Investigating a pharmacological agent against tobacco smoke addiction using the rat as animal model

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UITTREKSEL

Dit word wêreldwyd aanvaar dat die meerderheid sigaretrokers rook om die psigofarmakologiese effekte van die nikotien, wat in tabak teenwoordig is, te ervaar. Geneesmiddels wat misbruik word aktiveer 'n gedeelte van die brein, bekend as die nukleus akkumbens (Nacc), waarna verwys word as die brein se "beloningsentrum" of "plesiersentrum" (Melichar *et al.*, 2001 en Balfour & Fagerström, 1996). 'n Gemeenskaplike eienskap van geneesmiddels wat misbruik word, is hul vermoë om dopamienneurotransmissie in die brein te verhoog. Omdat daar geglo word dat die onderliggende meganismes van baie verslawende geneesmiddels ooreenstem, hipotetiseer ons dat nikotienamied-adeniendinukleotied (NAD), wat tans gebruik word vir die behandeling van alkoholisme, moontlik ook effektief aangewend kan word om die drang na tabakrook te termineer.

Die doel van hierdie studie was om gepaste metodes te vind om onttrekkingsimptome in 'n rotmodel van rookverslawing te bepaal en om vas te stel of NAD die drang na tabak sal termineer.

Twee metodes is in hierdie studie gebruik om onttrekkingsimptome te bepaal: lokomotoriese aktiwiteit en geïnisieerde akoestiese refleksmetode (ASR). Lokomotoriese aktiwiteit word algemeen gebruik om nikotien se invloed op die gedrag van rotte te bestudeer aangesien die dopamienaktivering wat deur nikotien veroorsaak word, geassosieer word met verhoogde lokomotoriese aktiwiteit in rotte. Die ASR is 'n refleksreaksie en bestaan uit 'n reflekssametrekking van die liggaamspiere in reaksie op 'n vinnige, intense stimulus.

'n Spesiale toestel is ontwerp om sigarette te rook en die verbindings op te vang wat gewoonlik deur rokers ingeasem word. Rotte is aan die rookekstrak blootgestel deur Alzet osmotiese minipompies, wat subkutaneus ingeplant is en vir 28 dae gelaat is om verslawing te bewerkstellig. Die minipompies is op dag 28 verwyder en die eksperimentele groep is vir 4 dae intraperitoneaal met NAD ingespuit (die kontrolegroep is met fisiologiese

soutoplossing ingespuit) waartydens die ASR van die rotte gemeet is. Die lokomotoriese aktiwiteit van die rotte is op spesifieke dae gedurende die eksperiment gemeet. Al die rotte se breine is op dag 42 verwyder. Die dopamien-, serotonien- en hul metabolietkonsentrasies is in die nukleus akkumbens bepaal met hoëdrukvloeistofchromatografie (HPLC) en elektrochemiese deteksie. Die komeetanalise (SCGE analise) is uitgevoer om die DNS skade in die striatums van die rotte te bepaal.

Die rotte wat aan die rookekstrak blootgestel is en met NAD ingespuit is, het 'n verhoging in lokomotoriese aktiwiteit getoon in vergelyking met die kontrolegroep (ook aan rookekstrak blootgestel) wat slegs met fisiologiese soutoplossing ingespuit is. Laasgenoemde dui daarop dat die kontrolegroep onttrekkingsimptome ervaar het. Die resultate van die ASR-eksperimente toon dat die NAD-behandelde groep 'n hewiger skrikreaksie getoon het as die fisiologiese soutoplossing-behandelde groep, wat daarop dui dat die NAD-behandelde groep 'n geringer drang na tabak ervaar het as die kontrolegroep. Die komeetanalise het getoon dat die behandelde rotte meer DNS-skade gehad het as die kontrolerotte. Dit kan moontlik toegeskryf word aan die hoë NAD-vlakke wat die elektrontransportketting aktiveer, wat sodoende lei tot 'n vrystelling van elektrone (hidroksi-radikale) en dus lei tot skade in die breinselle.

Die lokomotoriese aktiwiteit en die ASR wat gemeet is gedurende rookblootstelling, onttrekking en behandeling kan gebruik word as parameters vir tabakverslawing en om nuwe geneesmiddels se potensiaal te toets om verslawing te genees. Bogenoemde resultate dui daarop dat NAD belowende potensiaal toon om tabakverslawing te behandel.

ABSTRACT

It is widely accepted that the majority of people who smoke tobacco do so to experience the psychopharmacological effects of the nicotine present in the smoke. Drugs of abuse activate the brain area called the nucleus accumbens (Nacc), which is putatively the brain's "reward centre" or "pleasure centre" (Melichar *et al.*, 2001 and Balfour & Fagerström, 1996). A shared feature of drugs of abuse is their ability to increase dopamine neurotransmission in the brain. Because the underlying mechanism of many addictive drugs is thought to be similar, we hypothesized that nicotinamide adenine dinucleotide (NAD), which is currently being used in the treatment of alcoholism, may also be effective to terminate the craving for tobacco.

The purpose of this study was to find appropriate methods to determine withdrawal symptoms in tobacco smoke addiction in a rat model and to determine if NAD would terminate the craving for tobacco.

Two methods were used in this study to determine withdrawal symptoms: locomotor activity and acoustic startle response (ASR). Locomotor activity is widely used to study nicotine's behavioural actions in rodents and the dopamine activation produced by nicotine is associated with elevated locomotor activity in rats. The acoustic startle response (ASR) is a reflex response and consists of a reflex contraction of the skeletal musculature in response to an intense, abrupt stimulus.

A special device was designed to smoke cigarettes and to trap compounds that are usually inhaled by smokers. Rats were exposed to the smoke extract via subcutaneously implanted Alzet osmotic minipumps for 28 days to accomplish addiction. On day 28 the minipumps were removed and the experimental group was injected with NAD (the control rats were injected with saline) for four days during which time the ASR of the rats was measured. The locomotor activity of the rats was monitored on specific days throughout the

experiment with a Digiscan Animal Activity Monitor. All the rats were sacrificed on day 42 and their brains removed. The concentrations of dopamine, serotonin and their metabolites were determined in the nucleus accumbens by HPLC-analysis using an electrochemical detector. To determine DNA damage, the Single cell gel electrophoresis (Comet) assay was performed on the striata of all the rats.

The rats that received tobacco smoke extract and injected with NAD displayed an increase in locomotor activity after the osmotic minipumps were removed when compared to the control group (received tobacco smoke extract) injected with saline after removal of the minipumps, indicating that the control group was experiencing withdrawal symptoms. The results of the ASR experiments also showed that the NAD treated group experienced higher startle levels than the saline treated group, indicating that the treated group experienced less craving than the control group. According to the comet assay, the treated rats had more DNA damage than the control rats. This might be the result of the higher levels of NAD that activates the electron transport chain, causing a release of electrons (hydroxy-radicals) which may cause more damage to the brain cells.

The locomotor activity and acoustic startle response recorded during smoking, withdrawal and treatment can be used as parameters for addiction to tobacco smoke and to test novel drugs for their potential to cure addiction. The above results indicate that NAD shows definite potential for treating tobacco smoke addiction.

ABBREVIATIONS

A	
ACh	acetylcholine
ADP	adenine dinucleotide diphosphate
ALDH	aldehyde dehydrogenase
ALS	alkali-labile sites
AOD	alcohol and other drugs
ASR	acoustic startle response
ATP	adenine dinucleotide triphosphate
AUC	area under curve
C	
CNA	central nucleus of the amygdala
COMT	catechol-O-methyltransferase
Cpu	caudate-putamen
CSC	cigarette-smoke condensate
Cu	copper atom
D	
DA	dopamine
ddH_2O	double distilled water
DLPC	dorsal lateral prefrontal cortex
DNA	deoxyribonucleic acid
DOPA	3,4-dihydroxy-phenylalanine
DOPAC	3,4-dihydroxy-phenylacetic acid
DPN	diphosphopyridine nucleotide
DSB	double-strand breaks
E	
EPM	elevated plus-maze

F

FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide

G

GABA	gamma-aminobutyric acid

H

5-HIAA	5-hydroxyindoleacetic acid
5-HT	serotonin
HVA	homovanillic acid

M

MAO	monoamine oxidase
MGE	microgel electrophoresis

N

Nacc	nucleus accumbens
nAChRs	nicotinic acetylcholine receptors
NAD	nicotinamide adenine dinucleotide
NADH	The reduced form of NAD
NIDA	National Institute on Drug Abuse
NRT	nicotine replacement therapies

O

O_2	oxygen
8-OH-DPAT	8-hydroxy-2-dipropylaminotetralin
OTu	olfactory tubercle

P

PET	positron emission tomography
PLC	prefrontal cortex
PPI	prepulse inhibition

Q

Q	ubiquinone
QH_2	ubiquinol (or the reduced form of coenzyme Q)

R

RSD	relative standard deviation

S

SCGE	single cell gel electrophoresis assay
SSB	single-strand breaks
Std.	standard
STDEV	standard error deviation

T

TH	tyrosine hydroxylase
TIQ	1,2,3,4-tetrahydroisoquinoline
TNP	transdermal nicotine patch

U

UNDCP	United Nations International Drug Control Programme

V

VTA	ventral tegmental area

W

WHO	World Health Organisation

CHAPTER 1

INTRODUCTION

The burden of disease associated with cigarette smoke and the negative economic impact of tobacco addiction on society is considerable. It is estimated that 430 000 people die each year as a result of smoking-attributable medical illnesses such as lung cancer, chronic obstructive pulmonary disease, cardiovascular disease and stroke (George & O'Malley, 2004). The World Health Organization estimates that one-third of the global adult population smokes (Dani & De Biasi, 2001) and the World Bank estimates that in high-income countries, smoking-related healthcare accounts for 6-15% of all annual healthcare costs (Kenny & Markou, 2001). There is little question that the rising rate of adolescent cigarette smoking represents one of the largest public health concerns facing our society (Slotkin, 2002).

When tobacco is smoked, nicotine enters the bloodstream through the lungs and reaches the brain even faster than the drugs that are administered intravenously. It takes only 7 seconds for nicotine in the lungs to reach the brain compared to the 14 seconds it takes for blood to flow from the arm to the brain (Jain & Mukherjee, 2003). Typical signs and symptoms experienced during smoking cessation include irritability, anxiety, a depressed mood, increased hunger, restlessness, difficulty concentrating, sleep disturbances, weight gain, decreased heart rate and craving for tobacco (Kreek & Koob, 1998; Gäddnäs *et al.*, 2000 and Picciotto, 1998 and Hildebrand *et al.*, 1997 and Arinami *et al.*, 2000 and Walton *et al.*, 2001).

Smoking during pregnancy and nursing carries risk to the fetus and to the infant during the rapid phases of development. Nicotine is passed to the infant in the milk from nursing mothers who smoke, increasing the direct exposure to the drug. Evidence indicates that nicotine can abnormally alter cell proliferation and differentiation, and thereby affect synaptic and circuit activity (Dani & De Biasi, 2001).

It has been estimated that 80% of all regular smokers want to quit smoking and a majority of them have tried to quit and failed. Of the 17 million smokers that try to

quit each year, fewer than 1 out of 10 actually succeed. It has also been estimated that only 2.5% of unaided quit attempts are successful (Malin, 2001 and Cohen *et al.*, 2001).

Not all tobacco users respond to the treatment of tobacco dependence with nicotine replacement therapies (NRT) or sustained-release bupropion. Methods used as NRT include nicotine chewing gum, patch, inhaler and nasal spray. However, if these products are used in the absence of intensive behavioural support, the long-term success rates are typically only 10-20% (Rose *et al.*, 2001). Smoking cessation could prevent a large number of deaths each year and defer the onset of a large number of terminal illnesses.

But why do people smoke?

Smokers consistently report that smoking has a "calming" effect when they are exposed to stressful stimuli and that the desire to smoke is increased by exposure to these stimuli. The anxiolytic properties of nicotine have primarily been observed in nicotine-dependent people, in whom the drug may act by preventing or relieving the anxiogenic effects caused by nicotine withdrawal (Little, 2000 and Balfour & Fagerström, 1996).

Nicotine can improve performance in a variety of cognitive tasks and enhances the capacity of people to work and socialize. The most consistent effect of smoking appears to be on vigilance and rapid information processing (Warburton, 1992 and Balfour & Fagerström, 1996). Loss of the cognitive-enhancing effects of nicotine may contribute to an inability to concentrate during nicotine withdrawal, thus contributing to the difficulty smokers find in quitting (Picciotto, 1998). For most smokers, all the uncomfortable health consequences are extremely remote in time and are therefore of weaker influence. Most smokers do not stop until their motives to stop are strengthened, for example by a current health problem or financial crisis etc. (Russel, 1977).

Nicotine also decreases food consumption and metabolism in humans, making smoking a method of appetite control, and resulting in weight gain upon smoking cessation (Winders & Grunberg, 1990).

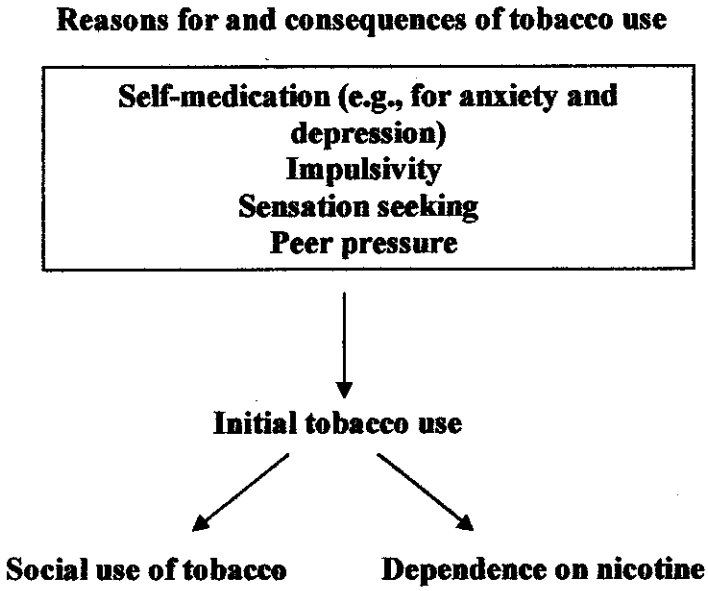


Figure 1.1. Reasons for and consequences of initial use of tobacco. The vast majority of smokers become nicotine dependent; only a few smokers maintain a pattern of social use (Little, 2000).

It generally has been accepted that nicotine is a major component in tobacco smoke responsible for addiction, but there are over 4000 chemicals in cigarette smoke, many of which potentially contribute to the reinforcing properties of tobacco. Some of the components of cigarette smoke are: tar, carbon monoxide, phenols, aldehydes, acrolein, oxides of nitrogen and sulphur, ammonia, hydrogen sulphide, nitrosamines, toxic metals and off course nicotine (Russel, 1977). Kenny and Markou (2001) pointed out that obtaining nicotine is probably not the exclusive reason for maintaining the tobacco habit in smokers. They observed that nicotine-containing and denicotinized cigarettes had similar measures of reinforcing efficacy in smokers when presented alone, although there was a preference for nicotine-containing cigarettes when smokers were offered a choice. This suggests that in addition to nicotine, sensory and conditioned reinforcing effects of smoking and possibly other reinforcing substances in cigarette smoke also play a role in maintaining the tobacco habit in smokers.

The development of more efficacious pharmacotherapies for the treatment of tobacco dependence is therefore of great importance. But to achieve this, it is necessary to understand the mechanisms by which tobacco addiction occurs.

CHAPTER 2

CRAVING AND ADDICTION

2.1. WHAT IS CRAVING?

Craving is primarily a subjective experience for each smoker. The United Nations International Drug Control Programme (UNDCP) and World Health Organisation (WHO) organised an Expert Committee meeting on drug craving, which defined drug craving as "the desire to experience the effects of a previously experienced psychoactive substance" (Miyata and Yanagita, 2001).

To be able to explain the neural systems that underlie this state, drug craving was defined scientifically by Markou and co-workers (1993) as the incentive motivation to self-administer a psychoactive substance that was previously consumed.

Many recent theories of addiction contain the concept of incentive motivational processes. Incentive motivation can be defined as a cognitive and affective state triggered by stimuli associated with the perception of unconditioned stimuli. According to the incentive motivation model, drug-related stimuli are able to elicit classically conditioned responses in drug abusers, both physiologically and subjectively (e.g., craving) (Franken, 2003).

Within this incentive motivational framework, craving is (just as food cravings) a conditioned appetitive motivational state. Drug craving and food craving are affective states that are results of the appetitive processes. Drug craving fits well within the definition of emotion as proposed by Gray (1972), "those states in the conceptual nervous system which are produced by reinforcing events or by stimuli which have, in the subject's previous experience, been followed by reinforcing events" (Franken, 2003).

Two important aspects enhance the incentive motivational value of the drug and thereby increases drug craving. The first is the dysphoric state (psychological

aspects) during withdrawal. These psychological aspects of withdrawal contribute significantly more to the motivation to continue drug administration than the somatic signs of withdrawal. The second is the conditioned aspects of the environment. This means that smoke-related cues, after repeatedly being paired with smoking, become conditioned stimuli. These cues elicit the same physiological and psychological response as smoking itself. These cue-induced responses result in craving if smoking does not occur immediately (Franken, 2003). According to Miyata and Yanagita (2001) there is a third aspect contributing to the development and maintenance of craving, which is the memory process. For drug craving to occur, memory of the fact that the drug has pleasurable effects, as well as that the dysphoric state during withdrawal is specifically alleviated by the drug, is required.

Withdrawal symptoms and craving appear 6-12hr (peak at 48hr) after smoking cessation, and whereas withdrawal symptoms disappear after 3-4 weeks, craving can still persist after 6 months (Teneggi *et al.*, 2002 and Gäddnäs *et al.*, 2000).

2.2. THE NEUROADAPTIVE MODEL OF CRAVING

This model combines psychological, behavioural and brain mechanisms.

Long-term drug use interferes with many brain functions. According to Robinson and Berridge (1993) a gradual and, perhaps, permanent adaptation of brain function (i.e., neuroadaptation) to the presence of a drug like alcohol is a central feature in the development of alcohol dependence (Olausson *et al.*, 2002).

Our bodies must maintain homeostasis with respect to critical bodily functions like blood pressure and body temperature. Thus, many cells (including neurons in the brain), adapt their activities in response to the prolonged presence of a drug to maintain this balanced state. According to Littleton (1998), these physiological mechanisms which maintain homeostasis in the person's body and brain are responsible for drug tolerance, but absence of the drug exposes these same homeostatic mechanisms and leads to the withdrawal syndrome. This neuroadaptation also leads to a condition that might be called reward memory. Reward memory has its roots in certain brain cells and is dependent on chemical

changes in those cells. The reward memory may be unconscious and gives heightened attention, or salience, to environmental cues that are commonly paired with the drug (e.g., in the case of smoke addiction, the sight or smell of a burning cigarette) or to drug use itself (Anton, 1999 and Little, 2000). Members of Alcoholics Anonymous are told to avoid "people, places and things" associated with alcohol, because any mood or circumstance associated with use of the drug may become a conditioned stimulus (cue).

Animal models of addiction and craving, as well as pharmacological studies in humans have indicated that several neurochemical systems contribute to neuroadaptation to drugs of abuse. For example, the neurotransmitters dopamine, glutamate, gamma-aminobutyric acid (GABA), endogenous opioids, as well as the neurons that respond to these molecules, may play a role in the development of reward memory. Stress, which may influence neuroadaptation, is also modulated by neurochemical systems, especially those involving the neurotransmitter serotonin (Anton, 1999).

Abnormalities in any of the neurotransmitter systems may result in the experience of craving because of the diverse functions of neurotransmitters. Such abnormalities in drug abusers can result from neuroadaptation to the presence of the drug, which occurs insidiously over many years. According to Anton (1999), the person is in most cases unaware of the neuroadaptation, and many alcoholics (particularly those who are in the early stages of alcohol dependence) are likely to deny any craving for alcohol. In fact, craving only fully emerges when a person is prevented from access to AODs or consciously attempts to quit AOD use (Tiffany, 1990). Craving seems to occur when there is a conflict between the need for a drug and the desire not to take the drug. This conflict appears to accentuate the urge to take the drug until the desire to take the drug becomes overpowering and irresistible (Li, 2000).

Brain mechanisms that have adapted to the chronic presence of the drug are left in an altered state during drug withdrawal. This imbalance can lead to physiological instability (e.g., anxiety and cardiovascular hyperactivity), sleep difficulties and possibly, subdued drive or reward states (e.g., depression, lack of motivation and concentration problems). The person experiences a subjective sense of discomfort, which may lead to a desire, urge or craving for the drug in order to "feel normal"

again. Some of the mechanisms underlying the craving of early abstinence may persist for weeks to months. However, if the person remains abstinent, the altered brain mechanisms eventually return to their original state, which leads to a renewed sense of well-being and a decrease in drug craving (Anton, 1999).

It is possible that people who have remained abstinent for many months or years can relapse to drug abuse. This craving for the drug that occurs later in recovery is most possibly caused by a long-term recollection of what if "felt like" to take the drug. Circumstances where the drug was previously used to relieve stress may activate this memory. Environmental events or changes in internal emotional states trigger a series of neurochemical reactions that through past experience have been programmed to activate various brain systems, thereby leading to the experience of craving (Anton, 1999).

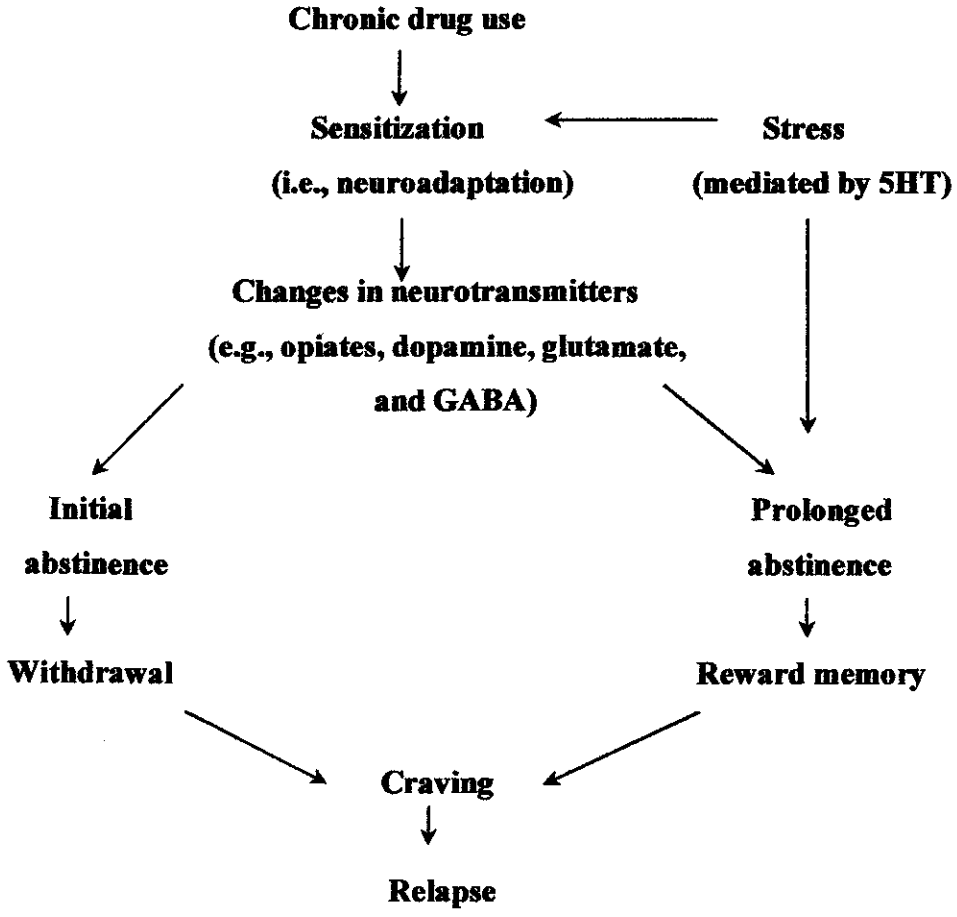


Figure 2.1. The neuroadaptive model of craving. This model proposes that chronic drug exposure leads to changes in brain cell function (i.e., sensitization or neuroadaptation) that are expressed as changes in the activity of various brain chemicals (i.e., neurotransmitters), such as

dopamine, glutamate, gamma-aminobutyric acid (GABA) and endogenous opioids. Neuroadaptation can contribute to certain characteristics of drug dependence, such as withdrawal, and to the development of a reward memory – that is, the memory of the importance of drug or drug-related stimuli to the abuser's wellbeing. During initial abstinence, when drug withdrawal may occur, neuroadaptation leads to an imbalance in brain function, which results in subjective feelings of discomfort and, subsequently, craving. During prolonged abstinence, situations or stimuli previously associated with drug taking may activate the reward memory, thereby also inducing craving. Craving, in turn, may result in relapse. Stress, which on a chemical level is mediated by the neurotransmitter serotonin, can enhance neuroadaptation as well as trigger the reward memory (Anton, 1999).

2.3. BRAIN NETWORKS ASSOCIATED WITH CRAVING

Drugs of abuse activate a brain area called the nucleus accumbens (Nacc), which is thought to be the brain's "reward centre" or "pleasure centre" (Melichar *et al.*, 2001 and Balfour & Fagerström, 1996). The mesocorticolimbic dopaminergic system (the dopaminergic neurons in the ventral tegmental area of the midbrain and their projections to the Nacc and thence to the Prefrontal Cortex) has long been seen as a key component of the reward pathway (Melichar *et al.*, 2001). Neurons located in the nucleus accumbens extend to both the amygdala and the frontal cortex areas. The amygdala is highly connected to brain regions that control emotions (i.e., the limbic system) and it plays a role in the modulation of stress and mood. The frontal cortex areas integrate incoming sensory information, such as smells, sights and sounds. One of those areas is the dorsal lateral prefrontal cortex (DLPC), where the memories for rewarding aspects of AOD use and their salience may be located (Kalivas *et al.*, 1998). Situations that are associated with tobacco use could be "remembered" with increased salience, because the DLPC is activated by both the information coming from those parts of the brain that control emotion and reward (i.e., the amygdala and the nucleus accumbens) and by the sensory information associated with these situations (Anton, 1999). The DLPC also sends information back to the nucleus accumbens and

therefore, researchers have hypothesized that in the case of recovering alcoholics, sensory information associated with alcohol-paired situations stimulates the DLPC, which, in turn, stimulates the nucleus accumbens and induces greater neural activity in that brain region (Kalivas *et al.*, 1998).

The orbitofrontal cortex controls the activities of the DLPC and other areas in the frontal cortex. The orbitofrontal cortex is an area of "executive function" that lies in front of the DLPC and which is involved in judgment (i.e., the evaluation of risk and reward). Genetic predisposition or injury may impair the orbitofrontal cortex and then it may no longer inhibit DLPC activity to the same extent, leading to impulsive and uncontrolled activity and behaviour. There is also a connection between the DLPC and another brain region called the basal ganglia, which plays a role in repetitive or stereotypic thought and behaviour patterns (Anton, 1999).

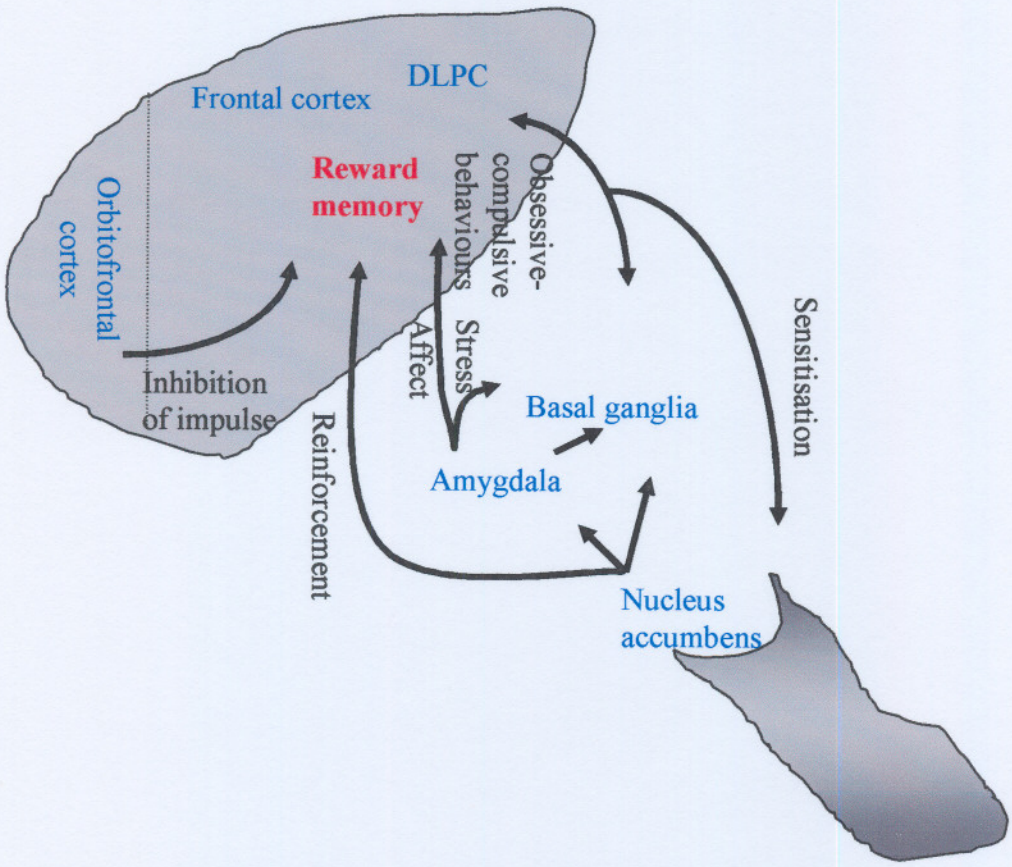


Figure 2.2. Brain regions involved in craving. The nucleus accumbens is the brain's "reward centre". Neurons in the Nacc send information to the amygdala, which plays a role in the modulation of stress and emotions;

the frontal cortex, including the dorsal lateral prefrontal cortex (DLPC), where the reward memory is thought to be located; and the basal ganglia, which plays a role in repetitive thought and behaviour patterns. Neurons located in the amygdala also send information to the DLPC and the basal ganglia. The DLPC sends information back to the basal ganglia and to the Nacc. The DLPC itself is controlled by the orbitofrontal cortex, which induces impulse control (Anton, 1999).

Koob and Roberts (1999) suggested the following roles for various neurotransmitters:

- Dopamine is involved in reinforcement mechanisms.
- Glutamate may play a role in sensitization mechanisms.
- GABA may be involved in sensitization mechanisms as well as in stress and affective mechanisms.
- Serotonin has been implicated in stress and affective mechanisms as well as in impulsivity and obsessive-compulsive mechanisms.
- Endogenous opiates may play a role in reinforcement mechanisms as well as in stress and affective mechanisms.

It is likely that many of these neurotransmitter systems, as well as other systems, play multiple and interconnected roles in the generation and maintenance of craving.

2.4. WHAT IS ADDICTION?

Drug addiction is "compulsive drug use without medical purpose and in the face of negative consequences", as described by the Director of NIDA (National Institute on Drug Abuse) (Betz *et al.*, 2000).

The pathway of addiction (the dopamine hypothesis of reward) has been evolving, with the mesocorticolimbic dopaminergic system now seen as key to natural rewards and drug-seeking behaviour. The perception of a common pathway has meant that the treatment for one drug of addiction can also serve as treatment for for other addictive drugs (Melichar *et al.*, 2001).

The addiction syndrome is remarkably similar between different drugs of abuse (Melichar *et al.*, 2001). Neurobiological research shows that drug-related stimuli are able to elicit an (classically conditioned) increase in dopamine levels in the brain. While it has long been postulated that dopamine acts by directly producing euphoric or pleasurable feelings, recent suggestions that dopamine primarily serves to draw a person's attention to events that predict or signal reward, such as a drug-related stimuli, have been made (Franken, 2003 and Powledge, 1999 and Little, 2000).

The most intuitive explanation for addiction is the traditional view that drugs are taken first because they are pleasant (positive reinforcement; which is critical for establishing self-administration behaviour). With repeated drug use homeostatic neuroadaptations lead to tolerance and dependence, which in turn leads to unpleasant withdrawal symptoms that ensue upon the cessation of use. Compulsive drug taking is maintained to avoid unpleasant withdrawal symptoms (negative reinforcement). Thus, negative reinforcement plays an important role in the maintenance of drug use after the development of dependence. The basic logic behind this hypothesis is that addictive drugs are taken initially simply to achieve pleasant drug "highs", and after addiction, to escape withdrawal "lows" (Robinson & Berridge, 2003 and Kreek & Koob, 1998 and Betz *et al.*, 2000 and Little, 2000).

Recently considerable attention has been paid to the role of learning in the transition to addiction, prompted in part by the realization that Nacc-related circuitry is involved in reward learning. For example, cues that predict the availability of rewards can powerfully activate Nacc-related circuitry in both animals and humans, sometimes even better than the reward itself (Robinson & Berridge, 2003).

2.5. INCENTIVE SENSITIZATION

The incentive sensitization theory of addiction focuses on how drug cues trigger excessive incentive motivation for drugs, leading to compulsive drug seeking, drug taking and relapse. The central idea is that addictive drugs enduringly alter Nacc-related brain systems that mediate a basic incentive-motivational function, the attribution of incentive salience. As a consequence, these neural circuits may become enduringly hypersensitive (or sensitized) to specific drug effects and to drug-

associated stimuli. The drug-induced brain change is called neural sensitization. Robinson and Berridge (2003) proposed that this leads psychologically to excessive attribution of incentive salience to drug-related representations, causing pathological "wanting" to take drugs. If the "wanting" system is activated implicitly it can instigate and guide behaviour without a person necessarily having conscious emotion, desire, or a declarative goal.

Robinson and Berridge (2003) suggest that this incentive-sensitization process is the fundamental problem in the transition to addiction and in relapse.

Sensitization is where a drug response gets progressively larger with repeated administration. In other words, the change in drug effect is in the opposite direction as seen with the development of tolerance (a decrease in a drug effect with repeated administration) (Kreek & Koob, 1998 and Robinson & Berridge, 2003 and Little, 2000). There are two major classes of drug effects that are sensitized by addictive drugs: psychomotor activating effects and incentive motivational effects (Robinson & Berridge, 2003).

2.6. PSYCHOMOTOR SENSITIZATION

In humans and animals many potentially addictive drugs can increase arousal, attention, and motor behaviour, producing heightened locomotion, exploration and approach. At higher doses psychomotor effects can also include intense repetitive stereotyped movements (Wise & Bozarth, 1987).

Sensitization is produced by many different drugs of abuse, including amphetamines, cocaine, opiates, methylphenidate, ethanol and nicotine. Sensitization is strongest when high or escalating doses are given, especially when the drug is administered rapidly and intermittently (continuous infusions are relatively ineffective) (Robinson & Berridge, 2003).

Another important feature of sensitization for addiction concerns individual differences in susceptibility to sensitization. Some individuals sensitize readily, whereas others are more resistant. Once sensitized, most individuals show cross-

sensitization, which means that sensitization to one drug can cause sensitized effects for other drugs as well. Even more intriguing, cross-sensitization can occur between drugs and nondrug stress. Animals previously exposed to stress may become sensitized to some potentially addictive drugs. Conversely, animals sensitized by drugs may become hypersensitive to stress (Robinson, 1988). Stress-drug cross-sensitization might be especially important in influencing stress-precipitated relapse, as well as initial susceptibility to addiction (Robinson & Berridge, 2003).

2.7. NEURAL SENSITIZATION

Behavioural sensitization is accompanied by an increase in the ability of a number of drugs to promote DA efflux in the Nacc. In addition, DA D1 receptors on neurons in the Nacc become hypersensitive after sensitization, presumably further potentiating the mesolimbic DA signal (Robinson & Berridge, 2003).

Consistent with circuit-level alterations, sensitization is also associated with persistent changes in the physical structure of neurons themselves. For example, cells in the Nacc and prefrontal cortex show changes in the length of dendrites and the extent to which dendrites are branched. At even finer level changes also occur in the density and types of dendritic spines, which are the primary site of excitatory glutamate synapses (Figure 2.3). These sensitization-related changes in dendritic structure may reflect changes in patterns of synaptic connectivity within these brain regions and therefore may alter information processing within Nacc-related circuitry.

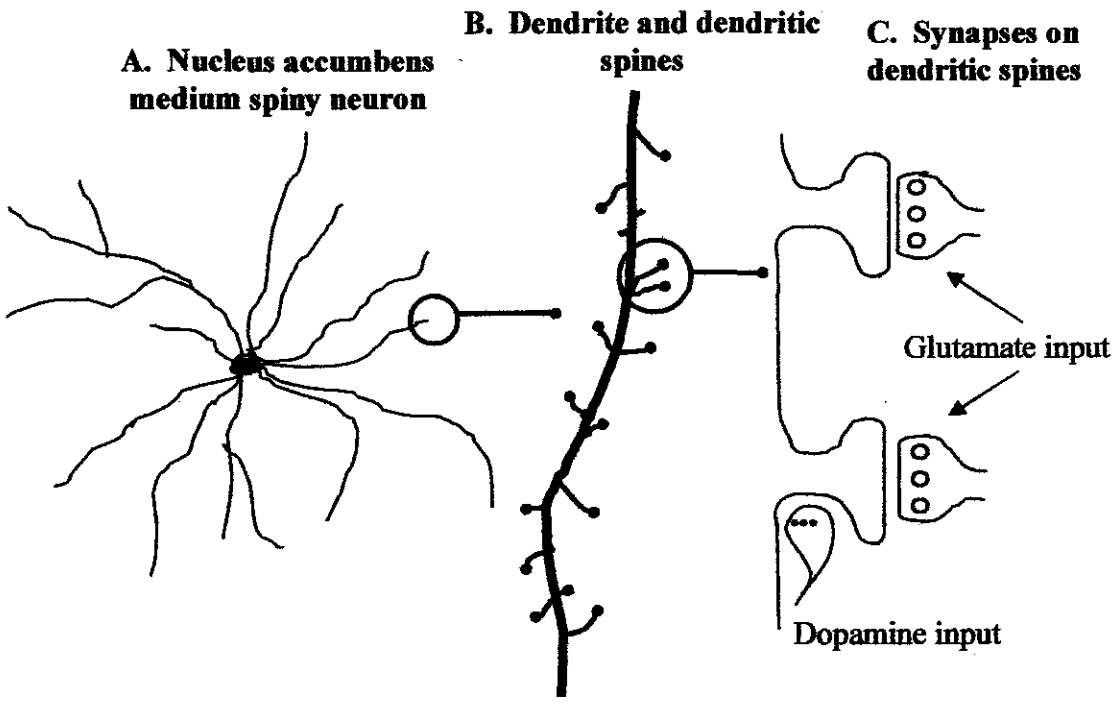


Figure 2.3. Graphic representation of the sites on neurons at which drugs have been shown to produce morphological changes. (A) The most common type of neuron in the nucleus accumbens, a medium spiny neuron. (B) Magnified view of a dendrite that is studded with many dendritic spines. (C) Dendritic spines are the site of synapses, and spines on the distal dendrites on medium spiny neurons receive both glutamate and DA inputs (Robinson & Berridge, 2003).

2.8. DECISION-MAKING AND LOSS OF INHIBITORY CONTROL

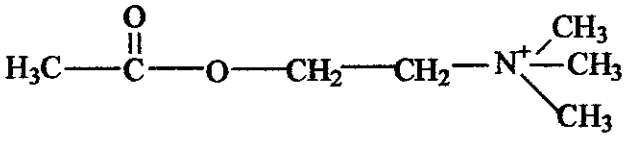
In addicts the excessive incentive salience posited by the incentive-sensitization theory can not only lead to the pathological pursuit of drugs but to apparently irrational choices to take drugs. Even if a person knows cognitively that the drug will not give much pleasure (e.g., the quality is poor), sensitised implicit "wanting" can overcome low expectations of "liking". The distinction between "wanting" and "liking" can sometimes result in strange dissociations in addicts. Goal-directed drug-seeking behaviour occurs in the absence of conscious awareness that pursuit is underway, and is dissociated from the ability of drugs to produce pleasure; that is, addicts will pursue drugs they do not like, as well as those they like (Robinson & Berridge, 2003).

CHAPTER 3

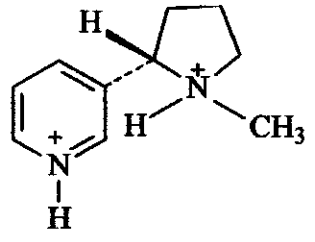
THE RELATIONSHIP OF NICOTINE TO ACETYLCHOLINE

3.1 INTRODUCTION

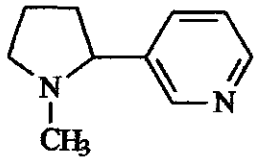
Nicotine and acetylcholine (ACh) can exist in remarkably similar molecular forms. The pyridine nitrogen of nicotine is an electronic donor similar to the keto oxygen of the acetyl group of ACh. The positive charge of the quaternary nitrogen of the choline group in ACh is similar to the positive charge of the pyrrolidine nitrogen of nicotine (Domino, 1998).



Acetylcholine



Diprotonated form of nicotine



Nicotine

At the pH of blood, nicotine exists in both charged and uncharged forms. The uncharged form can readily penetrate the blood-brain barrier, but ACh cannot (Domino, 1998).

Cholinergic receptors can be divided into muscarinic (mAChR) and nicotinic (nAChR), based on the agonist activities of the natural alkaloid muscarine and nicotine (Mihailescu & Drucker-Colin, 2000). The molecular basis for the behavioural and physiological effects of nicotine is binding of the drug to nAChRs and subsequent activation of these receptors (Picciotto, 1998 and Jain & Mukherjee, 2003). The endogenous neurotransmitter at nAChRs is ACh (Mihailescu & Drucker-Colin, 2000 and George & O'Malley, 2004).

3.2. NICOTINIC CHOLINERGIC RECEPTORS

Clinical and laboratory studies indicate the involvement of neuronal nicotinic acetylcholine receptors (nAChRs) in complex brain functions such as memory, attention and cognition (Mihailescu & Drucker-Colin, 2000). The specific sites for binding of nicotine to nAChRs in the brain are the hypothalamus, hippocampus, thalamus midbrain, brain stem and cerebral cortex. Nicotine also binds to receptors in the nigrostriatal and mesolimbic dopaminergic neurons (Jain & Mukherjee, 2003).

The cholinergic receptors are relatively large structures that consist of several components known as subunits. The different nicotinic receptors present in the brain are ligand-gated-ion channels made of five subunits. Different combinations make different types of receptors, which vary in terms of affinity and localization within the brain. One of these subunits, the β subunit, has recently been implicated as having a role in nicotine addiction (Jain & Mukherjee, 2003).

When the nicotinic receptors are stimulated they release ACh, nor-epinephrine, dopamine (DA), serotonin (5-HT), vasopressin, growth hormone and ACTH. Nicotine is one of the most potent stimulants of the midbrain dopamine reward pathway (Jain & Mukherjee, 2003 and Picciotto, 1998).

Table 3.1. Regional expression of the neuronal nAChRs subunits in the brain (Picciotto, 1998).

Brain area	Highly expressed	Slightly expressed
Anterior thalamus	α4, β2	α3
Reticular thalamus	α4, α6, β2, β3	α3
Mesolimbic DA system	α4, α5, α6, β2, β3	α3, α7
Interpeduncular nucleus	α2, α3, α5, α7, β2, β4	α4, α6, β3
Medial habenula	α2, α3, α4, α5, α7, β2, β3, β4	α6
Hippocampus	α3, α7, β2	α4
Cortex	α4, α5, α7, β2	α3

Stimulation by nicotine of presynaptic nACh receptors on the mesocorticolimbic DA-containing neurons increases neurotransmitter release and metabolism. Unlike most agonists, which down-regulate receptor numbers with chronic exposure, chronic administration of nicotine leads to desensitisation and inactivation of nACh receptors, and a paradoxical upregulation of nACh receptor sites. After overnight abstinence, these nACh receptors are likely to resensitise and are thought to be fully responsive to nicotine as an exogenous agonist. This might explain why most smokers report that the most satisfying cigarette of the day is the first one in the morning (George & O'Malley, 2004).

