Different nAChR subtypes. Acetylcholine binding sites are marked with a white arrow (57).

A variety of different subunit combinations, $\alpha 2$ to $\alpha 9$ and $\beta 2$ to $\beta 4$, have been identified, which means that there is a multitude of different subtypes of neuronal nicotinic receptors (36). The predominant neuronal receptor subtype is $\alpha 4$, $\beta 2$, which accounts for more than 90% of high affinity binding in the rat brain. The $\alpha 7$ -containing receptors are most likely responsible for low-affinity binding in the rat brain (42).

Not all subunits have the same characteristics; for instance subunits differ in their ability to form acetylcholine receptor binding sites (57). Also differences in sensitivity for nicotine have become apparent. The $\alpha 4$ subunits have high affinity for nicotine. The $\alpha 3$ subunits have lower affinity for nicotine compared to $\alpha 4$ subunits (57). Mice with $\beta 2$ subunit knocked out do not self-administer nicotine, and nicotine does not enhance dopamine levels in these mice (75).

Moreover differences in amount of desensitization are discovered. Prolonged exposure to 0.2 $\mu\text{mol/L}$ nicotine is very effective at inhibiting $\alpha 4\beta 2$, $\alpha 4\alpha 6\beta 2$ and $\alpha 7$ acetylcholine receptors, whereas $\alpha 3\beta 2$ acetylcholine receptors and $\alpha 3\alpha 6\beta 2$ acetylcholine receptors are relatively resistant to inactivation. Prolonged exposure to 0.2 $\mu\text{mol/L}$ nicotine also causes an increase in the amount of acetylcholine receptors.

$\alpha 4$ Acetylcholine receptors are more easily and extensively up-regulated than are $\alpha 3$ and $\alpha 7$ acetylcholine receptors, and $\alpha 3\beta 2$ acetylcholine receptors are more sensitive to up-regulation than are $\alpha 3\beta 4$ acetylcholine receptors (57). These differences in sensitivity for up-regulation may implicate a changing receptor pattern in people who begin to smoke. It is thought that diversity of nicotinic cholinergic receptors may explain the multiple effects of nicotine in humans (42).

3.1.3. Peripheral effects

In autonomic ganglia nicotine elicits a brief stimulation followed by a prolonged ganglionic blockade. Electrophysiologically, the nicotine effect is seen as an initial depolarization, followed by a repolarization with a persistent block of transmission. The ganglion-stimulating action of nicotine is reflected in an increased rate of synthesis of postganglionic transmitter, such as noradrenaline in sympathetic ganglia, after systemic doses of nicotine. Nicotine is able both to stimulate and to block sympathetic as well as parasympathetic ganglia, and also to directly affect catecholamine release from the adrenal glands and sympathetic nerve terminals. Yet, the most consistent acute effects of nicotine are elevated blood pressure and heart rate together with peripheral vasoconstriction (73) (42).

3.2. Tolerance and sensitisation

With prolonged or repetitive exposure to nicotine, the brain cells adapt to compensate for the actions of nicotine, i.e. return brain functioning to normal. This process is called neuroadaptation (6). Neuroadaptation is associated with an increasing number of nicotinic receptors in the brain, and results in the development of tolerance. Tolerance can be defined in two related ways: (1) decreased reactivity, both physical and psychological, to the same dose of a substance over time, or (2) the need for a larger dose of the substance over time to achieve the same physical and psychological effects. The development of tolerance is seen as the adaptation of the body to the effects of the substance. In general, acute and chronic tolerance develops to many effects of nicotine, including cardiovascular and toxic effects (nausea, vomiting, and dizziness). The development of chronic tolerance is believed to contribute to an increase in cigarette consumption, as individuals smoke more to obtain desired effects of nicotine over time (76) (36).

The time path of the development of chronic and acute tolerance during a smoking career is not yet clear. There are many studies, which show that smokers compared to non-smokers show chronic tolerance. Acute tolerance is usually apparent for both smokers and non-smokers (77) (78). Regular use of nicotine is associated with chronic functional tolerance and repeated nicotine exposure during a single episode produces acute tolerance. Chronic tolerance shows variability across subjective, behavioural and cardiovascular responses (79). From animal studies it is clear that tolerance develops rapidly. For instance tolerance to locomotor depressant effects developed within 6 days after daily injections of 0.32 mg/kg nicotine to rats (80), tolerance to anxiogenic effects of nicotine in rats developed within 7 days of daily 0.1 mg/kg injections (81), and tolerance to cardiovascular effects in rats developed after only 6 injections of 5 μ g/kg at 1 minute intervals and after 4 injections of 10 μ g/kg (82). No extrapolation, however, has been made to the human smoking career.

To understand the mechanisms underlying chronic tolerance and sensitisation to nicotine, knowledge of acetylcholine receptors and working mechanisms is necessary. The endogenous agonist for acetylcholine receptors is acetylcholine, but nicotine obtained exogenously from tobacco may also serve as an agonist (in other words both

nicotine and acetylcholine are capable to activate the receptor). Upon binding of an agonist, the acetylcholine receptor briefly enters the open conformation of the ion channel, which provides a water-filled pathway through the membrane for cations. After a couple of milliseconds, the receptor undergoes another conformational change that closes the channel, and the receptor returns to the resting conformation or enters into a desensitised conformation that is further unresponsive to agonists (83) (42) (31).

When examining the function of nicotinic acetylcholine receptors and the effect of smoking, it is important to consider that the probability of a nicotinic acetylcholine receptor being in a particular conformational state depends on the agonist concentration and the rate of agonist exposure. In synapses in the central nervous system, acetylcholine is delivered by the pre-synaptic terminal at a concentration of about 1 mM for a couple of milliseconds before the acetylcholine is hydrolysed by acetylcholinesterase. This rapid pulse of agonist causes synchronised activation of nearby nicotinic acetylcholine receptors with little or no desensitisation. Nicotine obtained from tobacco arrives much more slowly at a concentration near or below 0.1 μ M and it is present much longer, in part, because nicotine is not broken down by acetylcholinesterase. This longer exposure to a low concentration of agonist favours desensitisation. In fact, a slow application of a low agonist concentration can cause some desensitisation without activation because the desensitised conformation of the nicotinic acetylcholine receptor has a higher affinity for agonist than the resting or open conformation. The higher affinity of the desensitised receptor for agonist and the changing distribution of nAChRs among the various functional conformations must be considered to understand what happens during sustained nicotine use (83).

Nicotine from tobacco bathes all of the brain and, therefore, reaches nicotinic acetylcholine receptors at synaptic and non-synaptic locations. The diverse distributions and roles of nicotinic acetylcholine receptors ensures that nicotine from tobacco will influence many neuronal regions and functions (83).

There is considerable variability in desensitisation of the various nicotinic receptor types, leading to significant differences in the level of desensitisation even when comparing similar, neighbouring neurons under the same experimental conditions.

Another important piece of information about long-term nicotine exposure is that it causes an increase in the number of nicotinic acetylcholine receptors in the brains of humans, rats and mice (42) (83) (84) (44). In a study in which 3 H-nicotine binding in human post-mortem brain was investigated, a significant increase has been observed in 3 H-nicotine binding in both hippocampus and thalamus of subjects with life-long smoking histories. In the hippocampus, this change resulted from a change in total receptor number, with no change in receptor affinity. There was also a positive correlation between the degree of smoking, as measured by the average reported packs smoked per day, and the number of nicotine binding sites found in both the hippocampus and thalamus, showing that humans exhibit a dose-dependent increase in brain nicotinic receptor binding. Receptor levels in these brain regions after smoking cessation were at or below those found in the control population, which indicated that smoking-induced changes are reversible after cessation of nicotine treatment (84). Smokers who had quit at least 2 months before death had levels of 3 H-nicotine binding which were comparable to levels found in non-smoking subjects (84).

In addition, the number of nAChRs seems to increase because long exposure to nicotine causes nAChRs to enter states of desensitisation much more often. In those desensitised conformations, the nicotinic acetylcholine receptors are turned over in the cell membrane more slowly, leading to an overall increase in their number. Along

with other factors that alter excitation and inhibition of dopamine neurons, this increase in the number of nicotinic acetylcholine receptors may contribute to nicotine sensitisation. When nicotine is removed from the brain, the excess of nicotinic acetylcholine receptors recovers from desensitisation, resulting in an excess excitability of the nicotinic cholinergic systems of smokers. This hyperexcitability at cholinergic synapses could contribute to the unrest and agitation that contributes to the smoker's motivation for the next cigarette. In part, the next cigarette 'medicates' the smoker by desensitising the excess number of nicotinic acetylcholine receptors back toward a more normal level (83).

Alternative explanations include a two population model in which long-term exposure to nicotine could induce a fraction of low-affinity nicotinic acetylcholine receptors to isomerise to high-affinity nicotinic acetylcholine receptors by slow conformational transitions (time scale in hours, *in vitro*). Such a hypothesis has the advantage of providing a coherent framework to explain the full reversibility of the up-regulation process (58). It has also been proposed that brain nicotine receptors exist in two forms: a pharmacologically active low-affinity form and a desensitised high affinity form, and it is only the second form that is recognised as a binding site in most studies. Thus, an increased number of binding sites would imply desensitisation. Another explanation is that the receptor number increases as a compensatory response to desensitisation (73).

3.3 Conclusion

Most relevant to nicotine addiction are the neuronal nicotinic acetylcholine receptors. The subtype of a nicotinic acetylcholine receptor is defined by the combination of sub-units that form it. The predominant neuronal receptor subtype is α_4 , β_2 . There is considerable variability in desensitisation of the various nicotinic receptor types, leading to significant differences in the level of desensitisation even when comparing similar, neighbouring neurons under the same experimental conditions. Another important piece of information about long-term nicotine exposure is that it causes an increase in the number of nicotinic acetylcholine receptors in brains of humans, rats and mice. Differences in sensitivity for up-regulation may implicate a changing receptor pattern in people who smoke. It is thought that diversity of nicotinic cholinergic receptors may explain the multiple effects of nicotine in humans.

Appendix 4 The biological mechanism of nicotine addiction

4.1. NICOTINE RELEASES DOPAMINE IN THE BRAIN	54
4.2. GLUTAMATE AND GABA MODULATE NICOTINE EFFECTS IN THE BRAIN	55
4.3. OTHER BRAIN SYSTEMS INVOLVED IN NICOTINE ADDICTION.....	57
4.3.1. MONOAMINE OXIDASE	57
4.3.2. SEROTONIN	57
4.3.3. NORADRENALINE	58
4.3.4. NITROGEN OXIDE	58
4.4. CONDITIONED REINFORCERS.....	59
4.5. ANIMAL DATA ON YOUTH NICOTINE ADDICTION.....	60
4.5.1. IN ADOLESCENTS BRAIN DEVELOPMENT CONTINUES	60
4.5.2. HETEROGENEOUS EFFECTS OF NICOTINE ON ADOLESCENT BRAIN	60
4.6. CONCLUSION	60

4.1. Nicotine releases dopamine in the brain

The release of dopamine in the brain by the action of nicotine is thought to be the primary mechanism of nicotine addiction. The midbrain dopamine neurones are equipped with nicotinic receptors both on their cell bodies and their nerve terminals. In the normal physiological situation, nicotinic acetylcholine receptors are activated

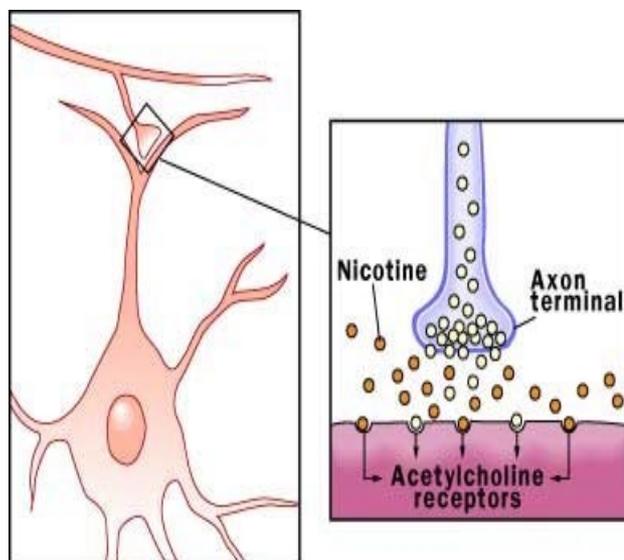


Figure 12 A synapse is the site where two neurons come into contact. The presynaptic neuron releases a neurotransmitter, which binds to receptors on the postsynaptic cell. This allows signals to be transmitted from neuron to neuron in the brain (85).

by the endogenous neurotransmitter acetylcholine (86). When a person smokes, nicotine (an exogenous substance) can also activate nicotinic acetylcholine receptors (see Figure 12). Many of these receptors are located on dopaminergic projections from the ventral tegmental area to the nucleus accumbens in the brain. The ventral tegmental area and the nucleus accumbens are different brain areas as seen in Figure 13 (40). The effect of activating the nicotinic acetylcholine receptors is activation of the dopamine neurons on which they are located. By activating these dopamine neurons nicotine evokes a sustained increase in dopamine release directly into the extracellular space which lies between the cells in the nucleus accumbens where it stimulates extra-synaptic dopamine receptors. Thus by stimulation of nicotinic acetylcholine receptors in the ventral tegmental area, nicotine causes a dopamine overflow in the shell of the nucleus accumbens. The ability to maintain increased dopamine overflow in the nucleus accumbens, during the period of exposure to the drug, is also thought to be fundamental to the potent addictive properties of amphetamine and cocaine (41). The reward associated with the increase in dopamine release, is the primary mechanism underlying the development of addiction. The shell of the nucleus accumbens has been related to the integration and expression of emotions with projections from the amygdala, lateral hypothalamus, and central grey matter (40). When nicotine addiction becomes chronic exposure to nicotine, the above described mechanism is still responsible for causing addiction, but a shift in receptor number and sensitisation will occur (see appendix 3).