The ability of nicotine to facilitate release of neurotransmitters may be due to the high permeability of brain nicotinic receptors to calcium (Rathouz & Berg, 1994). Activation of nAChRs by endogenous ACh or pharmacologically administered nicotine is likely to result in increases in the level of intracellular calcium, and this in turn may increase neurotransmitter release at the nerve terminal. Extremely low concentrations of nicotine, consistent with the levels found in the blood of moderate smokers, are sufficient to affect neurotransmitter release and the electrophysiological properties of neurons (Picciotto, 1998).

CHAPTER 4

NEUROTRANSMITTER SYSTEMS INVOLVED IN TOBACCO ADDICTION AND WITHDRAWAL

4.1 INTRODUCTION

DA, like most biologically important molecules, must be kept within strict bounds. Too little DA in certain areas of the brain triggers the tremors and paralysis of Parkinson's disease. Too much DA causes the hallucinations and bizarre thoughts of schizophrenia (Nash & Park, 1997). DA is associated with feelings of pleasure and elation, whereas serotonin is associated with feelings of sadness and well-being (Nash & Park, 1997).

In this study the focus will be on two of the neurotransmitter systems as mentioned above in section 3.2, and therefore only dopamine (DA) and serotonin (5-HT) will be discussed.

4.2. THE ROLE OF DOPAMINE

Dopamine is a neurotransmitter, a chemical that carries messages from one nerve cell, or neuron, to another or from one functional section of the brain to another. Dopamine is associated with body movement, awareness, judgement, motivation and pleasure (Swan, 1998).

DA receptors can be divided into five subtypes that cluster into two families, D1 and D5 and D2 to D4. These receptors are found in the caudate-putamen (Cpu), olfactory tubercle (OTu), nucleus accumbens (Nacc), cortex and hippocampus of the brain (Bahk *et al.*, 2002).

The striatum is the main target area of midbrain DA neurons. Although the striatum is morphologically homogeneous, it is often divided into dorsal (caudate-putamen, Cpu)) and ventral (nucleus accumbens, Nacc) compartments based on specifics of

input/output connections, peptide co-existence and presumed functional differences (Parent and Hazrati, 1995). According to Balfour *et al.* (1998), electrophysiological studies suggest that the DA neurons that innervate the Nacc are more sensitive to nicotine than those that innervate the caudate-putamen.

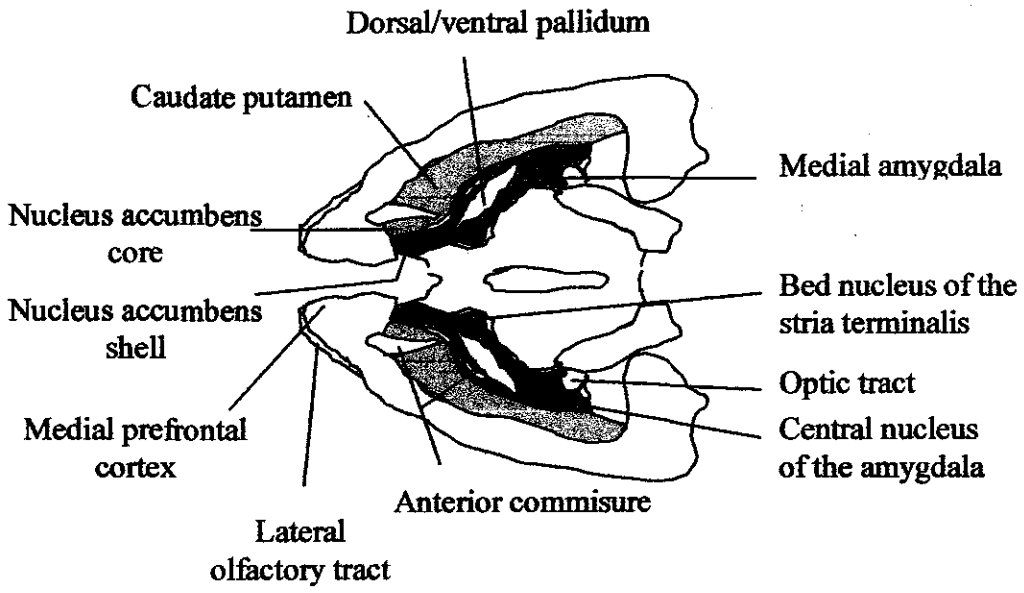


Figure 4.1. The striatal complex, nucleus accumbens subdivisions (shell and core) and the extended amygdala (Di Chiara, 2000).

The DA neurons of the midbrain are implicated in motor tasks related to normal movements, motivational as well as reward-related behaviour and cognitive functions. The mesolimbocortical DA neurons are profoundly implicated in reward-related behaviour and many drugs of abuse including nicotine are known to directly or indirectly cause an increased release of DA in terminal areas (Grillner & Mercuri, 2002 and Di Chiara, 2002). It is known that smokers have a reduced DA turnover (Reuter *et al.*, 2002).

Acute systemic administration of drugs of abuse produces a variety of neurochemical effects which are specific for each drug, but a shared feature of these substances is their ability to increase DA neurotransmission in the rodent brain. Nicotine has been shown to stimulate the DA neurons that project to the nucleus accumbens (Nacc) from the ventral tegmental area (VTA) of the midbrain, known as the mesolimbic DA system. Dopamine's stimulatory effects on these neurons mediate both the locomotor

stimulant properties of the drug and the reinforcing properties of acute nicotine (Balfour *et al.*, 1998 and Kenny & Markou, 2001 and Picciotto, 1998).

The role of the mesolimbic-DA system in reward is shifting from ''pleasure juice'' towards a role in "wanting" processes such as drug craving. This role of DA has been put forward in the Incentive Sensitization Theory. The DA signal relates to the curiosity about all salient stimuli (Powledge, 1999 and Franken, 2003). DA release, triggered by stimuli or actions that predict rewarding outcome, is necessary to focus the subject towards these cues, reducing the probability that these cues are ignored (Franken, 2003).

Repeated exposure to drugs of abuse progressively enhances the locomotor stimulatory properties of these substances. This phenomenon is generally referred to as behavioural sensitization and can be defined as an increased effect of a fixed drug dose, or a maintained effect even after dose reduction, occurring after recurrent drug exposures. Behavioural sensitization appears to be associated with drug-induced neural alterations that make the mesolimbic DA projection hypersensitive. These alterations occur both pre- and postsynaptically, and include augmented drug-induced elevation of the DA output in the ventral striatum and enhanced postsynaptic DA receptor function (Olausson *et al.*, 2002).

Initial acute exposure to nicotine stimulates dopamine preferentially in the nucleus accumbens shell. Intermittent discontinuous exposure to nicotine as in the case of peak smokers results in rapidly reversible desensitization, resulting in acute tolerance to nicotine-induced stimulation of dopamine release in the nucleus accumbens shell. Repeated continuous exposure to nicotine during the day, as in through-the-day smokers, results in a complex exposure to nicotine characterised by peaks, which correspond to cigarette smoking, on a baseline of nicotine that builds up in a stepwise manner at each cigarette smoking episode during the day to decrease during the night, when smoking ceases. The presence of a steady-state level of nicotine, while eventually insufficient to phasically stimulate DA release in the Nacc is sufficient to induce desensitization. However, as a result of a relative resistance to inactivation of nicotine acetylcholine receptors containing certain subunits, desensitization of DA transmission is not complete even in a chronic smoker. This allows DA release in

response to nicotine to take place also after chronic exposure. The steady-state level of nicotine in a chronic smoker progressively increases during the day and the phasic response of DA transmission in the Nacc to smoking should be minimal at night and maximal in response to the first morning cigarette (Di Chiara, 2000).

Hildebrand *et al.* (1999) have shown that besides an increase in somatic withdrawal signs, mecamylamine (a nicotinic receptor antagonist, acting centrally and peripherally) also significantly decreased accumbal DA release in rats chronically exposed to nicotine compared with control rats. Therefore, it is likely that deficits in DA transmission in the Nacc play a role in mediating nicotine withdrawal.

According to Rada *et al.* (2001) the neurochemical effect of nicotine withdrawal is an increase in extracellular ACh levels in the Nacc and a simultaneous decrease in extracellular DA levels. With respect to nicotine intake, the motivation for smoking may be directed to either restoring a homeostatic imbalance (smoking for the compensation of a DA deficit) or to induce goal-directed behaviour (smoking for the sake of enjoying the behaviour that leads to DA release).

The effect of nicotine withdrawal on dopamine transmission has also been examined in the central nucleus of the amygdala (CNA). Mecamylamine-precipitated nicotine withdrawal significantly reduced dopamine overflow in the CNA. Dopamine may possibly mediate an anxiolytic effect in this brain structure. Therefore, the reduction in dopamine output during nicotine withdrawal in the CNA may be involved in mediating the increase in anxiety associated with nicotine withdrawal. However, the precise role of CNA dopamine neurotransmission in mediating anxiety states is unclear (Kenny & Markou, 2001).

4.2.1. The synthesis of dopamine

The amino acid tyrosine is taken up by dopaminergic neurons, converted by the enzyme tyrosine hydroxylase to 3,4-dihydroxy-phenylalanine (DOPA), decarboxylated by the enzyme aromatic L-amino acid decarboxylase to DA and stored in vesicles (see figure 4.2). Drugs stimulate receptors on the cell bodies of dopaminergic neurons causing DA release and stimulation of post-synaptic DA receptors in the nucleus accumbens, which is thought to result in the perception of pleasure (Walton *et al.*, 2001 and Balfour *et al.*, 1998).

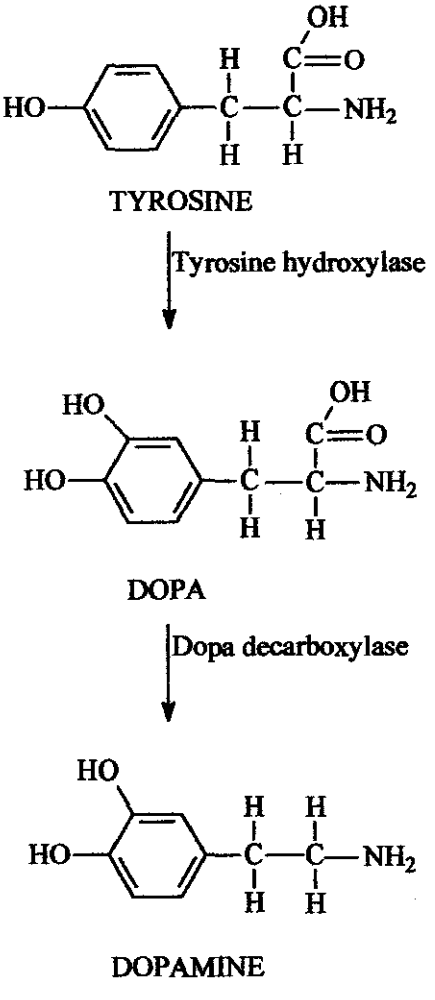


Figure 4.2. The synthesis pathway of dopamine (Katzung & Trevor, 1998).

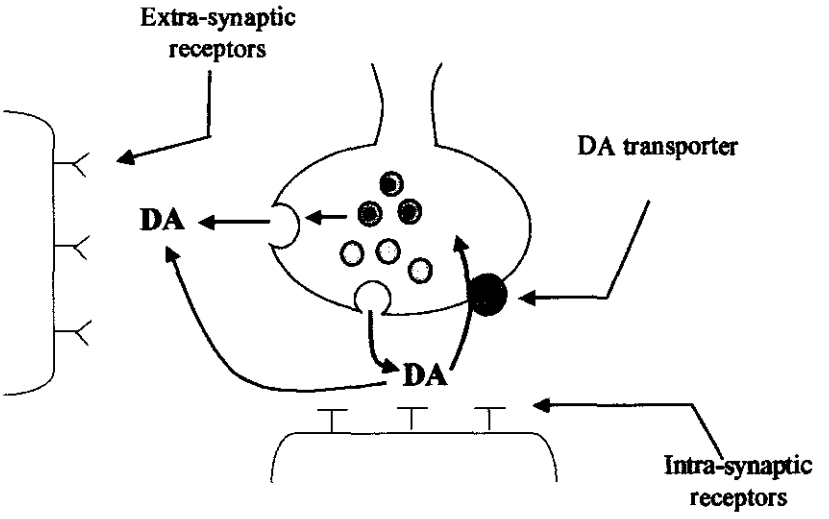


Figure 4.3. Diagrammatic representation of a DA terminal in the Nacc. This figure summarizes the two putative mechanisms by which DA may be released into the extrasynaptic space and gain access to extrasynaptic DA receptors. It may either escape from the synaptic cleft or be released directly into the extrasynaptic space from vesicles that release neurotransmitter preferentially in response to burst firing (Balfour *et al.*, 1998).

4.2.2. The metabolism of dopamine

The action of DA is terminated by two mechanisms:

1. Reuptake into nerve terminals.
2. Dilution by diffusion out of the junctional cleft and uptake at extraneuronal sites and metabolic transformation by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT). MAO is associated chiefly with the outer surface of the mitochondria and COMT is located largely in the cytoplasm. Dopamine released within the terminal is metabolized by MAO, while COMT plays an important role in the metabolism of endogenous circulating and administered catecholamines. It is known that smokers have a reduced dopamine turnover and a reduced sensitivity of nicotinic acetylcholine receptors (nAChR) (Reuter *et al.*, 2002).

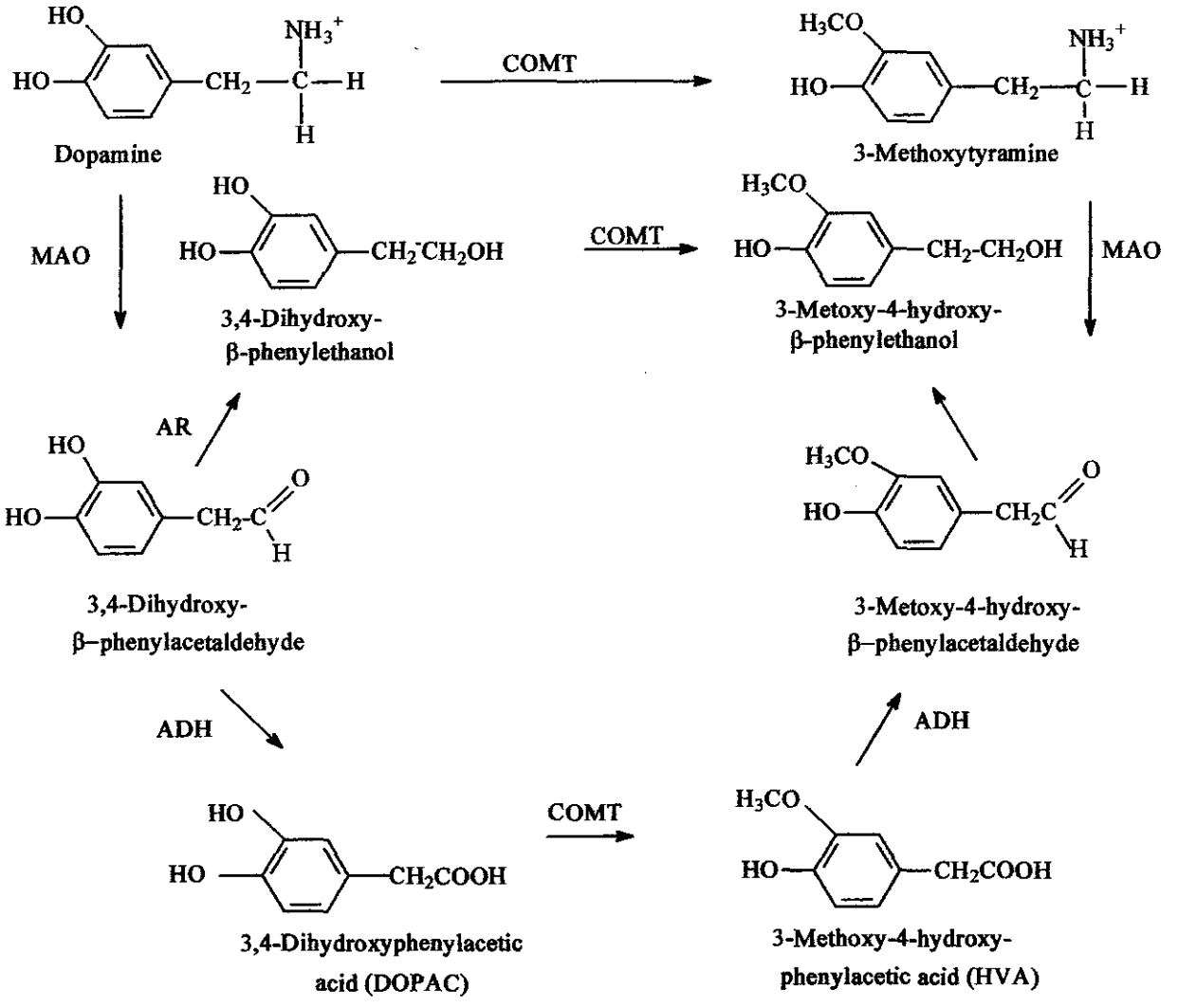


Figure 4.4. The metabolic pathway of dopamine (Brand, 1996).

Gäddnäs *et al.* (2000) studied the effects of chronic nicotine and its withdrawal on locomotor activity and brain monoamines by administering nicotine in the drinking water to male NMRI mice. They found that the increased locomotor activity in the nicotine-treated mice correlated to increased striatal concentrations of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), indicating enhanced striatal DA metabolism. In their experiments the dissected striatal tissue contained the dorsal striatum as well as the ventral striatum, which includes the nucleus accumbens.

4.3. THE ROLE OF SEROTONIN

According to Olausson *et al.* (2001) a lack of inhibitory control is a core feature of impulsivity and other factors such as decision time, persistence and sensation seeking are also important for the expression of impulsive behaviour in man. Clinical observations support the view that chronic intermittent exposure to nicotine impairs neural mechanisms involved in inhibitory control of behaviour, since cigarette smokers display impulsive behaviour when assessed in neuropsychological test paradigms and have higher scores in questionnaires that measure impulsivity. Drug addiction could be considered an impulse control disorder, since several items of the DSM-IV diagnostic criteria both for substance dependence and abuse contain elements of impulsivity (Olausson *et al.*, 2001).

A large number of experimental findings have implicated the brain serotonin (5-hydroxytryptamine, 5-HT) systems in the neuronal circuits that mediate inhibitory control of behaviour. In experimental animals, 5-HT depletion consistently produces an impulsive behavioural pattern, anticipatory responses in various animal models and aggressivity (Olausson *et al.*, 2001). A brain 5-HT depletion that reduces 5-HT neurotransmission increases responding for conditioned reward, whereas manipulations that facilitate brain 5-HT neurotransmission decreases responding for conditioned reinforcers.

Manipulations which decrease brain 5-HT neurotransmission (e.g., a neurotoxic 5-HT depletion), elevate self-administration of several different drugs in rats and compounds that facilitate 5-HT neurotransmission, like selective 5-HT reuptake inhibitors, decrease voluntary ethanol consumption in rats. Similar effects of 5-HT enhancing drugs have been reported on the intake of nicotine (Opitz & Weischer, 1988). These observations suggest that an increase in 5-HT neurotransmission could reduce drug consumption by means of strengthening inhibitory control.

According to Seth *et al.* (2002), nicotine increases 5-HT release in the cortex, striatum, hippocampus, dorsal raphé nucleus, hypothalamus and spinal cord. The effects in the cortex, hippocampus and dorsal raphé nucleus involve stimulation of 5-HT_{1A} receptors, and in the striatum, 5-HT_3 receptors. The 5-HT_{1A} receptors in the

dorsal raphé nucleus play a role in mediating the anxiolytic effects of nicotine and the 5-HT_{1A} receptors in the dorsal hippocampus and lateral septum mediate its anxiogenic effects. The increased startle and anxiety during nicotine withdrawal is mediated by 5-HT_{1A} and 5-HT_3 receptors. The locomotor stimulant effect of acute nicotine is mediated by 5-HT_{1A} receptors and 5-HT_2 receptors may play a role in the expression of a sensitised response after chronic nicotine treatment.

It has been reported by Helton *et al.* (1993) that nicotine withdrawal significantly increased the acoustic startle response in rats for approximately 4 to 5 days. This increased startle reactivity perhaps most closely resembles the increased irritability observed in smokers undergoing nicotine withdrawal. Systemic administration of 5-HT_{1A} receptor agonists such as 8-OH-DPAT exacerbates this response, whereas 5-HT_{1A} receptor antagonists, such as WAY-100635, alleviate this enhanced response. Electrophysiological investigations have demonstrated that the responsiveness to 8-OH-DPAT of neurons in the dorsal raphè nucleus was significantly increased during nicotine withdrawal. Therefore, one possibility is that nicotine withdrawal increases the inhibitory influence of somatodendritic 5-HT_{1A} autoreceptors located within the raphè nuclei and thereby decreases 5-HT release into forebrain and limbic brain sites which contributes to nicotine withdrawal signs (Kenny & Markou, 2001).

Contrary to the view that reduced serotonergic transmission contributes to nicotine withdrawal, Seth *et al.* (2002) have shown that administration of nicotine directly into the dorsal raphè nucleus, at a concentration that activates somatodendritic 5-HT_{1A} receptors, reversed the increase in anxiety observed in rats undergoing nicotine withdrawal as measured in the social interaction test. This observation suggests that there is enhanced serotonergic transmission during nicotine withdrawal that mediates the observed increases in anxiety.

4.3.1. The synthesis of serotonin

Serotonin is an indoleethylamine formed in biologic systems from the amino acid L-tryptophan. Figure 4.5 illustrates the hydroxylation of the indole ring followed by the

decarboxylation of the amino acid. Hydroxylation at C5 is the rate-limiting step (Katzung & Trevor, 1998).

4.3.2. The metabolism of serotonin

5-HT is metabolized by monoamine oxidase (MAO), and the intermediate product, 5-hydroxyindoleacetaldehyde, is further oxidized by aldehyde dehydrogenase. When the latter enzyme is saturated, eg., by large amounts of acetaldehyde from ethanol metabolism, a significant fraction of the 5-hydroxyindolaecetaldehyde may be reduced in the liver to the alcohol, 5-hydroxytryptophol. In humans consuming a normal diet, the excretion of 5-hydroxyindoleacetic acid (5-HIAA) is a measure of serotonin synthesis. See Figure 4.5.

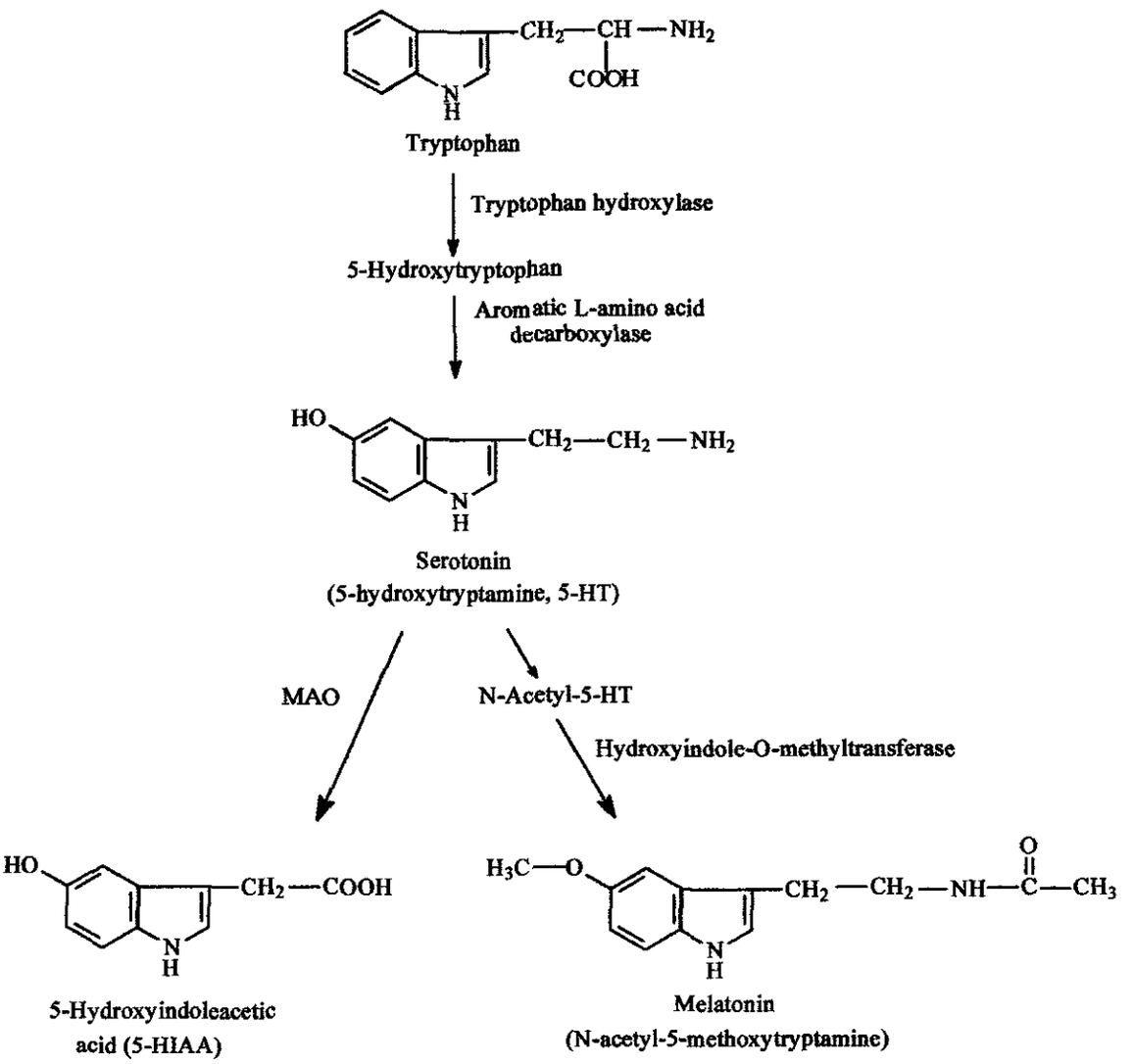


Figure 4.5. Synthesis and metabolism of serotonin (Katzung & Trevor, 1998).

Gäddnäs *et al.* (2000) reported that the chronic administration of nicotine in the drinking water to mice significantly increased the concentration of the 5-HT metabolite 5-HIAA in the striatum and hypothalamus. It also tended to increase the 5-HIAA concentration in the cortex.

CHAPTER 5

MONOAMINE OXIDASE, ALDEHYDE DEHYDROGENASE AND TYROSINE HYDROXYLASE

5.1. MONOAMINE OXIDASE

Monoamine oxidase (MAO) is an integral protein of outer mitochondrial membranes and occurs in neuronal and non-neuronal cells in the brain and in peripheral organs. It oxidizes amines from both endogenous and exogenous sources, thereby influencing the concentration of neurotransmitter amines as well as many xenobiotics (Fowler *et al.*, 2003).

MAO occurs as two subtypes, MAO A and MAO B which have different inhibitor and substrate specificities and which are encoded by separate genes that are closely linked on the X chromosome and share 70% similarity in amino acid sequence. MAO A preferentially oxidizes noradrenaline and serotonin. MAO B preferentially breaks down the trace amine phenethylamine. Both forms oxidize dopamine, tyramine and octopamine (Fowler *et al.*, 2003).

5.1.1. Monoamine oxidase levels and smoking

Studies have shown that smokers have lower levels of brain MAO A and B activity and lower MAO B platelet activity than non-smokers (Fowler *et al.*, 2003). It was not known at that time whether this was due to decreased MAO B synthesis in smokers, the presence of MAO inhibitory compounds in smoke or whether low MAO individuals are more vulnerable to smoking. A later study reported normal MAO levels in former smokers (Boulton, 1996), which provided evidence that low MAO B may be a pharmacological effect of the smoke rather than a biological characteristic of smokers (Fowler *et al.*, 2003 and Mihailescu & Drucker-Colin, 2000).

According to Essman (1977) the MAO catalyzed oxidation of serotonin, but not tyramine, was inhibited *in vitro* in the skin of mice following repeated exposure to

cigarette tobacco smoke. Yu and Boulton (1987) described the inhibition of rat lung mitochondrial MAO exposed to pH 7.5 phosphate-buffer cigarette smoke extracts, and irreversible MAO inhibition in rat lung tissue exposed to cigarette smoke. Independent ex vivo studies have shown that human blood platelet MAO B activity is significantly reduced in smokers.

Nicotine inhibits human platelet MAO B activity, but at concentrations 2000 times higher than that observed in the blood of heavy smokers. Using positron emission tomography (PET) imaging, Fowler *et al.* (1998), found that an acute dose of nicotine did not lower MAO B activity in the brain of a baboon treated with nicotine. However, dimethylsulfoxide cigarette smoke extracts inhibits MAO A and MAO B in cerebral homogenates in a dose dependent manner. Castagnoli *et al.* (2002) reported that neither (S)-nicotine nor (R,S)-N-methylanatabine (a minor tobacco alkaloid) administered via minipumps for 28 days to rats, led to a change in brain MAO A or MAO B activity. This shows that nicotine itself is not an MAO inhibitor in the concentrations normally achieved during smoking. The possibility arises that another component of cigarette smoke other than nicotine may be inhibiting the MAO (Jain & Mukherjee, 2003). This reinforces the need to look beyond nicotine as the only pharmacologically relevant substance in tobacco smoke.

Castagnoli *et al.* (2002) have isolated three MAO B inhibitors from tobacco leaf and characterized farnesylacetone as one of these compounds. Studies on crude extracts from the tobacco leaf and tobacco smoke showed the presence of both reversible and irreversible MAO B activity. Recently the fractionation of extracts from flue-cured tobacco leaves led to the isolation of a competitive inhibitor of human MAO A and MAO B. The chemical structure was found to be 2,3,6-trimethyl-benzoquinone by classical spectroscopic analysis and was confirmed by synthesis (Khalil *et al.*, 2000). Another MAO inhibitor, 2-naphthylamine, which is present in tobacco smoke, was identified. It also inhibits both MAO A and MAO B but is 10-fold less potent than the benzoquinone (Fowler *et al.*, 2003).

5.2. ALDEHYDE DEHYDROGENASE

The primary biotransformation reactions of Phase I and Phase II metabolism chemically modifies various endogenous and exogenous substrates to more watersoluble intermediates or products which can then be readily eliminated. As specific substrates are chemically altered by these pathways, oxygen may be added and/or electrons are removed resulting in intermediates and products that are substantially more oxidized than the parent compound (Vasiliou *et al.*, 2000).

Aldehydes are highly reactive electrophilic compounds, which interact with thiol and amino groups and the aldehyde-mediated effects vary from physiologic and therapeutic to cytotoxic, genotoxic, and mutagenic or carcinogenic. One of the most important pathways for aldehyde metabolism is their oxidation to carboxylic acids by aldehyde dehydrogenases (ALDHs). Oxidation of the carbonyl functional group is considered a general detoxification process. However, a number of ALDH-mediated oxidation forms products that are known to possess significant biologic, therapeutic and/or toxic activities. These include: retinoic acid, an important element for vertebrate development, γ-aminobutyric acid (GABA), an important neurotransmitter, and trichloroacetic acid, a potential toxicant (Vasiliou *et al.*, 2000).

The cytosolic ALDH1A1 and the mitochondrial ALDH1B1 may be involved in acetaldehyde metabolism. The ALDH2 encodes mitochondrial ALDH that exhibits the highest affinity for acetaldehyde (Vasiliou *et al.*, 2000). The ALDH2 enzyme plays a major role in the rapid clearance of acetaldehyde and individuals that carry the ALDH2*2 deficient and dominant allele exhibit the "alcohol flushing" syndrome attributable to elevated blood acetaldehyde (Peng *et al.*, 1999).

Helander *et al.* (1991) examined the effect of a cigarette-smoke condensate (CSC) and three CSC subfractions on the ALDH activity in human blood cells under physiological conditions in vitro. They found that incubation of intact or sonicated cells with different concentrations of crude CSC resulted in a dose-dependent reduction of the ALDH activity. The inactivation was only restored in part after extensive washing of the cells, indicating that the inhibition observed was mainly irreversible. These results, showing that the human blood cell ALDH is inactivated

by constituents of cigarette smoke in vitro, suggest that the blood ALDH activity reduction found in habitual smokers is also caused by components formed during the combustion of tobacco.

5.2.1. The role of acetaldehyde

Acetaldehyde (CH_3CHO) is one of the most common neurotoxins in the lives of millions of people. Acetaldehyde is electrophilic and reacts with nucleophilic groups of various macromolecules including DNA. Acetaldehyde inhibits synthetic and metabolic pathways; it interferes with the polymerization of tubulin and stimulates collagen synthesis (von Wartburg, 1987).

Circulating acetaldehyde is known to have a half-life of only a few minutes because it is rapidly oxidized by the oxidized form of nicotinamide-adenine dinucleotide (NAD^+)-dependent ALDH, which can be found in essentially all tissues in the body, including the brain (Quintanilla *et al.*, 2002).

Acetaldehyde promotes damage to brain structure and function through numerous pathways. There are four main routes that bring acetaldehyde into the human brain:

1. Alcohol consumption. Once in the body, an enzyme called alcohol dehydrogenase converts alcohol to acetaldehyde. Then ALDH must break the acetaldehyde down into acetate. However, the conversion of acetaldehyde to acetate does not always occur quickly and the body may have difficulty in rapidly detoxifying acetaldehyde (Cleary, 1986).
2. Candida (the yeast syndrome). Candida is known to occur in the intestinal tract of all humans to some degree. Candida is relatively harmless when it is being kept in small amounts by a healthy immune system and the so-called "friendly flora", such as *Acidophilus* and *Bifidus* bacteria. But due to the modern overuse of antibiotics and birth control pills, as well as excessive stress, sugar consumption and malnutrition, millions of people suffer from an excessive growth of Candida in their intestines, the so-called "yeast syndrome". Candida lives by fermenting sugars to produce energy. Unfortunately for humans who have large colonies of Candida in their gut, the waste by-product of the sugar fermentation is acetaldehyde. Research has

shown that the acetaldehyde may combine with red blood cells, proteins or enzymes and travel through the bloodstream to reach more distant parts of the body such as the brain (Truss, 1984).

3. Exhaust from cars and trucks. When oil, gasoline and diesel fuel are burned, acetaldehyde is produced. Thus, acetaldehyde can enter the body through inhaling air laden with vehicle and factory exhaust (Brain wave entrainment technology, 2002).
4. Cigarette smoking. Acetaldehyde is also produced through the burning of tobacco. Heavy cigarette smokers are at risk of inhaling acetaldehyde through the inhaled smoke. Although the amounts of acetaldehyde inhaled through auto exhaust and cigarette smoke may be small compared to that from alcohol, research shows that low-dose chronic acetaldehyde exposure may still be sufficient to gradually damage proteins, enzymes and other cellular structures in the brain (Brain wave entrainment technology, 2002).

There are many ways by which acetaldehyde can gradually damage brain structure and function through chronic, low-dose acetaldehyde exposure. The following are some of them:

Acetaldehyde alters red blood cell structure and decreases the ability of the protein tubulin to assemble into microtubules. Microtubules help provide structural support to the nerve cell, keeping the nerve cell and the dendrites semi-rigid. Microtubules also serve to transport nutrients and biochemical raw materials manufactured in the cell body to the dendrites. When this raw material transport is compromised, the dendrites will gradually atrophy and die off. Two classic examples of brain pathology involving degeneration of the dendrites in humans are chronic alcoholic brain damage and Alzheimer's disease. Acetaldehyde also induces deficiency of vitamin B1. Unfortunately, in detoxifying acetaldehyde through combination with it, vitamin B1 is destroyed. Moderately severe vitamin B1 deficiency in humans leads to a group of symptoms called Wernicke-Korsakoff syndrome. This syndrome is characterized by mental confusion, poor memory, poor neurotransmitter co-ordination and visual disturbances. Its primary accepted cause is chronic alcoholism (Dreyfus & Victor, 1971).

Acetaldehyde promotes addiction to toxic substances. Acetaldehyde may alter brain function by combining in the brain with brain biogenic amines to form tetrahydroisoquinolines (TIQs) (see Figure 5.1) (Brain wave entrainment technology, 2002 and Gant, 2000). In an experiment done by Cohen & Collins (1970) they found that when cow adrenal glands were perfused with dilute solutions of acetaldehyde, 1,2,3,4-tetrahydroisoquinoline (TIQ) alkaloids were formed. According to Cohen & Collins (1970), these substances are derived from a condensation reaction of acetaldehyde with the tissue catecholamines, adrenaline and noradrenaline. The various tetrahydro-isoquinolines are closely related in structure and function. These alkaloids could be involved in the development of alcohol dependence and withdrawal symptoms and successfully detoxifying alcoholics have been shown to excrete especially high levels of these chemicals in their urine. Thus, these acetaldehyde-generated, opiate-like biochemicals may at least partly explain why alcoholics are so addicted to alcohol, cigarette smokers to cigarettes and Candida-sufferers to sugar, since all three of these conditions promote chronic excessive body acetaldehyde levels (Brain wave entrainment technology, 2002 and Cohen & Collins, 1970).

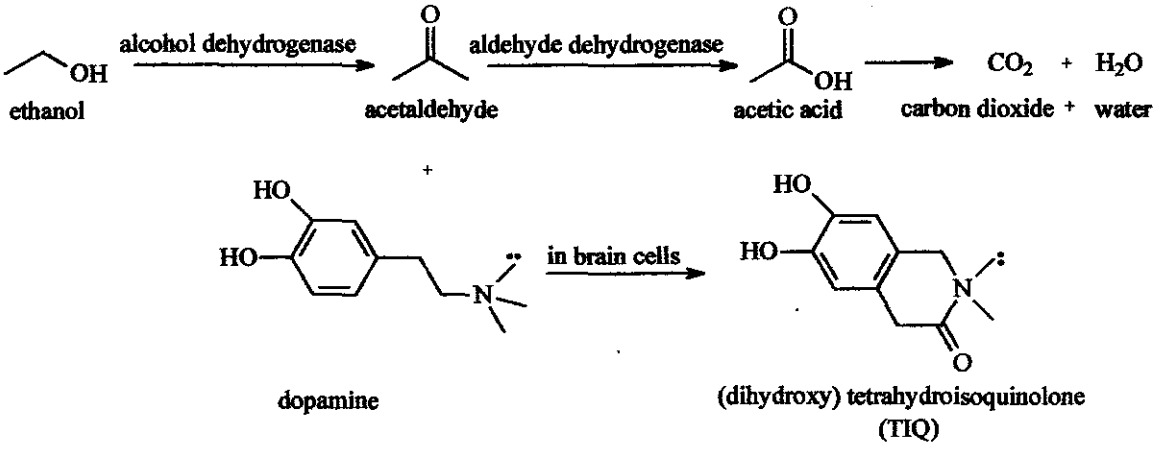


Figure 5.1. The reaction path for the dehydrogenase systems.

5.3. TYROSINE HYDROXYLASE

Tyrosine hydroxylase is the rate-limiting enzyme that catalyzes the hydroxylation of tyrosine to DOPA (see section 4.2.1.) in the synthesis of dopamine. Many drugs of abuse, including cocaine, morphine and ethanol, can alter the mRNA levels, protein levels or activity of tyrosine hydroxylase (TH) in the mesolimbic dopamine system (Ortiz *et al.*, 1995). Nicotine increases not only the protein level of TH but also the mRNA level of the TH gene in rat brain (Ishiguro *et al.*, 1997). Like acute cocaine administration, acute administration of nicotine can increase TH activity in the Nacc (Arinami *et al.*, 2000). In addition, chronic administration of nicotine can increase TH mRNA levels and activity in the locus coeruleus. These changes in the activity and levels of the rate-limiting biosynthetic enzyme for catecholamines are likely to be common markers for the development of dependence for drugs of abuse (Picciotto, 1998 and Jain & Mukherjee, 2003).

CHAPTER 6

PHARMACOLOGICAL TREATMENT OF TOBACCO ADDICTION

6.1. INTRODUCTION

Nicotine is a substance that causes physiological dependency on tobacco products that contain not only nicotine but also other harmful substances. There are nearly 1.1 billion users of nicotine and tobacco products worldwide. Although some cigarette smokers are able to quit, many are not, and standard medications to assist in smoking cessation are ineffective in many remaining smokers (George & O'Malley, 2004). Nevertheless, the goal of pharmacological intervention in tobacco dependence is to control the uncomfortable withdrawal symptoms (Froelicher & Kozuki, 2002).

6.2. CURRENT PHARMACOLOGICAL TREATMENTS FOR TOBACCO ADDICTION

There are at present two effective drugs for treating tobacco dependence: bupropion and nicotine (Lillington *et al.*, 2000) (See the structure of nicotine in section 3.1.). The treatment of smoking cessation has focused on the alleviation of the abstinence syndrome, which is often experienced when smokers first stop smoking. Nicotine substitution therapy is being used as the primary pharmacological intervention, but the success rates have been disappointingly low (Balfour *et al.*, 2000). Bupropion is the first and currently the only non-nicotine agent FDA-approved for smoking cessation (Nielsen & Fiore, 2000). Bupropion is an antidepressant but has also been approved as an aid to smoking cessation under the brand name of Zyban® (Fang *et al.*, 2000).

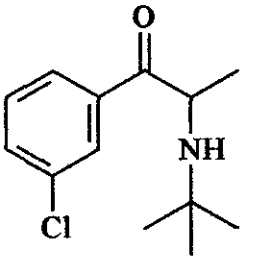


Figure 6.1. The chemical structure of bupropion (Fang *et al.*, 2000).

According to Walton *et al.* (2001), only a small proportion of individuals (20 %) respond to the best treatments that are currently available to aid long-term smoking cessation. See table 6.1 for current pharmacological treatments of nicotine dependence.

Table 6.1. Current pharmacotherapies for nicotine dependence (George & O'Malley, 2004).

Medication	**Clinical and pharmacological mechanisms**
Nicotine patch Nicotine gum Nicotine lozenge	Nicotine replacement therapy (NRT): reduces nicotine craving and withdrawal
Nicotine nasal spray Nicotine vapour inhaler	NRT: nACh receptor stimulation rapidly reduces nicotine craving and withdrawal symptoms
Bupropion hydrochloride (sustained-release)	Blocks re-uptake of DA and NA; high affinity, non-competitive nACh receptor antagonist reduces nicotine reinforcement, withdrawal and craving
Clonidine	α_2-Adrenoceptor agonist reduces nicotine withdrawal symptoms
Tricyclic antidepressants (nortriptyline, doxepin)	Blocks re-uptake of NA and 5-HT; probably reduces withdrawal symptoms and co-morbid depressive symptoms
Mecamylamine	Non-competitive, high-affinity nACh receptor antagonist combined with transdermal nicotine patch (TNP) reduces nicotine reinforcement, withdrawal and craving
Naltrexone	Endogenous mμ opioid peptide receptor antagonist appears to reduce nicotine craving and withdrawal in combination with TNP
Buspirone	Partial agonist of 5-HT_{1A} receptors reduces 5-HT release; might be effective in smokers with co-morbid anxiety symptoms
Moclobemide	Reversible MAO-A inhibitor increases NA and 5-HT levels; might be helpful for smokers with co-morbid mood disorders
Selegiline	Irreversible MAO-B inhibitor increases synaptic DA levels; might reduce nicotine reinforcement, withdrawal and craving

6.3. NICOTINAMIDE ADENINE DINUCLEOTIDE (NAD)

Nicotinamide adenine dinucleotide (NAD) is a ubiquitous biological molecule that participates in many metabolic reactions and is known chemically as 3-carbamyl-1-b-D-ribofuranolyl-pyridinium hydroxide, 5'-ester with adenosine-5'-pyrophosphate inner salt. NAD is also known as diphosphopyridine nucleotide (DPN), nadide and codehydrogenase I (National institute for occupational safety and health, 1997). The structure is as follows:

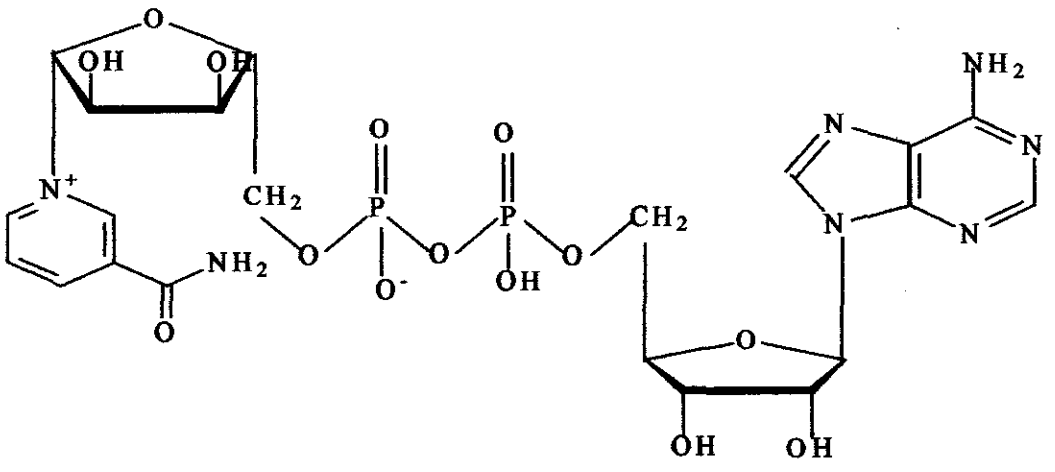


Figure 6.1. The chemical structure of NAD.