It is puzzling that injections of nicotine in the presence of nicotine concentrations commonly found in smokers may not necessarily result in the stimulation of the

dopaminergic neurones (due to desensitisation) which project to the nucleus accumbens and subsequently no dopamine overflow in the terminal field. If so, why

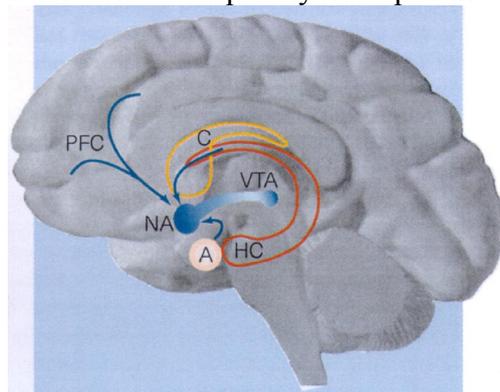


Figure 13 Some of the brain structures affected by drugs of abuse. The mesolimbic dopamine system originates in the ventral tegmental area (VTA) of the midbrain, and projects to the nucleus accumbens (NA). The amygdala (A), hippocampus (HC) and medial prefrontal cortex (PFC) send excitatory projections to the nucleus accumbens. C, caudate nucleus (striatum) (87).

do smokers continue to smoke? An explanation could be that other neurotransmitters also play an important part in the addiction process (see 4.2 and 4.3). Secondly, the reinforcing properties of tobacco smoke in habitual smokers, who maintain relatively high plasma nicotine levels for much of the day, may not depend upon stimulation of mesolimbic dopamine neurones by the nicotine present in smoke. The latter explanation implies that avoidance of withdrawal is a potent reinforcer in people who are modest or heavy smokers (see 4.4), but does not explain why smokers increase their smoking when given a dopamine receptor antagonist. (41).

4.2. Glutamate and GABA modulate nicotine effects in the brain

The link between nicotine and the release of dopamine appears to be more complicated than described in the previous paragraph. A single exposure to nicotine increases dopamine release for more than an hour in vivo (88), (89), (90). However, the nicotinic acetylcholine receptors on the dopamine neurones desensitise in seconds to minutes in the presence of physiologically relevant nicotine concentrations. This means that due to the desensitisation of the receptors no more dopamine can be released by the dopamine neurones if these receptors are the only mechanism for dopamine release. In stead dopamine release is increased for more than one hour after a single exposure to nicotine, implying that the existence of multiple regulation steps for dopamine release.

Mansvelder *et al.* (91) examined the role of glutamate and γ -amino butyric acid (GABA), two neurotransmitters of the brain, in nicotine addiction. There are indications that dopamine neurones may also be under excitatory (stimulation) glutaminergic control and this excitation may induce the long-term excitation of the dopamine system by nicotine, i.e. long-term release of dopamine. A single exposure to nicotine can induce long-term potentiation of glutaminergic inputs to the VTA dopamine neurones (92). In vivo biochemical data indicate that dopamine neurones are, besides the glutaminergic influences, also under tonic inhibitory control by GABA, and that removal of this inhibition leads to increased dopamine release and behavioural reinforcement (93) (94) (95). These different hypothesis on glutamate (enhances dopamine release) and on GABA (nicotine removes inhibition on dopamine neurones) were confirmed by studies using brain slices of naive rats (91).

How do these two neurotransmitters, GABA and glutamate affect dopaminergic neurons? The answer lies in the different subtypes of acetylcholine receptors. The GABAergic inputs to the dopamine neurons are also due to functional nicotinic acetylcholine receptors. These receptors show a similar pharmacology as those expressed by the dopamine neurons themselves and are likely to contain the $\alpha 4$ and $\beta 2$ sub-units. The nicotinic acetylcholine receptors on GABA neurons are, however, distinct from those found on the pre-synaptic terminals of glutaminergic fibers in the ventral tegmental area that contain the $\alpha 7$ subunit. As a result of different desensitisation properties of different nicotinic acetylcholine receptors subtypes, GABAergic and glutaminergic inputs respond differently to nicotine exposures that are experienced by smokers (91).

Mansvelder *et al.* (91) found that nicotine enhances GABAergic transmission transiently, which is followed by a persistent depression of the inhibitory inputs due to nicotinic acetylcholine receptor desensitisation. The normal inhibition of dopamine neurons by GABA stops due to desensitisation of the receptors by nicotine. Simultaneously, nicotine enhances glutaminergic transmission through nicotinic acetylcholine receptors that desensitise less than those on GABA neurons.

The time course of nicotinic modulation of GABAergic and glutaminergic inputs to VTA dopamine neurons is outlined in figure 14. During the first minute of cigarette

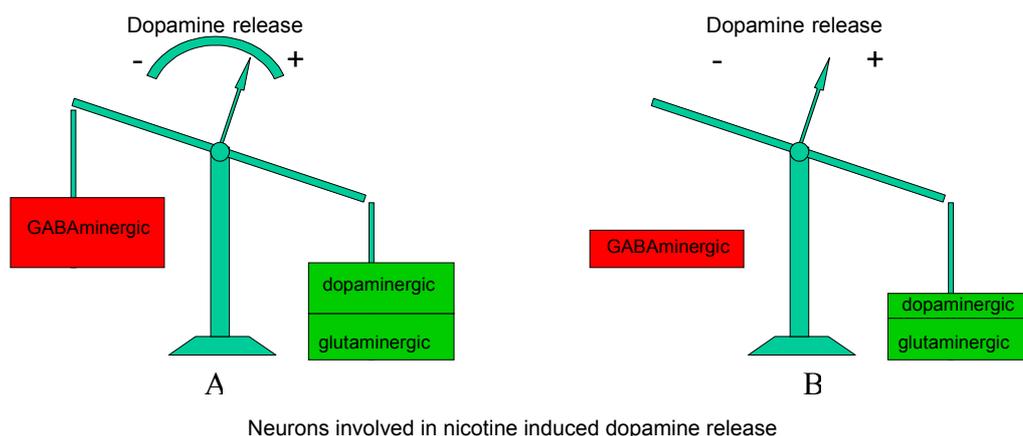


Figure 14 A: During the first minute of cigarette smoking nicotine activates not only the nAChRs on the dopamine neurons, but also the nAChRs on GABAergic and glutaminergic neurons. Due to this activation the dopamine neuron is inhibited by the GABAergic neuron and stimulated by the glutaminergic neuron. The net effect is dopamine release. (The green colour symbolises stimulation, the red colour symbolises inhibition). B: After the initial activation by nicotine, desensitisation occurs. The nAChRs on the dopamine neurons are desensitised rapidly. The GABAergic and the glutaminergic neurons differ in the amount of desensitisation which occur. The GABAergic neurons are more desensitised than the glutamate neurons. This means that the weight of inhibition by the GABAergic neurons is reduced more than the weight of stimulation by glutaminergic neurons (The size of the box represents the weight of the stimulation or inhibition). The net effect is that the dopamine neurons still release dopamine although their own nAChRs are desensitised. (The green colour symbolises stimulation, the red colour symbolises inhibition).

smoking, the arterial blood concentration of nicotine in humans rises rapidly above 250 nM (56). During this increase, the nicotinic acetylcholine receptors on the dopaminergic neurons (96;97) as well as on the GABAergic neurons become activated (Figure 14A) and desensitised rapidly thereafter (Figure 14B). The direct depolarisation (activation) of the dopamine neurons by nicotinic acetylcholine receptors coincides with the increase in GABA synaptic transmission, in other words

dopamine release is suppressed. Once these nicotinic acetylcholine receptors desensitise, the inhibitory input to a subset of dopamine neurons will be suppressed. In other words the dopamine release is no longer suppressed. At the same time, the $\alpha 7$ nicotinic acetylcholine receptor mediated enhancement of glutaminergic transmission increases during the nicotine exposure, as there is far less desensitisation of these receptors. If the dopamine neuron is depolarised sufficiently, the enhancement of glutaminergic transmission can induce a long-term potentiation of these inputs, this means a long-term release of dopamine (92). Complete recovery of the enhancement of GABA transmission takes more than an hour. There was a 13 minute refractory period during which nicotine did not enhance GABA transmission, which was followed by recovery of this effect with a time constant of 20 minutes. Thus the dopamine neurons receive a net increase in excitatory drive from the synaptic inputs that outlasts both the presence of nicotine and the time course of nicotinic acetylcholine receptor activation. These synaptic mechanisms may explain the prolonged excitation of mesolimbic dopamine system after a single nicotine exposure, as observed in vivo (91).

4.3. Other brain systems involved in nicotine addiction

4.3.1. Monoamine oxidase

MAO exists in MAO A and MAO B subtypes. In catecholamine neurons of the brain subtype A oxidises serotonin and noradrenaline, whereas in serotonergic neurons and in glia cells of the brain subtype B oxidises serotonin. Both forms oxidise dopamine (40) (20) (98). It has been shown that some undetermined constituents of tobacco smoke, other than nicotine, act as MAO inhibitor (99). Another study has shown that the MAO activity in smokers is significantly lower than in non-smokers or in former smokers (100) (98). It is generally accepted that MAO plays a major role in depressive disorders. A reason for the tobacco dependence may be the antidepressant properties of inhaling tobacco smoke, explaining why smoking is more common in depressed individuals (101) (45).

Studies in animals have reported that the simultaneous inhibition of both MAO subtypes produces large increases in serotonin outflow. This increase in serotonin is thought to have an antidepressant effect. Since both MAO A and MAO B break down dopamine, their simultaneous inhibition by smoke may combine to enhance brain dopamine which has been implicated in brain reward and reinforcement. This may enhance the behavioural and addictive properties of nicotine and other substances of abuse by preventing the breakdown of dopamine (102). Thus, it is not surprising that MAO A and MAO B inhibition by tobacco smoke has been suggested to contribute to the high rates of smokers failing to quit (40).

4.3.2. Serotonin

Serotonin exerts numerous effects on the central nervous system through the large family of serotonin receptors. Serotonin plays a role in depression, aggression, long term memory, mental fatigue during endurance exercise (103) (104) (105) (50)(106) (107)(108). Serotonin is furthermore involved in regulation of sleep, circadian rhythms, food intake (fat and energy intake) and regulation of the BBB (brain blood barrier) function (109) (91)(110)(111).

The prevalence of tobacco smoking is much higher in people suffering from depression. It is known that depression sensitises patients to the adverse effects of stressful stimuli, and that drugs that stimulate dopamine release in the forebrain can relieve this. This mechanism may contribute to the increased craving to smoke in abstinent smokers exposed to such stimuli, because they become conditioned to use

this property of nicotine to produce rapid alleviation of the adverse effects of the stress. Also, chronic exposure to nicotine elicits changes in serotonin formation and release in the hippocampus which are depressogenic. It is postulated that smokers are protected from the consequences of these changes, while they continue to smoke, by the antidepressant properties of nicotine. However, they contribute to the symptoms of depression experienced by many smokers when they first quit the habit (102).

Serotonin is expected to influence nicotine reward. There is some evidence that nicotine alters serotonin release and neuronal activity; nicotine reward related effects are modified by serotonin manipulations; and dopaminergic neurons are affected by serotonin processes. Yet, there is no direct evidence for distinct serotonin circuitry in nicotine reinforcement (112).

However, Serotonine has been associated with nicotine withdrawal. Serotonin reuptake inhibitors (SSRIs) and 5-HT antagonist were shown to be effective in diminishing the smoking withdrawal negative effects. It was shown in rats that sertraline (SSRI) can counteract the hyperphagia and rapid weight gain associated with nicotine withdrawal, and might therefore be a useful adjunct to smoking cessation (113). In another study it was shown that ondansetron, a selective 5-HT₃-receptor antagonist, may attenuate the aversion effect associated with nicotine withdrawal, and may be useful for the treatment of nicotine dependence (114).

4.3.3. Noradrenaline

Noradrenaline mechanisms may be relevant because they are able to modulate midbrain dopamine function (115). Moreover, nicotine releases noradrenaline in various central nervous system regions by action at distinct sites (116) and through several nicotinic acetylcholine receptor subtypes. Recent evidence implicates noradrenaline more directly in nicotine reinforcement. First, the noradrenaline reuptake inhibitors attenuate nicotine self-administration. This means that noradrenaline is not removed from the site of action and its function is thus prolonged. This observation is important because bupropion applied in smoking cessation treatment has some noradrenergic properties (112).

Second, noradrenaline secretion from the hypothalamic paraventricular nucleus (PVN), measured with microdialysis, has been shown to increase during continuous nicotine self-administration; nicotinic acetylcholine receptors in the tractus solitarius may be the locus of this effect (112;116).