NAD is one of the biologically active forms of nicotinic acid. NAD is distributed normally throughout the body tissues and the highest concentration is usually in the liver. It occurs in living cells primarily in the oxidized state. The main function of NAD is to act as a hydrogen carrier in anaerobic and aerobic oxidation and fermentation processes throughout the body. NAD serves as a coenzyme of the dehydrogenases, especially in the dehydrogenation of primary and secondary alcohols. The toxic metabolites of alcohol, such as acetaldehyde and aceto-acetic acid, are therefore removed from the nervous system (Alclin, 2003).

This coenzyme plays a central role in the regulation of energy metabolism in the cells. The more energy a cell needs, the more NADH it needs. Electrons are not transferred directly from fuel molecules (glucose, fatty acids), but instead these substrates transfer electrons to special carriers, which are either pyridine nucleotides (NAD) or flavins (FAD). The reduced forms of these carriers then transfer their high-potential

electrons to O_2 by means of a mitochondrial electron transport chain. ATP (adenosine triphosphate) is synthesized from ADP (adenosine diphosphate) and phosphorous by a process known as oxidative phosphorylation. NAD^+ is a major electron acceptor in the oxidation of fuel molecules, the reactive part being its nicotinamide ring (see section 6.3.1.).

NAD is converted to NADH ($NAD^+ + H^+ + 2e^- \longrightarrow$ NADH) mostly in catabolic reactions including glycolysis and the tricarboxylic acid (Krebs) cycle. The NAD : NADH ratio plays an important role in regulating the intracellular redox state (Lin & Guarente, 2003). The main purpose of the citric acid cycle is to convert NAD to NADH (including FAD to $FADH_2$), which then drives its energy potential to produce ATP via oxidative phosphorylation (Wan *et al.*, 1999 and Maiese & Chong, 2003).

NAD is synthesised via two major pathways in both prokaryotic and eukaryotic systems. In one pathway, NAD is synthesised from tryptophan and in the other pathway NAD is generated by recycling degraded NAD products such as nicotinamide (Lin & Guarente, 2003).

NAD is currently indicated for detoxification in the excessive use of alcohol, amphetamines, opiates and other analgesics. It is also indicated for the prevention, alleviation and removal of the acute and chronic symptoms associated with alcoholism and drug addiction (Alclin, 2003).

NADH stimulates tyrosine hydroxylase and dopamine biosynthesis in tissue culture and humans (Swerdlow, 1998). Niacin (vitamin B3) is a cofactor for the conversion of the amino acids tryptophan and tyrosine to serotonin and the catecholamines respectively (Gant, 2000). NAD is one of the biologically active forms of nicotinic acid (Alclin, 2003). Nicotinic acid (a form of vitamin B3) oxidizes alcohol to reduce acetaldehyde levels, thus reducing oxidative stress and the formation of tetrahydroisoquinolone (TIQ) (Gant, 2000).

6.3.1. The electron transport chain (ETC)

The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are integral proteins with prosthetic groups capable of accepting and donating either one or two electrons (table 6.2). Three types of electron transfers occur in oxidative phosphorylation: (1) direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+}; (2) transfer as a hydrogen atom ($H^+ + e^-$); and (3) transfer as a hydride ion (H^-), which bears two electrons.

Table 6.2. Protein components of the mitochondrial electron transfer chain (Nelson & Cox, 2000 and Greenstein & Greenstein, 1996 and Stryer, 1995).

Enzyme complex	**Prosthetic groups**
I NADH dehydrogenase	Flavin mononucleotide (FMN), Fe-S
II Succinate dehydrogenase	Flavin adenine dinucleotide (FAD), Fe-S
III Ubiquinone: cytochrome c oxidoreductase	Hemes, Fe-S
Cytochrome c*	Heme
IV Cytochrome oxidase	Hemes, Cu_A, Cu_B

*Cytochrome c is not part of an enzyme complex; it moves between Complexes III and IV as a freely soluble protein.

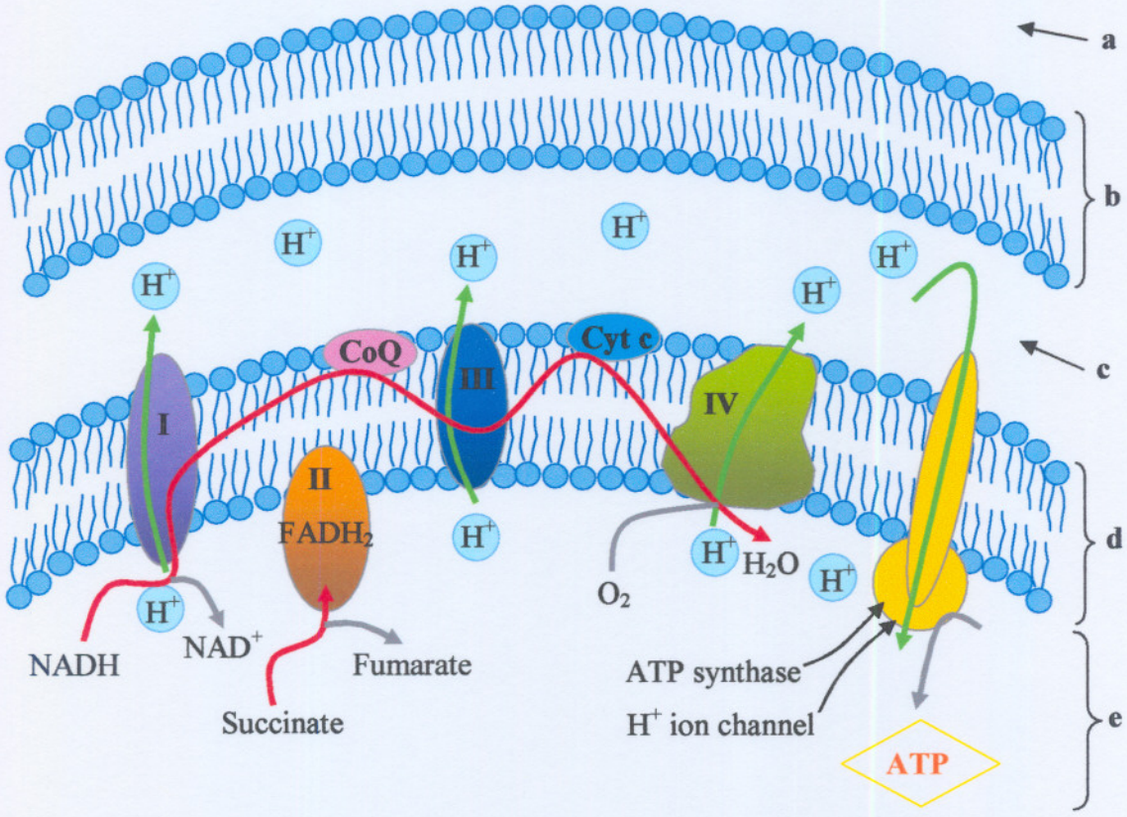


Figure 6.2. The electron transport chain. **(a)** Cytosol, **(b)** outer mitochondrial membrane, **(c)** intermembrane space, **(d)** inner mitochondrial membrane and **(e)** mitochondrial matrix.

Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II). Complex III carries electrons from ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O_2 (Nelson & Cox, 2000 and Stryer, 1995).

6.3.1.1. Complex I: NADH to Ubiquinone

Complex I is also known as NADH:Ubiquinone oxidoreductase. It is a large enzyme composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least six iron-sulfur (Fe-S) centres. Complex I catalyze two simultaneous and obligately coupled processes: (1) the transfer of a hydride ion from NADH and a proton from the matrix to ubiquinone:

$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2$$

and (2) the transfer of four protons from the matrix to the intermembrane space. Complex I is therefore a proton pump driven by the energy of electron transfer. The overall reaction is:

$$NADH + 5H^+ + Q \longrightarrow NAD^+ + QH_2 + 4H^+$$

Ubiquinol (QH_2) diffuses in the mitochondrial inner membrane from Complex I to Complex III, where it is oxidised to ubiquinone (Q) in a process that also involves outward movement of H^+ (Nelson & Cox, 2000 and Stryer, 1995).

6.3.1.2. Complex II: Succinate to Ubiquinone

Complex II is also known as succinate dehydrogenase. It contains two types of prosthetic groups and at least four different proteins. One protein has a covalently bound FAD and a Fe-S centre with four Fe atoms; a second iron-sulfur protein is also present. Electrons pass from succinate to FAD, then through the Fe-S centres to ubiquinone (Nelson & Cox, 2000 and Stryer, 1995).

6.3.1.3. Complex III: Ubiquinone to Cytochrome c

Complex III is also known as cytochrome bc_1 complex or ubiquinone:cytochrome c oxidoreductase. This complex couples the transfer of electrons from ubiquinol (QH_2) to cytochrome c with the vectorial transport of protons from the matrix to the intermembrane space of the mitochondrion (Nelson & Cox, 2000 and Greenstein & Greenstein, 1996). The overall reaction is:

$$QH_2 + 2\ cyt\ c_{1(oxidised)} + 2H^+ \longrightarrow Q + 2\ cyt\ c_{1(reduced)} + 4H^+$$

Cytochrome c is a soluble protein of the intermembrane space (Stryer, 1995). After its single heme accepts an electron from Complex III, cytochrome c moves to Complex IV to donate the electron to a binuclear copper centre in that enzyme (Nelson & Cox, 2000).

6.3.1.4. Complex IV: Cytochrome c to O_2

Complex IV is also known as cytochrome oxidase. It carries electrons from cytochrome c to molecular oxygen, reducing it to H_2O (Nelson & Cox, 2000 and Greenstein & Greenstein, 1996). Complex IV is a large enzyme (13 subunits) of the inner mitochondrial membrane.

Three subunits are critical to the function of Complex IV. Mitochondrial subunit II contains two Cu ions complexed with the –SH groups of two cysteine residues in a binuclear centre (called Cu_A) that resembles the 2Fe-2S centres of iron-sulfur proteins. Subunit I contains two heme groups, designated a and a_3, and another copper ion (Cu_B). Heme a_3 and Cu_B form a second binuclear centre that accepts electrons from heme a and transfers them to O_2 bound to heme a_3 (Nelson & Cox, 2000).

Electron transfer through Complex IV is from cytochrome c to the Cu_A centre, to heme a, to the heme a_3-Cu_B centre and finally to O_2. For every four electrons that pass through this complex, the enzyme consumes four H^+ from the matrix in converting O_2 to $2H_2O$ (Nelson & Cox, 2000 and Stryer, 1995). It also uses the energy of this redox reaction to pump one proton outward into the intermembrane space for each electron that passes through. The overall reaction catalyzed by Complex IV is:

$$4\ \text{cyt}\ c_{(\text{reduced})} + 8H^+ + O_2 \longrightarrow 4\ \text{cyt}\ c_{(\text{oxidised})} + 4H^+ + 2H_2O$$

This four-electron reduction of O_2 involves redox centres that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as hydrogen peroxide or hydroxyl free radicals (very reactive species that would damage cellular components). The intermediates remain tightly bound to the complex until completely converted to water (Nelson & Cox, 2000 and Stryer, 1995).

6.3.1.5. ATP synthesis

The only way for H^+ to cross the inner mitochondrial membrane is via a proton channel (F_0) which is coupled to ATP synthase. The ATP synthase enzyme is activated by the passage of H^+ and catalyses the phosphorylation of ADP (adenosine

diphosphate) to ATP (adenosine triphosphate) with the passage of three protons through the channel (Greenstein & Greenstein, 1996 and Nelson & Cox, 2000 and Stryer, 1995). The equation for ATP synthesis is:

$$ADP + P + H^+ \longrightarrow ATP + H_2O + H^+$$

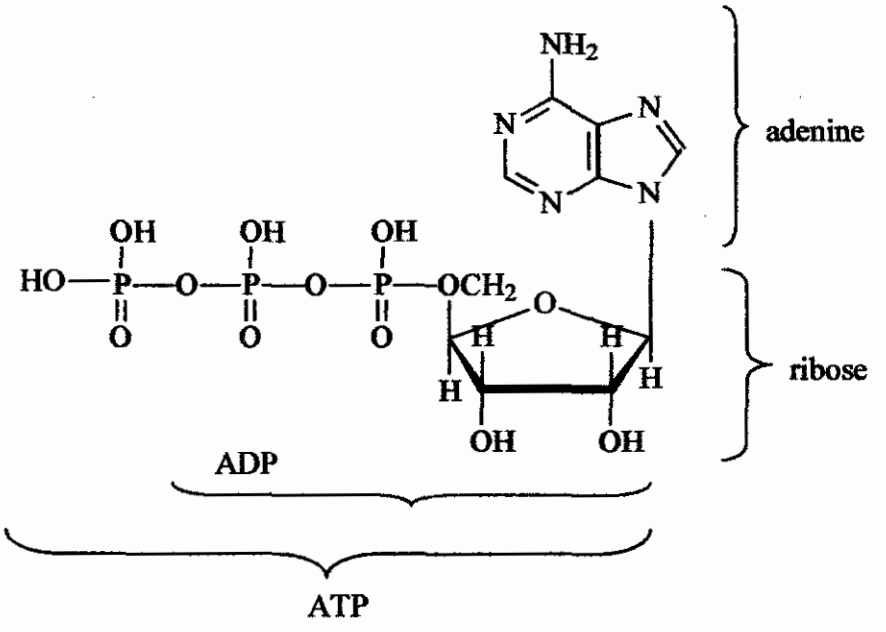


Figure 6.2. The structure of ATP (adenosine triphosphate).

CHAPTER 7

SINGLE CELL GEL ELECTROPHORESIS ASSAY

7.1. INTRODUCTION

A number of techniques for detecting DNA damage, as opposed to the biological effects (e.g., micronuclei, mutations, structural chromosomal aberrations) that result from DNA damage, have been used to identify substances with genotoxic activity. The Comet assay, also called the single cell gel electrophoresis (SCGE) assay or microgel electrophoresis (MGE) assay, primarily measures DNA strand breakage in single cells.

In 1984, Ostling and Johanson described a microelectrophoretic procedure for the direct visualization of DNA damage in individual cells. Mammalian cells were suspended in a thin agarose gel on a microscope slide and were lysed by detergents and salts at high concentrations, electrophoresed under pH neutral conditions and stained with a fluorescent DNA binding dye. This version of the comet assay, however, detects only double strand DNA breaks, which are characteristic effects of radiation and radiomemetic agents (Faust *et al.*, 2004). The continuity of the double-stranded DNA is not affected by occasional single-strand breaks (SSB) at neutral pH. This original neutral method appeared to be sensitive to measure changes in DNA supercoiling that resulted from single-strand breaks, but the lysis conditions were ineffective in removing all proteins (Cotelle & Férard, 1999 and Tice *et al.*, 2000). One of the goals of the microgel electrophoresis assay is to strip all proteins from DNA before it is electrophoresed in alkaline or neutral environment. This is essential in order to free the smaller broken pieces of DNA to move in an electric field (Singh, 2000).

Subsequently, in 1988 Singh and co-workers introduced a microgel technique involving electrophoresis under alkaline (pH >13) conditions for detecting DNA damage in single cells. This is the most frequently used version of the comet assay and detects DNA single-strand breaks (SSB), alkali-labile sites (ALS) and DNA

cross-linking in individual cells. (Tice *et al.*, 2000 and Cotelle & Férard, 1999 and Kassie *et al.*, 2000).

During electrophoresis, the broken and relaxed DNA fragments migrate further than the nucleus toward the anode. Normally low molecular weight DNA moves farther in electric current in agarose matrix. The resulting images, which were subsequently named for their appearance as comets, are measured to determine the extent of DNA damage (Singh, 2000). The sensitivity of the alkaline comet assay is greatly influenced by the pH of the lysis and electrophoresis buffer (Faust *et al.*, 2004).

7.2. ADVANTAGES OF THE SCGE ASSAY

The Comet assay is able to detect DNA damage induced by alkylating agents, intercalating agents and oxidative damage. Despite the fact that the Comet assay requires isolated cells of the same type, there has been increasing interest in this test in the past years, mostly because of its main advantages, sensitivity and rapidity (Cotelle & Férard, 1999). Compared to other genotoxicity assays, the advantages of the technique include:

(1) its demonstrated sensitivity for detecting low levels of DNA damage
(2) the requirement for small numbers of cells per sample
(3) flexibility
(4) low costs
(5) ease of application
(6) the ability to conduct studies using relatively small amounts of a test substance
(7) the relatively short time period (a few days) needed to complete an experiment and
(8) the collection of data at the level of the individual cell, allowing for more robust types of statistical analyses (Tice *et al.*, 2000).

7.3. THE BASIC STEPS OF THE SCGE ASSAY

At the International Workshop on Genotoxicity Test Procedures held in Washington, DC, March 25-26, 1999, an expert panel met to develop guidelines for the use of the single-cell gel (SCG)/Comet assay in genetic toxicology. The first consensus decision of the expert panel was that, in terms of a testing strategy for genetic toxicology, the alkaline (pH>13) version of the Comet assay is the method of choice. Generally, DNA is denatured and unwound at pH values above 12.0 because of the disruption of hydrogen bonds between double-stranded DNA. At pH conditions of 12.6 or higher, ALS (e.g., apurinic sites) are quickly transformed to strand breaks. A pH of >13 would be expected to maximize the expression of ALS as SSB. Preference for the pH>13 Comet assay does not mean that positive data obtained using other versions of the assay are not acceptable for identifying genotoxic agents. However, negative data may need to be considered with more caution (Tice *et al.*, 2000).

Once a suspension of cells is obtained, the basic steps of the assay include:

(1) preparation of microscope slides layered with cells in agarose
(2) lysis of cells to liberate DNA
(3) exposure to alkali (pH>13) to obtain single-stranded DNA and to express ALS as SSB
(4) electrophoresis under alkaline (pH>13) conditions
(5) neutralization of alkali
(6) DNA staining and comet visualization, and
(7) comet scoring (Tice *et al.*,2000).

Singh *et al.* (1994) reported that longer electrophoresis time is one of the most important factors that increase the sensitivity of the comet assay. The voltage and time of electrophoresis is related to the levels of damage to be detected. Comet data can be collected either by visual scoring or by using computer automated image analysis systems. Generally, cells with a high level of DNA damage exhibit increased comet parameters, which may be expressed as tail length, %DNA in the tail and tail moment (tail length x %DNA in the tail) or simply as high, medium and low damage (Faust *et al.*, 2004).

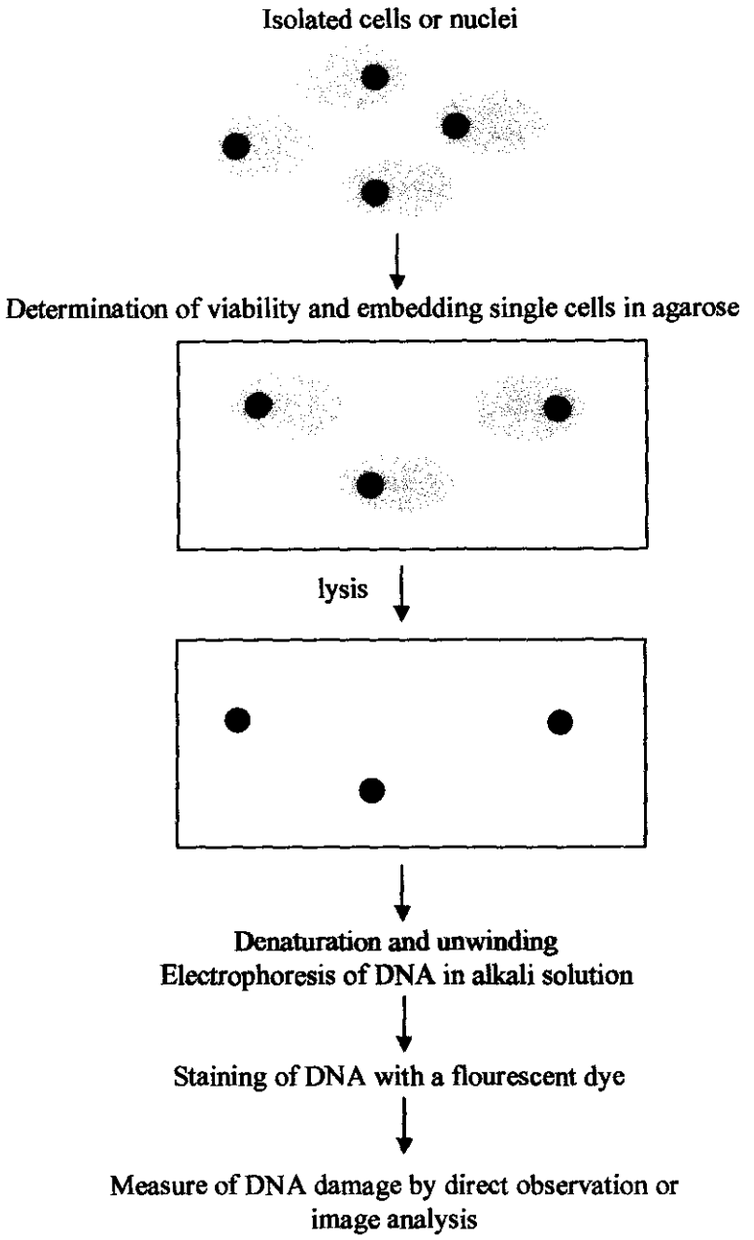


Figure 7.1. Procedure of the Comet assay (Singh *et al.*, 1988).

It is not appropiate to use this test when there is evidence that the test substance will not reach the target tissue. In conducting *in vivo* comet studies, care should be taken to avoid conditions that would lead to positive results that do not reflect genotoxicity but may arise from DNA damage associated with apoptosis or necrosis. The comet assay is capable of detecting various kinds of DNA damages (e.g., apoptosis, necrosis and alkali-labile sites as single-stranded DNA breaks) with high sensitivity, if the method is optimized (Tice *et al.*, 2000).

7.4. APOPTOSIS AND NECROSIS

Apoptosis (Ptosis=tosis=dropping off, *Greek*) or 'programmed cell death' in the tissues of an organism, is not associated with inflammation or scarring, unlike necrosis (*Greek*=dead). Apoptosis is a normal event that occurs both during and after development. Mild to moderate genotoxic and cytotoxic insults also induce apoptosis (Singh, 2000). Apoptosis is characterized by cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation and formation of apoptotic bodies (Carnevali *et al.*, 2003).

Apoptosis is generally considered an energy-dependent process requiring active participation of many proteins and other cellular macromolecules. Apoptosis seems to be induced by mild genotoxic stimuli, as the strength of stimuli increase the cell death mode shifts to necrosis (Singh, 2000). Apoptosis results in the extensive formation of DSB. Similarly, the DNA of necrotic cells also undergoes extensive degradation due to the induction of DSB. Such cells can be detected using either neutral or alkaline electrophoretic conditions.

Apoptic cells can be readily distinguished from necrotic cells in the alkaline SCG assay. Apoptic cells form comets with large fan-like tails and small heads (i.e., so-called hedgehogs), while necrotic cells form comets with relatively large heads and narrow tails of varying lengths (i.e., comets indistinguishable from those resulting from genotoxic damage) (Tice *et al.*, 2000).

7.5. THE EFFECT OF TOBACCO ON DNA DAMAGE

Among the different toxic effects of cigarette smoke on human tissues, oxidation of structural and functional molecules and modulation of cell turnover play a major role (Carnevali *et al.*, 2003).

According to Kassie *et al.* (2000) the effect of tobacco/cigarette smoking on DNA damage was investigated under two circumstances: either as a sole genotoxic factor or confounding factor. The habit of tobacco/cigarette smoking itself was found to

cause a significant increase in DNA strand breakage in blood cells. However, the extent of damage did not correlate either with the number of cigarettes smoked per day or with the condensate tar content. In one study in which DNA damage was monitored in newborn babies of smoking mothers, significant differences were not observed between the two groups.

Although cigarette smokers usually show higher genotoxic responses to mutagenic agents than non-smoking co-workers, the confounding effect of cigarette smoking in biomonitoring for genotoxic effects was found to be inconsistent (Kassie *et al.*, 2000). According to Faust and co-workers (2004) who reviewed 29 studies on the effect of tobacco smoking on DNA damage, nine reports found a significant relationship between DNA damage and the habit of tobacco smoking, whereas 16 reports did not find any association between tobacco smoking and a significant increase in DNA damage in blood cells. In four studies where people was exposed to a genotoxic agent significant DNA damage was only found in smokers, indicating an additive or synergistic effect between the genotoxic agent and tobacco constituents. Faust and co-workers (2004) also gave some reasons for what they think the discrepancies in the results obtained with smokers could be: differences in the brand of cigarettes smoked, absence of data on the number of cigarettes smoked per day or the amount of tar contained, genetic polymorphism of enzymes that metabolise genotoxins contained in tobacco and the slightly higher level of DNA damage among smokers compared to non-smokers which might be missed when the statistical power is weak.

CHAPTER 8

METHODS TO MEASURE WITHDRAWAL

8.1. LOCOMOTOR ACTIVITY

Locomotor activity is widely used to study nicotine's behavioural actions, especially psychomotor stimulant actions, in rodents. Horizontal activity has been interpreted to reflect general arousal and vertical activity is thought to indicate exploration (Faraday *et al.*, 2003).

Acute doses of nicotine can cause small increases in locomotor activity, but the stimulant effects are much greater after a period of chronic administration when there is sensitization to this response. The stimulant effects are mediated by activation of postsynaptic DA receptors in the Nacc (Olausson *et al.*, 2001 and Seth *et al.*, 2002).

In an experiment done by Suemaru and co-workers (1992), rats were chronically exposed to cigarette smoke for 20 minutes twice daily using a smoking machine. On days 1, 4 and 14, locomotor activity and rearing were measured for 15 minutes in an open-field apparatus. On day 1, exposure to cigarette smoke increased locomotor activity and rearing. This effect became more pronounced on days 4 and 14. Chronic cigarette smoke exposure for 21 days significantly decreased the norepinephrine levels in the hypothalamus, thalamus and pons medulla, but not the levels of DA, 5-HT, or their metabolites. These results suggest that repeated cigarette smoke exposure increasingly stimulates locomotor activity and rearing.

8.2. ELEVATED PLUS-MAZE

The elevated plus-maze (EPM) is a widely used behavioural paradigm that presumably measures fear-motivated avoidance behaviour and which has been extensively assessed as a model of anxiety (Hogg, 1996).

The EPM is an apparatus that is elevated above the floor with a small center area and four arms (in the shape of a plus sign). Two opposite arms are enclosed with side walls and the other two opposite arms have no walls. The rat is allowed to freely explore the maze (Olausson *et al.*, 2002).

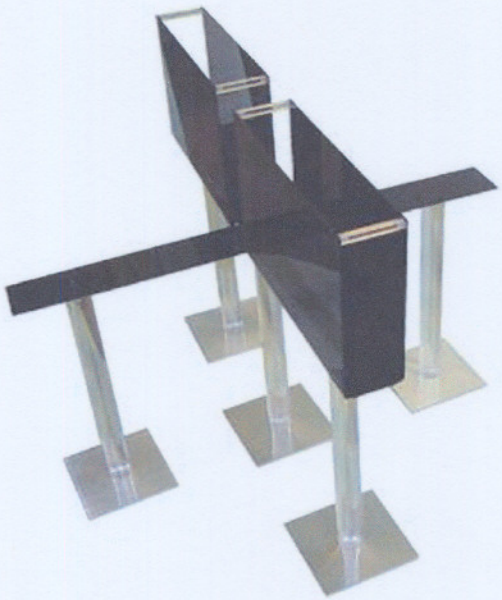


Figure 8.1. The elevated plus-maze (Coulbourn Instruments, 2004).

The critical determinants which are considered to be correlated with anxiety are the entries made onto the open arms and the time spent on these arms. Expression of the open arm data as percentages of the total number of arm entries or total time spent on either the open or closed arms corrects for overall changes in exploration of the maze (Hogg, 1996).

When rodents are introduced into a novel environment, they tend to move around the perimeter of the environment. They stop occasionally and rear up, sniffing the walls and the floor. At first, they spend very little time in the open center of the area. If they have a choice, they will spend more time in a dark area than in a brightly lit one. They will spend more time in a small, elevated area enclosed by walls than in an elevated area without walls. Anxiolytics usually increase the amount of time an animal spends in open, lighted areas away from walls (the open arms of the EPM) and the tendency to stay in the closed arms of the maze can be enhanced by compounds that increase the aversion towards the anxiety-provoking open arms, for example, anxiogenics (Olausson *et al.*, 2002 and Flint, 2003 and Hogg, 1996).

The EPM is currently being employed by a large number of investigators and aside from those who have published freely are the pharmaceutical companies who use it as a first screen for compounds with anxiolytic potential. While the behavioural profiles of compounds acting at the GABA/benzodiazepine receptor complex are seen to produce consistent and reproducible data, this is not the case for all putative anxiolytic or anxiogenic compounds (Hogg, 1996).

Studies of smokers have shown that nicotine can reduce anxiety and relieve stress (Gilbert *et al.*, 1989). According to Picciotto (1998) nicotine has anxiolytic-like actions in rodents in several different behavioural tests including the mirrored chamber, the EPM and fear-potentiated startle. Picciotto (1998) also suggests that nicotine can only relieve the anxiety caused by withdrawal in smokers, rather than having anxiolytic properties on its own. Increased anxiety has been reported on withdrawal from nicotine in animal tests, in smokers and in those withdrawing from nicotine gum (Irvine *et al.*, 2001).

Research with selectively bred Wistar rats and outbred Wistar rats has shown that these animals, although identical in strain, sex and age, can differ systematically in anxiety-like behaviour in the EPM. These behavioural differences were related to the neurotransmitter serotonin in the ventral striatum, a brain region which is critical for motivated behaviour, and a transmitter which is critical for anxiety (Pawlak *et al.*, 2003).

8.3. ACOUSTIC STARTLE RESPONSE

For over 50 years, the startle reflex has been studied systematically as a means of understanding the neural control of behaviour. A major advantage of startle response paradigms is that the same phenomena can be studied across species. In humans, the blink reflex component of the startle response elicited by acoustic (noise bursts) or tactile (air puff) stimuli is measured using electromyography of the orbicularis oculi muscle. In rats, a stabilimeter chamber measures the whole body flinch elicited by acoustic or tactile stimuli similar to those used in humans (Geyer & Swerdlow, 1998).

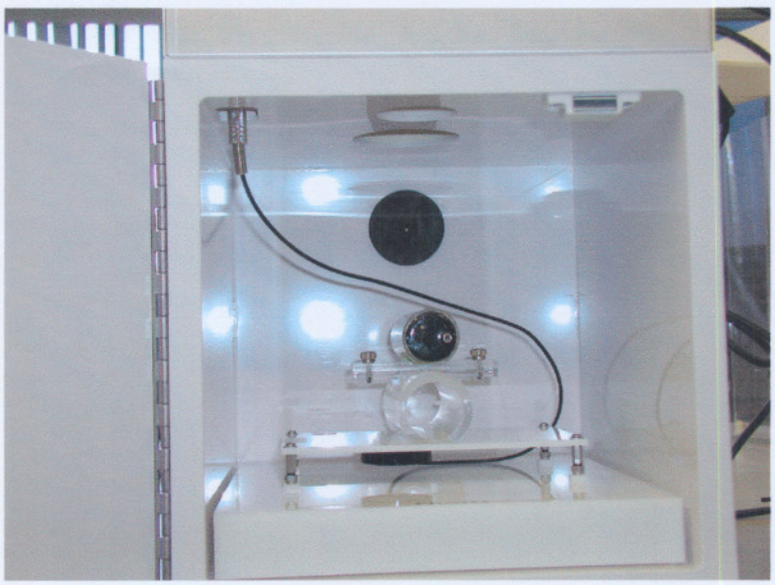


Figure 8.2. The startle chamber.

According to Duncan and co-workers (2001) the acoustic startle response (ASR) is a well characterized reflex response which is mediated by a simple three-synapse subcortical circuit. Different models have also been proposed using four or five synaptic relays and all investigators agreed on the following principle components: an initial central relay in the cochlear nuclear complex, an intermediate brain stem relay in the reticular formation, a long reticulospinal pathway via the medial longitudinal fasciculus and output via spinal cord and brain stem motor neurons (Leumann *et al.*, 2001).

Behaviourally, the startle response consists of rapid reflex contraction of head, neck, trunk and legs muscles in addition to the arrest of ongoing activity in response to an intense, abrupt stimulus (Duncan *et al.*, 2001 and Blaszczyk, 2003). The ASR is understood as a protective response to unexpected, aversive events. Thereby the eyes are covered first, the shoulders curl forward and up, the whole body length is shortened especially the dorsal neck region. At the same time the ongoing behaviour is disrupted and an acceleration of the heart rate is observed (Leumann *et al.*, 2001). Startle reactivity can be defined as the magnitude of the startle response either on initial stimulus presentation or over a number of startle trials (Geyer & Swerdlow, 1998).

Two behavioural measures dependent on DA function are the acoustic startle response (ASR) and prepulse inhibition (PPI) of the ASR. The ASR is a short-latency reflex behaviour that is elicited by a brief, intense acoustic pulse, while PPI is the reduction of the acoustic startle that is observed when the startling pulse is preceded by a weak prepulse (Vaillancourt *et al.*, 2002). Dopaminergic agents modify the ASR, with DA agonists enhancing the ASR (Bell *et al.*, 2003 and Lewis & Gould, 2003). Thus, pharmacological agents that enhance dopamine levels would be expected to enhance the startle reflex. Previous research suggests that nicotine's rewarding effects are mediated, in part, by an increase in DA release, and that presynaptic modulation of nicotinic acetylcholinergic receptors (nAChRs) can facilitate DA release and thus increase the ASR (Lewis & Gould, 2003).

The ASR can be used to examine the aversive effects of drug withdrawal. This is a defensive reflex that is increased by fear and by withdrawal from expected food reward. According to Fendt and Mucha (2001) it is still not certain whether the ASR is valid for testing drug withdrawal motivation. Alcohol and diazepam withdrawal increased the ASR as is known for aversive stimuli. In contrast, cocaine withdrawal had no effect. Nicotine and smoking withdrawal varied between an increase and no effect (Fendt & Mucha, 2001). Based on the available literature, it remains unclear how the ASR is affected by nicotine and nicotine withdrawal. According to Kenny and Markou (2001), nicotine withdrawal significantly increased the acoustic startle response in rats for approximately 4 to 5 days. In the rat, an increase (Acri *et al.*, 1995 and Faraday *et al.*, 1999), no effect (Mirza *et al.*, 2000) and a decrease (Faraday *et al.*, 1999 and Mirza *et al.*, 2000) of auditory gating has been reported for nicotine.

Acri *et al.* (1991) tested the ASR in three groups of rats: a group receiving 6 mg nicotine/kg/day, a group receiving 12 mg nicotine/kg/day and a group receiving saline via osmotic minipumps. They expressed their data as percentages calculated from the following equation:

$$\frac{\text{Nicotine group mean for day/nicotine group mean at baseline}}{\text{Saline group mean for day/saline group mean at baseline}} \times 100$$

The mean startle amplitude (using a stimulus of 98 dB) of animals receiving 12 mg/kg/day and 6 mg/kg/day nicotine was 152 % and 139 % of control, respectively, during the period of nicotine admininstration. During days 1 through to 3 of nicotine

cessation, the 12 mg/kg/day and 6 mg/kg/day groups were only slightly higher than baseline at 106 % and 105 %, respectively.

For the 124 dB stimulus, there was also a positive dose-response effect of nicotine on ASR amplitude during nicotine administration. In the first 3 days after cessation of nicotine, the startle amplitude of the 12 mg and 6 mg/kg nicotine groups decreased to 92 % of control (Acri *et al.*, 1991).

A major advantage of the startle reflex over other behavioural measures is the exquisite sensitivity of this behaviour to stimulus parameters directly controlled by the experimenter. This tight stimulus control affects each aspect of the experimental design, and the presence or absence of an effect of a specific drug or experimental manipulation under one set of conditions does not necessarily predict their presence or absence under another set of conditions (Geyer & Swerdlow, 1998).

The ASR can be elicited by pulses as short as 6 milliseconds, and further elongation of the stimulus has no effect on the magnitude of the response. It is not the duration of the stimulus but its sudden onset that is essential to elicit the ASR (Blaszczyk, 2003).

CHAPTER 9

EXPERIMENTAL PROCEDURES

This study protocol was approved and done in accordance with the guidelines stipulated by the Ethics Committee for Use of Experimental Animals at the North-West University.

9.1. ANIMALS

Male Wistar rats used in this study were bred in the Northwest University and housed in the Animal Research Centre (ARC) of the Northwest University. Male rats initially weighing 200-365 g were used. At the end of the study, the animals weighed between 270-405 g.

The rats were housed in the ARC under constant conditions of temperature (25°C) and humidity (55 ± 5%) and with a 12:12h light-darkcycle. After implantation of the Alzet osmotic mini-pump, each rat was housed in its own cage with free access to food and water.

Wistar rats were chosen for this study because different rat strains respond differently to nicotine. In a study comparing ASR in three rat strains it was noted that the response amplitude differed across strains, with the Wistar rats showing the highest values, followed by Sprague-Dawley and the Long-Evans rats (Mohammed, 2000). In an experiment done by Hildebrand *et al.* (1997), male Wistar rats were used to determine abstinence signs following withdrawal from 7 days of continuous nicotine infusion with Alzet osmotic minipumps. They found that there was a significant reduction in locomotor activity in the group withdrawn from nicotine.

9.2. PREPARATION OF TOBACCO SMOKE EXTRACT

Peter Stuyvesant cigarettes (king size, filtered) containing 15 mg tar and 1.4 mg nicotine per cigarette were used for this experiment.

A special smoking device was designed to smoke cigarettes and to trap compounds that are usually inhaled by smokers. Individual cigarettes were puffed every 30 s for a duration of 3 s. These compounds were collected in acetone (200 ml of acetone were used for sixty cigarettes) and then evaporated using a rotary evaporator to give a solid extract. After weighing the solid extract (average: 7 mg/cigarette), the extract was dissolved in propylene glycol to achieve a concentration of 80 mg/ml. This solution was used to fill the Alzet osmotic pumps.

Figure 9.1. The smoking device.

9.3. ALZET OSMOTIC PUMPS

Alzet osmotic pumps are miniature, implantable pumps used for research in laboratory animals. These infusion pumps continuously deliver drugs and other test agents at controlled rates from day one to day 28 without the need for external connections or frequent painful handling. All the rats were implanted with the Alzet pumps subcutaneously at the back of the neck during anaesthesia for systemic administration of the smoke extract solution or placebo (propylene glycol).

Table 9.1. Technical description of the Alzet osmotic pump Model 2ML4 (Alzet osmotic pump Model 2ML4 leaflet, 2000).

Pumping Rate	2.5 µl/hr (± 0.05 µl/hr)
Duration	28 days
Reservoir Volume	2000 µl

The filling of the pumps was accomplished with a small syringe and the blunt-tipped, 25 gauge filling tube that was provided with the osmotic pumps. A 0.22 µm syringe-end filter was used. The pump was held in an upright position and the filling tube was inserted through the opening at the top of the pump. Filling of the pump was stopped when the solution appeared at the outlet, the tube was then removed and the excess solution wiped off. The flow moderator was inserted into the pump and the pumps were primed by placing them in a beaker with saline for 12 hours at 25°C before implantation.

A video supplied by the Alzet company was used as guideline to implant the osmotic pumps. Vigorous hand washing with an antiseptic-containing preparation preceded the subcutaneous implantation of the osmotic pumps. Sterile gloves were worn during implantations. The skin of the rats was cleaned with 2% chlorhexidine and sterile technique was used during the implantation procedure. A small incision was made in the skin between the scapulae and a small pocket was formed by spreading the subcutaneous connective tissues apart. The pump was inserted into the pocket with the flow moderator pointing away from the incision and the skin was closed with silk stitches (Alzet osmotic pump Model 2ML4 leaflet, 2000).

The forty-eight rats were divided into six groups as shown in Figure 9.3. Each group consisted of eight rats. In groups 1, 3 and 5 (3 x 8 rats) 2ml of the smoke extract solution (SR) was administered subcutaneously via Alzet osmotic pumps. In groups 2, 4 and 6 (3 x 8 rats) 2ml of a placebo (propylene glycol) was administered subcutaneously via Alzet osmotic pumps. The pumps immediately started release of the solution upon wound closure. The rats were exposed to the smoke extract for 28 days to accomplish addiction, during which time locomotor activity was monitored. The Alzet osmotic pumps of groups 1,2,3 and 4 were removed on day 28 and groups 1 and 2 were injected with saline for four days (day 28, 29, 30 and 31), whereas groups

3 and 4 were injected with NAD for four days (day 28, 29, 30 and 31). Groups 5 and 6 were not treated with NAD or saline, but were sacrificed on day 28 to evaluate the difference in monoamine levels between smoking and non-smoking rats.

9.4. LOCOMOTOR ACTIVITY

Locomotor activity was measured for two hours during the night (21h00 to 23h00) because rodents are nocturnal animals, on days 2, 27, 29, 32 and 37. We also wanted disturbances (cleaning of cages and changing of food and water) to be kept to a minimum during the monitoring of the locomotor activity.

Horizontal and vertical activity was determined using a Digiscan Animal Activity Monitor (DAAM, AccuScan Instruments, Colombus, OH, USA). A single rat is free to move around in the cage while the locomotor activity or movement is being determined via infrared light beams. The monitor cages (42 x 42 x 30 cm) are surrounded by a series of horizontal infrared light beams (16 beams spaced 2.5 cm apart), with one set of beams at ground level and a second set 10 cm above the first. This array of infrared beams enables the computerised collection of all locomotor activity by a digital analyser that effectively determines the position of the animal 100 times/s (AccuScan Instruments Incorporated, 2000).

Figure 9.2. A locomotor cage.

Horizontal activity represents the total number of horizontal beam "breaks" that occured during the given time period, whereas vertical activity represents the total number of vertical beam "breaks" that occurred during the given time period.

The rats were allowed an acclimatization period of two hours in the locomotor cages before the measuring begun. All cumulative horizontal and vertical activity were recorded by the analyser of the DAAM at intervals of 10 minutes over a period of 120 minutes. This outomated method provides continual computerised monitoring of the animal's behaviour that is more sensitive than simple observation and, above all, is without the risks of investigator bias (Sanberg *et al.*, 1987). The test cages were cleaned thoroughly with water after every testing period.