4.3.4. Nitrogen oxide

Bronchodilatation induced by inhaled nitrogen mono-oxide (NO) allows the smoke an easier and deeper passage in the lungs, exposing the body and brain to increased amounts of nicotine (117).

It is unlikely that NO from cigarette smoke reaches the brain, because it is rapidly bound and subsequently inactivated by haemoglobin. However, nicotine quickly crosses the blood-brain barrier, and after entry it causes the release of NO from neuronal sites. This endogenously released NO subsequently acts both as a cerebral vasodilator and as a non-conventional neurotransmitter. Endogenously produced NO that is released by nicotine inhibits dopamine re-uptake or facilitates its release in *in vitro* and *in vivo* preparations. This could be a physiologically important mechanism in nicotine addiction, since it directly enhances dopaminergic receptor stimulation and thus, presumably, the acute rewarding effects of nicotine (117).

The nicotine-induced release of NO seems to be associated with stress reduction, which may contribute to nicotine's psychoactive effects (117).

4.4 Conditioned reinforcers

It has also been hypothesised that reinforcing properties³ of tobacco smoke are important for nicotine addiction. Habitual smokers maintain relatively high plasma nicotine levels for much of the day and this results in desensitisation of the nicotinic acetylcholine receptors of the mesolimbic dopaminergic neurones. If this is the case, conditioned reinforcers⁴ may contribute to maintain the addiction. This explanation includes the possibility that avoidance of withdrawal symptoms is a potent reinforcer in people who are modest or heavy smokers.

In the beginning of the addiction, nicotine and the conditioned reinforcers are connected. Later on, when desensitisation of the nicotinic acetylcholine receptors occurs the reinforcers may be enough to maintain the addiction. For cigarette smokers, the salience of the conditioned reinforcers paired with the nicotine in cigarette smoke are likely to be re-established each day on occasions when they smoke following a period of abstinence which permits reactivation of the receptors responsible for the dopamine response. One such time will be the first cigarette in the morning following the abstinence associated with sleep. Interestingly, many smokers report that this cigarette is the most pleasant of the day. Thus, even for people who smoke in a way, that sustains desensitising nicotine concentrations (i.e. high concentrations) through much of the day, the hypothesis predicts that the primary reinforcing properties of nicotine continue to play a central role in maintaining the addiction by repetitively re-establishing the salience of the conditioned reinforcers present in the smoke (41).

Table 9 Nicotine withdrawal symptoms (6).

Nicotine withdrawal syndrome	
Restlessness	Loss of energy/ fatigue
Eating more than usual	Dizziness
Excessive hunger	Stomach or bowel problems
Anxiety / tension	Headaches
Impatience	Sweating
Irritability/anger	Insomnia
Difficulty concentrating	Heart palpitations
Depression	Tremors
Disorientation	Craving for cigarettes

The reinforcers can be divided in positive and negative reinforcers. Negative reinforcement refers to the above-described relief of nicotine withdrawal symptoms in the context of physical dependence (see Table 9). Positive reinforcing effects that are reported include relaxation, reduced stress, enhanced vigilance, improved cognitive function, mood modulation, and lower body weight (42).

³ **Reinforcement** A reinforcer increases the likelihood of the act that produced it being repeated. The resultant strengthening of behaviour can be seen as a form of memory, by which voluntary actions with certain outcomes are consolidated into long-term memory. (87)

⁴ **Conditioning** Forms of learning and association. They may be either instrumental (in which voluntary actions are controlled by their outcomes; see reinforcers below) or Pavlovian (in which a temporal correlation between events is detected and learned by an animal, even in the absence of voluntary control).

4.5. Animal data on youth nicotine addiction

4.5.1. In adolescents brain development continues

The ability of nicotine to produce unique effects in adolescents likely stems from the fact that brain development continues in this period. Apoptosis, synapse formation and the functional programming of behavioural responses are all consolidated during adolescence, refuting the now-outdated view that brain development is essentially complete in early childhood (34).

Nicotine given during adolescence produces a pattern of nicotinic receptor up-regulation in brain regions associated with addiction and reward pathways. With adolescent nicotine treatment, male rats show prolonged nicotinic receptor up-regulation that remains evident even a month after the termination of drug exposure, a much longer span than in foetal or adult rats exposed to comparable or even higher levels of nicotine (35). Furthermore, adolescent nicotine exposure produces long-term alterations in cell number, and gene expression, commensurate with brain cell damage. Ultimately, animals exposed to nicotine during adolescence display long-term changes in the functioning of the reward pathway (34).

4.5.2. Heterogeneous effects of nicotine on adolescent brain

What mechanisms underlie the different patterns of effects of nicotine on different brain regions in adolescent rats? Trauth *et al.* (35) give different answers to this question. One potential explanation is the heterogeneous expression of nicotinic receptor subtypes, the responses that are linked to the various subtypes, and their differential propensities to desensitisation. In vitro experiments indicate that, even within a specified region (i.e. the shell versus the core of the nucleus accumbens), chronic nicotine treatment can enhance or repress dopamine turnover selectivity in different areas. Similarly, desensitisation and the resultant tolerance to nicotine are non-uniform, with some neurons in the ventral tegmental area of the midbrain and hippocampus exhibiting decreases in their firing rates while others are activated. A third possibility for regional or gender-selective differences resides in the different rates of adjustment of tyrosine hydroxylase activity, the rate limiting enzyme in catecholamine biosynthesis. By evoking the acute release of noradrenaline and dopamine, nicotine may relieve end-product inhibition of this enzyme, whereas, with long-term changes in neuronal firing rates, allosteric modification of the enzyme and enzyme induction can participate. In this regard also, there are differential rates for each process according to brain region and potentially, gender (35).

4.6. Conclusion

The release of dopamine in the brain by the action of nicotine is thought to be the primary mechanism of nicotine addiction. When a person smokes, nicotine activates nicotinic acetylcholine receptors. By stimulation of nicotinic acetylcholine receptors in the ventral tegmental area, nicotine causes a dopamine overflow in the shell of the nucleus accumbens. The reward associated with the increase in dopamine release, is the primary mechanism underlying the development of addiction.

There are indications that dopaminergic neurons may also be under excitatory (stimulation) glutaminergic control and this excitation may induce the long-term excitation of the dopamine system by nicotine, i.e. long-term release of dopamine. In vivo biochemical data indicate that dopamine neurons are, besides the glutaminergic influences also under tonic inhibitory control by GABA, and that removal of this inhibition leads to increased dopamine release and behavioural reinforcement.