9.5. ELEVATED PLUS-MAZE

The elevated plus-maze was used to assess anxiety. The apparatus consisted of four arms of black perspex arranged in the shape of a plus sign and elevated 50 cm from the floor. Two opposing open arms (50 x 15 cm) and two opposing enclosed arms (50 x 15 x 40 cm) extended from a common central platform (see figure 8.1).

Rats were placed individually on the centre platform and allowed to freely explore the maze for 5 minutes. Behaviour of the rat was then videotaped for 5 minutes for later scoring. The percent time spent in the open arms [(time spent in open arms / total time) x 100] was calculated. An arm entry was registered only when the rat had exited the central square and entered one of the arms with all four paws.

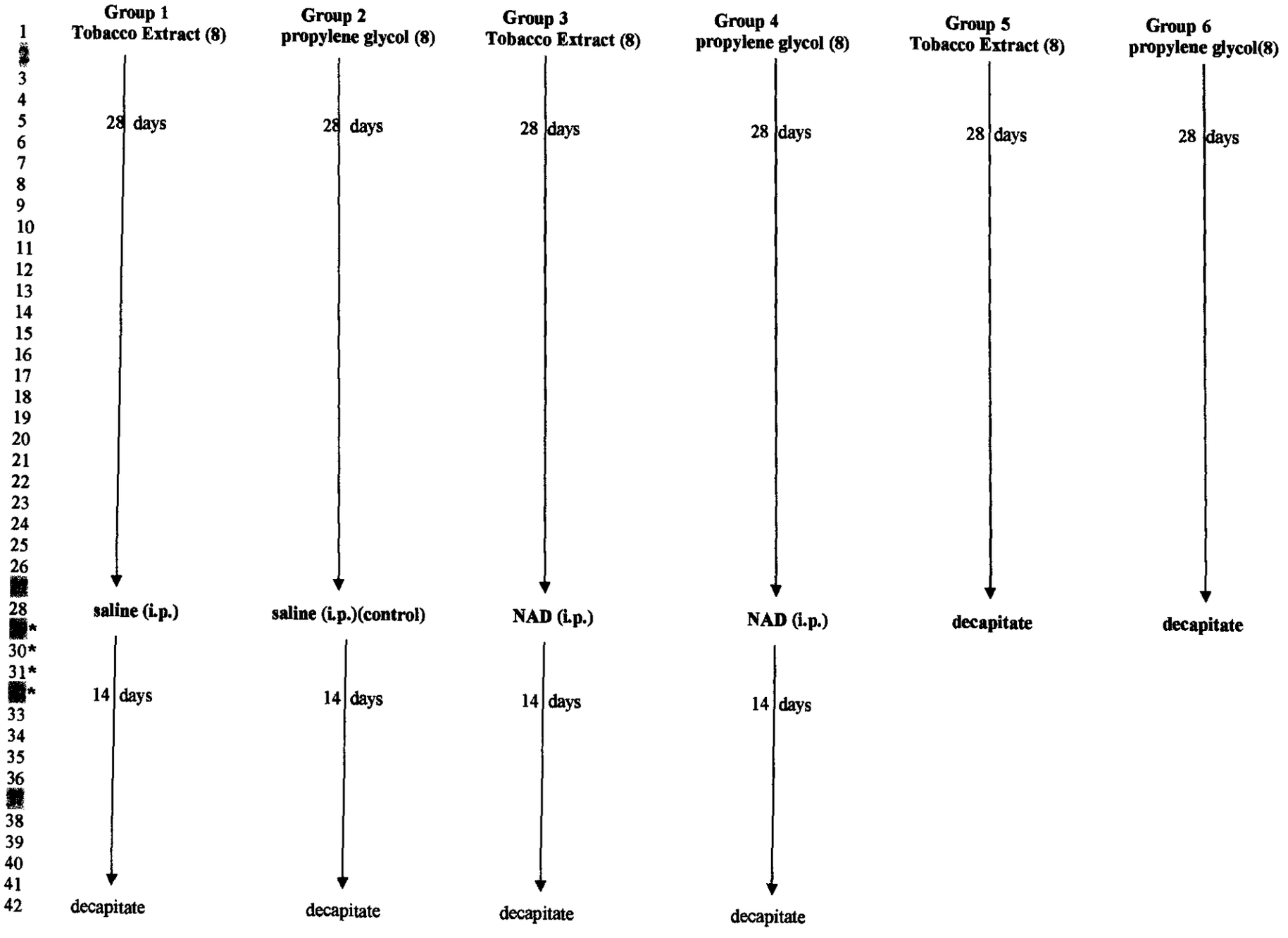


Figure 9.3. Schematic presentation of the study.

9.6. ACOUSTIC STARTLE REFLEX

Acoustic startle response was measured using a SR-LAB startle apparatus (San Diego Instruments, San Diego, CA, USA). It consisted of a clear Plexiglas chamber (8 cm diameter, 16 cm long), resting on a platform within a lit, sound-attenuated enclosure. A piezo-electric transducer detected the vibrations caused by movement of the animal and sent an analog signal to the computer. The analog measure was then digitized and stored in the computer. A speaker located in the ceiling of the chamber produced continuous background white noise at 65 dB and the required acoustic stimuli (Vaillancourt *et al.*, 2002).

The whole-body startle of the rat in response to acoustic stimuli was measured for four days (days 29, 30, 31 and 32) in 40 minute sessions. Each session consisted of 10 consecutive blocks of four stimulus intensities (95, 100, 105 and 110 dB).

Startle testing took place between 8h00 and 11h00. The startle session began with a 5 min acclimatisation period in the presence of 65 dB background noise, which continued throughout the session. After this habituation period, the 10 consecutive blocks of four stimulus intensities (95, 100, 105 and 110 dB) were delivered. Each stimulus was presented as a 50 ms burst of white noise. The inter-stimulus interval was 30 seconds. The inter-trial interval was 75 seconds. The Plexiglas chamber was cleaned after each rat was tested.

Startle amplitude was defined as the maximal peak accelerometer output voltage over a 250 ms period beginning at the onset of each startle stimulus (Winslow *et al.*, 2002).

9.7. DECAPITATION, DISSECTION AND STORAGE

On day 28 the animals in groups 5 and 6 were decapitated. The animals in groups 1, 2, 3 and 4 were decapitated on day 42.

The brains were quickly removed. The nucleus accumbens and striatum (also called the neostriatum or caudate putamen) were subsequently dissected out on ice. The

nucleus accumbens of all the rats were transferred individually to previously weighed and marked polypropylene containers, frozen with liquid nitrogen and subsequently transferred to a -86°C freezer until analysis. The striatum of all the rats were also individually transferred to polypropylene containers and kept on ice for the comet analysis which was performed directly after decapitation.

9.8. SINGLE CELL GEL ELECTROPHORESIS ASSAY (COMET ASSAY)

9.8.1. Instrumentation

Table 9.1. The istrumentation used during the Comet assay.

System	Specifics
Sandblasted microscope slides (76 x 26 mm)	RESY
Power supply unit (30 V, 300 mA)	Bio-Rad Model 200/2.0 Power supply
Fluorescent microscope	Olympus IX-70
Software	Olympus IX-70 software

9.8.2. Chemicals and reagents

Table 9.2. Chemicals and reagents used for comet assay and the suppliers of the chemicals.

Reagents	Supplier
Dimethylsulfoxide (DMSO)	Sigma
EDTA	Sigma
Ethidium bromide	Sigma
Potassium chloride (KCl)	Sigma
$(KH_2P)O_4$	Merck
Sodium chloride (NaCl)	Sigma
(Na_2HPO_4)	Merck
Sodium hydroxide (NaOH)	Sigma
High melting point agarose (HMPA)	Sigma
Low melting point agarose (LMPA)	Sigma
Triton X-100	Sigma
Tris HCl	Sigma

9.8.3. Preparation of phosphate-buffered saline (PBS)

8 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4 and 0.2 g KH_2PO_4 were dissolved in 1000 ml of double distilled water (ddH_2O) and kept in the refrigerator. The pH of this solution was between 7 and 8.

9.8.4. Preparation of lysing solution

A mixture containing 500 ml of NaCl (5 M), 250 ml of EDTA (0.4 M), 10 ml Triton X-100 and 100 ml DMSO (10 %) was prepared. The 0.4 M EDTA was titrated with 5 M NaOH to a pH of 7-8 before its use in the above solution. 140 ml of ddH_2O was added to adjust the volume of the solution to 1000 ml.

This buffer solution can be re-used for four weeks.

9.8.5. Preparation of electrophoresis buffer solution

20 ml 0.05 M EDTA was added to 500 ml of 0.6 M NaOH. 480 ml of ddH_2O was added to adjust the volume of the solution to 1000 ml.

9.8.6. Preparation of Tris HCl buffer solution

63.04 g of Tris HCl was dissolved in 500 ml of ddH_2O. The solution was then stored in the refrigerator at 4°C for at least an hour before the pH was adjusted, because of the buffer's temperature gradient.

After an hour, the pH of the solution was adjusted to 7.5 with 0.6 M NaOH (about 65-70 ml NaOH was added). ddH_2O was then added to adjust the volume of the buffer solution to 1000 ml.

The Tris HCl buffer solution was freshly prepared on each day.

9.8.7. Preparation of ethidiumbromide dye solution

0.005 g of ethidiumbromide was dissolved in 1000 ml of ddH_2O. The solution was kept in the refrigerator at 4°C for at least an hour before it was used. The container was covered with foil. Ethidiumbromide dye solution is carcinogenic and should only be handled if you are wearing gloves and a mask.

9.8.8. Preparation of high melting point agarose (HMPA)

0.5 g of HMPA was dissolved in 50 ml EDTA (0.1 M). The solution was heated until all the HMPA was dissolved.

9.8.9. Preparation of low melting point agarose (LMPA)

A 0.5 % solution was prepared: 0.25 g LMPA was dissolved in 50 ml of EDTA. The solution was heated until all the LMPA was dissolved and then kept in a water bath at 42°C.

9.8.10. Method for placing the rat striatum cells on the glass micro plates

Directly after decapitation the striatum of all the rats were individually transferred to polypropylene containers and kept on ice. A small volume of PBS was added to the polypropylene container and the striatum of each rat was minced and kept on ice for 10 minutes. The polypropylene container with the minced striatum was then centrifuged for about 10 seconds and the clear supernatant was removed with a Pasteur pipette and transferred to another polypropylene container. 250 µl PBS was added to the fluid and it was again centrifuged for 10 seconds. All the clear fluid was removed with a Pasteur pipette and thrown away. 250 µl PBS was added to the cells that were left in the polypropylene container and it was vortexed. 50 µl of the cell and PBS mixture was then added to 150 µl of LMPA. The mixture was quickly vortexed and 130 µl of the cell and LMPA mixture was spread on a micro plate.

The micro plates were soaked in electrophoresis buffer for 30 minutes. The apparatus was then turned on and electrophoresis took place for one hour at 30 Volt and 300 mA.

After electrophoresis the micro plates were soaked in Tris HCl buffer for 15 minutes and subsequently washed with ddH_2O. The plates were then soaked in ethidiumbromide dye for 15 minutes and transferred to the fluorescene microscope.

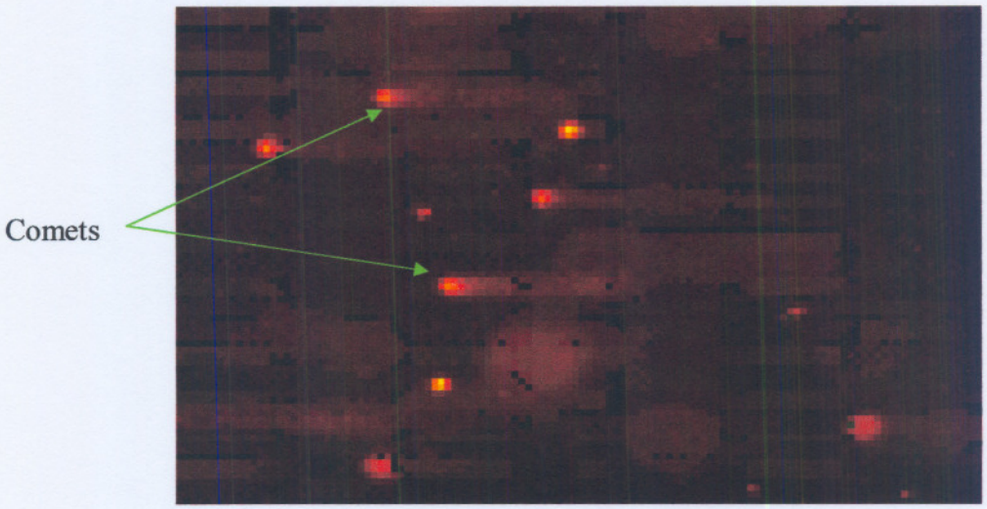


Figure 9.4. A microscope slide with comets under a fluorescene microscope.

9.8.11. Comet scoring

The computer program, CASP, was used to score the comets. Two parameters were used to describe the DNA damage: Tail DNA% (% of DNA that migrated from the head of the cell) and Tail Moment (%DNA x Tail length).

9.9. DETERMINATION OF CATECHOLAMINES

The quantification of monoamines and their metabolites in the nucleus accumbens was performed by high-performance liquid chromatography with electrochemical detection (HPLC-EC). This method is commonly used for the quantification of monoamines and is both selective and sensitive (Brand, 1996). The biogenic amines, dopamine (DA), noradrenaline (NA), serotonin (5-HT) and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyphenylacetic acid (5-HIAA) and vanillylmandelic acid (VMA) were

determined. The method performed was a modified version of the HPLC-EC method done by Brand (1996).

9.9.1. Chemicals and reagents

All the chemicals and reagents used for HPLC analyses were of HPLC grade. Distilled deionized water was used in the preparation of all the buffers and mobile phase. See table 9.3.

Table 9.3. Chemicals and reagents used for HPLC analyses and the suppliers of the chemicals.

Chemical or reagent	**Supplier**
Noradrenalinebitartrate-monohydrate	Merck
Dihydroxyphenylacetic acid (DOPAC)	Aldrich
5-Hydroxy-indoleacetic acid (5-HIAA)	Aldrich
Dopamine hydrochloride	Aldrich
Homovanillic acid	Aldrich
Serotonine creatininesulphate	Merck
Isopropyl-DL-noradrenaline hydrochloride (isoprenaline)	Aldrich
Methanol (MeOH)	Merck
Acetonitrile	BDH Licrosolv
Sodium hydroxide (NaOH)	Merck
Formic acid (HCOOH)	Sigma-Aldrich
Sodium heptanesulphonic acid monohydrate	Merck
Disodiumedethate (Na_2EDTA)	Merck
Perchloric acid ($HClO_4$) 70%	Merck
Sodium methabisulphite ($Na_2S_2O_3$)	Merck

9.9.2. Instrumentation

The analysis was done on a model LC-304 Bioanalytical system (BAS). Experimental conditions are summarised in table 9.4.

Table 9.4. Analytical conditions in the HPLC analysis of striatal monoamines.

Column	75 × 4.6 mm C18 Luna column (reversed phase, particle size 5 μm).
Detector	GBC LC 1260 electrochemical detector. The glassy carbon working electrode was set at an applied potential of + 0.70 V vs. Ag/AgCl as reference electrode. Sensitivity of electrode was set at 2 ηA/V.
Pump	GBC LC 1120 pump. Operating pressure, 2500 PSI. 50 μl loop Rheodyne 7725 injector for manual injection.
Flow rate	1.2 ml/min
Temperature	Room temperature was constant between 22-24°C.
Integrator	Spectra-Physics (SP-4290). Chart speed 0.5 cm/min and attenuation was set at 4.
Guard-column	95 mm × 4 mm guard-column (SecurityGuard, HPLC Guard cartridge system, Phenomenex), packed with HC Pellosil ® (High capacity silica gel bonded to 30-38 μm glass bead).

9.9.3. Mobile phase

The mobile phase consisted of 0.1 M sodium formate buffer, 0.5 M ethylenediaminetetracetic acid (EDTA), 5 mM sodium heptane sulphonic acid, 6 % v/v methanol and 4 % v/v acetonitrile. The EDTA forms complexes with any free metallic ions in the samples, preventing it from interfering with detection. The pH of the buffer was adjusted to 3 by the addition of 6 ml of concentrated phosphoric acid (H_3PO_4) to 1000 ml of mobile phase. Before use, the mobile phase was filtered through a 0.22 μm Millipore filter. The only variation from the method described by Brand (1996) was that we used phosphoric acid to adjust the pH of the buffer instead of formic acid.

9.9.4. Preparation of standard solutions

Stock solutions of the biogenic amines and their metabolites were prepared. Standard solutions of all the monoamines and the internal standard were prepared from the stock solutions to calibrate the system prior to analysis. Isoprenaline was used as internal standard. All dilutions were done with the 0.1 M perchloric acid solution (containing 0.5 mM sodium metabisulphite and 0.3 mM Na_2EDTA) that was used for tissue homogenization (further on referred to as the homogenization solution)(Brand, 1996).

Stock solution 1 was prepared as follow:
Dopamine: 5 mg of 3-hydroxythyramine hydrochloride (MM = 189.64),
Serotonin: 11.504 mg of serotonin creatinine sulphate (MM = 405.43),
Noradrenaline: 6.08 mg of Na-noradrenaline (MM = 169.18),
DOPAC: 5 mg DOPAC (MM = 168.15),
5-HIAA: 5 mg 5-HIAA (MM = 191.19) and
HVA: 5 mg HVA (MM = 182.18) were dissolved in the homogenization solution.
Isoprenaline: 5 mg isoprenaline was dissolved in 50 ml of the homogenization solution. 30 µl of this solution was then diluted to 2 ml with the homogenization solution to give a concentration of 1500 ng/ml.

Stock solution 2 was prepared as follow:
40 µl of stock solution 1 was diluted with 1960 µl of the homogenization solution to give a concentration of 10 µg/ml.

Table 9.5. The composition of the standard solutions (containing noradrenaline, DA, 5HT, DOPAC, 5-HIAA and HVA).

Volume of stock solution 2 (µl)	Volume of 0.1 M perchloric acid solution (µl)	Total volume (ml)	Concentration (ng/ml)
2	1998	2	10
5	1995	2	25
10	1990	2	50
15	1985	2	75
20	1980	2	100
40	1960	2	200
60	1940	2	300
80	1920	2	400
100	1900	2	500
150	1850	2	750

The integrator was calibrated daily with the 25 ng/ml and 200 ng/ml solutions prior to analysis to account for inter-day variations in experimental conditions. All solutions (stock-, standard- and calibration solutions) were stored in a refrigerator to prevent degradation.

9.9.5. Sample preparation

After decapitation, the nucleus accumbens (Nacc) of each rat was placed individually into polypropylene containers and frozen with liquid nitrogen. On the day of sample preparation the weight of the Nacc was determined.

1 ml of perchloric acid solution (containing 0.5 mM sodium methabisulphate and 0.3 mM Na_2EDTA) was added to each Nacc for extraction of monoamines out of the tissue and to precipitate proteins. Each sample was then homogenised by sonication of the sample for 2 x 12 s at an amplitude of 14 micron. The homogenate was left on ice for 20 minutes in order to ensure perchlorate precipitation of proteins and extraction of monoamines. Following this period, the homogenate was centrifuged at

4 °C in a Beckman ultracentrifuge (model L8-70) for 15 minutes at 16 000 rpm (20 000 x g) (Brand, 1996).

The supernatant was transferred to clean polypropylene containers and immediately frozen with liquid nitrogen. The containers were then kept in the freezer at –86 °C until analysis on the following day.

9.9.6. HPLC analysis

For analysis of the monoamines and their metabolites, samples were analysed individually to minimise degradation. The tissue extract was thawed at room temperature just prior to injection onto the HPLC column. 200 µl of the extract was mixed with 20 µl isoprenaline (internal standard) to give a total volume of 220 µl. The solution was vortexed for 2 s. 50 µl of the solution was injected manually into the HPLC-EC system. Results were expressed as ng/g wet mass.

STANDARD CURVES

A standard curve was constructed for each monoamine and metabolite. The standard curve represents AUC-standard/AUC-internal standard, against concentration. The monoamines and metabolites were identified based on their retention time and quantified by comparing the AUC-sample/AUC-internal standard to the standards, utilizing the linear equation obtained from the standard curve.

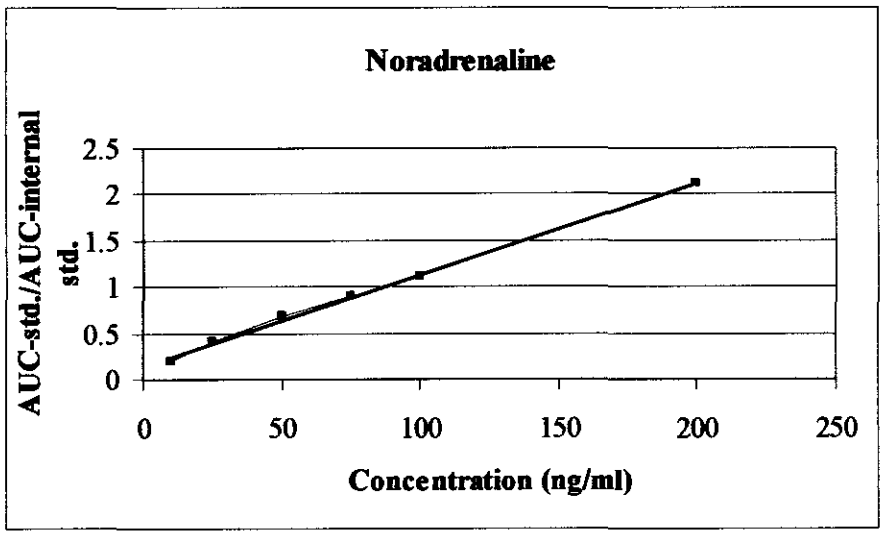


Figure 9.5. Linear curve of AUC ratio for noradrenaline against a concentration range of standard solutions.

The linear equation obtained from the noradrenaline standard curve was: **y = 0.0099x + 0.1398**. The regression coefficient was 0.9981.

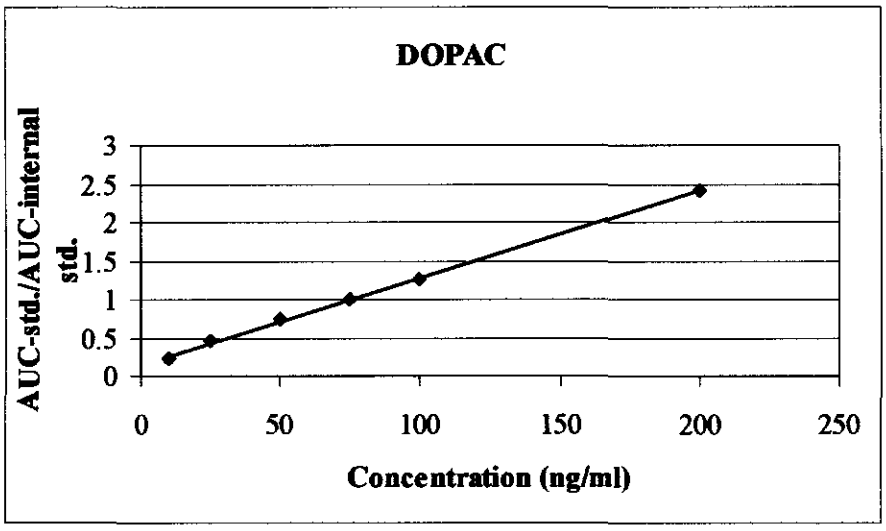


Figure 9.6. Linear curve of AUC ratio for DOPAC against a concentration range of standard solutions.

The linear equation obtained from the DOPAC standard curve was: **y = 0.0113x + 0.1472**. The regression coefficient was 0.9986.

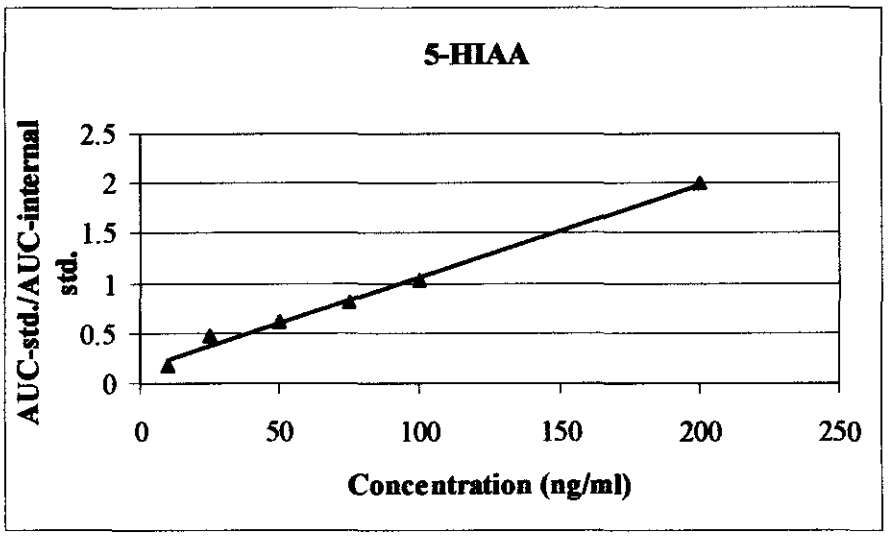


Figure 9.7. Linear curve of AUC ratio for 5-HIAA against a concentration range of standard solutions.

The linear equation obtained from the 5-HIAA standard curve was: **y = 0.0092x + 0.1439**. The regression coefficient was 0.9923.

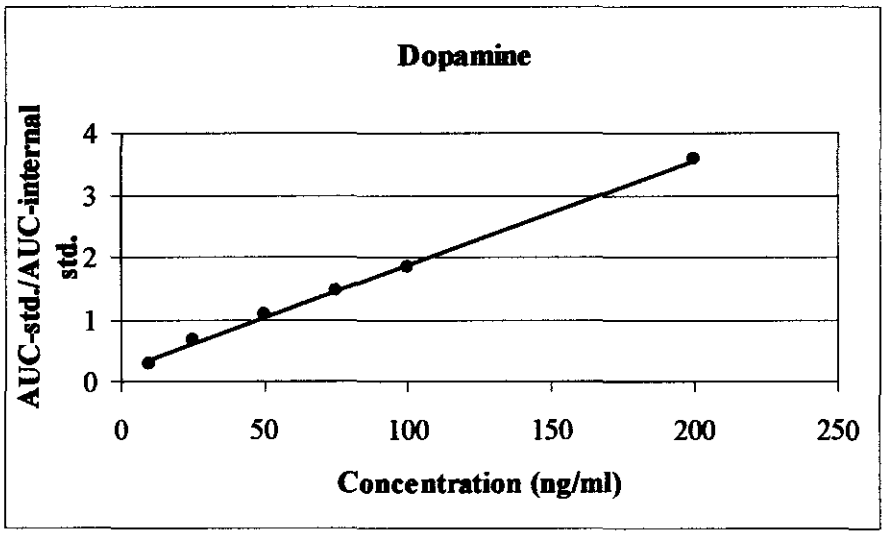


Figure 9.8. Linear curve of AUC ratio for dopamine against a concentration range of standard solutions.

The linear equation obtained from the dopamine standard curve was: **y = 0.0169x + 0.1888**. The regression coefficient was 0.9982.

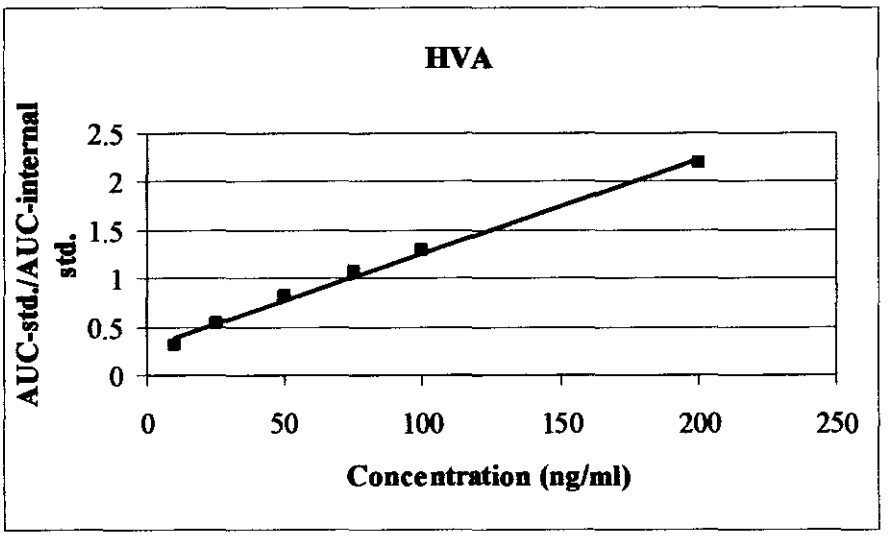


Figure 9.9. Linear curve of AUC ratio for HVA against a concentration range of standard solutions.

The linear equation obtained from the HVA standard curve was: **y = 0.0097x + 0.2863**. The regression coefficient was 0.9946.

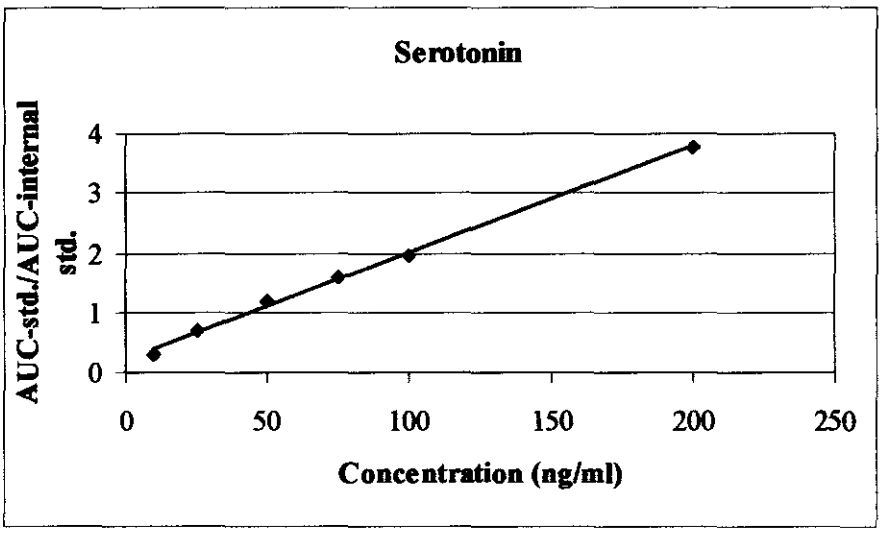


Figure 9.10. Linear curve of AUC ratio for serotonin against a concentration range of standard solutions.

The linear equation obtained from the serotonin standard curve was: **y = 0.0179x + 0.2016**. The regression coefficient was 0.9972.

9.9.7. Validation of method

Validation in general has to do with the provision of the certainty that a process or procedure is performing consistently and is capable of delivering specified results. To validate therefore means to provide documented evidence that all causes for variation have been accounted for and, as far as possible eliminated, and that any variation present will not be excessive beyond the expected or "normal value". The above method was validated in terms of its selectivity, linearity, range and precision.

9.9.7.1. Specificity and selectivity

Specificity and selectivity refers to the ability of an analytical method to accurately analyse a component in the presence of other compounds such as biological materials and other metabolic products. Chromatographs of the standard solution as well as a brain sample are shown in figure 9.5 and 9.6, respectively.

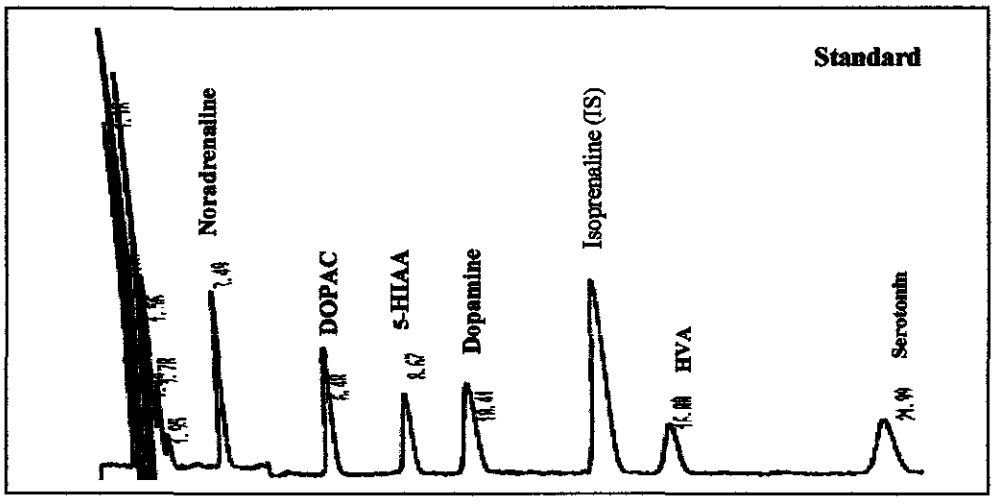


Figure 9.11. Chromatograph of the standard solution.

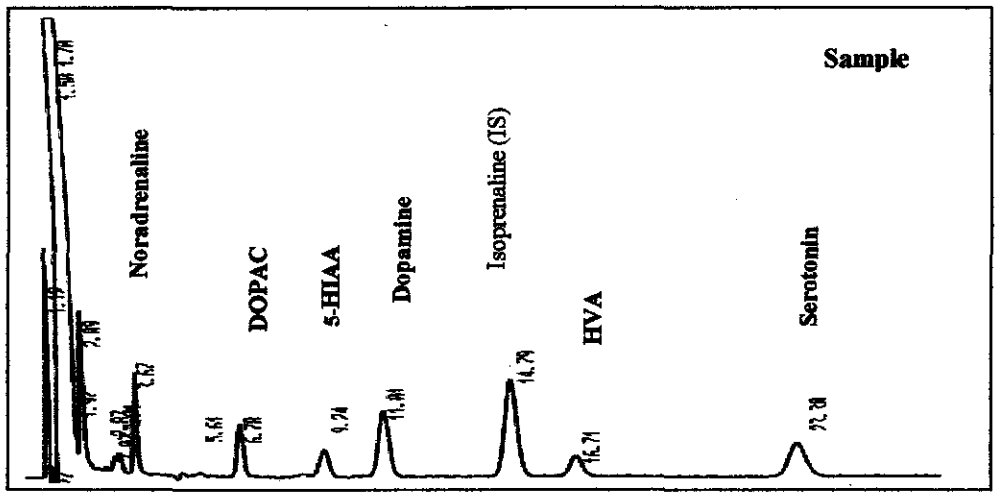


Figure 9.12. Chromatograph of the brain sample.

This method proved to be both sensitive and selective since there was no overlap of compounds in the standard solution or in the sample.

9.9.7.2. Linearity

The linearity of an analytical procedure is its ability (within a given range) to obtain test results, which are directly proportional to the concentration of analyte in the sample. The peak surface area ratio is plotted against concentration. The best straight line through these points is drawn. The following equation is used:

$$y = mx + c$$

Where y = peak surface area ratio; m = gradient; x = concentration in ng/ml; c = y intercept was used and the values are depicted in Table 9.6.

Table 9.6. The gradient, y-intercept and regression values of the standard curves.

Monoamine/Metabolite	Slope (m)	y – intercept (c)	Correlation coefficient (r^2)
Noradrenaline	820.81	12266	0.9969
DOPAC	938.05	12893	0.9987
5-HIAA	765.38	12478	0.9948
Dopamine	1399.3	16807	0.9982
HVA	777.37	24724	0.9928
Serotonin	1484.4	17918	0.9966

The linearity was acceptable, since r^2 was calculated to be between the specified values of 0.98-1.00.

9.9.7.3. Range

The range of an analytical procedure is the interval between the upper and lower concentration of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. This method proved sensitive enough to detect a minimum of 10 ng/ ml and the maximum limit detected was 200 ng/ ml of the standard solutions.

9.9.7.4. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

The repeatability was determined by injecting three concentrations of every sample that was to be analysed, into the HPLC consecutively. This was done by injecting three solutions with three different concentrations six consecutive times. The surface area ratio was calculated using the area under the curve (AUC) of the standard divided by the area under the curve of the internal standard.

Surface area ratio = AUC standard / AUC internal standard

The % RSD (relative standard deviation) was calculated for each concentration after which the mean % RSD was calculated for each compound.

% RSD = Standard error variation (STDEV) / Mean AUC ratio

For a method to be repeatable according to USP (2002) specifications, the average % RSD must be smaller than 10%.

Table 9.7. The average % RSD of the monoamines and their metabolites.

Monoamine/Metabolite	Average % RSD
Noradrenaline	5.822253
DOPAC	5.148422
5-HIAA	7.115169
Dopamine	5.918645
HVA	5.268425
5-HT	5.460373

CHAPTER 10

RESULTS AND DISCUSSION

The aim of this study was to find appropriate methods to determine withdrawal symptoms in tobacco smoke addiction and to determine if NAD, which is currently being used in the treatment of alcoholism, will be effective to terminate the craving for tobacco smoke.

Groups 1, 3 and 5 received the smoke extract via osmotic pumps for 28 days, whereas groups 2, 4 and 6 received a placebo (vehicle – propylene glycol) for 28 days. The osmotic pumps of groups 1, 2, 3 and 4 were removed on day 28 and groups 1 and 2 were injected with saline for four days (day 28, 29, 30, 31), whereas groups 3 and 4 were injected with NAD for four days (day 28, 29, 30, 31). Groups 5 and 6 were not treated with NAD or saline, but were sacrificed on day 28 to evaluate the difference in monoamine levels between smoking and non-smoking rats.

10.1. LOCOMOTOR ACTIVITY

The data from the locomotor activity experiments were analysed using a two-way analysis of variance (ANOVA) with group and repeated measures over time as factors followed by post hoc comparisons using Tukey's studentised range (HSD) test. A probability of $p \leq 0.05$ was employed to declare statistically significant differences.

10.1.1. Results

See appendix A for the number of vertical/horizontal/total movements over a period of two hours for each rat in groups 1, 2, 3 and 4 on days 2, 27, 29, 32 and 37. The means and standard error of the mean (S.E.M.) of each group are shown in figures 10.1 – 10.15 and $n = 8$ for all groups.

10.1.1.1. Day 2

The vertical activity of each group of rats on day 2 is shown in figure 10.1. There was no statistically significant difference between the vertical activity of the four groups on day 2 ($p > 0.05$).

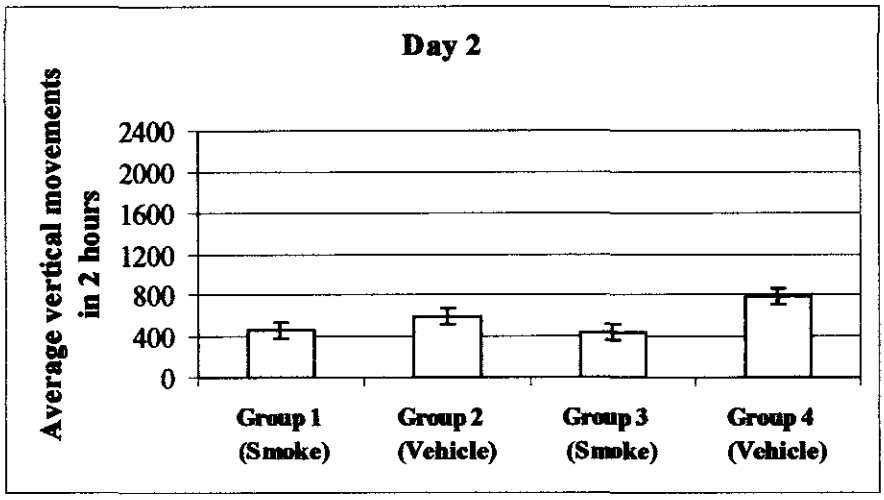


Figure 10.1. The average vertical activity of the rats on day 2.

Figure 10.2 represents the horizontal activity of the four groups on day 2. There was no statistically significant difference between the horizontal activity of the four groups on day 2 ($p > 0.05$).

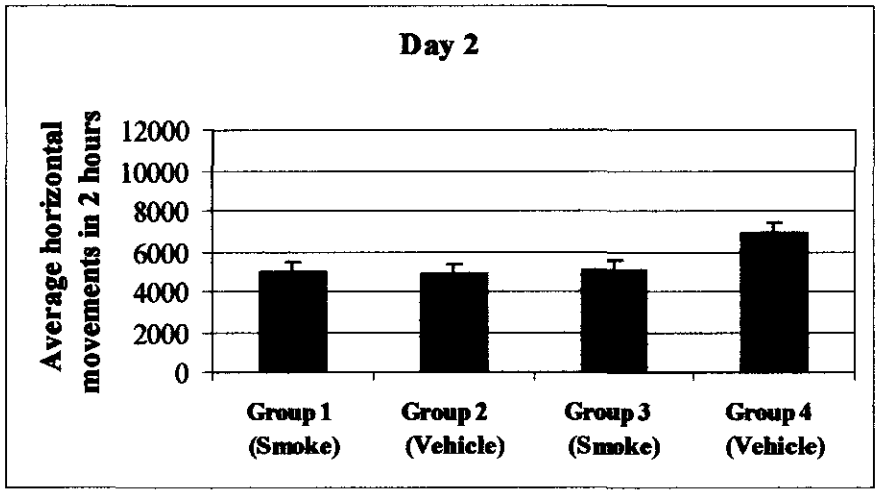


Figure 10.2. The average horizontal activity of the rats on day 2.

The total activity (sum of the horizontal and vertical activities) of each group on day 2 is shown in figure 10.3. There was no statistically significant difference between the total activity of the four groups on day 2 ($p > 0.05$).

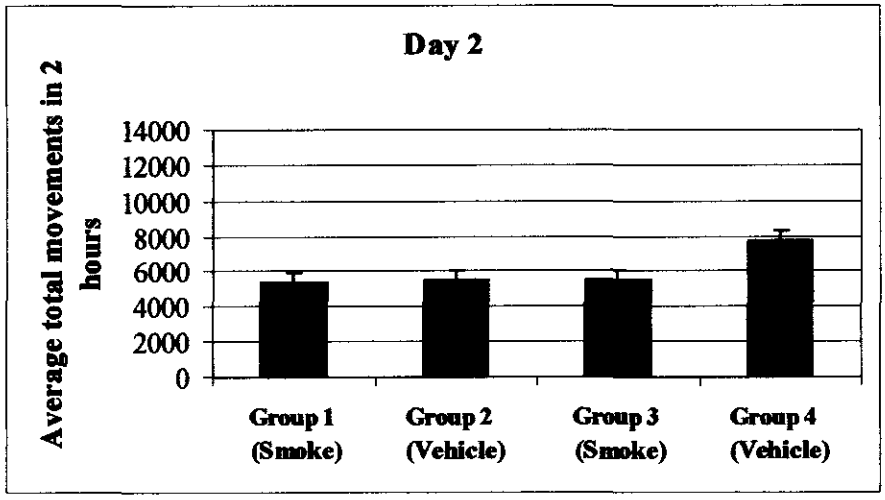


Figure 10.3. The average total activity of the rats on day 2.

10.1.1.2. Day 27

The vertical activity of the four groups on day 27 is shown in figure 10.4. There was no significant difference in vertical activity between groups 1, 2, 3 and 4 ($p > 0.05$).