Other brain mechanisms that could be involved in nicotine addiction include monoamine oxidase, serotonin, noradrenaline and nitrogen oxide. It has also been

hypothesised that reinforcing properties of tobacco smoke are important in the process of nicotine addiction. Nicotine given during adolescence produces a pattern of nicotinic receptor up-regulation in brain regions associated with addiction and reward pathways.

Appendix 5 Genetics

5.1 INTRODUCTION.....	64
5.2. DOPAMINE PATHWAY RELATED GENES.....	64
5.2.1. INTRODUCTION	64
5.2.2. DOPAMINE D2 RECEPTOR POLYMORPHISMS.....	65
5.2.3. DOPAMINE TRANSPORTER GENE.....	65
5.2.4. DOPAMINE β -HYDROXYLASE	65
5.2.5. CATECHOL O-METHYL TRANSFERASE.....	65
5.3. NICOTINE METABOLISM AND CYP2A6 POLYMORPHISMS.....	66
5.3.1. INTRODUCTION	66
5.3.2. ETHNIC DIFFERENCES IN METABOLISM.....	66
5.3.3. THE FIRST DISCOVERED CYP2A6 ALLELES.....	67
5.3.4. CYP2A6 ALLELE FREQUENCIES AMONG DIFFERENT ETHNIC GROUPS	69
5.4. OTHER RELATED GENES	69
5.4.1. CHOLECYSTOKININ POLYMORPHISM	69
5.4.2. SEROTONINE ASSOCIATED POLYMORPHISMS.....	69
5.4.3. PERSONALITY TRAITS.....	70
5.5. CONCLUSION	70

5.1 Introduction

It is now generally accepted that nicotine causes addiction and that the mechanisms underlying nicotine addiction and smoking behaviour are more complex than originally thought. Genes that are polymorphic, that is, in different individuals the same gene has slight variations called alleles, could cause a part of the variation in smoking behaviour as seen in the general population.

There are several ways to investigate genetic influences on smoking behaviour and nicotine addiction. First of all there are numerous twin studies available on this subject (paragraph 2.5.1). If genetic factors are involved, identical twins, who share the same genes, will be more similar in their use of tobacco products than fraternal twins, who on average only share half of their genes.

Another possibility to study genetic influences on nicotine addiction or smoking behaviour is by screening for candidate genes in groups of smokers compared to non-smokers. These candidate genes do not only involve the dopamine pathway (appendix 5.2), but also differences in metabolism (appendix 5.3) or other related genes (appendix 5.4).

However, several factors exist that contribute to the difficulty in finding reproducible associations between candidate genes and nicotine dependence. One is the multiple comparison problem: in searching for associations between candidate genes, there will be false positive and false negative associations. A second factor is that it is difficult to detect multiple genes of modest effect because association and linkage studies have low statistical power to detect these types of associations. A third factor is differences between studies in the definition of a smoker (16).

5.2. Dopamine pathway related genes

5.2.1. Introduction

In paragraph 2.5.1. on twin studies it has become clear that genetic factors are involved in smoking initiation and in smoking persistence. The next step is to identify which genes are responsible for the genetic influence in smoking. There is evidence that genetically determined variations in dopaminergic transmission predisposes to nicotine dependence (59). The relevant genes in the dopamine reward pathway which have been identified are summarised in Table 10. Habit-forming actions of nicotine appear to be triggered primarily at nicotinic receptors on the cell bodies of dopaminergic neurons in the mesolimbic reward system of the brain. Important aspects of the dopaminergic pathway include synthesis of dopamine in dopaminergic neurons, release of dopamine by presynaptic neurons, receptor activation of postsynaptic neurons, dopamine re-uptake by presynaptic neurons, and metabolism of released dopamine (18).

Table 10 Role of identified genes in the dopaminergic reward pathway (18)

Function	Relevant genes
Dopamine synthesis	Tyrosine hydroxylase (TH)
Receptor activation	Dopamine receptors (DRD1, DRD2, DRD3, DRD4, DRD5)
Re-uptake	Dopamine transporter (DAT1)
Metabolism	Catechol-o-methyltransferase (COMT) Monoamine oxidase A and B (MAO A, MAO B) Dopamine β -hydroxylase (DBH)

Studies comparing ever smokers versus never smokers have shown that positive associations with smoking initiation are the dopamine D2 receptor (DRD2) and the

dopamine D4 receptor (DRD4) genes. Candidate genes that may influence persistent smoking include the dopamine transporter (DAT1), DRD2 and DRD4 genes (19). Another question concerns the extent to which genotype influences the amount of cigarettes smoked. Positive associations have been shown with DRD1, DRD2, DRD4, dopamine hydroxylase and monoamine oxidase (MAO) A and B (19).

5.2.2. Dopamine D2 receptor polymorphisms

A polymorphism (in different individuals the same gene has slight variations called alleles) of the dopamine receptor gene DRD2 is approximately twice as common in smokers compared with non-smokers. The allele is more common in smokers who start at an early age and in those who are unable to stop. Individuals with one or more of the variant alleles have reduced numbers of dopamine receptors in the corpus striatum. If these changes are also present in the central dopamine reward pathway, it could be that this allele is linked to impaired perception of reward. This means that less reward sensations are perceived. Nicotine stimulates dopamine release and thereby restores dopamine function to normal levels. Individuals with this allele would be more susceptible to nicotine addiction because smoking would restore dopamine levels to normal. Although this is the prevailing theory, the converse could also be true; perhaps this allele is a marker for enhanced dopaminergic transmission. Individuals with this form of the gene could be particularly sensitive to the rewarding effect of tobacco because they experience a greater hedonic effect from nicotine-stimulated dopamine release (59). This polymorphism is thought to be important as well as in smoking initiation as in smoking persistence (19).

5.2.3. Dopamine transporter gene

Several studies suggest a similar link between an allele of the dopamine transporter gene and smoking behaviour. In this case, the variant allele, which is related to low scores for novelty seeking and extraversion in personality questionnaires, seems to protect individuals from persistent smoking. On a molecular level, the mechanism of action of this allele has not yet been determined, but it is thought to enhance dopaminergic transmission, thereby reducing the need to use nicotine to augment dopamine function (59).

5.2.4. Dopamine β -hydroxylase

McKinney *et al.* (2000) (20) found an association between dopamine β -hydroxylase and the amount of cigarettes smoked. Dopamine β -hydroxylase is responsible for the break down of dopamine in the synaptic cleft. The investigated polymorphism of the dopamine β -hydroxylase gene was found more in heavy smokers (>20 cig/day) than in light smokers (<10 cig/day) (20). Walton *et al.* (59) state that although it is tempting to speculate that genetically regulated variations in activity might influence susceptibility to nicotine withdrawal, more information is required on the molecular effects of this allele.