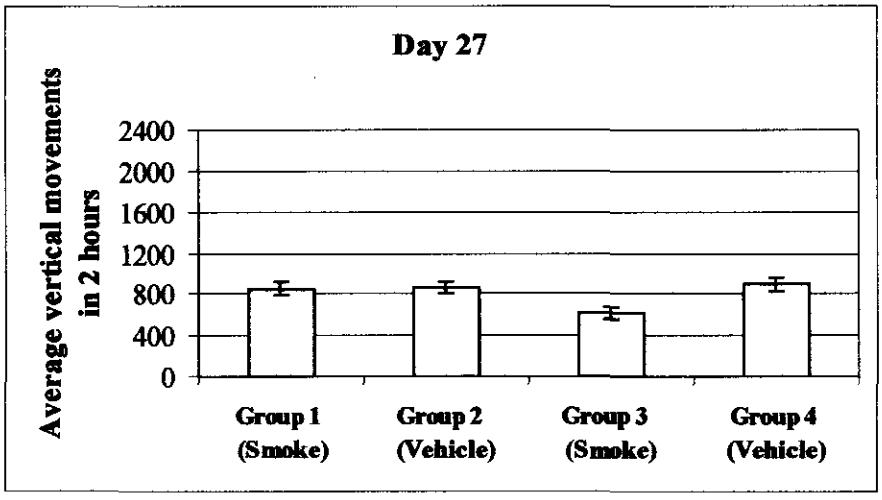


Figure 10.4. The average vertical activity of the rats on day 27.

Figure 10.5 represents the horizontal activity of the four groups on day 27. There was no significant difference in horizontal activity between the four groups ($p > 0.05$).

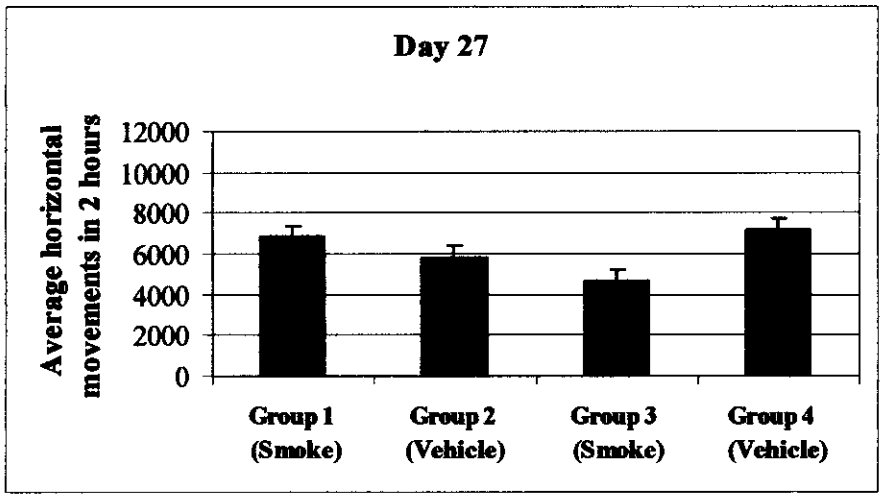


Figure 10.5. The average horizontal activity of the rats on day 27.

The total activity of the four groups on day 27 is shown in figure 10.6. There was no significant difference in total activity between the four groups ($p > 0.05$).

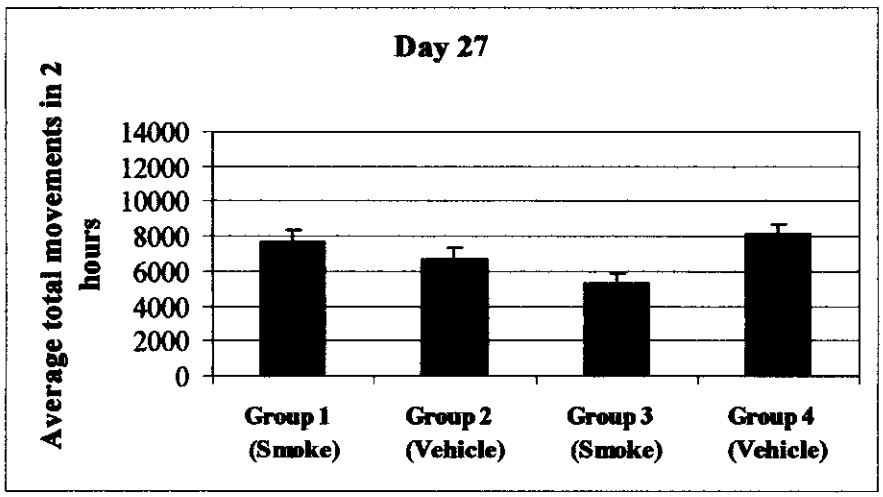


Figure 10.6. The average total activity of the rats on day 27.

10.1.1.3. Day 29

Figure 10.7 represents the average vertical activity of the four groups on day 29. The two-way ANOVA showed a significant difference on day 29 and Tukey's test revealed that the significant difference was between groups 1 and 3 ($p < 0.05$).

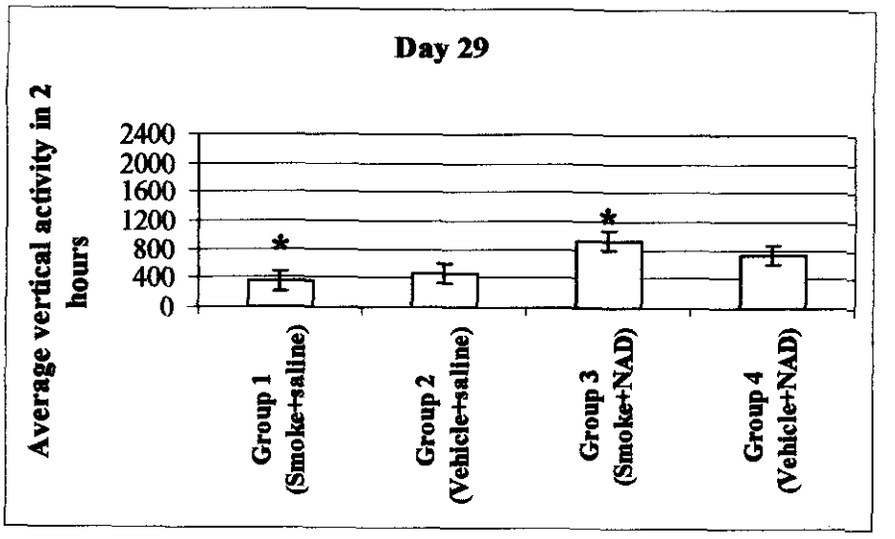


Figure 10.7. The average vertical activity of the rats on day 29 (* $p < 0.05$).

The average horizontal activity of the four groups on day 29 is shown in figure 10.8. There was no significant difference between the groups.

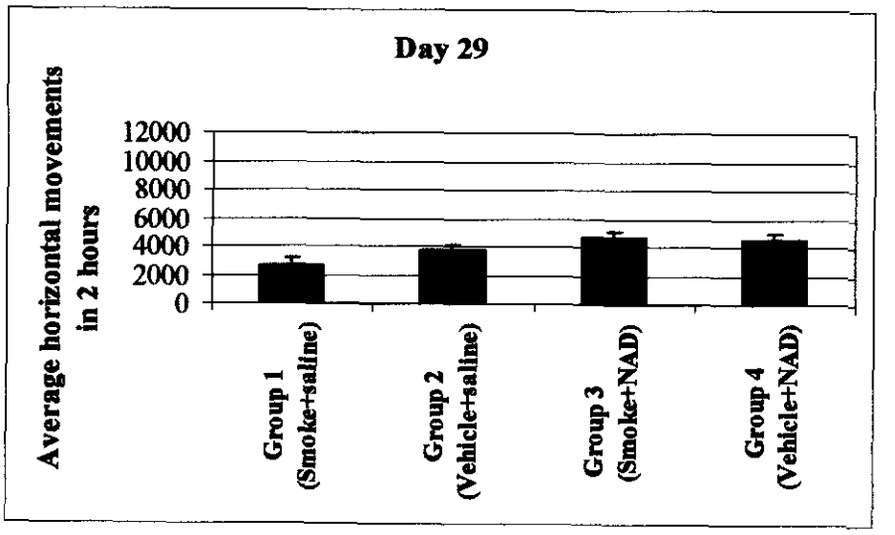


Figure 10.8. The average horizontal activity of the rats on day 29.

Figure 10.9 represents the average total activity of the four groups on day 29. The two-way ANOVA showed a significant difference on day 29 and Tukey's test revealed that the significant difference was between groups 1 and 3 ($p < 0.05$).

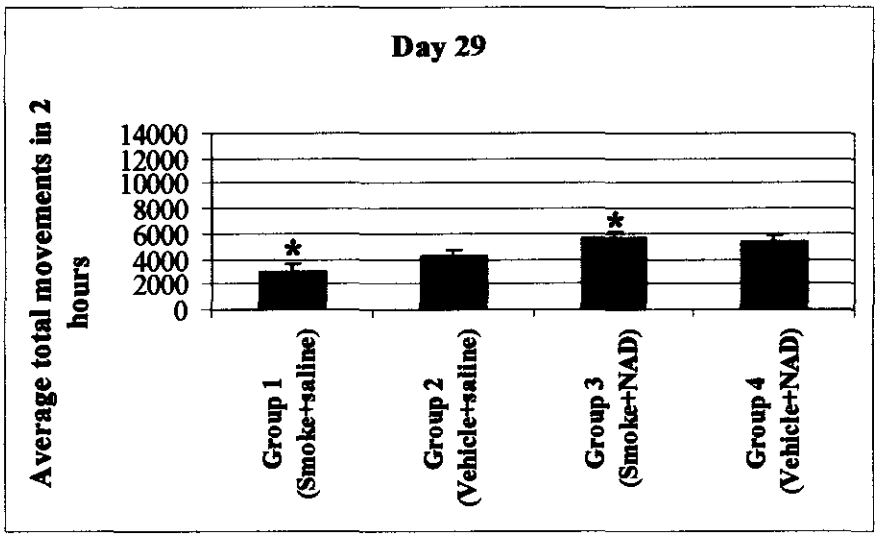


Figure 10.9. The average total activity of the rats on day 29 (* $p < 0.05$).

10.1.1.4. Day 32

The vertical activity of the rats on day 32 is shown in figure 10.10. There was no significant difference between the different groups ($p > 0.05$).

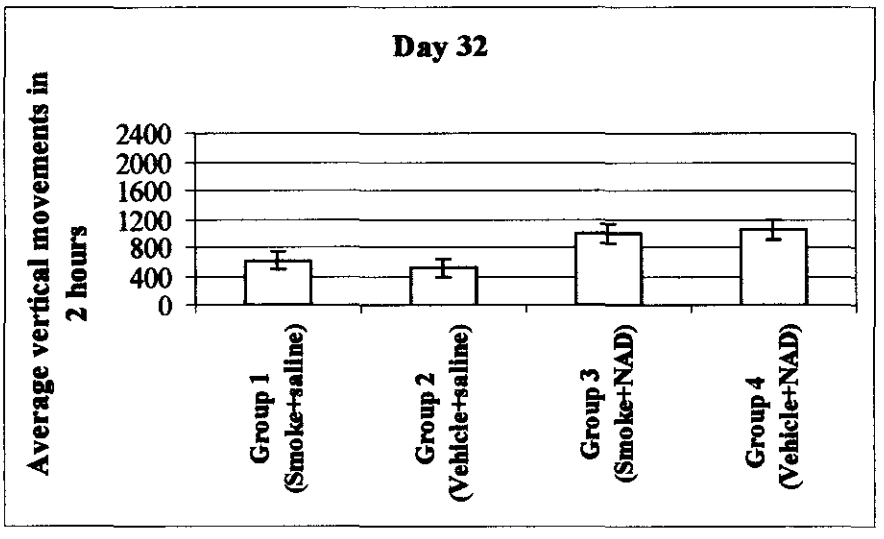


Figure 10.10. The average vertical activity of the rats on day 32.

Figure 10.11 represents the horizontal activity of the rats on day 32. There was no significant difference between the different groups ($p > 0.05$).

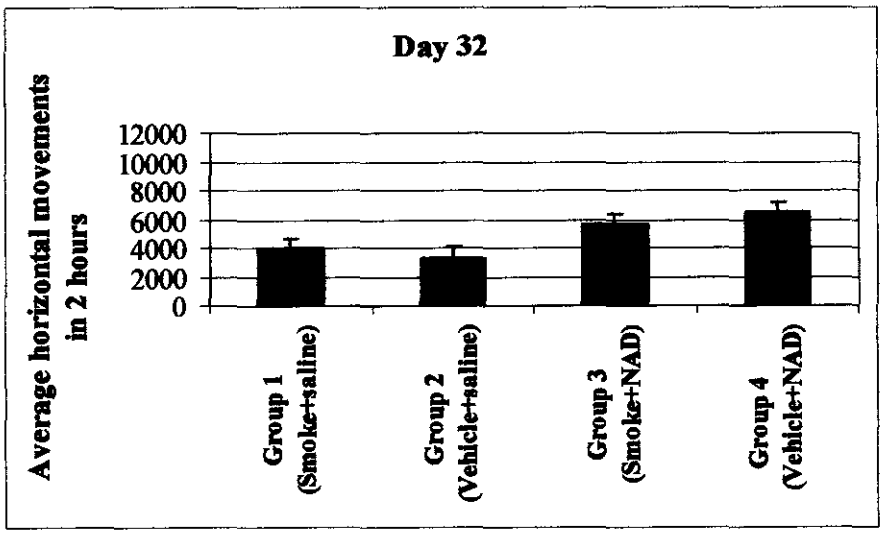


Figure 10.11. The average horizontal activity of the rats on day 32.

The total activity of the rats on day 32 is shown in figure 10.12. There was no significant difference between the different groups ($p > 0.05$).

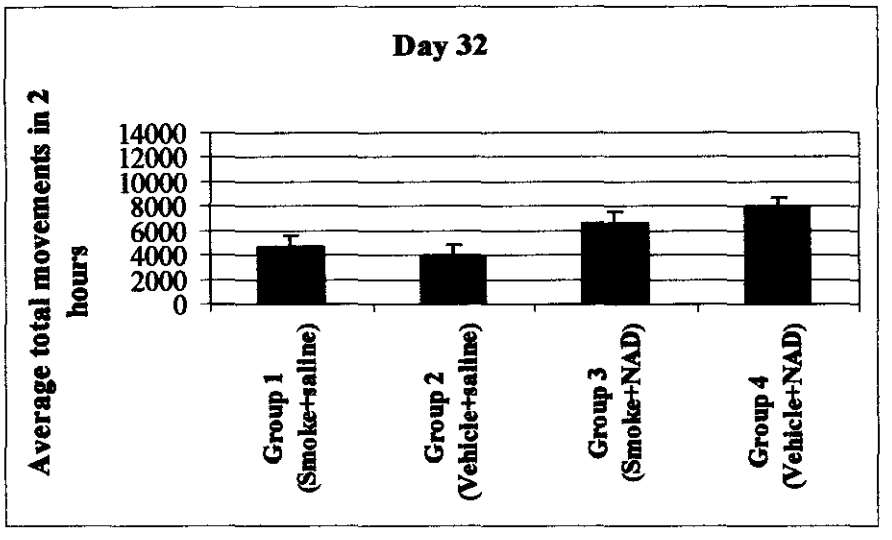


Figure 10.12. The average total activity of the rats on day 32.

10.1.1.5. Day 37

Figure 10.13 represents the average vertical activity of each group on day 37. The two-way ANOVA showed that there was a significant difference and Tukey's test revealed that the significant difference was between groups 1 and 4 and also between groups 3 and 4 ($p < 0.05$).

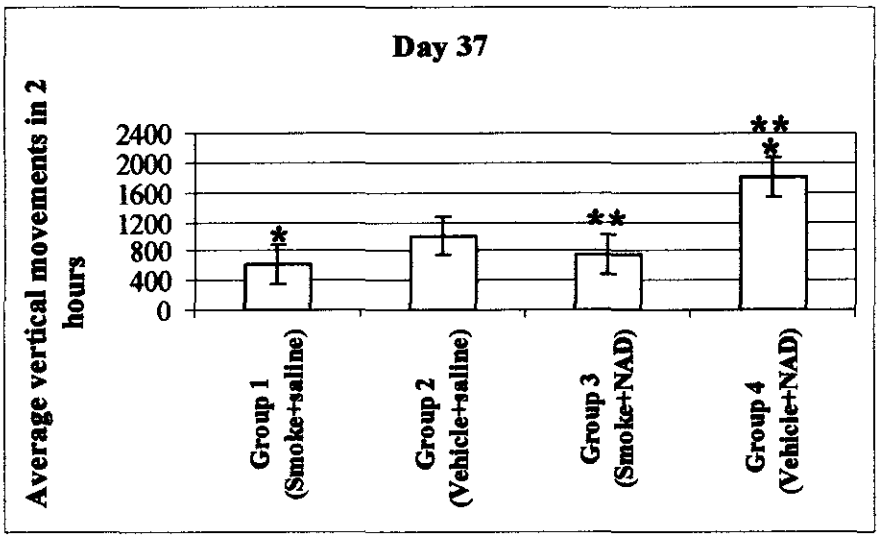


Figure 10.13. The average vertical activity of the rats on day 37 (* and ** $p < 0.05$).

The average horizontal activity of each group on day 37 is shown in figure 10.14. The two-way ANOVA showed that there was a significant difference and Tukey's test revealed that the significant difference was between groups 1 and 4 and also between groups 3 and 4 ($p < 0.05$).

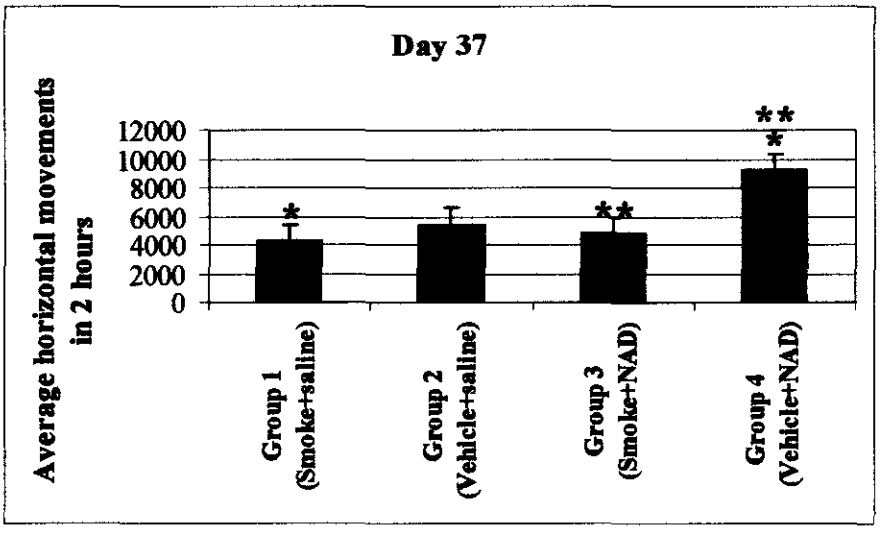


Figure 10.14. The average horizontal activity of the rats on day 37 (* and ** $p < 0.05$).

Figure 10.15 represents the average total activity of each group on day 37. The two-way ANOVA showed that there was a significant difference and Tukey's test revealed

that the significant difference was between groups 1 and 4 and also between groups 3 and 4 ($p < 0.05$).

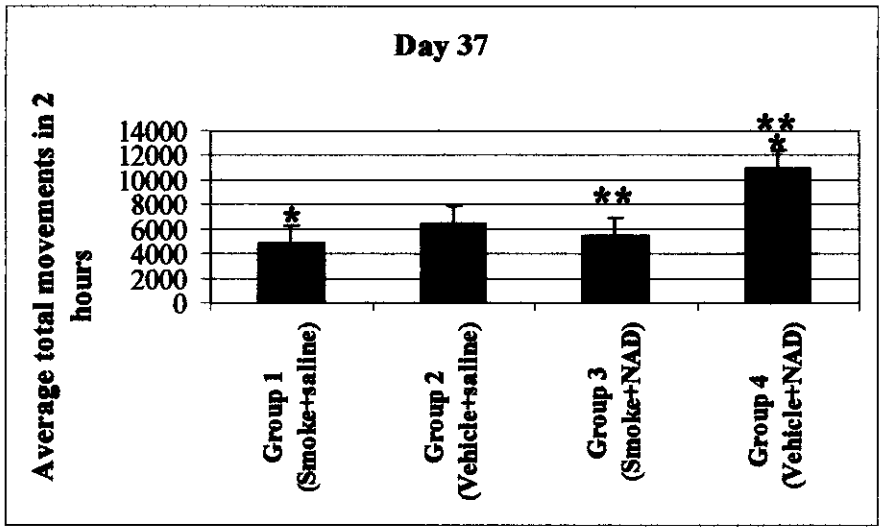


Figure 10.15. The average total activity of the rats on day 37 (* and ** $p < 0.05$).

10.1.2. Discussion

Locomotor activity is widely used to study nicotine's behavioural actions, especially psychomotor stimulant actions, in rodents (Faraday *et al.*, 2003). On day 2 it is to be expected that there would be no significant difference in locomotor activity between the four groups because all the rats had surgery on day 1 (to implant the Alzet osmotic pumps) and the activity can be interpreted as baseline activity.

On **day 27** there was no significant difference in vertical/horizontal/total activity between the four groups, but the vertical activity of groups 1 and 3 were slightly higher than on day 2. Acute as well as chronic administration of nicotine increases locomotor activity in rats (Hildebrand *et al.*, 1999 and Olausson *et al.*, 2001). In an experiment done by Suemaru and co-workers (1992), rats were chronically exposed to cigarette smoke for 20 minutes twice daily using a smoking machine. They also found that chronic cigarette smoke exposure for 21 days increasingly stimulated locomotor activity and rearing.

Group 1 and 3 received smoke extract via minipumps and group 2 and 4 were implanted with minipumps containing only the vehicle. The Alzet osmotic pumps in all four groups were removed on day 28 and the rats in groups 3 and 4 were injected

with NAD on day 28, 29, 30 and 31 while the rats in groups 1 and 2 were injected with saline on the same days.

A significant difference in vertical activity between groups 1 and 3 was revealed with Tukey's test on **day 29**. According to Faraday and co-workers (2003), vertical activity is thought to indicate exploration. In an experiment done by Gäddnäs *et al.* (2000), the effects of chronic nicotine and its withdrawal on locomotor activity were studied by administering nicotine in the drinking water to male NMRI mice. They found that when the nicotine solution was replaced with tap water, the locomotor activity of the mice dropped and tended to be less than that of the control mice that drank tap water during the entire experiment. Hildebrand and co-workers (1999) also confirmed that locomotor activity was markedly reduced during nicotine withdrawal. We therefore suggest that the higher vertical activity of the rats in group 3 compared to group 1 indicates that NAD relieves the symptoms of tobacco withdrawal in the rats of group 3. On day 29 there was no significant difference between the horizontal activity of the four groups. Horizontal activity has been interpreted to reflect general arousal (Faraday *et al.*, 2003) and we suspected that the effect of withdrawal would be on the vertical activity of the rats because vertical activity indicate exploration. There was a significant difference in the total activity (horizontal + vertical) between groups 1 and 3 on day 29, indicating that the NAD injected group 3 had higher locomotor activity than group 1 which experienced tobacco withdrawal.

On **day 32** no significant difference was found in the vertical/horizontal/total activity between the four groups. The nicotine withdrawal syndrome in humans is prompt in onset and reaches peak intensity within 24 hours (Gäddnäs *et al.*, 2000 and Hildebrand *et al.*, 1997). According to Malin (2001), abstinence signs in rats reach a peak at 18-22 hours post-cessation. In an experiment done by Gäddnäs and co-workers (2000), mice withdrawn from nicotine for 12-14 hours were found to be less active than the control mice drinking tap water throughout the experiment, but no differences could be found between the mice withdrawn for 23-25 hours and the control mice. We therefore suggest that the abstinence syndrome of the rats in group 1 might have faded and that the vertical/horizontal/total activity of the rats on day 32 were not influenced by withdrawal symptoms anymore. This could explain the lack of any significant difference in activity between the rats in groups 1 and 3.

On **day 37** (the Alzet osmotic pumps were removed on day 28) we suspect that the withdrawal symptoms would have disappeared and should not have had any effect on the locomotor activity. It is interesting to note that there was no significant difference between the vertical/horizontal/total activity of groups 1 and 3. After NAD treatment the activity of group 3 was the same as the activity of group 1 after the disappearance of withdrawal symptoms.

Tukey's test revealed a significant difference in vertical/horizontal/total activity between groups 1 and 4. Group 1 received smoke extract for 28 days and was then injected with saline for 4 days, the vertical/ horizontal/total activity of this group on day 37 could be considered normal. Group 4 received vehicle for 28 days and was then injected with NAD for 4 days. NAD is converted via the citric acid cycle to NADH. The more energy a cell needs, the more NADH it needs that will be available for oxidative phosphorylation, which then augment ATP production (Wan *et al.*, 1999 and Maiese & Chong, 2003). Because of the higher levels of NAD we suggest that these rats had more energy than the rats in group 1 on day 37 and therefore had higher levels of vertical/horizontal/total activity. Tukey's test also revealed a significant difference in vertical/horizontal/total activity between groups 3 and 4. Group 3 received smoke extract for 28 days and was then injected with NAD for 4 days. The vertical/horizontal/total activity of group 3 was significantly lower than that of group 4. We suggest that the NAD, which was injected into the rats in group 3, diminished withdrawal symptoms after the Alzet osmotic pumps were removed. The NAD thus increased the vertical/horizontal/total activity of the rats in group 3 to a normal level. Because the rats in group 4 did not receive tobacco extract and thus did not experience withdrawal symptoms, the NAD was not used by their bodies to diminish withdrawal symptoms and explains why the rats in group 4 had higher levels of activity than group 3.

10.2. ELEVATED PLUS MAZE

Different groups were used in the pilot study:
Group 1: Received smoke extract for 28 days and was injected with NAD on days 28, 29, 30 and 31.
Group 2: Received propylene glycol for 28 days and was injected with saline on days 28, 29, 30 and 31.
Group 3: Received smoke extract for 28 days and was injected with saline on days 28, 29, 30 and 31.

During the pilot study four rats were housed per cage, which resulted in some of the rats' stitches being bitten open by the other rats in the cage. These rats, numbered 4, 11, 13, 17, 19, 27 and 31, did not receive the same volume of smoke extract or propylene glycol as the other rats and thus were excluded from the processed results. Rat 33 had an abscess and was not included in the processing of the results. None of the rats explored the open arms of the maze. We therefore concluded that this was not an appropriate method to measure withdrawal. See Appendix B for the results.

10.3. ACOUSTIC STARTLE REFLEX

10.3.1. Results

Acoustic startle reflex was measured on days 29, 30, 31 and 32 between 08h00 and 11h00 in the morning. The data from the acoustic startle experiments were analysed using a one-way analysis of variance (ANOVA) with groups as factor followed by post hoc comparisons using Tukey's studentised range (HSD) test. A probability of $p \leq 0.05$ was employed to declare statistically significant differences.

Figure 10.16 represents the average startle amplitude of the 8 rats in each group on day 29. There was no significant statistical difference in startle amplitude between the four groups at the four startle intensities ($p > 0.05$).

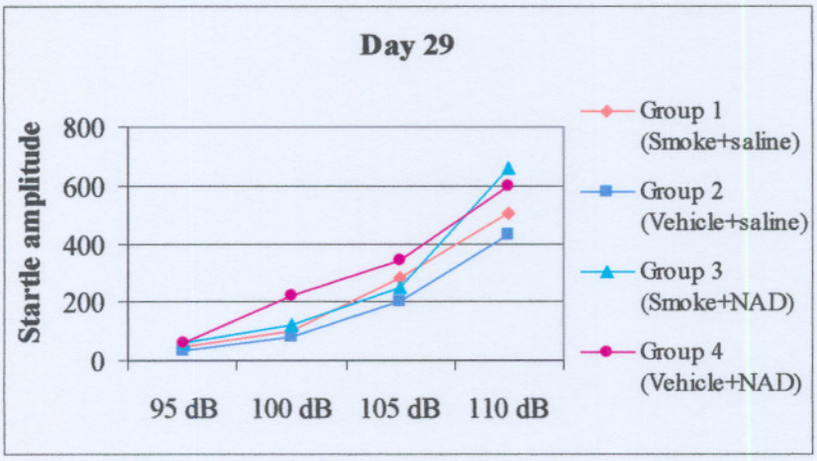


Figure 10.16. Average acoustic startle amplitude for all four groups of rats on day 29.

The average startle amplitude of the rats on day 30 is shown in figure 10.17. There was no significant difference in startle amplitude between the groups at 95 dB and 100 dB. According to Tukey's studentized range (HSD) test, there was a significant difference in startle amplitude between groups 1 and 4 and also between groups 2 and 4 at 105 dB. There were no statistical differences at 110 dB.

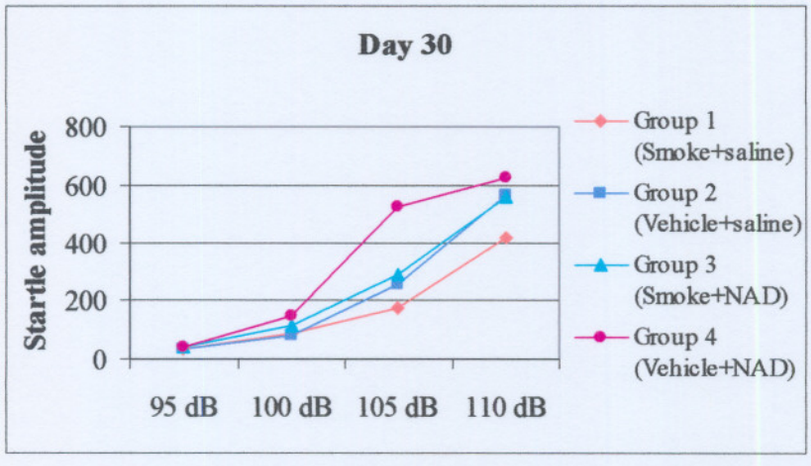


Figure 10.17. Average acoustic startle amplitude for all four groups of rats on day 30.

Figure 10.18 represents the average startle amplitude of the four groups of rats on day 31. There was no significant statistical difference in startle amplitude between the four groups at the four startle intensities ($p > 0.05$) on day 31.

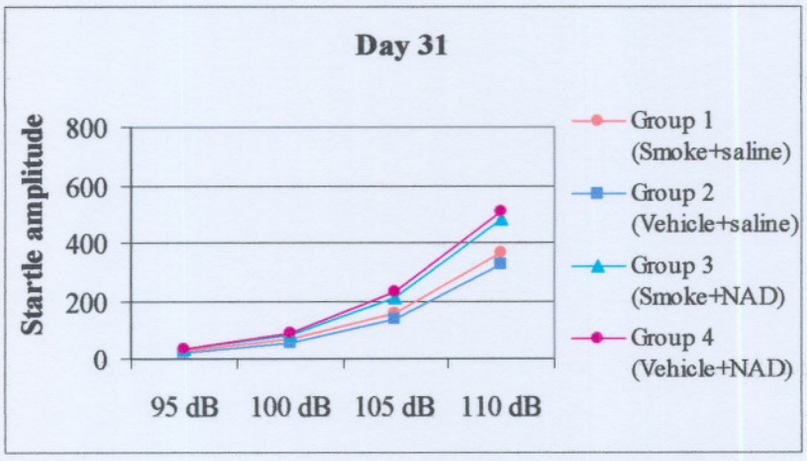


Figure 10.18. Average acoustic startle amplitude for all four groups of rats on day 31.

The average startle amplitude of the four groups on day 32 is shown in figure 10.19. According to Tukey's test there was a significant statistical difference in average startle amplitude between group 2 and 3 at 105 dB.

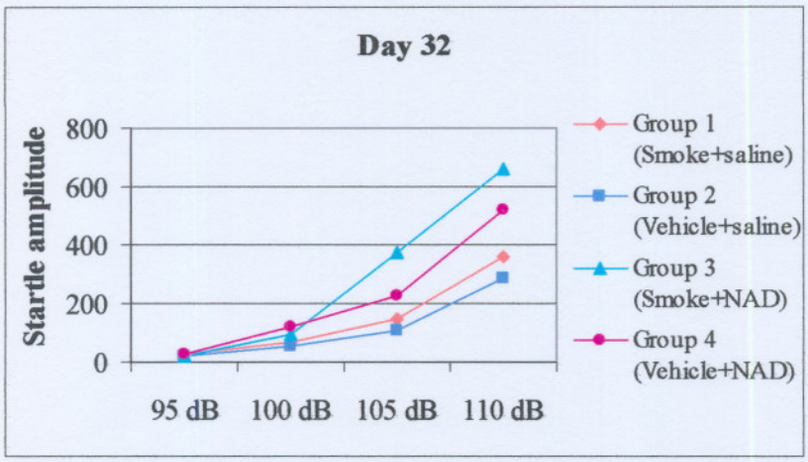


Figure 10.19. Average acoustic startle amplitude for all four groups of rats on day 32.

10.3.2. Discussion

On **day 29**, the acoustic startle response (ASR) of groups 3 and group 4 were slightly higher than the ASR of groups 1 and 2, although not statistically. We argue that the higher ASR in groups 3 and 4 was due to the NAD enhancing dopamine (DA) levels. Dopaminergic agents modify the ASR, with DA agonists enhancing the ASR (Bell *et al.*, 2003 and Lewis & Gould, 2003). Thus, pharmacological agents that enhance dopamine levels would be expected to enhance the startle reflex. The acoustic startle response was measured between 08h00 and 11h00 in the morning and the rats in

groups 3 and 4 were injected with NAD at 11h00. On day 29 the withdrawal symptoms in group 1 had not reached a peak and therefore there was not a significant difference between groups 1 and 2. Alternatively, peak effects of nicotine cessation might have occurred earlier than 24 hrs post-cessation and were waning by the time of evaluation. According to Malin (2001), abstinence signs in rats reach a peak at 18-22 hours post-cessation. The response of the rats in group 2 could be considered "normal", for these rats did not receive smoke extract or NAD. The difference in the ASR between groups 1 and 3 at 110 dB, can be ascribed to higher DA levels in group 3 (injected with NAD) compared to group 1, which were experiencing withdrawal symptoms. Shoaib and co-workers have shown that there is a decrease in DA in the Nacc during nicotine withdrawal (Shoaib *et al.*, 2004 and Hildebrand *et al.*, 1998).

On **day 30** the rats in group 1 were clearly experiencing withdrawal symptoms when compared to the ASR of the rats in group 2 (control group). Nicotine increases the ASR in rats (Lewis & Gould, 2003 and Acri *et al.*, 1995). We thus assume that nicotine withdrawal would decrease the ASR in rats because of lower DA levels. Findings by Lewis & Gould (2003) corroborate this conclusion. They injected mice intraperitoneally with nicotine and subsequently with a nicotinic receptor antagonist, mecamylamine. Mecamylamine blocked the nicotine enhancement of the startle response and the startle response of these mice was lower than saline-treated mice. The difference in ASR between group 1 and 3 indicates that the DA levels of the rats in group 3 were higher than in group 1, indicating that group 3 experienced less craving than group 1. According to Acri *et al.* (1991), there are decreases in startle responsivity following nicotine cessation when compared to rats receiving nicotine. Acri and co-workers (1991) found that during the first 3 days after cessation of nicotine, the ASR of rats receiving nicotine decreased to 92 % of the control group (which only received saline).

The curve of group 1 moved closer to the curve of the control group (group 2) on **days 31 and 32**, indicating that the withdrawal symptoms experienced by the rats in group 1 were disappearing and their DA levels were beginning to normalise. Groups 3 and 4 were injected with NAD on days 28, 29, 30 and 31. The higher ASR in groups 3 and 4 when compared to groups 1 and 2 could be explained by the increase in DA caused by the NAD.

A possible shortcoming of this experiment may have been that the ASR was not measured on day 37. We suspect that the ASR of groups 1, 2 and 3 would have been the same on day 37 (as in the case of the locomotor activity on day 37) whereas group 4 might have shown higher ASR on day 37. The effect of NAD would have diminished and the ASR of group 3 would have normalised. The rats in group 4 did not receive tobacco extract and thus did not experience withdrawal symptoms; the NAD was not used by their bodies to diminish withdrawal symptoms, explaining why the rats in group 4 had higher levels of activity than group 3.

Although we could not find any statistical differences in the ASR response, the trend shown in the graphs does suggest that NAD is able to diminish withdrawal symptoms.

10.4. SINGLE CELL GEL ELECTROPHORESIS (SCGE) ASSAY

10.4.1. Results

The median of the tailmoments of each group of rats is shown in figure 10.20. The equation for calculating the tailmoment is: Tailmoment = tail length x % DNA in the tail. The median, non-outlier range and outliers are shown. Groups 1, 5 and 6 had low damage compared to groups 2, 3 and 4 (see Appendix D for raw data).

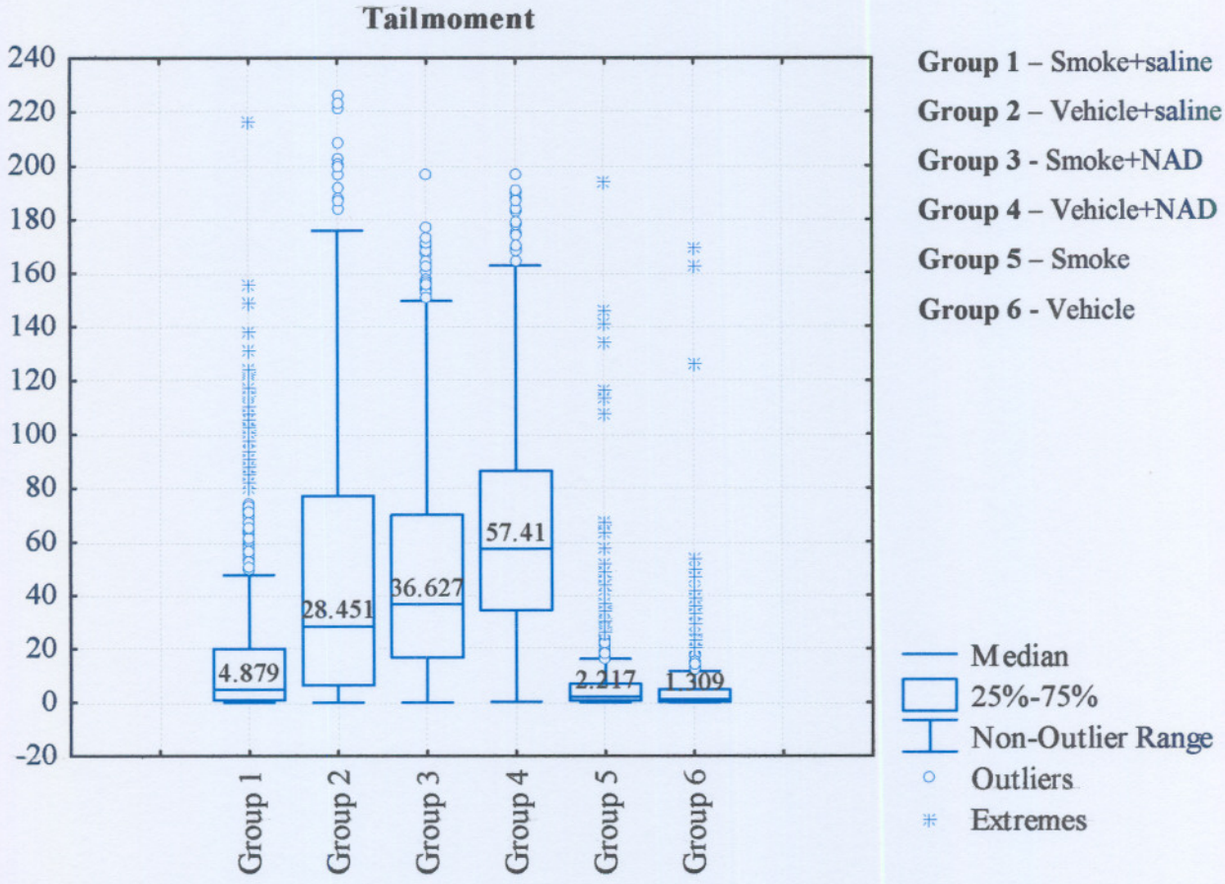


Figure 10.20. Representation of the tailmoment of each group of rats. Tailmoment = tail length x amount of DNA in the tail.

The median of the percentage of DNA in the tail for each group of rats is shown in figure 10.21. The median, non-outlier range and outliers are shown. Groups 1, 5 and 6 again had less damage than groups 2, 3 and 4 (see Appendix D for raw data).

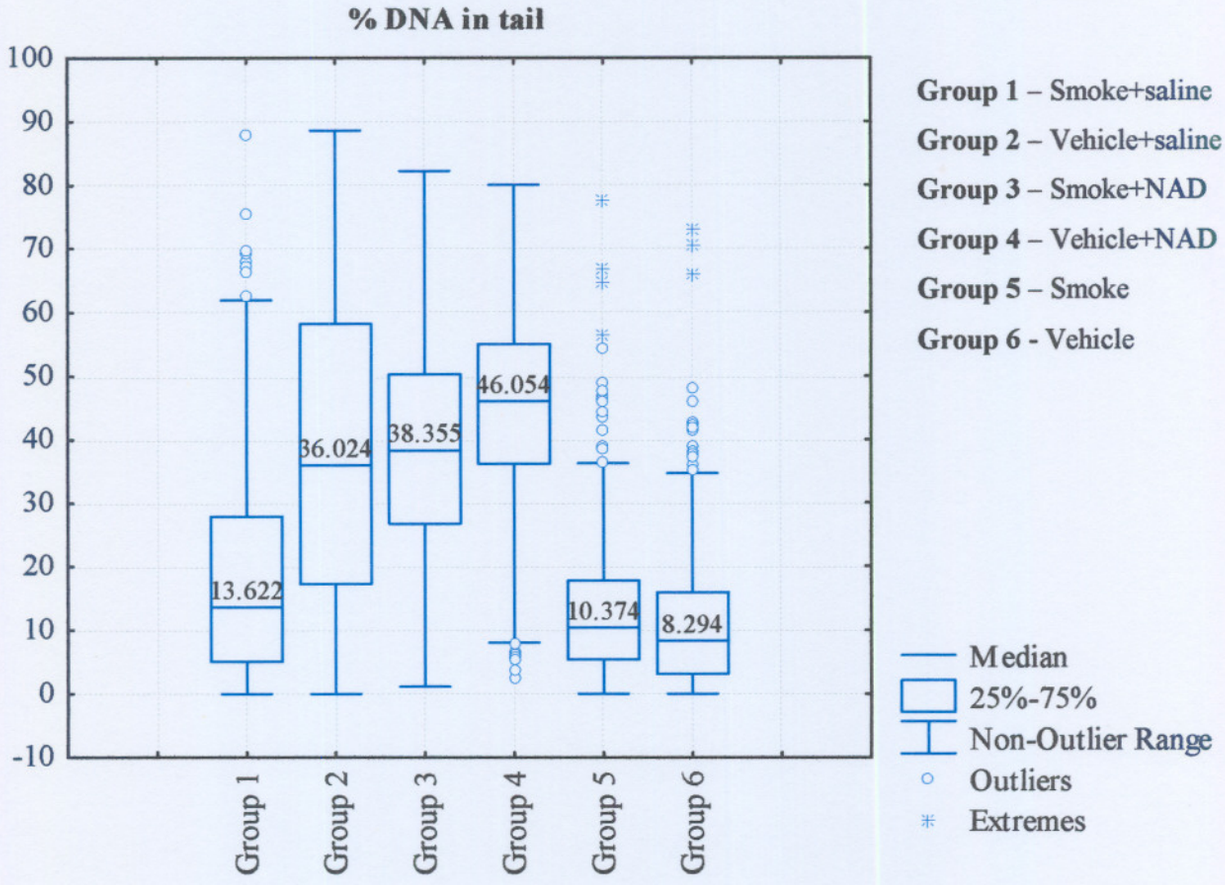


Figure 10.21. Representation of the percentage of DNA in the tail of the comet for each group of rats.

10.4.2. Discussion

According to George & O'Malley (2004), 430 000 people die each year as a result of smoking-attributable medical illnesses such as lung cancer, chronic obstructive pulmonary disease, cardiovascular disease and stroke. These are all diseases of the periphery. Alternatively, cigarette smokers are 50 % less likely to have Parkinson's disease or Alzheimer's disease than non-smokers. This negative association suggests that cigarette smoke exerts an undefined, neuroprotective influence against the development of Parkinson's disease and Alzheimer's disease (Fratiglioni & Wang, 2000). The SCGE assay was done on the striata of the rats. We suggest that the smoke extract exerted a neuroprotective function in Group 1 (received smoke extract for 28 days and was injected with saline for four days) and Group 5 (received smoke extract for 28 days and was then sacrificed), which explains the low damage in the striata of these rats. According to Tsuda *et al.* (2000), liver, skin and upper digestive

tract are target organs when orally or dermally exposed to cigarette smoke condensate, but not kidney, brain or bone marrow.

Group 6 received propylene glycol (vehicle) for 28 days and was then sacrificed. According to Leal-Klevezas *et al.* (2000), a 20 % ethylene glycol-propylene glycol solution preserves cells and improves DNA yield and quality. This might explain the low DNA damage of group 6. Villard and co-workers (1998) confirm that propylene glycol reduce DNA single-strand breaks in the lung and liver, induced after exposure to a 7-fold dilution of cigarette smoke for 8 days.

The higher damage in Group 3 (received smoke extract for 28 days and was injected with NAD for four days) and Group 4 (received vehicle for 28 days and was injected with NAD for four days) could be explained by the fact that the more energy a cell needs, the more NADH it needs (Wan *et al.*, 1999). Thus, NAD increases metabolism and the electron transport chain is activated. Complexes I and II catalyze electron transfer to ubiquinone from two different electron donors: NADH (Complex I) and succinate (Complex II). Complex III carries electrons from ubiquinone to cytochrome c, and Complex IV completes the sequence by transferring electrons from cytochrome c to O_2 (Nelson & Cox, 2000 and Stryer, 1995). We suspect that some of these electrons might have been released from the electron transport chain and caused damage to the striata cells.

A shortcoming of this study is that the SCGE assay was done on the striata of the rats, but smoking-attributable medical illnesses such as lung cancer, chronic obstructive pulmonary disease and cardiovascular disease are mostly diseases of the periphery. It would therefore be more reasonable to determine the DNA damage in the periphery (whole-blood samples or the lungs). It should also be noted that only DNA damage was measured and not DNA recovery. We suspect that DNA recovery would take place more readily in groups 3 and 4.

10.5. CATECHOLAMINES

10.5.1. Results

Groups 1, 2, 3 and 4

The data from the catecholamines were analysed using a one-way analysis of variance (ANOVA) with groups as factor followed by post hoc comparisons using Tukey's studentised range (HSD) test. A probability of $p \leq 0.05$ was employed to declare statistically significant differences.

Figure 10.22 represents average noradrenaline concentrations in the nucleus accumbens of the rats in groups 1, 2, 3 and 4 (see Appendix E for raw data). $n = 8$ for all groups. There was no significant difference between the noradrenaline concentrations in the four groups ($p > 0.05$, $p = 0.284$).

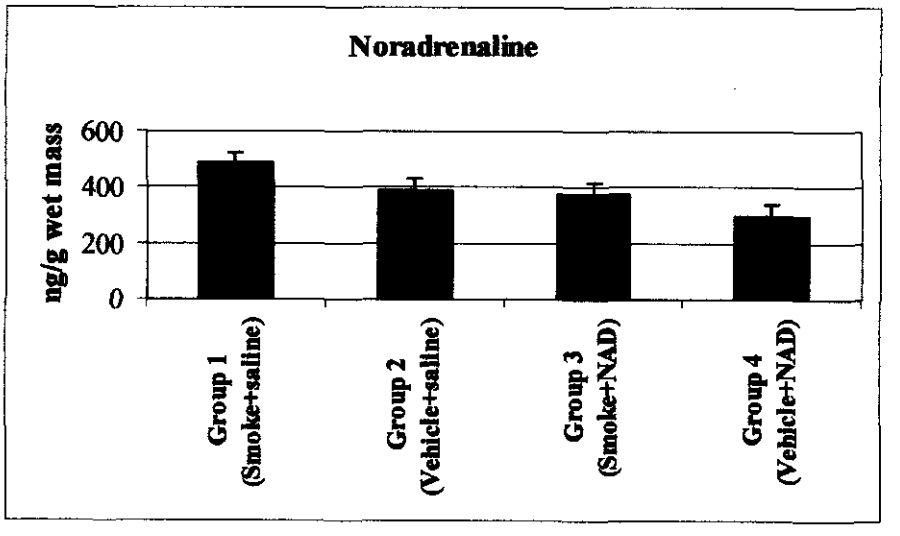


Figure 10.22. Average noradrenaline concentrations in the nucleus accumbens of the rats on day 42.

Figure 10.23 represents average DOPAC concentrations in the nucleus accumbens of the rats in groups 1, 2, 3 and 4. Tukey's test revealed that there was a significant difference in DOPAC concentrations between groups 3 and 4 ($p < 0.05$, $p = 0.017$).

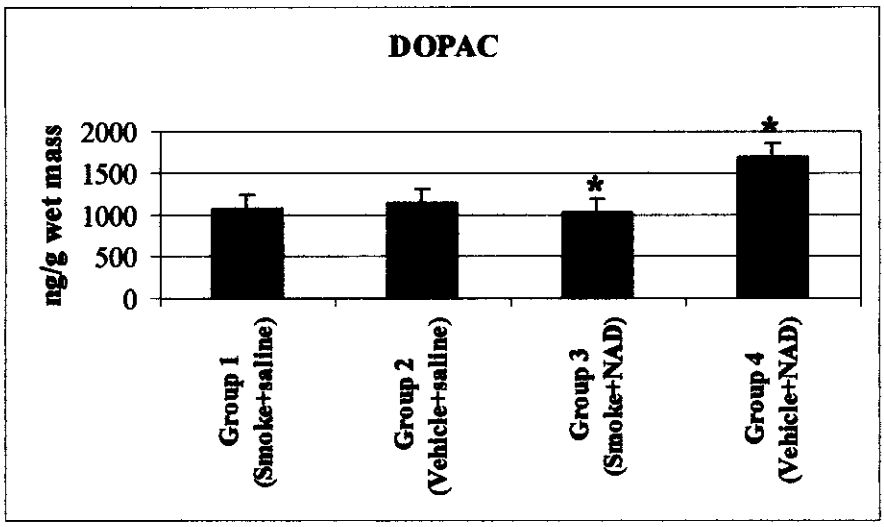


Figure 10.23. Average DOPAC concentrations in the nucleus accumbens of the rats on day 42 (* $p < 0.05$).

Figure 10.24 represents average HVA concentrations in the nucleus accumbens of the rats in groups 1, 2, 3 and 4 (see Appendix E for raw data). $n = 8$ for all groups. There was no significant difference in HVA concentrations between the four groups ($p > 0.05$, $p = 0.439$).

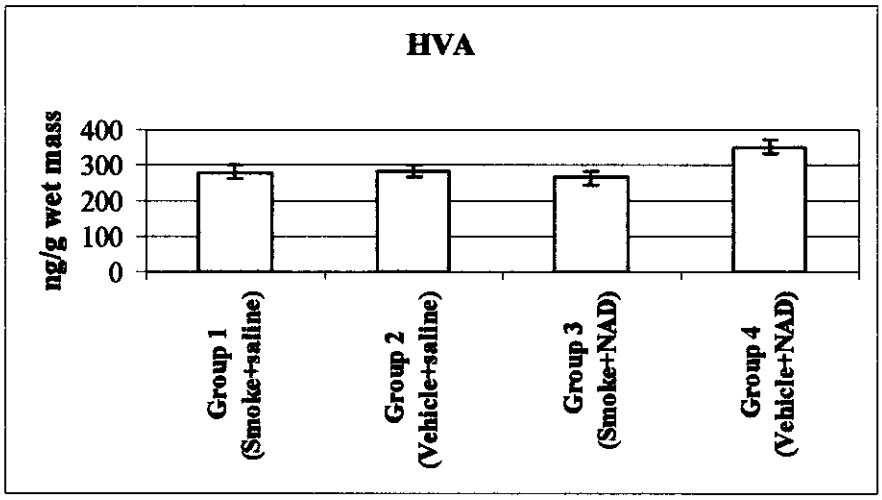


Figure 10.24. Average HVA concentrations in the nucleus accumbens of the rats on day 42.

Figure 10.25 represents average dopamine concentrations in the nucleus accumbens of the rats in groups 1, 2, 3 and 4. There was no significant difference in DA concentrations between the four groups ($p > 0.05$, $p = 0.785$).

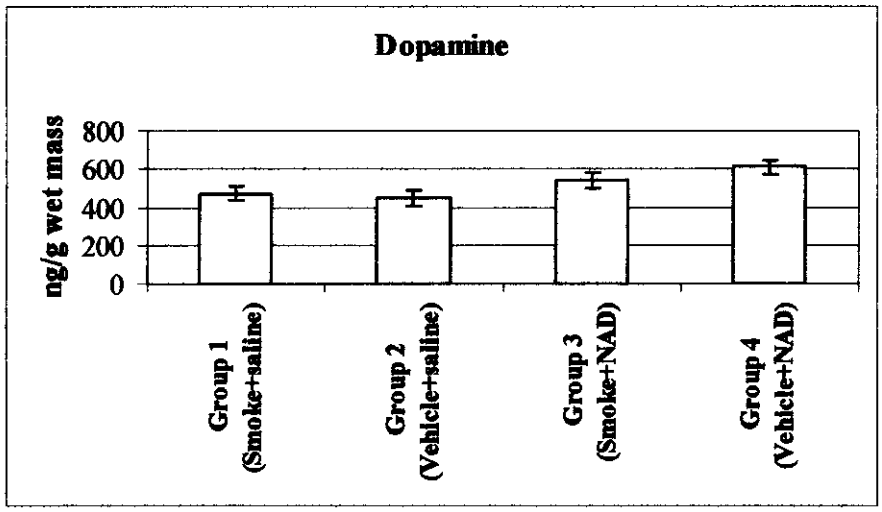


Figure 10.25. Average dopamine concentrations in the nucleus accumbens of the rats on day 42.

Figure 10.26 represents average 5-HIAA concentrations in the nucleus accumbens of the rats in groups 1, 2, 3 and 4. There was no significant difference in 5-HIAA concentrations between the four groups ($p > 0.05$, $p = 0.428$).

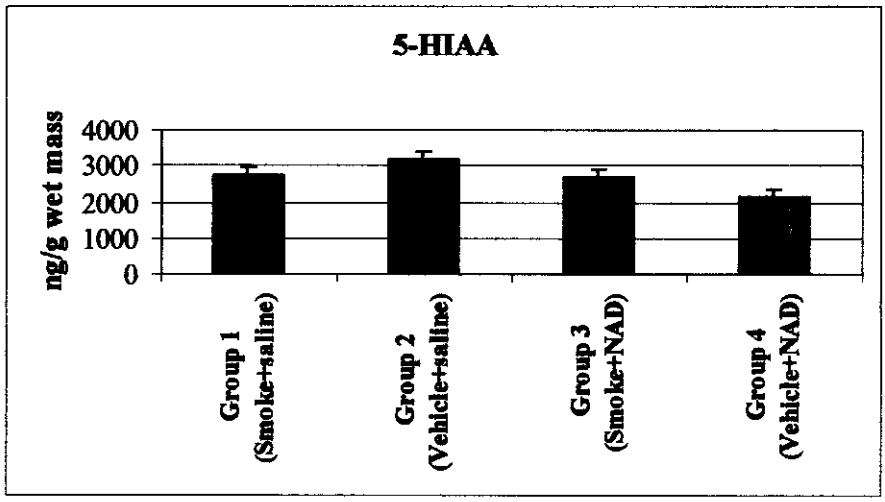


Figure 10.26. Average 5-HIAA concentrations in the nucleus accumbens of the rats on day 42.

Figure 10.27 represents average 5-HT concentrations in the nucleus accumbens of the rats in groups 1, 2, 3 and 4. There was no significant difference in 5-HT concentrations between the four groups ($p > 0.05$, $p = 0.053$).

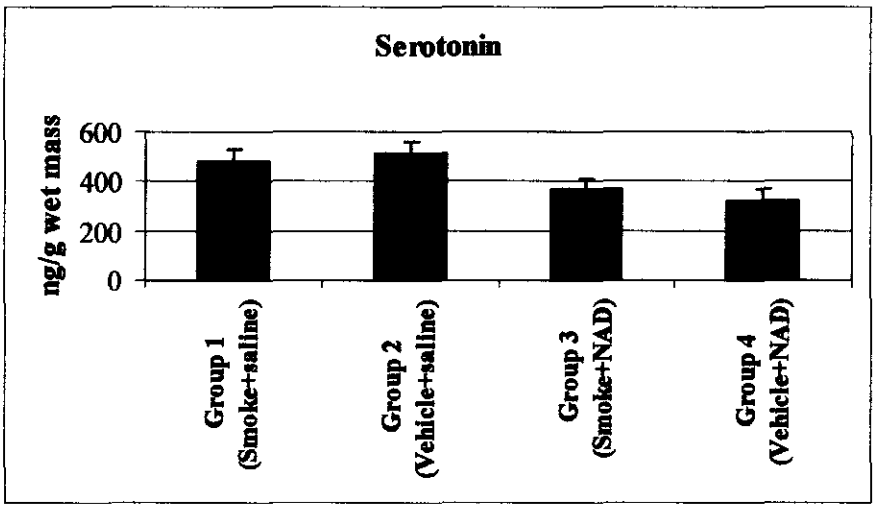


Figure 10.27. Average serotonin (5-HT) concentrations in the nucleus accumbens of the rats on day 42.

Groups 5 and 6

The data from the catecholamines were analysed using Student's T test. A probability of $p \leq 0.05$ was employed to declare statistically significant differences.

Figure 10.28 represents average noradrenaline concentrations in the nucleus accumbens of the rats in groups 5 and 6 (see Appendix E for raw data). $n = 8$ for both groups. There was no significant difference in noradrenaline concentrations between the two groups ($p > 0.05$, $p = 0.702$).

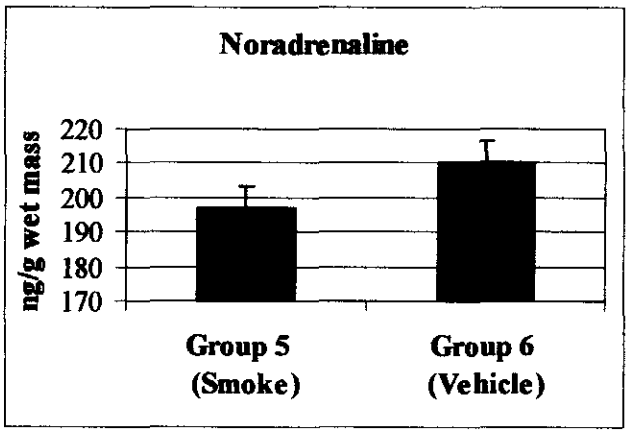


Figure 10.28. Average noradrenaline concentrations in the nucleus accumbens of the rats on day 28.

Figure 10.29 represents average DOPAC concentrations in the nucleus accumbens of the rats in groups 5 and 6 (see Appendix E for raw data). n = 8 for both groups. There was no significant difference in DOPAC concentrations between the two groups ($p > 0.05$, $p = 0.549$).

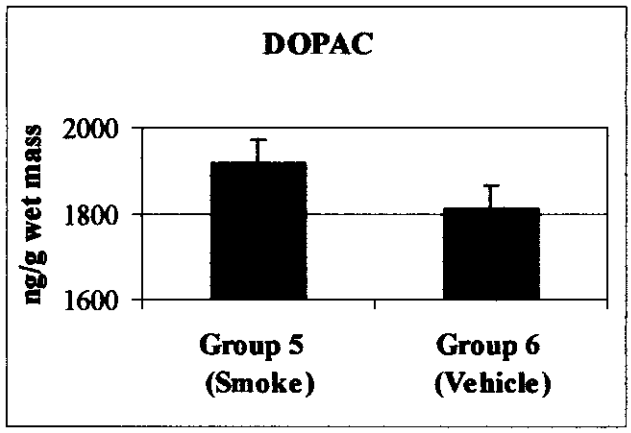


Figure 10.29. Average DOPAC concentrations in the nucleus accumbens of the rats on day 28.

Figure 10.30 represents average HVA concentrations in the nucleus accumbens of the rats in groups 5 and 6 (see Appendix E for raw data). n = 8 for both groups. There was no significant difference in HVA concentrations between the two groups ($p > 0.05$, $p = 0.839$).

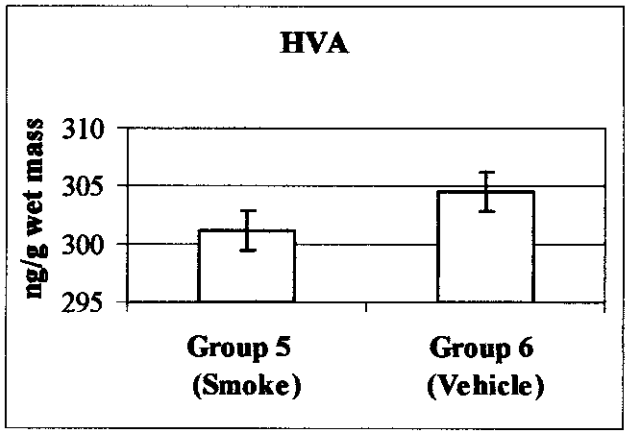


Figure 10.30. Average HVA concentrations in the nucleus accumbens of the rats on day 28.

Figure 10.31 represents average dopamine concentrations in the nucleus accumbens of the rats in groups 5 and 6 (see Appendix E for raw data). n = 8 for both groups.

There was no significant difference in dopamine concentrations between the two groups ($p > 0.05$, $p = 0.983$).

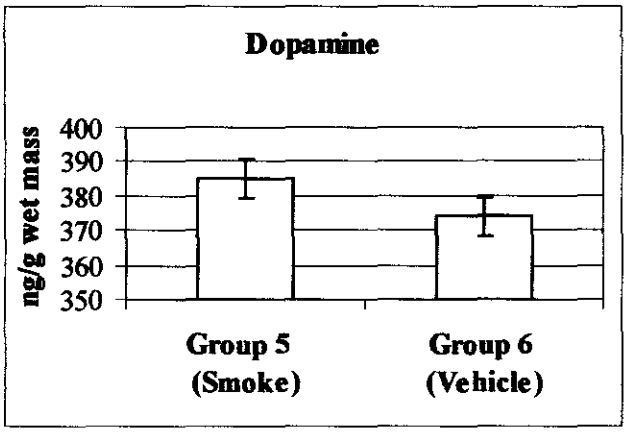


Figure 10.31. Average dopamine concentrations in the nucleus accumbens of the rats on day 28.

Figure 10.32 represents average 5-HIAA concentrations in the nucleus accumbens of the rats in groups 5 and 6 (see Appendix E for raw data). $n = 8$ for both groups. Tukey's test revealed that there was a significant difference in 5-HIAA concentrations between groups 5 and 6 ($p < 0.05$, $p = 0.026$).

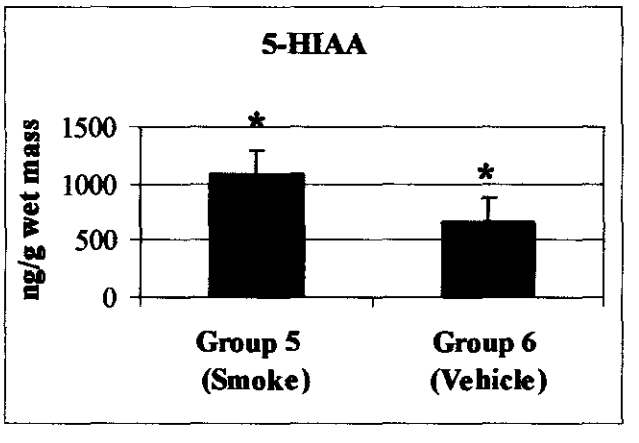


Figure 10.32. Average 5-HIAA concentrations in the nucleus accumbens of the rats on day 28 (* $p < 0.05$).

Figure 10.33 represents average serotonin (5-HT) concentrations in the nucleus accumbens of the rats in groups 5 and 6 (see Appendix E for raw data). $n = 8$ for both groups. There was no significant difference in 5-HT concentrations between the two groups ($p > 0.05$, $p = 0.108$).

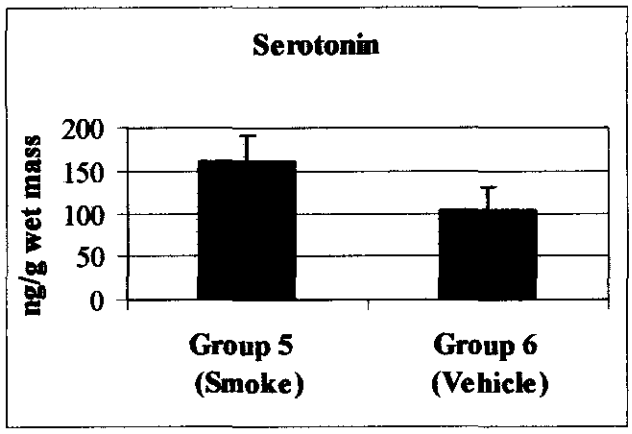


Figure 10.33. Average serotonin concentrations in the nucleus accumbens of the rats on day 28.

10.5.2. Discussion

Groups 1, 2, 3 and 4

There was no significant difference in the nucleus accumbens NA, DOPAC, HVA, DA, 5-HIAA and 5-HT concentrations of groups 1, 2 & 3 on day 42, indicating that group 3's (smoke & treated with NAD) concentrations of NA, DOPAC, HVA, DA, 5-HIAA and 5-HT reversed to control levels (group 1 & 2 treated with saline) within 10 days after treatment (figures 10.22 - 10.27). The NAD-induced elevation of nucleus accumbens DOPAC concentrations of group 4 (vehicle & treated with NAD) was still significant at 10 days after treatment with NAD (figure 10.23)

The smoke induced elevation of the nucleus accumbens NA concentration in group 1 (received smoke & treated with saline) was still visible at fourteen days after withdrawal, although not statistically significant.

The nucleus accumbens 5-HT concentrations of groups 3 (smoke & treated with NAD) and 4 (no smoke & treated with NAD) were lower compared to the concentrations of group 2 (no smoke & treated with saline) and group 1 (smoke and

treated with saline) after 10 days of treatment (although not statistically), indicating that NAD might influence 5-HT levels in the nucleus accumbens.

A shortcoming of this experiment is that we sacrificed the rats on day 42, fourteen days after nicotine cessation and ten days after treatment with NAD. At this stage all monoamine levels would have normalised. A possible solution could be to use larger groups of rats, sacrificing rats on days 1, 2, 3, 4, 5 and 6 after smoking cessation (or during treatment with NAD) to determine NA, DA, DOPAC, HVA, 5-HT and 5-HIAA concentrations. This would indeed give a clearer picture of the monoamine levels and their metabolites during withdrawal and treatment with NAD.

Groups 5 and 6

In an experiment by Gäddnäs *et al.* (2000), the effects of chronic nicotine and its withdrawal on brain monoamines were studied by administering nicotine in the drinking water to male NMRI mice. Monoamines were measured post mortem on the 50th day of nicotine administration. They found that during chronic nicotine administration the **striatal** DOPAC and 5-HIAA, **hypothalamic** 5-HIAA and NA as well as **cortical** NA concentrations were elevated compared to the control mice that drank tap water throughout the experiment. These effects disappeared during withdrawal. We found no significant difference in the **nucleus accumbens** DOPAC and HVA concentrations between groups 5 and 6 although the trend of the graphs show that group 5 (smoke-treated) has higher levels of DOPAC than group 6 (no-smoking).

In vivo studies revealed that injections of nicotine increased NA turnover in rodent brain (Balfour & Fagerström 1996). In contrast to the effects of nicotine injections, the chronic infusion of nicotine from subcutaneous minipumps resulted in decreased NA levels in the **frontal cortex** (Kirch *et al.*, 1987). Unpublished observations of Balfour and co-workers have shown that nicotine infusions also abolish the increase in NA overflow in the hippocampus evoked by nicotine injections (Balfour & Fagerström 1996). Gäddnäs and co-workers (2000) found that **hypothalamic** and **cortical** concentrations of NA were increased on the 50th day of nicotine administration. We found no significant difference in the **nucleus accumbens** NA

concentrations between groups 5 and 6 although the trend of the graphs show that group 5 (smoke-treated) has higher levels of NA than group 6 (no-smoking).

During chronic nicotine administration, the **striatal** 5-HIAA concentrations were increased significantly (Gäddnäs *et al.*, 2000). We found a significant difference in the nucleus accumbens 5-HIAA concentrations between groups 5 and 6 ($p < 0.05$, $p = 0.026$). No difference was found in the serotonin concentrations, although the trend of the graph show that group 5 (smoke-treated) had higher levels of serotonin than group 6 (no-smoking). Interestingly, studies with postmortem tissue taken from human subjects who were habitual smokers resulted in a reduction in the concentrations of 5-HT and 5-HIAA in the hippocampus of the human brain (Balfour & Fagerström, 1996). According to Reuben & Clarke (2000), nicotine significantly increased 5-HT release from striatal synaptosomes.

Our results might be attributed to the fact that Gäddnäs *et al.* (2000) exposed the mice to nicotine for 50 days; whereas in this study we only exposed the rats to the smoke extract for 28 days and also because we determined monoamines in a different brain region, the nucleus accumbens. We thus conclude that differences in the monoamine levels in the nucleus accumbens might have resulted if the rats in the smoking group (group 5) would have been exposed longer to the smoke extract.

CHAPTER 11

CONCLUSION

Smoking is more than a bad habit or a social problem; it is the most common cause of avoidable illness and death. Despite the availability of effective treatments for nicotine addiction like nicotine replacement therapies and bupropion, smoking remains prevalent with serious health consequences. Reducing the number of current smokers is the single most important behavioural change that would substantially reduce morbidity and mortality associated with cigarette smoking. It is therefore of great importance to study new pharmacological agents against smoke addiction.

The aim of this study was to find appropriate methods to determine withdrawal symptoms in tobacco smoke addiction and to determine if NAD, which is currently being used in the treatment of alcoholism, would terminate the craving for tobacco smoke.

The results indicate that NAD shows definite potential for treating tobacco smoke addiction, although the exact mode of action of NAD in smoking cessation is unclear. Because the underlying mechanisms of addiction of many drugs of abuse are so similar, we also suggest that NAD could be useful for the treatment of other kinds of addictions.

The locomotor activity and acoustic startle response recorded during smoking, withdrawal and treatment can be used as parameters for addiction to tobacco smoke and to test novel drugs for their potential to cure addiction.

Proposals for future studies:

- To determine the mechanism of action of NAD as well as the effect of NAD on monoamine oxidase and tyrosine hydroxylase. Studies have shown that smokers have lower levels of brain MAO A and B activity and lower MAO B platelet activity than non-smokers (Fowler *et al.*, 2003 and Mihailescu & Drucker-Colin, 2000). Tyrosine hydroxylase is the rate-limiting enzyme that catalyzes the hydroxylation of tyrosine to DOPA in the synthesis of DA.

- To compare the anti-addictive properties of NAD, bupropion and nicotine replacement therapy in rats.
- To use whole blood samples to perform the SCGE (Comet) assay. In this study the SCGE assay was done on the striata of the rats, but smoking-attributable medical illnesses such as lung cancer, chronic obstructive pulmonary disease and cardiovascular disease are mostly diseases of the periphery. It could therefore be highly informative to determine the DNA damage not only in the brain, but also in the periphery.
- Acoustic startle response, locomotor activity and dopamine levels could be determined every 2 hours after removal of the osmotic minipumps for 2 days to draw a curve and to accurately determine when withdrawal symptoms reach a peak in rats.

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APPENDIX A – LOCOMOTOR ACTIVITY RAW DATA

GROUP 1

Table A.1. Horizontal activity of the 8 rats in Group 1.

Day	2	27	29	32	37
Rat nr.	Total number of horizontal movements in 2 hours				
1	5184	7869	1756	5012	5518
2	7248	10308	3516	5901	5400
3	6000	8430	3855	6162	2399
4	4004	4971	3636	5039	2136
5	3749	5707	1392	1938	3508
6	4703	6314	2654	4003	5573
7	3990	6315	2712	1890	5758
8	4791	4768	1602	2351	3367
Sum	**39669**	**54682**	**21123**	**32296**	**33659**
Average	**4958.625**	**6835.250**	**2640.375**	**4037.000**	**4207.375**

Table A.2. Vertical activity of the 8 rats in Group 1.

Day	2	27	29	32	37
Rat nr.	Total number of vertical movements in 2 hours				
1	491	1011	364	949	924
2	705	1223	532	1022	368
3	698	813	686	718	131
4	529	869	859	1133	320
5	251	576	41	25	596
6	205	796	62	356	910
7	237	968	128	120	1077
8	599	568	106	623	633
Sum	**3715**	**6824**	**2778**	**4946**	**4959**
Average	**464.375**	**853.000**	**347.250**	**618.250**	**619.875**

Table A.3. Total activity of the 8 rats in Group 1.

Day	2	27	29	32	37
Rat nr.	**Total number of movements in 2 hours**				
1	5675	8880	2120	5961	6442
2	7953	11531	4048	6923	5768
3	6698	9243	4541	6880	2530
4	4533	5840	4495	6172	2456
5	4000	6283	1433	1963	4104
6	4908	7110	2716	4359	6483
7	4227	7283	2840	2010	6835
8	5390	5336	1708	2974	4000
Sum	**43384**	**61506**	**23901**	**37242**	**38618**
Average	**5423.000**	**7688.250**	**2987.625**	**4655.250**	**4827.250**

GROUP 2

Table A.4. Horizontal activity of the 8 rats in Group 2.

Day	2	27	29	32	37
Rat nr.	**Total number of horizontal movements in 2 hours**				
9	5606	6533	3110	1500	5643
10	5880	8432	3514	2090	8207
11	4592	4556	2199	3201	4065
12	5349	3749	4160	1564	3214
13	6561	7625	4330	5096	4535
14	3054	5675	6698	4534	4565
15	5199	5282	3325	3791	6676
16	2536	4621	2529	5245	6633
Sum	**38777**	**46473**	**29865**	**27021**	**43538**
Average	**4847.125**	**5809.125**	**3733.125**	**3377.625**	**5442.250**

Table A.5. Vertical activity of the 8 rats in Group 2.

Day	2	27	29	32	37
Rat nr.	**Total number of vertical movements in 2 hours**				
9	710	794	451	16	944
10	1014	1167	285	73	1363
11	543	637	163	94	514
12	542	710	617	100	470
13	661	1212	431	1340	1398
14	413	1110	1253	1000	727
15	568	652	286	699	1426
16	324	614	247	770	1229
Sum	**4775**	**6896**	**3733**	**4092**	**8071**
Average	**596.875**	**862.000**	**466.625**	**511.500**	**1008.875**

Table A.6. Total activity of the 8 rats in Group 2.

Day	2	27	29	32	37
Rat nr.	**Total number of movements in 2 hours**				
9	6316	7327	3561	1516	6587
10	6894	9599	3799	2163	9570
11	5135	5193	2362	3295	4579
12	5891	4459	4777	1664	3684
13	7222	8837	4761	6436	5933
14	3467	6785	7951	5534	5292
15	5768	5934	3611	4490	8102
16	2860	5235	2776	6015	7862
Sum	**43553**	**53369**	**33598**	**31113**	**51609**
Average	**5444.125**	**6671.125**	**4199.750**	**3889.125**	**6451.125**

GROUP 3

Table A.7. Horizontal activity of the 8 rats in Group 3.

Day	2	27	29	32	37
Rat nr.	**Total number of horizontal movements in 2 hours**				
17	2251	4460	4250	4832	4056
18	6075	1992	6657	9255	2431
19	4450	4398	7021	8405	4880
20	2960	2367	2723	3637	3254
21	3931	3831	3017	4677	3591
22	7471	7948	3775	5512	6829
23	6756	8315	2314	4835	6989
24	6622	4196	7587	4174	6191
Sum	**40516**	**37507**	**37344**	**45327**	**38221**
Average	**5064.500**	**4688.375**	**4668.000**	**5665.875**	**4777.625**

Table A.8. Vertical activity of the 8 rats in Group 3.

Day	2	27	29	32	37
Rat nr.	**Total number of vertical movements in 2 hours**				
17	46	181	800	637	494
18	244	58	914	1431	42
19	215	189	1839	1305	320
20	166	111	435	368	267
21	254	1302	1010	1179	1608
22	850	1000	359	1199	853
23	1366	1475	1122	1414	1561
24	381	585	997	439	922
Sum	**3522**	**4901**	**7476**	**7972**	**6067**
Average	**440.250**	**612.625**	**934.500**	**996.500**	**758.375**

Table A.9. Total activity of the 8 rats in Group 3.

Day	2	27	29	32	37
Rat nr.	**Total number of movements in 2 hours**				
17	2297	4641	5149	5469	4550
18	6319	2050	7571	10686	2473
19	4665	4587	8860	9710	5200
20	3126	2478	3158	4005	3521
21	4185	5133	4027	5856	5199
22	8321	8948	4134	6711	7682
23	8122	9790	3436	6249	8550
24	7003	4781	8584	4613	7113
Sum	**44038**	**42408**	**44919**	**53299**	**44288**
Average	**5504.750**	**5301.000**	**5614.875**	**6662.375**	**5536.000**

GROUP 4

Table A.10. Horizontal activity of the 8 rats in Group 4.

Day	2	27	29	32	37
Rat nr.	**Total number of horizontal movements in 2 hours**				
25	10876	12129	5820	4263	9420
26	5290	11082	4677	2933	10277
27	4592	6570	4245	3900	7929
28	3795	7621	3430	2309	9203
29	8161	5900	3792	17347	20095
30	7990	6785	7096	4208	5019
31	8506	3846	4143	10507	5420
32	6075	3693	3140	6615	6030
Sum	**55285**	**57626**	**36343**	**52082**	**73393**
Average	**6910.625**	**7203.250**	**4542.875**	**6510.250**	**9174.125**

Table A.11. Vertical activity of the 8 rats in Group 4.

Day	2	27	29	32	37
Rat nr.	**Total number of vertical movements in 2 hours**				
25	1220	1741	1188	470	1243
26	282	1794	903	317	1841
27	228	686	430	180	1296
28	662	1400	626	109	2075
29	979	486	608	3884	4753
30	768	530	1155	747	973
31	1030	200	511	1599	1178
32	1131	312	398	1070	1038
Sum	**6300**	**7149**	**5819**	**8376**	**14397**
Average	**787.500**	**893.625**	**727.375**	**1047.000**	**1799.625**

Table A.12. Total activity of the 8 rats in Group 4.

Day	2	27	29	32	37
Rat nr.	**Total number of movements in 2 hours**				
25	12096	13870	7008	4733	10663
26	5572	12876	5580	3250	12118
27	4820	7256	4675	4080	9225
28	4457	9021	4056	2418	11278
29	9140	6386	4400	23231	24848
30	8758	7315	8251	4955	5992
31	9536	4046	4654	12106	6598
32	7206	4005	3538	7685	7068
Sum	**61585**	**64775**	**42162**	**62458**	**87790**
Average	**7698.125**	**8096.875**	**5270.250**	**7807.250**	**10973.750**

APPENDIX B – ELEVATED PLUS-MAZE DATA

GROUP 1

Table B.1. Open arm exploration of the rats in Group 1 on day 27. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 27			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
1	300	0	0 %
2	300	0	0 %
3	300	0	0 %
4	300	0	0 %
5	300	0	0 %
6	300	0	0 %
7	300	0	0 %
8	300	0	0 %
9	300	0	0 %
10	300	0	0 %
11	300	0	0 %
12	300	0	0 %

Table B.2. Open arm exploration of the rats in Group 1 on day 34. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 34			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
1	300	0	0 %
2	300	0	0 %
3	300	0	0 %
4	300	5	1.67 %
5	300	0	0 %
6	300	0	0 %
7	300	0	0 %
8	300	0	0 %
9	300	0	0 %
10	300	0	0 %
11	300	0	0 %
12	300	0	0 %

Table B.3. Open arm exploration of the rats in Group 1 on day 41. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 41			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
1	300	0	0 %
2	300	0	0 %
3	300	0	0 %
4	300	0	0 %
5	300	0	0 %
6	300	0	0 %
7	300	0	0 %
8	300	0	0 %
9	300	0	0 %
10	300	0	0 %
11	300	0	0 %
12	300	0	0 %

GROUP 2

Table B.4. Open arm exploration of the rats in Group 2 on day 27. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 27			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
13	300	0	0 %
14	300	0	0 %
15	300	0	0 %
16	300	0	0 %
17	300	0	0 %
18	300	0	0 %
19	300	0	0 %
20	300	0	0 %
21	300	0	0 %
22	300	0	0 %
23	300	0	0 %
24	300	0	0 %

Table B.5. Open arm exploration of the rats in Group 2 on day 34. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 34			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
13	300	0	0 %
14	300	0	0 %
15	300	0	0 %
16	300	0	0 %
17	300	0	0 %
18	300	0	0 %
19	300	0	0 %
20	300	0	0 %
21	300	0	0 %
22	300	0	0 %
23	300	0	0 %
24	300	13	4.33 %

Table B.6. Open arm exploration of the rats in Group 2 on day 41. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 41			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
13	300	0	0 %
14	300	0	0 %
15	300	0	0 %
16	300	0	0 %
17	300	5	1.67 %
18	300	0	0 %
19	300	0	0 %
20	300	0	0 %
21	300	0	0 %
22	300	0	0 %
23	300	0	0 %
24	300	0	0 %

GROUP 3

Table B.7. Open arm exploration of the rats in Group 3 on day 27. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 27			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
25	300	0	0 %
26	300	0	0 %
27	300	0	0 %
28	300	0	0 %
29	300	0	0 %
30	300	0	0 %
31	300	0	0 %
32	300	0	0 %
33	300	0	0 %
34	300	0	0 %
35	300	0	0 %
36	300	0	0 %

Table B.8. Open arm exploration of the rats in Group 3 on day 34. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 34			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
25	300	0	0 %
26	300	0	0 %
27	300	0	0 %
28	300	0	0 %
29	300	0	0 %
30	300	0	0 %
31	300	0	0 %
32	300	0	0 %
33	300	0	0 %
34	300	0	0 %
35	300	0	0 %
36	300	0	0 %

Table B.9. Open arm exploration of the rats in Group 3 on day 41. (The values in blue represent the rats that did not receive the correct volume of smoke extract).

Day 41			
Rat nr.	**Total time spent on EPM (s)**	**Time spent on open arms (s)**	**Percentage of time spent on open arms**
25	300	0	0 %
26	300	0	0 %
27	300	0	0 %
28	300	0	0 %
29	300	0	0 %
30	300	0	0 %
31	300	25	8.33 %
32	300	0	0 %
33	300	27	9.10 %
34	300	0	0 %
35	300	0	0 %
36	300	0	0 %

APPENDIX C – ACOUSTIC STARTLE REFLEX RAW DATA

GROUP 1

Day 29

Table C.1. Startle amplitudes of the 8 rats in Group 1 during the 95 dB trials on day 29.

Day 29								
Trial 95								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	34	24	15	20	54	15	488	39
2	20	15	29	44	5	29	10	68
3	24	39	29	5	44	29	20	103
4	10	10	5	24	29	34	49	308
5	15	63	5	34	117	39	24	205
6	10	63	20	20	410	39	5	24
7	15	5	10	5	10	15	68	54
8	15	49	10	15	5	15	15	15
9	15	10	5	10	10	15	93	73
10	24	5	107	5	10	122	5	10
Average Startle amplitude of the 8 rats								**45.05**

Table C.2. Startle amplitudes of the 8 rats in Group 1 during the 100 dB trials on day 29.

Day 29								
Trial 100								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	15	20	15	24	200	24	98	112
2	24	39	122	49	10	44	29	273
3	63	122	15	15	430	78	273	303
4	63	20	20	29	39	88	381	63
5	15	88	15	29	298	88	63	366
6	5	63	10	54	542	15	239	132
7	88	15	49	5	195	59	15	215
8	83	54	5	15	190	15	54	161
9	127	171	5	5	698	29	132	225
10	29	15	34	10	254	63	15	103
Average Startle amplitude of the 8 rats								**103.188**

Table C.3. Startle amplitudes of the 8 rats in Group 1 during the 105 dB trials on day 29.

Day 29								
Trial 105								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	88	59	39	269	698	889	674	151
2	20	107	156	88	220	317	542	674
3	20	39	20	20	1230	449	498	952
4	24	20	20	29	601	73	430	811
5	15	78	117	10	273	703	98	1040
6	93	39	78	34	752	654	752	454
7	29	151	73	137	361	220	68	913
8	103	78	39	24	312	29	264	205
9	83	103	44	396	601	630	103	24
10	34	591	10	601	371	498	98	59
Average Startle amplitude of the 8 rats								**283.363**

Table C.4. Startle amplitudes of the 8 rats in Group 1 during the 110 dB trials on day 29.

Day 29								
Trial 110								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	29	161	103	205	234	732	894	479
2	78	234	425	376	264	347	845	1245
3	229	703	703	137	1250	1484	688	1460
4	78	112	122	479	117	1587	444	1343
5	44	146	156	259	630	142	161	1221
6	142	659	293	308	557	1235	1436	1064
7	49	63	493	786	742	444	405	923
8	386	635	210	156	347	112	605	703
9	215	679	93	29	669	620	1211	952
10	347	176	156	171	854	137	312	396
Average Startle amplitude of the 8 rats								**501.45**

Day 30

Table C.5. Startle amplitudes of the 8 rats in Group 1 during the 95 dB trials on day 30.

Day 30								
Trial 95								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	195	34	15	20	10	132	29	29
2	88	34	15	142	15	24	10	39
3	10	44	10	5	24	5	39	15
4	5	34	29	29	15	20	15	112
5	15	5	83	54	5	44	15	15
6	20	34	10	5	88	29	15	176
7	5	88	5	5	34	10	24	5
8	49	39	10	5	15	20	34	5
9	49	15	15	5	24	20	29	29
10	5	10	5	5	15	15	15	10
Average Startle amplitude of the 8 rats								**30.925**

Table C.6. Startle amplitudes of the 8 rats in Group 1 during the 100 dB trials on day 30.

Day 30								
Trial 100								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	107	93	20	29	34	44	200	112
2	234	151	24	78	107	15	200	88
3	15	15	78	29	63	5	39	63
4	98	117	54	54	5	20	835	44
5	20	10	39	24	171	98	298	117
6	29	93	20	68	20	347	137	63
7	59	181	10	5	59	20	166	39
8	44	288	5	5	244	195	273	15
9	10	122	10	5	34	10	20	98
10	190	10	20	5	10	127	10	171
Average Startle amplitude of the 8 rats								**88.55**

Table C.7. Startle amplitudes of the 8 rats in Group 1 during the 105 dB trials on day 30.

Day 30								
Trial 105								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	161	342	54	98	88	93	337	122
2	249	503	54	20	10	20	195	195
3	68	59	63	44	151	5	264	249
4	103	137	98	49	488	137	615	308
5	39	34	34	24	137	342	259	459
6	20	83	15	107	151	303	132	322
7	112	259	78	15	566	29	132	103
8	59	503	29	10	298	376	352	63
9	59	122	200	5	869	88	161	205
10	63	78	73	5	229	352	317	68
Average Startle amplitude of the 8 rats								**171.475**

Table C.8. Startle amplitudes of the 8 rats in Group 1 during the 110 dB trials on day 30.

Day 30								
Trial 110								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	39	298	15	264	679	210	674	264
2	68	166	117	308	44	103	703	420
3	103	371	225	63	376	264	1182	459
4	527	396	337	137	737	713	605	620
5	98	181	171	269	376	1304	244	991
6	29	312	186	29	347	684	469	1504
7	928	288	78	303	459	39	483	708
8	59	493	254	146	835	361	381	171
9	508	234	112	10	630	239	273	625
10	2588	54	166	24	1157	532	430	1069
Average Startle amplitude of the 8 rats								**416.475**

Day 31

Table C.9. Startle amplitudes of the 8 rats in Group 1 during the 95 dB trials on day 31.