5.2.5. Catechol O-methyl transferase

Catechol O-methyl transferase (COMT) inactivates released dopamine and is present in dopaminergic brain regions. A functional polymorphism (COMT 1947A>G) resulting in increased enzyme activity has been associated with alcoholism and polysubstance abuse. In a study with 266 current smokers, 270 ex-smokers and 265 lifetime non-smokers (never smokers), matched for age and gender, the COMT 1947A>G polymorphism was examined. Smoking status was ascertained by self-

report. There was no difference in genotype frequencies between never smokers and ever smokers (current + ex-smokers); between non-smokers (never + ex-smokers) and current smokers; or between current smokers and ex-smokers. These data suggest that the COMT 1947A>G polymorphism is not associated with smoking initiation, smoking persistence or smoking cessation (118).

5.3. Nicotine metabolism and CYP2A6 polymorphisms

5.3.1. Introduction

Based on the idea that smokers regulate nicotine levels in their bodies by adjusting how many cigarettes they smoke or their smoking pattern, it is reasonable to speculate that smokers who metabolise nicotine more rapidly may need to take in more cigarette smoke, and vice versa. Liver CYP2A6 is responsible for the conversion of nicotine to the nicotine iminium ion, which is the rate-limiting step in nicotine metabolism (see appendix 2.7). Several scientists investigated the idea that polymorphisms in the CYP2A6 gene may account for differences in nicotine metabolism.

It has been postulated that individuals lacking full functional CYP2A6, who therefore have impaired nicotine metabolism, are significantly protected against becoming tobacco-dependent smokers. When they start smoking they will have high levels of nicotine during a longer period of time and therefore experience the negative effects of nicotine during a longer time. Later on in their smoking career, smokers whose nicotine metabolism is thus impaired smoke fewer cigarettes than those with normal nicotine metabolism do (31)(108). The question if these smokers actually differ in nicotine dependence has not been answered.

5.3.2. Ethnic differences in metabolism

Ethnic differences in nicotine metabolism have recently been demonstrated. Afro-Americans show in several studies (29) (30) to have higher levels of cotinine, normalised for cigarettes smoked per day. Recently, Perez-Stable *et al.* (31) (32) administered deuterium-labelled nicotine and cotinine to Afro-American and Caucasian smokers. Afro-American metabolise cotinine more slowly than Caucasians due to slower oxidation to trans-3'-hydroxycotinine and slower N-glucuronidation.

Black smokers smoke differently than whites. They smoke fewer cigarettes per day than whites and they have a predominant preference for mentholated cigarettes. Despite smoking fewer cigarettes per day, black smokers have higher levels of serum cotinine after controlling for number and yield of cigarettes. Blacks have had an overall higher prevalence of cigarette smoking since 1965, but data for 1994 show similar overall rates, with 26.3 % of whites and 27.2% of blacks reporting current cigarette smoking. However, sex differences persists as black men continue to smoke at higher rates than white men (33.9% vs 28.0%), while black women smoke at lower rates than white women (21.8% vs 24.7%) (32).

Besides the slower metabolism of cotinine it has also been suggested that the higher levels of cotinine are due to smoking mentholated cigarettes. Blacks prefer mentholated cigarettes compared to whites. There are conflicting reports on the effect of mentholated cigarettes on smoking behaviour. Some report that African-Americans take in 20% more nicotine per cigarette, which means an intake of 20% more tobacco smoke per cigarette (31). Other studies measuring puffing behaviour and puff volumes however, do not support the statement that menthol cigarettes via their cooling action facilitate deep inhalation. Persons smoking mentholated cigarettes take fewer puffs with a lower average of total volume of smoke, but with an increased carbon monoxide boost compared with persons smoking non-mentholated cigarettes (32). This effect appeared to be race and cigarette preference independent (119).

Instead of slower cotinine metabolism there are also ethnic groups with slower nicotine metabolism. Benowitz *et al.* (33) administered deuterium-labelled nicotine and cotinine to Chinese-Americans, Latinos and whites. Total and non-renal clearance of nicotine via the cotinine pathway were similar in Latinos and whites and significantly lower in Chinese-Americans (35% slower). The fractional conversion of nicotine to cotinine and the clearance of nicotine were also significantly lower in Chinese-American smokers than in Latinos and whites. The half-life of nicotine was significantly longer in Chinese-Americans than in members of the other ethnic groups. In other words in this ethnic group metabolism of nicotine appears to be slower than in Caucasian smokers. In contrast to the situation in African-Americans, the intake of nicotine per cigarette by Chinese-Americans (0.73 mg) was significantly lower than by Latinos (1.05 mg) or whites (1.10 mg). The rate of nicotine metabolism and the intake of nicotine from cigarette smoke demonstrated a statistically significant correlation. This observation is consistent with the hypothesis that slow nicotine metabolism is partially responsible for the lower intake of nicotine among Chinese-American smokers. This corresponds with the observation that Latinos and Asians are less likely than whites to smoke cigarettes, and those who do smoke fewer cigarettes per day on average (33).

It has been reported that the prevalence of genes for mutant alleles of CYP2A6 is greater in Asians than in whites (see also appendix 5.3.4.). It is unclear whether these mutations, which occur at a relatively low frequency even in Asians, can explain the considerable population difference that has been observed in nicotine metabolism between Chinese-Americans and whites. Asian smokers also start smoking at a relatively later age than Caucasians, which might be a factor in the lower levels of nicotine per cigarette. The finding that CYP2A6 activity is lower in Chinese-Americans than in whites may have implications for cancer risk, in addition to its implications for the intake of cigarette smoke. CYP2A6 is involved in the metabolic activation of 4-(methyl-nitrosamine)-1-(3-pyridyl)-1-butanone, a tobacco-specific nitrosamine that is believed to contribute to smoking-related lung cancer, as well as in the metabolic activation of other potential carcinogens, including aflatoxin B, N-nitrosodiethylamine, and 1,3-butadiene (33).

A remark on this study was the fact that the ethnic groups differed in their smoking behaviour. The whites in this experiment were significantly older than the Chinese-Americans, smoked significantly more cigarettes per day than Chinese-Americans or Latinos, had smoked for significantly more years than Chinese-Americans or Latinos and had significantly higher Fagerström tolerance scores, suggesting a higher level of dependence, than Chinese-Americans or Latinos (33).

5.3.3. The first discovered CYP2A6 alleles

Three CYP2A6 alleles have been identified: the wild-type (CYP2A6*1) and two null, or inactive, alleles (CYP2A6*2 and CYP2A6*3). Each individual has two copies of the CYP2A6 gene, one from the maternal and one from the paternal side. An individual can have two active forms of the gene and have normal nicotine removal (metabolism), one active and one defective copy and have reduced nicotine removal, or two defective copies, which will drastically reduce their nicotine inactivation to cotinine (31) (108) (111).