Day 31								
Trial 95								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	49	98	20	49	127	20	73	225
2	15	15	10	20	127	10	10	20
3	10	24	5	39	10	20	29	195
4	10	63	20	39	10	10	15	10
5	10	24	29	20	24	15	10	10
6	15	20	5	5	5	10	34	15
7	10	54	5	5	10	10	15	10
8	5	15	39	10	34	29	44	10
9	15	10	5	5	34	15	29	5
10	5	15	5	5	176	10	5	20
Average Startle amplitude of the 8 rats								**28.9**

Table C.10. Startle amplitudes of the 8 rats in Group 1 during the 100 dB trials on day 31.

Day 31								
Trial 100								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	24	20	44	88	15	20	10	117
2	10	10	15	49	151	49	59	20
3	44	68	10	98	73	63	29	249
4	24	29	98	200	15	347	107	34
5	44	78	39	5	234	288	122	29
6	68	49	29	20	88	107	68	10
7	10	20	5	5	34	73	29	63
8	54	34	39	15	20	29	59	181
9	20	39	44	146	49	171	166	498
10	15	15	10	15	98	200	24	24
Average Startle amplitude of the 8 rats								**70.8875**

Table C.11. Startle amplitudes of the 8 rats in Group 1 during the 105 dB trials on day 31.

Day 31								
Trial 105								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	15	161	10	34	298	107	127	361
2	10	83	20	54	10	39	24	127
3	98	68	54	161	249	78	229	63
4	112	49	186	39	342	322	117	698
5	181	171	176	78	117	273	225	708
6	29	142	181	29	54	1538	654	146
7	39	107	78	20	210	122	229	171
8	39	142	161	34	234	117	654	98
9	15	24	34	29	39	54	107	146
10	44	54	15	15	10	293	103	229
Average Startle amplitude of the 8 rats								**158.913**

Table C.12. Startle amplitudes of the 8 rats in Group 1 during the 110 dB trials on day 31.

Day 31								
Trial 110								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	88	1226	68	83	288	684	649	371
2	68	356	88	601	107	273	49	244
3	103	117	59	195	439	49	674	723
4	786	146	166	34	161	161	645	1099
5	117	366	239	88	132	1357	825	161
6	327	210	762	59	73	923	522	596
7	146	439	303	142	171	430	142	1382
8	303	1099	293	34	405	552	991	288
9	83	39	205	195	103	352	137	317
10	283	190	1079	293	327	161	195	498
Average Startle amplitude of the 8 rats								**364.175**

Day 32

Table C.13. Startle amplitudes of the 8 rats in Group 1 during the 95 dB trials on day 32.

Day 32								
Trial 95								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	15	24	29	10	10	15	10	59
2	15	34	44	54	63	10	10	24
3	15	68	44	15	29	15	44	15
4	10	29	54	20	20	15	15	10
5	10	5	15	10	20	20	20	10
6	10	10	29	39	24	10	15	5
7	39	156	5	54	78	29	24	10
8	5	5	15	10	15	78	34	78
9	10	15	5	5	10	34	29	10
10	5	5	5	5	34	10	15	5
Average Startle amplitude of the 8 rats								**24.1625**

Table C.14. Startle amplitudes of the 8 rats in Group 1 during the 100 dB trials on day 32.

Day 32								
Trial 100								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	20	327	34	176	15	78	68	112
2	15	20	10	44	29	249	386	5
3	39	15	49	5	54	54	68	376
4	20	54	205	5	88	44	24	39
5	24	229	15	5	10	39	415	93
6	15	15	29	24	156	10	83	59
7	73	24	5	20	83	54	39	83
8	15	24	5	88	15	5	59	68
9	5	10	20	5	342	39	195	39
10	15	93	5	10	44	54	59	24
Average Startle amplitude of the 8 rats								**69.5875**

Table C.15. Startle amplitudes of the 8 rats in Group 1 during the 105 dB trials on day 32.

Day 32								
Trial 105								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	381	117	98	5	44	127	713	293
2	78	117	132	49	425	171	283	312
3	34	591	54	10	195	54	718	415
4	68	29	63	15	63	93	225	142
5	24	249	34	29	195	54	254	190
6	88	49	63	24	181	20	200	122
7	49	166	39	15	54	249	63	244
8	508	210	15	98	220	195	93	405
9	83	49	10	103	146	186	93	117
10	34	190	44	10	181	20	156	39
Average Startle amplitude of the 8 rats								**149.675**

Table C.16. Startle amplitudes of the 8 rats in Group 1 during the 110 dB trials on day 32.

Day 32								
Trial 110								
Session	**Rat 1**	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
1	107	249	117	63	254	327	269	176
2	15	483	88	83	215	884	3569	156
3	171	142	107	488	400	381	361	889
4	166	205	186	54	93	132	1006	444
5	205	205	112	44	449	146	474	381
6	269	2842	366	44	244	137	376	312
7	132	39	430	137	405	620	15	669
8	498	44	312	88	522	493	674	225
9	386	24	215	264	762	381	190	327
10	107	151	68	269	566	103	107	454
Average Startle amplitude of the 8 rats								**357.038**

GROUP 2

Day 29

Table C.17. Startle amplitudes of the 8 rats in Group 2 during the 95 dB trials on day 29.

Day 29								
Trial 95								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	54	24	20	29	24	34	24	15
2	73	24	20	142	5	15	10	5
3	20	24	15	88	5	15	59	5
4	29	20	63	24	10	10	15	29
5	34	10	44	20	5	5	234	10
6	39	59	29	29	15	5	381	44
7	29	15	10	24	5	54	24	15
8	10	10	10	10	29	5	63	10
9	20	39	10	10	15	29	15	29
10	10	10	15	15	5	5	161	15
Average Startle amplitude of the 8 rats								**32.975**

Table C.18. Startle amplitudes of the 8 rats in Group 2 during the 100 dB trials on day 29.

Day 29								
Trial 100								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	78	73	376	68	15	54	127	24
2	34	63	34	171	73	10	200	63
3	44	171	132	29	5	15	39	10
4	15	210	146	24	10	15	151	44
5	20	39	10	29	49	10	239	459
6	34	83	34	186	73	20	44	210
7	34	15	10	93	10	15	220	15
8	15	54	44	200	20	24	479	293
9	20	34	181	34	10	10	117	49
10	49	15	59	39	10	10	20	20
Average Startle amplitude of the 8 rats								**78.5**

Table C.19. Startle amplitudes of the 8 rats in Group 2 during the 105 dB trials on day 29.

Day 29								
Trial 105								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	49	610	366	137	112	15	659	127
2	186	151	88	229	63	39	63	449
3	44	386	151	83	29	29	127	449
4	34	54	371	39	34	49	103	39
5	249	381	127	103	400	132	972	142
6	63	361	190	122	54	83	464	112
7	98	312	161	103	63	24	474	117
8	59	239	352	54	39	10	532	420
9	44	186	601	298	10	15	122	264
10	640	171	78	537	24	88	718	15
Average Startle amplitude of the 8 rats								**202.338**

Table C.20. Startle amplitudes of the 8 rats in Group 2 during the 110 dB trials on day 29.

Day 29								
Trial 110								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	723	522	1450	190	259	59	815	234
2	288	796	630	576	195	171	117	391
3	542	317	269	439	356	20	391	435
4	132	337	386	127	293	73	474	396
5	366	493	415	229	366	107	1177	117
6	132	391	1099	103	215	215	371	459
7	264	1021	430	444	98	552	474	469
8	171	1016	703	381	142	15	1494	391
9	166	469	649	303	20	195	483	332
10	273	1147	454	1299	171	444	942	239
Average Startle amplitude of the 8 rats								**428.863**

Day 30

Table C.21. Startle amplitudes of the 8 rats in Group 2 during the 95 dB trials on day 30.

Day 30								
Trial 95								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	161	10	137	34	10	15	112	10
2	68	10	20	107	20	10	20	10
3	39	10	29	20	10	88	39	78
4	59	10	29	73	15	10	73	15
5	10	10	20	10	15	15	83	127
6	15	10	5	98	20	20	44	5
7	15	24	29	117	5	29	59	5
8	5	10	20	34	63	10	24	10
9	10	10	20	49	24	49	132	10
10	54	15	10	5	10	15	10	20
Average Startle amplitude of the 8 rats								**34.5**

Table C.22. Startle amplitudes of the 8 rats in Group 2 during the 100 dB trials on day 30.

Day 30								
Trial 100								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	88	93	220	63	24	20	122	5
2	54	63	24	200	44	24	49	20
3	156	39	39	39	39	5	59	20
4	68	161	78	59	34	10	49	39
5	88	20	73	54	10	20	327	10
6	10	54	54	54	220	44	186	298
7	161	400	10	664	39	29	200	190
8	24	34	20	127	39	278	88	20
9	15	29	117	54	10	20	161	29
10	39	63	112	88	39	15	44	15
Average Startle amplitude of the 8 rats								**83.775**

Table C.23. Startle amplitudes of the 8 rats in Group 2 during the 105 dB trials on day 30.

Day 30								
Trial 105								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	93	171	2725	195	63	49	415	54
2	679	1211	98	469	54	15	151	39
3	176	132	146	127	161	39	718	293
4	103	293	249	308	146	20	483	24
5	288	386	156	54	68	20	410	15
6	142	454	435	166	137	63	186	933
7	205	161	205	200	20	20	479	200
8	215	483	146	361	54	68	435	73
9	20	469	693	142	63	156	610	15
10	288	181	449	215	63	88	68	10
Average Startle amplitude of the 8 rats								**258.338**

Table C.24. Startle amplitudes of the 8 rats in Group 2 during the 110 dB trials on day 30.

Day 30								
Trial 110								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	39	557	4639	234	610	356	371	54
2	615	356	1865	317	229	34	928	210
3	820	444	601	249	396	127	801	225
4	1367	547	640	537	361	117	361	220
5	757	566	1001	215	142	93	518	5
6	391	991	2212	78	278	44	552	1445
7	840	659	415	679	68	264	493	103
8	430	815	957	630	137	283	874	527
9	342	566	986	347	361	34	1392	29
10	1055	532	1416	63	488	112	806	20
Average Startle amplitude of the 8 rats								**565.475**

Day 31

Table C.25. Startle amplitudes of the 8 rats in Group 2 during the 95 dB trials on day 31.

Day 31								
Trial 95								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	63	20	34	161	5	15	10	10
2	39	39	29	15	15	15	10	10
3	10	15	34	63	29	15	15	15
4	5	20	15	5	5	5	34	10
5	29	54	20	5	15	10	29	15
6	29	15	44	44	10	10	63	10
7	5	29	5	10	10	5	20	20
8	10	10	73	39	5	10	5	15
9	5	10	15	5	15	10	29	5
10	24	24	83	5	15	10	10	24
Average Startle amplitude of the 8 rats								**21.8375**

Table C.26. Startle amplitudes of the 8 rats in Group 2 during the 100 dB trials on day 31.

Day 31								
Trial 100								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	63	59	10	24	15	146	93	10
2	73	20	73	122	20	39	20	15
3	29	49	29	166	24	34	10	15
4	73	59	68	83	29	10	439	44
5	44	98	39	24	10	10	112	171
6	68	29	15	59	10	10	259	190
7	10	24	5	5	34	5	39	10
8	24	5	59	39	83	10	5	39
9	20	15	117	20	10	10	24	225
10	44	24	54	29	24	98	98	24
Average Startle amplitude of the 8 rats								**54.2875**

Table C.27. Startle amplitudes of the 8 rats in Group 2 during the 105 dB trials on day 31.

Day 31								
Trial 105								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	288	20	103	195	44	107	898	88
2	54	34	151	137	10	15	107	34
3	132	49	68	166	29	24	259	146
4	112	44	132	78	93	88	356	542
5	190	312	34	34	10	10	200	210
6	142	225	88	49	20	20	513	449
7	54	49	132	103	137	10	59	39
8	39	137	488	889	44	10	10	137
9	34	195	15	142	59	24	234	39
10	146	88	44	68	73	98	317	49
Average Startle amplitude of the 8 rats								**138.388**

Table C.28. Startle amplitudes of the 8 rats in Group 2 during the 110 dB trials on day 31.

Day 31								
Trial 110								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	874	59	254	967	293	195	371	464
2	298	117	161	49	44	161	166	273
3	791	190	337	127	117	68	635	591
4	127	68	303	93	205	68	1191	244
5	723	225	737	49	176	103	879	439
6	10	225	620	103	396	132	825	166
7	273	537	435	430	220	298	151	63
8	200	464	73	386	732	176	24	591
9	73	586	308	117	288	244	791	181
10	347	93	503	1221	181	34	317	205
Average Startle amplitude of the 8 rats								**328.138**

Day 32

Table C.29. Startle amplitudes of the 8 rats in Group 2 during the 95 dB trials on day 32.

Day 32								
Trial 95								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	10	29	29	10	10	10	29	10
2	39	15	5	15	10	10	10	39
3	5	5	20	15	5	24	20	15
4	5	10	29	24	49	5	29	10
5	20	15	103	5	10	5	10	10
6	10	5	5	49	34	10	20	20
7	5	5	5	5	15	10	15	5
8	5	15	5	44	10	5	10	20
9	5	5	15	29	181	10	15	10
10	5	54	5	10	10	10	98	10
Average Startle amplitude of the 8 rats								**19.15**

Table C.30. Startle amplitudes of the 8 rats in Group 2 during the 100 dB trials on day 32.

Day 32								
Trial 100								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	10	20	29	176	20	10	20	59
2	15	10	59	39	10	10	44	112
3	20	63	34	78	39	20	176	10
4	73	5	5	29	15	15	44	29
5	24	24	63	5	15	15	15	39
6	5	63	34	93	15	24	24	146
7	10	5	34	39	68	5	420	34
8	215	156	5	20	10	10	29	59
9	44	24	20	332	10	15	24	117
10	142	220	39	29	49	15	112	29
Average Startle amplitude of the 8 rats								**53.025**

Table C.31. Startle amplitudes of the 8 rats in Group 2 during the 105 dB trials on day 32.

Day 32								
Trial 105								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	78	44	142	39	20	63	591	24
2	181	98	20	24	34	10	195	234
3	15	20	49	366	107	24	264	59
4	20	20	39	20	15	39	112	127
5	54	15	151	137	39	10	73	503
6	146	44	34	205	54	34	73	210
7	24	59	20	137	88	24	562	112
8	29	68	146	88	103	10	34	366
9	54	39	190	78	10	20	103	142
10	161	249	29	249	332	15	103	24
Average Startle amplitude of the 8 rats								**106.788**

Table C.32. Startle amplitudes of the 8 rats in Group 2 during the 110 dB trials on day 32.

Day 32								
Trial 110								
Session	**Rat 9**	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
1	44	166	39	15	195	161	205	337
2	171	34	117	283	215	107	396	576
3	34	15	83	1279	73	459	132	117
4	195	93	127	581	112	210	283	98
5	229	195	229	312	464	146	303	229
6	444	15	186	571	864	83	190	342
7	273	156	117	215	371	68	728	107
8	601	288	356	415	557	220	293	376
9	322	298	234	312	635	142	215	386
10	117	112	1006	454	645	376	430	312
Average Startle amplitude of the 8 rats								**286.138**

GROUP 3

Day 29

Table C.33. Startle amplitudes of the 8 rats in Group 3 during the 95 dB trials on day 29.

Day 29								
Trial 95								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	10	63	24	498	34	78	10	15
2	83	34	10	20	10	156	5	498
3	39	20	20	10	5	29	20	15
4	171	15	20	39	122	176	10	15
5	5	34	5	10	20	15	10	88
6	20	34	29	20	39	88	24	15
7	10	5	10	20	161	107	34	942
8	15	34	15	34	34	78	20	44
9	10	15	10	5	39	151	10	127
10	20	10	5	24	10	15	34	10
Average Startle amplitude of the 8 rats								**59.85**

Table C.34. Startle amplitudes of the 8 rats in Group 3 during the 100 dB trials on day 29.

Day 29								
Trial 100								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	78	171	63	229	205	249	20	49
2	49	132	59	93	24	63	39	142
3	54	166	39	39	24	44	10	29
4	39	205	39	459	83	73	10	210
5	39	220	15	146	215	88	20	63
6	54	54	24	49	107	54	15	366
7	513	215	98	132	127	132	103	200
8	103	156	24	73	308	127	93	376
9	73	44	29	112	542	269	10	571
10	161	29	10	10	29	44	29	24
Average Startle amplitude of the 8 rats								**118.538**

Table C.35. Startle amplitudes of the 8 rats in Group 3 during the 105 dB trials on day 29.

Day 29								
Trial 105								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	132	405	322	112	459	54	83	137
2	420	850	210	400	347	103	49	63
3	171	771	161	542	557	186	117	112
4	20	361	244	156	767	29	151	156
5	234	68	63	264	361	73	127	249
6	176	381	107	54	488	400	49	254
7	312	273	322	78	49	249	127	205
8	439	400	396	347	474	405	210	479
9	488	132	161	132	527	439	10	244
10	151	112	132	205	190	34	34	405
Average Startle amplitude of the 8 rats								**251.95**

Table C.36. Startle amplitudes of the 8 rats in Group 3 during the 110 dB trials on day 29.

Day 29								
Trial 110								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	1021	1816	2422	1357	352	352	63	654
2	1353	840	542	1260	1064	93	161	132
3	903	474	1597	903	1221	269	107	34
4	898	347	269	488	522	210	396	2803
5	342	386	156	532	596	161	200	1362
6	664	410	679	234	1436	181	205	938
7	1240	806	732	327	249	396	498	1084
8	1187	1484	601	410	864	908	127	1328
9	723	327	312	488	684	542	78	156
10	913	210	137	601	347	522	63	791
Average Startle amplitude of the 8 rats								**656.75**

Day 30

Table C.37. Startle amplitudes of the 8 rats in Group 3 during the 95 dB trials on day 30.

Day 30								
Trial 95								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	132	39	59	15	15	981	39	24
2	15	29	39	10	10	78	88	29
3	24	88	15	20	20	181	15	29
4	73	20	73	20	20	63	10	34
5	5	15	10	10	10	29	5	107
6	15	29	10	24	10	24	78	20
7	15	54	10	15	59	29	15	54
8	15	20	34	24	10	10	15	10
9	24	44	39	5	34	15	20	29
10	15	10	54	15	20	10	10	54
Average Startle amplitude of the 8 rats								**43.6875**

Table C.38. Startle amplitudes of the 8 rats in Group 3 during the 100 dB trials on day 30.

Day 30								
Trial 100								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	366	137	59	88	39	127	59	39
2	59	308	151	15	15	137	54	78
3	166	347	220	63	112	68	10	132
4	425	117	117	34	34	54	29	73
5	73	200	24	54	425	98	78	15
6	107	54	15	78	44	117	68	93
7	98	327	405	20	20	20	98	63
8	112	68	103	49	5	635	29	132
9	83	68	54	78	20	29	142	103
10	63	137	63	151	98	151	161	83
Average Startle amplitude of the 8 rats								**111.788**

Table C.39. Startle amplitudes of the 8 rats in Group 3 during the 105 dB trials on day 30.

Day 30								
Trial 105								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	156	132	83	381	137	391	68	342
2	176	98	537	166	112	78	332	88
3	186	273	273	386	59	615	44	171
4	366	1333	312	361	54	396	366	220
5	10	20	449	552	171	1528	122	15
6	151	649	352	151	142	352	78	29
7	112	278	698	259	234	371	83	195
8	1221	312	249	93	10	137	29	566
9	49	352	234	132	146	327	63	127
10	625	708	1392	186	205	161	127	220
Average Startle amplitude of the 8 rats								**292.05**

Table C.40. Startle amplitudes of the 8 rats in Group 3 during the 110 dB trials on day 30.

Day 30								
Trial 110								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	195	220	186	332	176	303	122	3306
2	127	205	688	283	283	1050	288	1396
3	78	635	977	532	239	957	317	654
4	483	830	391	317	811	122	347	1128
5	532	381	142	850	708	718	322	503
6	767	444	469	63	552	1387	186	366
7	293	576	854	166	649	1064	303	1890
8	400	259	518	186	1045	205	303	527
9	464	1040	796	967	962	654	122	195
10	537	425	610	273	493	312	356	869
Average Startle amplitude of the 8 rats								**558.513**

Day 31

Table C.41. Startle amplitudes of the 8 rats in Group 3 during the 95 dB trials on day 31.

Day 31								
Trial 95								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	39	166	34	20	10	20	15	63
2	24	24	10	15	10	132	10	112
3	34	15	20	10	10	49	10	127
4	24	29	15	10	10	34	10	73
5	15	10	24	10	5	24	20	63
6	10	29	29	10	10	10	5	15
7	29	10	15	49	10	63	5	63
8	34	20	15	10	10	20	29	15
9	20	63	15	10	10	15	15	10
10	522	39	20	10	10	73	10	5
Average Startle amplitude of the 8 rats								**33.9625**

Table C.42. Startle amplitudes of the 8 rats in Group 3 during the 100 dB trials on day 31.

Day 31								
Trial 100								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	93	117	15	88	10	54	10	156
2	220	107	44	49	54	78	15	49
3	93	229	49	15	68	20	15	59
4	98	49	225	156	15	39	34	78
5	68	132	78	10	20	24	15	49
6	366	49	229	54	117	34	29	44
7	161	63	20	88	39	49	15	176
8	142	54	161	54	29	234	15	132
9	137	68	24	59	10	93	93	44
10	88	29	122	34	34	220	15	83
Average Startle amplitude of the 8 rats								**78.8**

Table C.43. Startle amplitudes of the 8 rats in Group 3 during the 105 dB trials on day 31.

Day 31								
Trial 105								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	34	176	186	532	220	225	34	181
2	122	122	669	98	103	264	49	430
3	195	278	264	54	10	244	112	103
4	103	269	356	469	73	293	44	73
5	254	283	181	312	78	63	332	54
6	195	59	337	630	63	205	88	356
7	347	117	405	142	20	49	83	801
8	239	107	239	142	68	195	49	303
9	376	332	151	78	317	645	244	366
10	283	49	171	327	63	190	24	88
Average Startle amplitude of the 8 rats								**211.063**

Table C.44. Startle amplitudes of the 8 rats in Group 3 during the 110 dB trials on day 31.

Day 31								
Trial 110								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	103	542	190	581	518	928	29	376
2	537	1133	435	1016	103	49	215	562
3	1157	347	283	850	205	337	361	410
4	1216	508	576	981	684	688	303	420
5	1025	405	420	1079	34	635	278	10
6	430	142	483	288	845	381	361	278
7	508	186	659	513	20	15	869	488
8	400	688	557	454	366	464	332	654
9	312	220	244	376	288	508	151	220
10	3042	220	210	215	762	83	391	410
Average Startle amplitude of the 8 rats								**482.025**

Day 32

Table C.45. Startle amplitudes of the 8 rats in Group 3 during the 95 dB trials on day 32.

Day 32								
Trial 95								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	20	24	15	24	5	59	39	44
2	15	39	15	54	5	15	10	34
3	10	24	10	20	10	15	10	39
4	34	15	10	10	20	34	5	39
5	15	5	10	10	5	34	20	20
6	10	10	10	15	146	34	15	5
7	10	54	15	29	10	20	10	15
8	54	20	156	24	20	39	15	5
9	15	10	15	5	10	10	5	29
10	63	20	10	15	15	24	5	10
Average Startle amplitude of the 8 rats								**23.0375**

Table C.46. Startle amplitudes of the 8 rats in Group 3 during the 100 dB trials on day 32.

Day 32								
Trial 100								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	44	39	39	161	10	146	39	327
2	10	54	122	371	15	122	15	156
3	98	312	278	293	5	220	10	249
4	24	20	68	20	24	112	10	420
5	20	68	59	54	10	54	44	107
6	49	98	39	29	73	54	63	88
7	186	34	39	29	98	117	24	20
8	49	29	54	59	39	195	15	146
9	54	78	20	29	39	44	10	112
10	103	493	122	20	356	44	29	122
Average Startle amplitude of the 8 rats								**94.375**

Table C.47. Startle amplitudes of the 8 rats in Group 3 during the 105 dB trials on day 32.

Day 32								
Trial 105								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	39	283	151	49	68	103	103	103
2	93	83	190	117	103	503	54	190
3	34	283	195	137	29	132	20	2236
4	186	205	78	146	200	112	29	2065
5	132	278	332	49	522	356	176	117
6	112	4795	332	137	303	78	73	44
7	254	225	156	20	39	215	400	107
8	498	342	4414	54	112	190	112	186
9	200	3076	146	54	410	186	10	68
10	112	220	288	244	684	98	156	244
Average Startle amplitude of the 8 rats								**370.938**

Table C.48. Startle amplitudes of the 8 rats in Group 3 during the 110 dB trials on day 32.

Day 32								
Trial 110								
Session	**Rat 17**	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
1	986	801	229	327	225	132	244	1450
2	254	522	415	210	2529	190	122	581
3	166	205	249	244	15	1250	29	1821
4	156	195	161	137	576	366	215	5322
5	244	586	801	273	376	1387	278	586
6	249	73	571	396	737	835	312	312
7	693	5645	415	352	396	439	254	166
8	571	449	293	332	454	562	239	352
9	176	640	225	78	679	220	308	205
10	864	298	249	527	396	181	254	7744
Average Startle amplitude of the 8 rats								**662.45**

GROUP 4

Day 29

Table C.49. Startle amplitudes of the 8 rats in Group 4 during the 95 dB trials on day 29.

Day 29								
Trial 95								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	220	44	39	308	24	15	146	29
2	10	15	44	259	112	39	34	68
3	20	15	20	59	68	10	34	39
4	15	10	20	73	20	5	20	20
5	59	24	34	259	107	63	49	20
6	73	15	10	15	15	68	132	29
7	63	44	63	63	49	63	63	107
8	73	10	10	220	15	10	10	181
9	63	10	73	73	15	20	15	68
10	171	15	10	186	5	15	73	93
Average Startle amplitude of the 8 rats								**59.6875**

Table C.50. Startle amplitudes of the 8 rats in Group 4 during the 100 dB trials on day 29.

Day 29								
Trial 100								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	566	49	176	122	127	78	63	6313
2	98	73	107	15	98	103	34	112
3	59	24	361	20	15	63	303	215
4	44	15	49	762	88	29	137	200
5	44	20	49	273	15	366	88	435
6	166	15	234	190	39	200	98	342
7	405	20	503	29	63	83	44	103
8	117	10	117	166	15	210	259	112
9	93	10	234	278	34	39	10	190
10	200	444	200	190	15	15	117	93
Average Startle amplitude of the 8 rats								**218.875**

Table C.51. Startle amplitudes of the 8 rats in Group 4 during the 105 dB trials on day 29.

Day 29								
Trial 105								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	283	298	229	117	142	83	317	1069
2	107	132	186	137	78	303	127	439
3	146	34	371	103	10	435	386	293
4	93	20	59	176	83	200	151	244
5	439	63	449	171	239	586	425	234
6	381	93	732	1172	68	1011	107	1152
7	1377	527	747	298	49	454	620	93
8	264	298	420	498	107	420	640	293
9	1270	132	581	396	132	156	93	220
10	1807	34	254	439	20	464	186	73
Average Startle amplitude of the 8 rats								**344.188**

Table C.52. Startle amplitudes of the 8 rats in Group 4 during the 110 dB trials on day 29.

Day 29								
Trial 110								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	376	205	483	9995	59	566	527	1260
2	371	225	449	562	117	654	98	1079
3	34	93	522	356	117	78	410	1006
4	312	728	347	293	59	220	254	728
5	425	205	547	366	156	405	293	688
6	225	288	659	483	264	1147	718	811
7	264	107	1191	522	107	684	742	127
8	732	425	713	1196	171	591	381	229
9	537	913	625	835	59	591	811	142
10	1011	1089	537	928	161	718	54	347
Average Startle amplitude of the 8 rats								**597.538**

Day 30

Table C.53. Startle amplitudes of the 8 rats in Group 4 during the 95 dB trials on day 30.

Day 30								
Trial 95								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	78	29	39	20	29	15	34	78
2	59	59	20	142	54	15	29	20
3	39	20	68	151	15	15	20	59
4	10	10	63	20	24	29	15	15
5	98	24	10	49	39	20	146	15
6	29	98	10	34	10	15	68	44
7	15	24	98	34	15	15	10	293
8	39	10	15	20	15	10	10	88
9	137	15	44	15	15	117	10	15
10	29	59	5	20	5	15	39	20
Average Startle amplitude of the 8 rats								**40.9125**

Table C.54. Startle amplitudes of the 8 rats in Group 4 during the 100 dB trials on day 30.

Day 30								
Trial 100								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	34	29	44	918	190	229	73	269
2	24	49	68	151	49	156	107	562
3	513	44	259	127	103	293	59	918
4	83	29	352	63	15	107	220	142
5	44	54	39	49	166	151	361	132
6	63	15	20	59	10	15	259	181
7	98	371	220	132	73	190	220	117
8	59	39	117	59	34	146	44	112
9	137	166	210	44	20	552	283	29
10	127	20	54	49	10	73	83	39
Average Startle amplitude of the 8 rats								**147.8**

Table C.55. Startle amplitudes of the 8 rats in Group 4 during the 105 dB trials on day 30.

Day 30								
Trial 105								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	195	2124	776	1533	98	1182	366	728
2	552	176	103	2061	93	127	596	181
3	122	107	244	3486	39	557	127	5146
4	420	54	283	161	127	39	63	288
5	898	283	244	156	151	767	518	117
6	1016	181	552	2031	44	225	1113	210
7	298	269	752	171	137	488	303	308
8	381	107	513	317	88	684	327	1035
9	493	103	269	88	39	166	278	620
10	1118	151	312	132	54	757	44	386
Average Startle amplitude of the 8 rats								**523.1**

Table C.56. Startle amplitudes of the 8 rats in Group 4 during the 110 dB trials on day 30.

Day 30								
Trial 110								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	356	566	342	430	269	518	1118	747
2	1777	386	1045	166	430	205	850	527
3	1553	200	1406	493	322	1064	537	513
4	63	112	469	142	381	146	122	146
5	273	366	1807	269	376	522	620	391
6	1016	278	303	1108	308	679	679	278
7	2417	1865	1353	225	449	1460	776	381
8	1606	625	620	874	112	605	620	581
9	1792	195	718	210	298	864	737	1069
10	625	278	435	103	122	649	425	361
Average Startle amplitude of the 8 rats								**626.55**

Day 31

Table C.57. Startle amplitudes of the 8 rats in Group 4 during the 95 dB trials on day 31.

Day 31								
Trial 95								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	39	15	73	29	20	34	39	78
2	39	10	24	24	63	39	29	49
3	49	10	20	49	10	34	176	29
4	15	127	24	20	10	20	24	24
5	20	39	15	49	54	10	78	68
6	59	20	54	20	20	15	10	44
7	44	10	15	24	10	20	20	98
8	39	10	10	24	10	15	15	20
9	15	15	24	117	15	10	63	34
10	63	10	20	10	5	20	63	54
Average Startle amplitude of the 8 rats								**34.275**

Table C.58. Startle amplitudes of the 8 rats in Group 4 during the 100 dB trials on day 31.

Day 31								
Trial 100								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	103	34	24	39	24	88	342	283
2	137	98	78	59	98	137	117	127
3	186	24	59	73	93	20	39	78
4	15	59	54	176	59	10	127	103
5	44	39	117	107	20	24	68	176
6	103	146	59	186	78	103	54	156
7	234	78	93	34	10	63	210	151
8	88	146	24	24	225	59	63	73
9	24	20	49	20	83	49	220	98
10	39	39	190	34	15	39	59	181
Average Startle amplitude of the 8 rats								**89.7125**

Table C.59. Startle amplitudes of the 8 rats in Group 4 during the 105 dB trials on day 31.

Day 31								
Trial 105								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	142	151	78	73	278	254	234	1597
2	181	312	146	474	278	98	107	117
3	610	176	103	127	54	513	132	132
4	215	166	59	728	10	220	342	225
5	156	508	288	479	73	166	146	425
6	264	112	337	83	107	83	273	127
7	547	73	78	132	464	112	78	63
8	200	83	171	371	225	186	239	225
9	68	215	161	20	146	83	146	200
10	303	68	117	317	54	88	522	562
Average Startle amplitude of the 8 rats								**228.45**

Table C.60. Startle amplitudes of the 8 rats in Group 4 during the 110 dB trials on day 31.

Day 31								
Trial 110								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	181	474	459	254	317	176	513	1377
2	498	952	850	503	161	503	63	435
3	205	234	171	298	625	669	273	293
4	1064	63	308	88	239	190	718	918
5	757	396	293	483	205	2939	786	786
6	752	332	273	93	244	361	513	327
7	391	273	283	366	811	264	190	469
8	1162	122	1348	381	605	537	200	1763
9	498	234	439	645	552	259	283	190
10	2422	229	137	757	210	78	425	654
Average Startle amplitude of the 8 rats								**509.863**

Day 32

Table C.61. Startle amplitudes of the 8 rats in Group 4 during the 95 dB trials on day 32.

Day 32								
Trial 95								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	20	15	24	34	15	39	44	83
2	63	44	59	34	10	20	88	44
3	20	10	34	10	20	34	15	29
4	20	15	20	34	10	15	15	24
5	20	15	15	44	15	24	15	29
6	5	10	44	29	29	24	20	34
7	15	10	10	39	10	15	34	44
8	29	10	10	10	24	39	54	34
9	10	54	15	10	15	15	20	24
10	15	20	10	24	39	15	59	20
Average Startle amplitude of the 8 rats								**26.0625**

Table C.62. Startle amplitudes of the 8 rats in Group 4 during the 100 dB trials on day 32.

Day 32								
Trial 100								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	122	59	24	347	34	303	156	312
2	39	49	29	34	20	317	39	186
3	59	54	24	63	10	20	24	127
4	93	34	34	112	15	254	49	29
5	15	34	44	137	29	239	93	54
6	88	10	29	29	24	278	1040	1802
7	15	15	29	63	15	34	142	15
8	327	10	49	195	24	220	68	410
9	205	156	20	78	29	49	29	88
10	78	156	54	15	34	132	78	15
Average Startle amplitude of the 8 rats								**123.288**

Table C.63. Startle amplitudes of the 8 rats in Group 4 during the 105 dB trials on day 32.

Day 32								
Trial 105								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	1201	15	59	928	24	215	176	254
2	112	122	63	59	83	161	308	161
3	59	29	68	24	29	703	88	107
4	142	234	801	225	49	439	73	83
5	15	20	229	44	73	1108	410	117
6	49	15	215	166	127	425	249	205
7	342	44	127	15	93	1553	122	39
8	400	107	44	73	176	249	312	190
9	620	24	288	366	59	259	122	225
10	796	161	83	44	93	283	312	44
Average Startle amplitude of the 8 rats								**227.825**

Table C.64. Startle amplitudes of the 8 rats in Group 4 during the 110 dB trials on day 32.

Day 32								
Trial 110								
Session	**Rat 25**	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
1	283	24	430	405	137	479	137	376
2	2656	420	161	117	83	283	894	186
3	640	195	132	186	312	171	327	181
4	332	518	469	4224	103	923	200	1055
5	293	63	78	117	83	815	708	889
6	244	171	68	420	273	1099	396	605
7	483	195	122	566	278	1221	913	1914
8	801	1060	542	317	659	718	44	928
9	557	1353	308	771	29	728	278	254
10	405	200	264	850	54	586	44	635
Average Startle amplitude of the 8 rats								**517.975**

APPENDIX D - SCGE ASSAY RAW DATA

GROUP 1

Table D.1. Tailmoments of the striata cells (comets) of the rats in Group 1.

Tailmoment							
Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
0.541998	4.98923	3.30352	18.774	16.6261	0.253161	22.0499	0.242546
2.00044	3.59059	1.89801	16.4771	11.8454	12.9371	0.1303	0.233997
0.539655	2.28217	1.60615	14.9222	1.10054	0.678663	23.5271	0.189861
0.878196	3.56952	1.33926	5.47763	5.91898	3.3072	74.5591	0.192945
0.21796	4.62353	2.1993	23.9852	12.5552	0.34186	29.4355	0.244178
0.581066	2.5872	2.96546	46.8043	66.9842	0.224163	58.6189	0.151117
1.39055	2.57555	0.882423	8.27207	0.461807	0.44484	38.0741	0.387774
0.523621	1.3563	1.28184	97.7986	28.0033	0.199498	0.0939777	0.0311435
4.99397	6.92357	6.43571	44.0527	0.161148	0.212131	38.6361	0.239925
0.383114	3.93282	2.47951	6.19191	0.367254	101.187	30.7693	0.164495
42.2787	4.46474	3.00857	40.8847	1.43427	103.319	56.008	0.130132
1.04212	1.78857	10.412	6.65533	0.106173	138.184	61.1525	0.246398
2.12235	5.67719	2.99807	17.7197	53.0386	13.732	15.499	0.0920821
0.447593	6.07869	3.65296	7.16356	123.569	100.967	0.192655	0.00015952
0.878915	2.13136	2.10247	17.0768	0.956516	124.384	0.204235	1.33665
39.563	1.78311	2.67529	8.18194	0.109847	216.752	0.158544	0.352886
19.136	4.9828	7.64086	7.1224	23.0334	82.1936	35.5574	0.193851
2.11683	2.21201	9.00211	17.9453	0.344373	0.0736348	16.4948	1.51736
19.1882	19.1427	3.18592	101.534	1.45023	0.0414203	28.8234	0.258151
0.430984	4.28132	1.31151	23.5929	0.128184	3.80381	25.9532	0.75666
29.1783	2.32468	4.59526	27.0892	1.03884	0.728406	28.9789	0.152722
3.45813	3.23832	6.1421	11.6249	0.428302	0.256594	36.5451	0.0342181
0.716715	8.26596	3.60699	9.48623	2.73636	0.254518	18.3985	0.10193
137.664	4.82425	3.07945	16.5094	2.88048	0.0874807	0.0795988	0.242436
0.93419	4.08986	3.37626	7.72034	0.162865	116.868	30.8803	0.267038
0.150313	7.05075	6.54085	11.0625	0.972765	0.114258	32.3321	0.570445
1.2293	3.60618	7.46198	23.5883	0.322596	0.849803	25.8822	0.394482
70.7489	5.04196	2.72159	14.625	3.09314	0.329356	37.9031	0.197093
95.8651	2.85181	3.77349	29.1723	0.262715	0.329787	14.6894	0.696285
8.94777	4.60821	6.34085	16.5261	1.34156	24.967	18.0667	0.00020433
0.462855	72.6192	3.56237	12.748	21.6035	25.3455	44.4919	0.24815
1.24466	5.88318	1.48288	27.3782	0.182173	0.356709	10.9602	0.360584
1.2254	5.3885	14.7397	59.0354	0.817316	0.109477	0.0297704	0.0032595
9.28554	9.32761	9.19187	15.993	0.242304	20.089	39.3889	21.5062
0.65197	5.27706	1.82758	13.9844	3.02454	0.654285	8.45159	0.0003858
0.437789	2.73108	15.0223	21.2274	0.292561	0.343146	100.135	0.0637982
0.212923	8.61135	3.87897	8.99774	0.250279	0.242843	24.3437	0.104069

2.00318	9.50904	4.06982	10.9049	0.325425	0.386712	97.6392	0.199089
2.15969	10.8285	6.5112	5.85893	0.71673	0.0962826	73.2174	0.313309
0.091708	8.0521	3.75287	9.81183	5.75057	0.245444	49.5445	0.298919
2.44142	6.26864	3.85251	15.9787	12.7692	0.431612	115.266	0.0671471
8.76905	3.29396	9.83684	22.307	0.332686	82.3417	88.678	0.19083
0.476824	3.78556	1.02104	64.7452	30.9157	0.559505	89.3806	0.217591
16.2982	6.01029	0.983382	55.6893	0.540759	0.0882633	26.3258	0.00029308
0.976214	3.24019	2.13157	8.92036	30.8258	0.170506	1.67462	0.0805574
26.0314	16.4064	1.34434	3.82708	0.214555	0.492899	117.046	0.314128
0.696941	13.7642	6.37518	122.318	0.0514754	0.0808595	6.13902	0.396416
99.5723	4.93383	1.55852	55.2171	0.197105	0.10862	88.7041	0.312664
0.054689	2.95803	1.65802	56.6507	0.270642	46.2297	47.2907	0.605887
0.545038	1.48101	1.53024	72.4223	80.2325	46.1198	63.343	0.0662362
2.06241	3.88253	1.7996	11.2175	0.327864	41.6872	64.4216	0.0789713
0.071493	2.70696	2.2902	19.7271	5.41661	0.0850176	22.3959	0.480692
148.636	4.77392	2.94264	27.1793	0.24781	0.0245241	90.4784	0.492185
0.136782	1.09711	0.827859	107.448	0.191882	0.428718	0.259065	0.139611
3.4526	1.51101	21.6513	10.5047	0.178866	0.0849286	18.1577	0.152247
2.51789	5.54778	1.66606	19.9343	0.118256	30.8446	73.2451	0.256755
0.71103	2.40388	1.80992	18.0564	0.165893		91.2675	0.443255
2.35026	1.358	2.15565	22.2924	0.503506		60.6903	0.142521
3.98058	2.30519	0.410175	20.7303	0.355432		87.5515	0.0717291
0.061796	1.71285	3.01815	7.01658	0.768586		0.174276	1.05941
	12.6028	1.46783	26.3243	0.22508		117.501	0.00025556
	3.76618	7.56111	16.4871	4.00789		138.468	1.09761
	2.19285	5.78829	12.5847	0.262271		101.255	0.128096
	3.86583	11.7687	20.0913	0.279026		71.326	
	1.78934	10.5452	22.9389	0.687917		38.7809	
	3.02465	4.61402	36.7964	0.386933		56.7707	
	9.81875	2.40561	23.336	3.71024		65.428	
	5.26474	6.74727	11.0531	0.834419		52.8796	
	4.08212	6.2752	38.6681	0.285327		16.6935	
	6.64896	9.86506	22.64	0.527599		50.36	
	3.27886	4.76635	15.8957	0.837924		0.122973	
	3.96793	10.2933	21.6595	0.441399		105.256	
	2.56821	2.13154	20.2875	2.11968		47.6493	
	15.9366	2.68223	25.4351	4.17956		61.4071	
	3.13096	1.05462	6.04641	3.24828		7.70667	
	27.1656	99.8883	56.4673	0.312197		10.2197	
	7.56804	6.14456	57.677	0.465837		18.8741	
	17.0709	3.18955	40.5628	0.999606		40.8545	
	5.11828	11.5215	15.3351	0.256201		31.4891	
	7.87847	3.92749	18.1114	0.355738		0.0512584	
	6.75352	3.9698	12.7282	3.59452		100.925	
	2.59853	1.83312	5.91123	8.0862		6.88945	
	4.31309	3.89748	11.709	0.330173		56.4039	
	4.29165	4.78519	18.1284	0.239123		37.6878	
	13.4464	3.76901	29.5918	5.05224		21.2896	

3.74167	2.132	28.0695	0.918634	36.8106
4.14094	5.80976	23.6144	0.396282	102.974
5.62819	37.1185	23.5585	0.190523	0.0775937
27.1574	2.58551	24.6066	0.118594	0.559932
9.97554	12.4849	12.3513	0.254595	60.3795
7.53795	5.58995	59.1311		9.5162
4.51441	5.97983	28.1053		34.0612
4.20597	0.875349	22.8013		61.2114
5.68735	2.99757	20.0538		23.6936
3.55614	4.49809	24.3364		131.063
5.53158	1.02117	16.3289		110.143
4.15273	1.27094	93.7094		105.249
12.1842	3.06301	8.37152		
10.6693	2.78482	8.5775		
34.0173	1.32496	86.331		
3.26897	5.58675	30.1345		

Table D.2. Percentage of DNA in the tail of the striata cells (comets) of the rats in Group 1.