Table 11 CYP2A6 allele nomenclature adapted from (120) and <http://www.imm.ki.se/CYPalleles>.

Allele	Nucleotide change	Trivial name	Effect	Enzyme activity
CYP2A6*1A	none			normal
CYP2A6*1B			gene conversion in 3' flanking region	normal
CYP2A6*1X2			CYP2A6 gene duplication	
CYP2A6*2	479T>A	v1	L160H	absent
CYP2A6*3	2A6/2A7 hybrid	v2	?	?
CYP2A6*4A	gene deletion	CYP2A6del, E-type	deletion	absent
CYP2A6*4B	gene deletion	D-type	deletion	absent
CYP2A6*4C	Identical to CYP2A6*4A			
CYP2A6*4D	gene deletion		deletion	absent
CYP2A6*5	1436G>T		G479V	absent
CYP2A6*6	383G>A		R128Q	decreased in vitro
CYP2A6*7	1412T>C; gene conversion in 3' flanking region		I471T	decreased
CYP2A6*8	1454G>T; gene conversion in 3' flanking region		R485L	normal
CYP2A6*9	-48T>G		TATA box	decreased
CYP2A6*10	1412T>C; 1454G>T; gene conversion in 3' flanking region		I471T; R485L	decreased
CYP2A6*11	670T>C		S224P	decreased

Several scientists showed that even a single CYP2A6 null allele (that is, heterozygosity) is sufficient to significantly reduce the risk of tobacco dependence. Within the tobacco-dependent group those who had one defective allele and one active allele smoked significantly fewer cigarettes per day (12.5 versus 18; accompanied by significantly lower carbon monoxide levels) and per week (129 versus 159) than smokers without impaired nicotine metabolism. These results suggest that CYP2A6 genotype has a major influence on nicotine kinetics and smoking behaviour (108;111). Furthermore, using nicotine as a substrate *in vitro* with Caucasian human livers, it has been shown that heterozygous livers (CYP2A6*1/*2 and CYP2A6*1/*3) have 50% of the CYP2A6-mediated nicotine metabolism. They also have 50% of the nicotine to cotinine V_{\max} values, when compared with homozygous wild-type (CYP2A6*1/*1) livers (111).

Very recently, improvements have been made to the original genotyping methods and new variant alleles of CYP2A6 have been found. CYP2A6*3, originally thought to be a true variant allele, has later been shown to be lacking in different populations. One reason for the original misclassification turned out to be due to the very common CYP2A6*1B allele exhibiting a gene conversion between 3' flanking regions of the

CYP2A6 and CYP2A7 genes. As a result of this, CYP2A7 sequences are also amplified when the original genotyping method is used, resulting in misclassification of this allele. All different CYP2A6 alleles currently known are presented in Table 11 (120).

5.3.4. CYP2A6 allele frequencies among different ethnic groups

The frequencies of CYP2A6 alleles vary considerably among different ethnic populations, the deletion alleles being most common in Orientals (up to 20%). The frequency of point mutations are low in all populations studied thus far (<3%). Initial studies were carried out using the first published genotyping method, which gives an overestimate of CYP2A6*2 allele frequencies and also detects the (probably) non-existent CYP2A6*3 allele. Thus the genotyping results obtained did not reflect the actual situation and the results need to be interpreted with caution (120).

Xu *et al.* (121) described that individuals possessing CYP2A6*7 appear to have decreased rates of nicotine metabolism. CYP2A6*8 is unlikely to affect catalytic activity *in vivo*. The combination allele (CYP2A6*10) appears to decrease activity of the enzyme to a much greater extent than either allelic substitution alone. The allele frequencies differed substantially among different ethnic groups. The frequencies of *7 and *8 were similar in Japanese and Chinese (*7 a little bit higher in Japanese). The *10 allele is found at a low frequency in both Chinese and Japanese. Neither *7, *8 nor *10 allele was found in 602 Caucasian alleles tested (121).

5.4. Other related genes

5.4.1. Cholecystokinin polymorphism

A major detriment to smoking cessation, especially in women, is the fear of gaining weight. It has been suggested that genetic variants in the cholecystokinin (CCK) gene might be a possible risk factor for smoking. CCK is a satiety neuropeptide. Animal studies have shown that both acute and chronic exposure to nicotine results in weight loss which is associated with an increase in hypothalamic CCK and that CCK antagonists ameliorate symptoms of nicotine withdrawal. In humans a diagnosis of nicotine dependence was significantly associated with the CCK polymorphism allele and with gender (males > females), but not with Body Mass Index. The allele was present in 15.9% of never smokers and 24.7% of ever-smokers. These results are consistent with a role of the CCK gene as a risk factor for smoking (122).

5.4.2. Serotonine associated polymorphisms

Genes in the serotonin system are plausible candidates because of serotonin's role in mood regulation. The association of smoking behaviour with a polymorphism in the tryptophan hydroxylase (TPH) gene, which codes for a rate limiting enzyme in the biosynthesis of serotonin, has been examined. A polymorphism in intron 7 has been linked with a variety of traits involving poor impulse control. There was no association of TPH alleles with smoking status. However, case series analysis indicated that individuals with the homozygous dominant A/A genotype started smoking at age 15.6 years, compared with 17.3 years among smokers with other genotypes. This association was significant in a multivariate regression model controlling for age, education, body mass index, alcohol use, and medication use. This finding is consistent with previous studies relating the A-allele to impulsive behaviour and suggests that it may predispose to early smoking initiation (123). In a population-based case control study of 780 genotyped subjects this polymorphism was also linked to early smoking initiation (124).

5.4.3. Personality traits

The most consistently reported association between smoking behaviour and personality relates to the personality dimension of sensation seeking, extraversion and neuroticism. These personality traits are heritable and there is increasing support for the notion that the genetic influence on smoking initiation is mediated by personality (19).

5.5. Conclusion

Studies comparing ever smokers versus never smokers have shown that positive associations exist between smoking initiation and the dopamine D2 receptor (DRD2) and the dopamine D4 receptor (DRD4) genes. Candidate genes that may influence persistent smoking include the dopamine transporter (DAT1), DRD2 and DRD4 genes. Positive associations between the amount of cigarettes smoked and genes have been shown with DRD1, DRD2, DRD4, dopamine hydroxylase and monoamine oxidase (MAO) A and B.

It has been postulated that individuals lacking full functional CYP2A6, who therefore have impaired nicotine metabolism, are significantly protected against becoming tobacco-dependent smokers. When they start smoking they will have high levels of nicotine during a longer period of time and therefore experience the negative effects of nicotine during a longer time. Later on in their smoking career, smokers whose nicotine metabolism is thus impaired smoke fewer cigarettes than those with normal nicotine metabolism do. The frequencies of CYP2A6 alleles vary considerably among different ethnic populations.

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