TailDNA%							
Rat 1	**Rat 2**	**Rat 3**	**Rat 4**	**Rat 5**	**Rat 6**	**Rat 7**	**Rat 8**
4.16922	14.6742	13.2141	28.8831	23.7516	2.8129	25.3447	3.03183
7.69401	11.2206	9.49006	23.5388	17.9476	19.9033	1.44778	2.92497
4.15119	7.86955	8.03073	24.4626	5.50269	4.84759	27.3571	2.37326
5.48873	10.1986	7.04876	18.8884	11.838	11.8114	52.1392	2.75636
2.42178	10.508	9.16376	32.4125	16.0964	3.4186	27.2551	2.71309
4.15047	7.84	11.8618	34.6699	49.9882	2.80203	42.4775	2.15882
9.27036	8.8812	6.78787	15.9078	3.84839	3.707	29.0642	3.52522
5.23621	6.45856	6.40918	49.145	21.3766	2.49372	1.56629	0.778588
11.6139	14.1297	14.9668	25.0299	2.30212	2.65163	31.1581	2.99906
4.25682	11.5671	8.85538	19.3497	3.33867	62.8494	28.2287	2.34993
49.1613	13.9523	9.40179	30.511	5.97613	57.0825	38.3617	2.16887
5.21058	7.7764	19.2814	19.5745	1.76956	69.7897	43.6804	3.07998
11.1703	17.7412	9.36898	24.6108	46.1205	20.1942	18.2341	1.84164
4.97326	12.1574	10.744	15.2416	69.4208	67.3113	2.40819	0.005317
5.49322	10.6568	9.14117	28.4613	3.18839	75.3841	2.55294	6.68324
38.7872	6.36826	11.6317	17.0457	2.19693	88.1105	2.26491	3.52886
29.9	13.1126	17.7694	13.4385	25.5927	51.371	32.325	2.7693
7.2994	9.21672	18.7544	16.3139	3.13067	1.4727	19.18	7.58679
29.9816	28.151	13.2747	48.8146	7.63279	1.03551	26.9378	3.22689
3.59154	14.2711	7.71479	15.6245	2.13641	9.75336	25.6962	4.45094
31.0407	8.30244	13.1293	29.1281	4.94686	4.55254	26.1072	1.69691
11.9246	11.5654	16.1634	24.2185	3.56918	2.85104	29.955	1.1406
3.98175	19.6809	11.6355	22.5863	11.4015	3.18148	26.6645	2.0386
68.1507	13.4007	9.62328	25.796	11.5219	1.74961	1.59198	3.03045

6.22794	12.7808	10.8912	16.4263	2.32664	59.024	32.5056	2.67038
2.50522	16.3971	19.2378	21.6912	4.42166	1.9043	35.1436	4.75371
7.23118	11.2693	16.959	26.5037	2.93269	4.99884	30.4496	3.94482
41.8633	14.0055	7.77597	28.6765	12.3726	2.99414	32.6751	2.81561
54.1611	9.50603	11.4348	33.9213	2.91906	3.29787	21.6021	4.35178
13.7658	13.5536	17.6135	21.4624	6.38836	28.0528	25.8096	0.006811
3.56042	35.252	12.284	20.235	24.2736	28.4781	41.1962	3.10187
6.22331	14.3492	6.74036	35.5561	2.27716	3.56709	21.4906	3.60584
7.20824	12.2466	18.4246	46.1214	2.47672	2.18954	0.992346	0.10865
16.5813	20.728	11.7844	31.3587	3.0288	19.6951	38.9989	20.679
5.43308	15.9911	8.30716	25.4261	6.43519	4.3619	15.6511	0.01286
1.98995	9.75387	17.6733	24.122	2.08972	3.81274	57.8817	0.911403
2.66154	19.5712	11.0828	18.3627	2.50279	2.42843	32.4582	1.73448
6.07026	16.6825	13.1285	19.4731	3.25425	3.86712	60.2711	2.84412
10.7984	21.2324	16.278	15.4182	4.47956	1.92565	44.6448	3.13309
1.01897	17.8936	12.5096	18.8689	12.5012	2.72716	41.9869	3.73648
8.13806	13.3375	13.2845	23.8488	16.3708	3.92374	59.1106	1.67868
13.4909	9.41131	13.8547	35.9791	3.69651	47.3228	51.259	2.72614
3.97353	12.2115	6.3815	46.5793	30.6096	2.94476	49.6559	2.41767
21.1665	15.0257	7.56448	34.3761	4.50633	1.47106	29.9157	0.009769
6.10134	11.1731	10.6578	20.2735	30.5206	2.13133	5.07462	2.01394
19.7207	26.4619	7.07546	12.3454	2.38395	2.73833	61.9293	3.14128
2.78776	21.8479	11.3842	49.7226	1.28689	1.61719	13.6423	3.60378
46.3127	13.7051	8.65845	35.6239	2.46381	1.81034	51.2741	3.47404
0.60765	11.8321	7.89532	41.0512	3.00713	31.8826	38.4477	4.32777
4.1926	8.7118	8.50133	41.8626	47.1956	37.8031	39.5894	1.32472
9.82102	14.3797	6.92155	24.9278	2.98058	29.3572	41.8322	1.31619
1.42987	12.8903	9.54248	27.7846	10.4166	1.70035	29.8612	4.36993
57.6109	17.0497	11.3179	32.3563	2.4781	0.81747	52.6037	4.47441
1.95403	7.31404	4.86976	51.9074	2.39853	3.57265	3.23832	1.74514
11.9055	9.44383	25.7754	19.8202	2.23583	2.12321	24.8735	2.53746
10.0716	16.8115	7.57301	27.6865	1.31396	38.5558	44.3909	2.56755
5.07879	10.0162	7.23968	29.6007	2.3699		57.0422	3.69379
10.683	7.14739	8.62261	29.7232	5.59451		44.2995	2.37535
12.8406	10.9771	2.7345	34.5505	3.2312		57.2232	1.43458
0.882798	10.0756	11.6083	16.7061	3.20244		2.48965	5.57584
	30.0066	7.7254	37.6061	2.8135		59.0457	0.008519
	12.5539	16.8025	26.17	8.34978		66.5711	6.45651
	10.9642	14.4707	23.305	2.91413		59.9141	2.13494
	13.3304	19.947	29.1178	3.10029		44.8591	
	11.1834	20.2793	29.0366	2.99094		32.3174	
	13.7484	11.2537	39.1451	3.51757		40.8422	
	25.8388	7.76004	37.6388	7.72966		49.945	
	15.9537	17.3007	24.0286	6.4186		36.2189	
	15.119	16.5137	33.9194	4.07609		22.8678	
	20.778	17.9365	31.0137	4.39666		39.9683	
	14.9039	12.882	33.8207	5.23702		2.04956	
	12.7998	20.1829	32.8175	4.01272		57.2042	

11.1661	8.88142	28.177	9.63491	47.6493
31.2483	9.93417	36.3358	11.2961	40.9381
12.0422	5.022	17.7836	12.4934	12.0417
35.2801	40.4406	43.4364	3.12197	19.2824
18.9201	15.3614	40.6176	3.58336	25.5055
24.7404	10.2889	39.0027	5.55337	40.8545
15.5099	18.8877	28.3983	2.3291	28.8891
21.2932	12.2734	29.2119	2.96448	1.28146
16.0798	10.4468	25.9759	10.8925	55.1504
11.298	8.33235	16.8892	17.9693	14.0601
13.07	12.1796	24.3937	3.66859	40.0027
13.844	11.963	27.8898	2.98904	32.772
24.9007	12.5634	36.5331	10.9831	28.0126
14.9667	8.528	38.4513	4.83491	30.9333
13.8031	16.5993	33.7349	3.04832	55.0661
17.5881	34.6902	26.771	2.11693	1.55187
35.2694	9.57598	34.6571	1.97656	3.29372
19.5599	21.5257	19.2989	2.82883	38.4583
17.1317	14.7104	38.3968		16.4072
14.1075	16.1617	38.5004		37.8457
12.0171	5.14911	33.0453		44.6799
15.7982	9.66959	25.71		28.2066
12.2625	13.2297	34.2767		60.6773
15.8045	4.25488	31.4018		59.2166
14.8312	6.05207	47.5682		59.4625
27.076	10.21	19.0262		
17.7821	10.3141	19.9477		
31.2085	6.6248	47.4346		
11.6749	13.3018	38.634		

GROUP 2

Table D.3. Tailmoments of the striata cells (comets) of the rats in Group 2.

Tailmoment							
Rat 9	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
61.1946	126.849	20.8552	30.7881	3.39766	2.4305	0.47506	5.10902
52.4756	101.749	12.8227	30.4998	9.07044	30.62	0.22173	35.2077
40.1807	123.444	6.02949	68.4659	4.08436	0.00024	0.068175	0.665715
58.5204	123.56	18.678	49.7385	16.1008	40.907	15.5409	0.439119
55.2625	110.048	20.7238	173.323	176.02	1.50419	4.98729	2.40432
67.3283	138.491	19.4038	14.9829	0.123077	0.845777	1.08632	1.71849
67.2322	122.674	22.8262	32.736	1.48642	14.0593	1.80989	12.6631
8.76069	139.104	22.8831	202.963	3.10899	106.894	10.3704	6.67009
81.4157	143.885	11.0712	10.8495	184.529	9.32517	4.02469	0.593526
88.1741	158.62	15.6449	226.277	163.909	6.33764	0.007293	7.81523
112.308	166.347	3.87585	15.7391	15.2546	7.28969	0.164998	0.857837
84.2991	115.744	15.758	152.024	2.08023	0.079151	0.286504	2.75753
67.896	124.634	10.1978	200.347	199.859	1.42974	16.3358	11.8763
100.378	131.188	5.60943	188.116	14.4901	73.662	67.8635	0.629427
4.35379	126.203	156.299	111.974	3.52383	33.3611	1.79802	13.8125
10.3313	137.773	136.164	81.3727	10.2084	35.4157	0.178356	8.85625
109.207	127.882	14.0522	65.6035	149.373	3.73896	0.82798	1.4269
85.1518	154.616	128.388	131.467	9.27422	4.17169	0.22352	4.11296
126.044	162.338	137.219	24.6513	223.414	4.91563	0.724239	61.8665
72.389	142.271	108.969	165.637	187.065	10.0756	0.18019	3.87089
1.82775	159.33	128.046	12.3175	1.32153	0.622141	0.642036	4.61802
57.0584	150.861	89.2929	38.048	3.78325	1.26251	0.045664	80.2451
53.807	153.597	5.64753	8.54138	163.827	63.0689	0.680038	6.47541
9.0917	191.886	6.92092	3.59064	6.72819	0.738859	0.082975	3.9825
70.8875	174.885	153.585	8.20541	4.14961	2.28325	0.733654	0.988884
9.22956	196.859	120.511	4.68665	129.115	2.9807	0.614361	0.090829
81.8219	208.729	11.9906	5.77658	8.45516	2.05621	1.10191	1.04177
91.6678	199.759	9.77295	12.6143	9.20604	2.74031	0.761056	0.223545
78.6099	200.87	25.0746	16.4154	16.2449	54.9964	0.486277	0.785079
43.8474	132.737	95.7603	64.5849	10.0195	48.9499	0.519954	0.368881
31.9504	137.432	7.05938	6.74441	3.03836	2.31579	0.311026	0.647844
56.2948	87.7261	9.85306	3.12917	19.2726	3.9498	0.515622	6.13688
12.6245	131.163	7.86333	18.3674	15.2047	18.0064	0.663303	0.05006
43.8952	103.979	14.7659	19.8312	5.76709	80.6256	0.456503	1.44122
27.1558	128.16	16.0587	3.82252	28.4743	10.7984	1.67616	3.00937
19.73	119.7	18.3796	21.7443	11.7034	2.96613	6.19511	1.60197
31.755	133.677	10.183	3.70597	1.61067	63.7715	0.203558	41.9136
44.485	149.044	12.0284	5.88311	3.97867	2.10673	0.721483	2.69562
41.7492	144.602	32.1467	6.71633	3.90346	10.0657	0.692455	2.25757
38.4202	161.831	14.4589	10.2118	6.86097	56.5406	0.269802	3.55243

34.2635	184.305	25.7143	6.8261	19.3855	3.28557	5.14783	20.7193
49.2967	159.129	20.6191	19.8244	1.38906	11.2316	42.1589	0.233144
15.836	154.864	30.6047	23.6522	4.15059	16.9806	11.7962	0.596568
28.9578	130.544	8.72568	5.6949	4.10777	12.7278	1.86438	1.8534
34.3582	170.108	5.80083	7.97196	15.3624	25.2892	0.607619	30.2319
32.1211	141.175	17.8735	9.76075	8.74726	5.80947	5.20036	0.848681
29.0251	161.514	5.25254	4.52898	2.28766	38.2125	9.09042	0.804159
16.4744	172.461	9.55948	84.1563	9.95932	3.20728	6.9891	0.640595
36.8912	143.815	19.2618	132.071	8.7376	2.37669	0.930136	1.90618
18.2768	139.276	12.3265	186.688	5.75406	5.57882	34.9549	1.33799
17.4548	136.782	9.49418	18.5538	15.4179	15.2429	1.68696	1.01539
19.5964	138.029	103.666	13.7516	15.2373	31.769	2.20537	1.88457
13.9412	132.551	153.589	156.297	26.1602	5.21767	4.6292	8.00976
9.10478	144.921	132.009	26.12	19.0461	1.17843	8.6736	1.37949
49.2236	133.516	155.583	8.49806	13.4142	7.25145	2.32837	0.851082
42.8499	105.442	99.2679	5.71177	38.105	3.60771	0.949261	1.69281
41.395	122.373	119.986	11.396	8.26233	1.791	2.61936	0.482657
26.0318	106.125	112.86	11.4099	3.59151	34.1031	1.35735	0.535642
34.3379	111.671	138.205	6.46016	0.992467	26.0069	9.80234	1.68492
37.19	114.51	96.46	23.1751	8.23172	1.95215	0.320837	1.07535
38.8145	124.891	88.6367	3.71094	9.04807	34.1634	3.60822	1.395
49.0545	127.049	8.97258	16.7838	13.5051	8.1139	24.0601	14.1361
54.6682	103.462	84.5266	9.33396	12.1444	6.04272	2.29665	9.78647
28.4678	117.754	118.42	17.2286	8.03114	22.6195	4.18627	6.75267
41.1788	113.462	115.017	125.093	19.6809	34.4378	1.77689	9.33512
48.7295	141.584	110.651	11.7355	13.5983	8.58644	0.741469	
54.2069	124.395	94.5582	22.7002	11.518	1.15856	3.24776	
45.9468	77.9568	101.447	6.29176	4.17244	12.42	1.30527	
43.3708	118.846	68.9669	2.71041	3.11377	7.87416	5.44384	
35.8843	116.675	83.7554	21.0511	6.67876	21.4355	0.035896	
64.3703	84.3987	91.9142	13.164	13.2849	6.43034	0.000195	
42.7393	105.807	49.0996	4.45948	13.286	30.9916	0.141249	
76.4972	99.95	76.7728	8.37623	3.94255	12.2775		
35.9413	103.529	81.7318	96.9357	11.5826	16.5435		
112.136	97.8696	77.1503	23.6249	0.653659	3.19668		
66.3073	110.913	111.315	14.5156	7.47956	6.02287		
67.5027	94.1518	106.559	8.29828	0.651615	3.54489		
58.772	56.5798	81.1792	23.8636	10.1392	2.80103		
59.1621	68.1513	39.6251	21.5388	21.6163	11.983		
66.8214	69.7417	78.5023	42.5054	0.716652	24.263		
55.9095	77.0201	104.227	12.0494	0.950874	2.88853		
34.4183	89.6508	98.7029	18.5843	15.0096	24.4408		
43.3195	45.2202	75.4998	82.6336		4.16791		
79.1936	69.7381	52.7038	8.49842		4.23579		
75.9641	74.3834	73.9236	16.744		4.26677		
40.5861	88.4926	76.9284	140.367		1.04266		
69.7635	70.3835	114.942			1.38412		
45.1852	77.4051	109.558			61.4067		

25.6764	80.5798	94.7068	1.50371
10.2427	54.7763	79.3596	37.7504
73.5422	167.576	69.9521	81.7665
85.8527	134.374	73.4019	7.08637
12.5713	156.364	107.649	2.23001
74.3671	154.456	106.847	22.0452
76.7704	160.609	41.8556	36.4941
67.7338	163.752	137.308	2.00236
96.8475	133.114	45.3399	1.41719
95.3508	142.424	65.4331	27.2302
99.0096	132.049	81.0768	2.62825
88.3525	94.297	42.4918	32.2757
76.3592	36.8879	1.77206	0.688769

Table D.4. Percentage of DNA in the tail of the striata cells (comets) of the rats in Group 2.

TailDNA%							
Rat 9	**Rat 10**	**Rat 11**	**Rat 12**	**Rat 13**	**Rat 14**	**Rat 15**	**Rat 16**
9.61973	41.4471	6.81563	10.4148	1.36753	0.007993	0.00651	1.0012
13.414	45.2202	9.64007	11.2207	3.25807	1.97878	0.243115	1.29756
13.5392	47.949	16.0225	13.552	3.84505	2.66109	0.358958	1.5543
13.5483	49.2995	17.5085	13.6051	5.51271	2.99465	1.14159	1.59675
16.9365	52.8471	18.2129	13.89	5.83804	3.45634	1.3635	2.16989
16.9883	55.8702	18.7124	14.2537	6.79196	3.84444	1.38292	3.01661
17.1307	56.1642	19.45	14.9804	7.70455	4.32538	1.64998	3.37784
17.4143	58.1809	20.3992	15.2901	7.77374	4.61208	1.76561	3.70251
17.484	58.208	20.7754	15.4622	8.17097	5.13723	2.22945	3.81085
17.7299	59.0201	20.9734	16.4386	8.3884	5.48918	2.25238	3.95684
18.4872	59.3642	21.5747	16.774	9.90945	5.79282	2.46367	3.97712
18.9295	59.647	21.8426	17.1241	10.1279	6.51402	2.54447	4.27063
19.045	59.7826	22.137	17.2799	10.4311	7.36515	2.794	4.4381
20.4855	59.9667	22.3933	17.2877	11.1206	7.36521	2.86504	4.46368
21.7664	60.4528	22.6737	17.3033	11.3672	7.44756	2.9978	4.61811
22.1939	60.8494	23.5851	17.6269	11.5855	7.51856	3.07181	5.0461
23.2882	61.203	23.7159	17.8493	12.0403	7.9223	3.16707	5.31926
23.4595	61.4739	24.0434	18.4748	12.1432	8.01521	3.22264	5.36106
23.9599	62.2458	25.8364	18.5812	12.2427	8.14093	3.45584	5.48302
25.5967	62.953	26.2265	18.8846	12.3898	8.20954	3.56485	5.4938
26.9028	63.352	26.893	19.1895	12.436	8.51628	3.77668	5.65973
30.3071	63.3982	27.1515	19.2697	12.6108	8.78171	3.80419	6.34621
32.3028	63.4016	27.9063	19.9192	13.2509	8.9069	3.99965	6.52831
33.5669	63.4468	29.1346	20.188	14.4177	9.34804	4.1399	6.79475
33.7848	63.653	30.5302	20.4409	14.6021	10.028	4.5265	6.89747
35.8369	64.3555	30.7451	21.2746	14.7724	10.1277	4.61637	7.04207

35.8727	64.6636	30.8981	21.2776	14.8235	10.281	4.73788	7.12846
36.9743	65.2236	31.5257	21.901	15.2644	10.4261	4.86277	7.32574
38.1787	65.5483	31.6473	22.7537	15.3026	10.5129	4.89103	7.3421
39.5386	66.7744	32.2341	23.7905	15.6138	10.5336	5.06349	7.47171
40.6747	66.8913	32.8612	23.9321	15.96	10.5387	5.43158	7.58539
41.5053	67.3296	33.815	24.1335	16.0195	10.5933	5.43611	7.6284
41.6978	67.4034	33.8847	24.5631	17.1048	10.9014	5.54987	7.69459
41.9046	67.6863	34.3392	24.7065	17.7581	10.9325	5.58389	8.28775
42.4309	67.9311	34.6496	24.7739	18.0552	10.9613	5.66699	8.56622
42.585	67.965	34.9103	24.9452	18.5906	11.1187	6.17891	8.66447
42.7873	68.0112	36.2411	25.5103	18.6112	11.2786	6.64383	9.19178
43.321	68.0268	36.7075	25.5119	18.8501	11.3296	6.88693	9.29525
43.8072	68.0287	37.2671	26.1751	19.2163	11.5709	6.91545	10.018
44.6304	68.0665	38.2907	26.1888	19.5993	11.588	7.25152	10.3771
44.6891	68.5097	38.5235	26.7765	20.873	11.7369	7.60473	10.8703
45.0006	68.5687	38.6207	26.813	20.8739	11.9655	7.76825	11.4249
45.1468	69.0708	38.904	27.0065	20.9228	12.9359	7.86908	12.4453
45.8873	69.2096	38.9125	27.3722	21.5727	13.1297	7.98439	12.903
46.302	69.3422	39.1016	27.4773	22.0358	13.1413	8.03317	13.1943
46.3822	69.4097	40.8063	27.4894	22.1318	13.2368	8.3156	13.2798
46.4624	69.8159	40.8946	30.614	22.357	13.3534	8.38082	15.7937
46.5129	69.9517	40.9153	30.8842	22.5085	13.3637	9.56939	16.1023
46.576	70.0382	41.2542	32.2765	22.6201	14.4935	9.87159	16.8817
47.0382	70.4715	41.4412	32.7418	22.6638	15.5209	11.3348	17.0469
47.076	70.5112	41.5022	34.2155	23.2575	15.8927	11.3885	18.0273
47.1804	70.7941	43.0813	35.0073	23.6379	15.9834	11.5991	18.3523
47.5804	70.8148	43.6688	35.654	23.782	16.278	12.9829	18.6077
47.7911	70.8988	43.746	36.0567	23.9958	16.4107	13.9542	18.8482
47.9144	71.1491	44.2889	36.346	24.0904	16.5985	14.0798	19.536
48.2137	71.1998	44.6838	36.4886	24.3821	16.8152	14.4329	21.2162
48.3168	71.4427	45.0286	37.7873	24.6011	16.9	15.1218	21.7477
48.3919	71.4848	46.5894	39.156	24.6135	17.0137	15.5995	21.9932
48.4923	71.6491	47.8373	39.6964	25.5479	17.3793	15.7587	23.0209
48.9997	71.7206	48.9648	40.2672	25.5499	17.9912	18.8894	28.5099
49.0601	71.7496	49.4316	40.8125	25.8198	18.225	20.1269	35.9904
49.0611	71.8373	49.8467	41.4488	26.453	18.6853	21.3094	36.9988
49.1118	72.1309	50.024	42.6035	26.4869	19.0828	22.257	38.6897
49.169	72.9221	50.3253	42.9168	27.5903	19.2373	22.8253	42.7689
49.1883	72.9706	50.3777	42.9347	27.7956	19.2791	23.3088	45.5938
49.2342	73.0439	50.6112	45.2971	29.911	19.4926	23.9091	
49.6173	73.2894	50.6388	45.7632	30.0227	19.79	26.348	
49.7622	73.6064	50.7109	46.3769	31.5003	19.997	29.277	
49.7918	73.8175	51.0255	47.8783	32.121	20.0847	30.3956	
49.8676	73.9	51.2632	50.081	32.3091	20.5359	30.8463	
49.9767	73.9134	51.3604	50.3087	33.1097	20.9425	31.4254	
50.2058	73.9421	51.8524	50.8509	33.5433	21.2288	53.4359	
50.2815	74.0714	51.9145	55.2627	44.8294	21.4289		
50.491	74.1315	52.0635	59.8794	63.6035	21.4739		

50.6995	74.2874	52.0922	60.3064	69.1253	21.5621
50.9136	74.5218	52.2738	61.4332	69.1542	21.564
50.9854	74.6838	53.0541	61.9512	69.1597	22.2028
51.0244	74.8797	53.0734	72.0804	75.4292	22.6095
51.0398	75.177	53.3144	72.6479	76.5679	22.7283
51.0798	75.1911	53.3411	73.3788	79.6252	22.7362
51.227	75.3443	53.3914	73.4418	80.0093	23.2129
51.2746	75.5388	53.4074	80.8617	86.2603	23.6336
51.7268	75.708	53.7687	82.1438		23.6926
51.768	75.7292	53.7979	84.737		23.9896
51.7821	75.7589	53.8619	86.0368		24.0008
52.1922	76.1073	53.9861	88.6491		24.6067
52.3558	76.4165	54.1394			25.0514
52.475	76.6488	54.1616			25.2188
52.6249	76.6776	54.2879			25.767
52.7468	76.8696	54.5962			26.0184
52.7949	77.2782	54.7582			26.0713
52.8858	77.6077	54.7769			27.0045
53.0072	78.6113	55.1681			27.066
53.1607	78.658	55.4364			27.1335
53.3558	78.7765	55.4797			27.3209
53.3589	79.2688	56.034			27.9602
53.5333	79.2917	56.3141			28.0789
53.6702	79.9498	57.0625			28.7792
54.525	79.9693	57.185			28.9683
54.6689	80.1117	57.3451			29.0022
54.7548	80.9285	57.3667			29.2937

GROUP 3

Table D.5. Tailmoments of the striata cells (comets) of the rats in Group 3.

Tailmoment							
Rat 17	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
11.0554	0.248627	73.4556	83.5099	0.989227	8.27712	84.6641	34.9033
17.6644	29.0363	154.015	79.2766	1.92458	10.0595	104.545	38.8433
28.4252	4.82718	56.5061	54.3289	0.53852	108.561	101.492	109.572
11.1331	62.3827	104.621	78.594	2.49841	124.695	31.6242	56.5815
31.9999	21.9238	79.9534	48.4223	4.7875	139.087	146.517	168.8
5.96808	124.952	140.091	115.702	12.4376	139.786	92.4059	152.793
16.373	103.467	62.666	13.3569	6.01897	126.811	119.382	56.6319
13.7783	135.717	18.6918	16.3133	0.456441	145.623	110.472	196.658
3.28911	69.8332	37.9225	35.5206	0.10059	144.53	58.2514	52.0083
21.9021	85.1317	18.4175	113.094	20.7116	107.558	42.2565	30.2948
7.77243	102.132	83.4687	38.1375	23.5797	128.157	33.5679	122.322
10.8753	126.399	80.6742	53.26	0.345524	83.9764	89.0318	56.7323
6.86383	58.1757	70.5809	79.3901	4.15894	2.55319	67.6937	86.0988
7.18669	97.3956	170.021	68.4879	5.58664	116	137.814	48.3249
21.221	65.1044	64.3758	27.0224	0.302769	84.8538	58.5833	47.3274
7.34838	101.27	23.6397	7.10283	0.607167	0.956347	45.8538	37.5326
32.3068	125.322	103.645	38.3288	18.8128	2.0584	104.81	98.7358
25.4221	89.469	15.0335	59.2136	7.35592	32.213	84.0649	42.1302
6.83289	37.3983	95.9463	56.9114	4.8907	126.951	44.0553	35.1594
21.6433	22.1585	45.2396	6.14138	43.4185	25.7852	24.41	89.588
48.0019	101.147	53.4451	30.0787	47.3093	6.3797	14.0799	58.1919
23.9017	86.0907	35.9029	14.7052	19.6676	79.2082	6.57174	83.7186
10.4316	87.0749	78.0108	39.5741	7.66893	97.2974	44.1858	101.523
1.91463	37.4907	82.8197	39.0085	22.3322	57.7374	41.8092	79.6912
21.9722	20.626	52.5514	80.8891	0.736824	87.7623	22.469	56.3136
9.73345	31.9142	81.1288	123.722	10.2583	87.7517	32.8228	23.4761
23.0077	38.2477	57.3758	48.1822	29.4053	62.1397	59.431	158.051
26.1558	5.9286	93.2688	7.46227	7.60412	5.22359	64.97	89.2965
29.7513	52.7829	17.6331	6.54945	27.5265	6.0691	24.1225	156.307
23.4591	70.2326	76.4186	59.6703	6.19471	40.2723	26.3501	109.937
4.83301	72.9104	154.714	3.8515	1.67614	4.35929	18.445	21.3579
34.9727	54.8347	87.7169	44.0845	0.23678	2.27078	34.9059	32.6124
20.9737	8.26385	76.0359	42.9821	15.0065	118.69	12.5805	147.388
21.9913	22.4559	126.934	6.40587	2.01703	143.509	48.0116	119.038
6.45876	1.85936	70.2586	33.5073	23.0092	168.124	18.4627	67.2732
47.971	35.822	15.2075	5.36077	2.44815	160.355	19.9808	149.443
44.3055	51.7948	109.27	20.5009	3.34782	9.17527	78.8349	177.435
41.1156	45.5618	64.2243	9.20593	17.5182	4.60925	12.2992	124.294
41.0482	51.0426	104.166	55.8308	12.9882	93.3779	69.2767	170.846
36.7187	8.73274	73.1986	39.1589	0.825415	71.1821	21.3357	150.764

42.5028	70.7499	55.8859	37.2369	12.1903	167.316	61.6275	173.608
8.59522	63.8409	99.5422	3.0123	38.0513	120.117	16.8521	137.307
3.78735	62.9835	61.733	28.9335	17.208	139.587	23.5337	23.5861
1.77933	33.1724	106.208	46.8522	2.21754	117.155	22.2376	53.9391
12.6662	26.1587	73.372	36.6272	15.5282	65.2893	29.8994	124.783
10.7118	17.0654	66.9638	2.49651	14.5983	110.616	28.6683	139.472
42.6509	23.5681	96.6354	35.4123	14.8725	164.042	27.995	63.5804
46.1115	24.4932	117.495	27.9604	10.6804	65.3483	18.2432	14.6712
63.6875	28.9178	117.014	23.2688	19.7436	46.958	29.5105	155.944
40.8531	37.8234	77.5078	44.124	5.1818	95.9788	81.2795	143.087
30.1641	28.5983	67.6476	43.8322	5.31239	120.192	22.0645	42.7916
12.5717	23.2994	45.1108	30.8601	1.1486	106.937	70.9584	31.6703
8.68259	65.8345	80.5013	2.93344	2.066	108.39	74.7263	93.6266
9.00217	10.2905	73.0073	25.7685	42.9891	37.1759	26.5979	149.164
13.8833	50.7988	77.9608	36.6571	19.7308	24.5701	33.9284	96.4361
26.1802	6.47578	87.3444	31.2908	2.37335	75.7913	55.1918	24.9805
36.6553	17.399	81.9042	28.6405	9.21567	103.529	69.8674	123.756
52.3944	62.427	70.1372	43.4447	8.6457	50.9539	17.3093	94.8879
35.3399	7.66256	126.804	43.465	3.8948	145.698	41.0707	79.4263
40.4511	21.7852	16.1077	63.8146	3.51773	68.2031	15.4027	75.7865
57.4039	94.798	114.363	31.0261	28.6781	37.3251	12.0147	66.236
56.048	45.2688	45.7426	27.3791	9.77005	50.8553	134.194	34.3271
9.49266	114.498	135.328	24.8767	1.7126	27.8539	38.0465	20.0636
16.7464	8.97482	59.84	9.41948	4.36015	128.845	10.0692	55.3769
83.3901	4.34914	39.1141	9.08892	11.3606	95.0847	13.3064	14.8247
40.2827	61.6929	141.09	32.9893	48.8599	82.0729	37.695	68.7102
7.55814	4.00292	131.862	28.5788	16.3841	5.35129	16.5196	17.4308
43.8116	148.014	63.4411	10.3623	3.44763	75.2853	5.08203	28.6751
	112.387	15.2719	50.9255	17.4609	49.7116	3.7671	162.288
	76.8185	81.3442	37.2844	27.0543	11.2122	20.4478	17.1986
	1.43935	75.2721	28.9319	64.733	10.1848	25.6974	108.475
	3.32616	36.6799	68.1511	32.528	7.9142	30.1057	142.882
	17.4366	40.1293	20.1901	21.3763	31.832	0.999577	75.4202
	55.3458	49.7931	40.1673	42.809	26.0792	50.8473	131.627
	85.7481	67.3669	23.559	7.84036	41.5772	30.2942	162.178
	116.028	15.5257	12.4482	9.24941	110.884	25.2841	9.46247
	85.5909	75.4832	90.2927	11.1319	41.3228	34.3009	88.0038
	105.325	46.5127	45.3967	12.2149	24.5699	18.3095	48.7445
	56.1717	148.458	27.5675	82.1997	95.9182	23.8348	85.2556
	13.1274	68.1703	15.3463	23.2874	51.1854	16.6158	72.1496
	70.2933	13.3	30.0335	6.19327	43.8293	14.0171	126.831
	2.54542	95.203	33.9562	7.01669	90.6601	10.4231	118.118
	102.718	91.4587	62.5805	0.977326	19.5599	16.0964	109.435
	88.7971	107.789	50.6838	13.7489	31.7384	26.0832	95.5433
	164.925	22.0083	27.3304	29.1537	33.0223	1.96668	122.615
	1.72975	66.9658	59.9318	70.297	15.6191	22.73	64.2334
	25.1887	71.9655	15.4118	64.4399	31.5614	106.471	45.7056
	93.448	91.8563	15.1707	17.0667		19.3667	65.3242

90.1653	54.8999	73.1836	67.3524	15.2051	47.5263
49.9169	15.8402	26.7928	19.9816	11.0627	64.6469
3.42954	45.1557	3.94865	75.4146	28.9759	36.5037
46.9863	115.249	21.1371	104.901	74.6416	54.4338
112.542	78.2524	15.5564	15.7664	14.0073	55.9773
44.9654	96.2416	7.92279	82.2395	11.5641	27.3852
89.3367	85.8663	18.9929	59.8775	11.788	86.0679
45.5501	13.2237	37.0409	36.8192		58.6101
53.1934	45.3508	16.6171	24.5743		65.1384
112.673	86.6726	3.31461	26.3019		108.177
62.3369	77.8045	52.8831	0.967887		66.3687
6.28268	49.0089	2.53145	15.6496		9.29469
3.08699	89.9444	7.10338	39.4622		8.75276

Table D.6. Percentage of DNA in the tail of the striata cells (comets) of the rats in Group 3.

TailDNA%							
Rat 17	**Rat 18**	**Rat 19**	**Rat 20**	**Rat 21**	**Rat 22**	**Rat 23**	**Rat 24**
6.13563	3.10783	11.487	8.16597	1.11767	4.34703	4.75989	18.1971
9.57315	5.40548	12.742	8.72265	2.15254	7.0922	10.3509	19.7759
13.7421	7.74733	13.1455	9.24633	2.75244	7.3251	12.7051	19.8926
13.8086	8.36373	13.3384	10.4766	3.14112	7.62371	14.6039	23.1637
14.0272	8.51686	14.715	10.7582	3.51108	10.0201	15.6962	24.9256
15.5293	8.57386	17.3939	11.7698	3.75188	11.6332	17.6686	25.0795
15.6624	9.35453	17.7222	11.8408	4.60515	12.4551	19.7436	25.262
16.7269	9.79006	17.8494	11.9656	4.67052	12.5092	21.7148	25.2951
17.6513	11.0222	18.9898	12.036	5.37715	12.7648	22.0038	27.0353
17.9229	11.0719	19.1399	12.7945	5.3852	14.7806	22.0262	30.428
18.0249	12.7929	20.9233	12.9152	5.42959	15.1728	22.5532	30.4789
18.0627	14.1976	22.3928	13.6447	5.81898	16.097	22.8736	31.357
18.4536	14.6826	23.2015	14.4956	6.04526	16.427	23.3618	32.1591
18.613	15.06	23.8624	14.561	6.52218	16.8503	23.576	33.0276
18.8752	15.6016	24.0413	14.8768	6.95527	17.05	23.6002	34.2198
19.9293	16.0265	24.1818	14.891	7.61883	23.8557	23.9546	34.5483
20.5334	17.123	24.2476	15.3561	7.91115	27.6855	24.5838	34.6855
21.8608	18.7815	25.2592	15.4459	8.01907	28.7645	25.9394	35.0277
23.4559	19.1564	25.4303	15.6722	8.26401	28.9243	26.6756	35.0772
23.8723	19.7895	25.5486	16.857	9.06722	31.1011	27.151	35.5846
24.1763	21.5735	27.1562	17.3132	9.51444	31.2078	27.2402	36.0652
26.8601	21.8223	27.7245	17.7037	9.85036	31.4498	27.2771	37.4036
27.4517	23.6378	27.9053	17.7673	10.0507	31.8039	27.2821	38.3553
27.6385	24.1652	29.0678	18.7748	10.41	32.059	27.3276	38.7336
27.965	26.3368	29.289	18.8721	11.0763	32.6395	28.4125	39.3801
28.2947	26.4229	29.7938	18.9353	11.5151	32.8704	29.1506	40.0064
28.5463	26.7758	30.7303	19.8098	11.5487	33.0582	29.1806	40.124
29.3927	28.6235	30.9977	19.8146	12.1712	35.2124	29.2288	40.4617

30.7715	28.6472	32.8649	20.5741	12.4108	38.787	29.9069	41.4815
31.294	28.6479	34.7954	20.7776	12.4576	40.0436	30.0507	42.5264
31.7517	28.8887	35.0898	21.4079	13.3913	41.1655	30.0931	42.6373
32.2045	31.0023	36.8952	22.0473	13.5853	41.5731	30.9543	43.1367
32.8054	31.1368	38.0776	22.0688	14.0809	42.3623	31.7155	43.192
33.306	31.6026	38.1374	22.1892	14.3412	42.7309	31.7471	43.2238
34.3545	32.018	38.2699	22.6112	15.099	42.8429	31.8443	43.3932
34.4987	32.1329	39.1141	23.1903	15.1613	45.1138	31.9496	43.563
34.636	32.1409	40.0673	23.2215	17.1285	45.607	32.3664	43.6403
34.8602	32.2626	40.2594	23.4871	17.2035	45.7012	32.659	44.9492
36.3845	32.6006	40.3076	23.7411	18.2334	46.3217	32.7439	45.2076
36.8735	33.5156	40.4419	23.8187	18.2393	46.958	33.4927	45.2632
37.2411	33.5735	40.7975	23.8516	18.9379	47.0755	33.5034	45.5895
37.4547	33.729	40.9464	24.3714	19.3585	48.2598	33.8743	45.7125
38.1087	34.3849	41.0392	24.413	19.4337	48.4813	34.1403	46.1474
38.2487	35.9905	41.592	24.5414	19.8809	48.5188	34.3146	46.2479
38.5183	36.7081	42.0508	24.6188	20.2397	49.7833	34.5427	46.4117
38.5813	38.4508	42.2016	24.7657	20.4901	50.2853	34.8715	46.8857
38.8586	38.6117	42.3055	25.1579	20.585	50.4833	34.9613	47.6819
39.026	38.8273	42.9656	25.1854	21.3609	50.8107	35.1168	48.0411
39.1464	39.7924	43.2664	25.2609	21.5099	50.9769	35.1758	48.4933
39.6632	40.3165	43.9167	25.8293	21.9272	51.4089	35.3365	49.2807
40.2827	41.0111	44.5212	26.1977	22.7961	52.2862	35.3618	49.4976
40.4253	41.097	45.0816	26.2592	23.0392	53.5071	35.6909	49.7239
40.5114	41.5614	45.2396	26.3763	23.0428	54.0897	35.7344	50.0633
41.0008	43.0225	45.3783	26.7432	23.2257	54.2056	36.2057	50.9596
41.0105	43.2565	45.6817	26.8214	23.2392	55.7804	36.2347	51.3536
42.1051	43.406	45.6897	26.8278	23.8294	56.0489	36.5971	51.7469
43.0974	43.9503	46.0294	26.9302	23.9025	56.3348	36.743	53.0089
43.6372	44.1714	46.1344	26.9474	24.0943	57.0476	37.205	53.0512
44.4144	45.3624	46.2764	27.0905	24.1715	57.2115	37.4002	54.0315
44.6982	45.6007	46.5411	27.3367	24.381	57.4439	37.7656	54.1332
44.7398	45.7403	46.6761	28.1301	24.3811	58.2619	38.8148	54.3234
45.6758	46.0381	46.8292	28.4156	24.9284	58.4908	40.4177	55.0781
45.8187	46.0424	47.2951	28.4735	25.2412	59.2988	40.5352	55.5235
47.0991	46.2422	47.6715	28.5192	25.258	59.3321	40.6641	55.8733
48.3713	48.5263	48.2289	28.6343	25.4297	59.6507	41.3761	56.6236
48.5133	50.0431	48.3261	29.2151	25.4561	60.1802	42.3439	57.2367
49.4557	50.3218	48.6587	29.3906	25.9676	60.3862	42.4881	57.4282
50.95	50.5385	48.9381	29.4447	26.1997	60.9616	43.0357	58.2101
	50.5707	49.0967	29.5467	26.694	61.0313	43.2583	58.9366
	51.389	49.4862	29.5487	26.9586	61.3288	44.9849	59.267
	52.4074	49.6091	29.6009	27.254	61.987	45.4335	59.5219
	52.8336	50.0136	29.8089	27.5439	63.486	45.8444	59.9306
	53.0384	50.2738	30.2192	27.7389	63.5099	45.9907	60.2721
	53.1424	50.2851	30.5065	27.7409	63.8921	46.6479	60.5732
	53.4922	50.6564	30.7026	27.8066	64.276	46.7727	60.6004
	53.8664	50.6896	30.735	28.1431	64.4201	46.796	61.9051

	53.9529	50.7529	30.9893	28.6244	65.7389	47.3589	62.6208
	54.1343	51.1233	31.0013	29.1142	65.8926	47.5424	62.8459
	54.2537	51.2836	31.1423	29.2826	67.1461	47.5641	64.0693
	55.2199	51.5189	31.2559	29.3375	67.2049	51.0492	64.1223
	55.4374	51.5611	31.3755	29.449	67.449	53.4893	64.5701
	55.5708	51.5883	31.4214	29.9145	67.7607	54.1863	64.6542
	56.2609	51.7142	31.5564	30.3386	68.3509	54.2438	64.9463
	56.2981	52.1598	31.6589	30.5836	68.9033	54.9579	65.079
	56.5422	52.2149	31.9452	30.6413	69.1532	56.148	65.1281
	56.6352	52.2178	32.0285	30.679	71.8095	57.1283	65.2159
	57.1654	52.2298	32.0437	30.7306	72.232	58.882	65.3364
	58.5347	52.4085	32.1189	30.9243		59.2007	66.8157
	58.6841	52.4385	32.1668	31.1718		59.6598	69.4767
	59.0333	52.5184	32.2015	31.2636		60.4118	70.0546
	59.5057	52.8209	32.3251	31.2682		63.7553	70.0885
	59.7856	52.8497	32.5096	31.4585		71.822	70.3333
	59.9519	52.88	32.7892	32.6575		72.1921	70.4366
	60.7927	52.9558	33.169	32.757		72.2038	70.5256
	61.4322	53.3742	33.4911	33.0731		75.5581	70.7066
	61.5582	53.7034	33.6062	33.4797			71.8414
	61.9562	54.0031	33.6502	33.6612			72.545
	62.9848	54.0039	33.6556	33.852			72.6418
	63.4193	54.9693	33.754	34.1183			72.9446
	64.6763	54.9701	34.0659	34.3741			76.4431
	65.1541	55.517	34.0922	34.6042			76.4944

GROUP 4

Table D.7. Tailmoments of the striata cells (comets) of the rats in Group 4.

Tailmoment							
Rat 25	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
41.8229	57.9617	3.20343	102.366	67.2323	57.3937	52.3569	75.2691
39.0158	62.258	142.781	133.303	109.709	18.7304	20.2106	41.084
18.8579	77.7745	3.08456	183.114	58.1917	34.9088	81.9272	69.99
13.0086	37.7987	9.67201	120.895	115.752	64.7911	53.434	88.0251
59.7921	60.6472	182.743	124.693	14.824	59.509	58.8833	61.2537
18.9625	39.4348	123.818	105.077	58.6796	112.823	35.1151	56.721
62.6061	46.6309	81.1908	43.9296	33.5512	50.0109	44.7322	30.9055
18.6451	51.4064	168.567	149.333	125.692	77.1529	18.1013	51.264
21.759	62.2996	136.66	92.7264	136.869	10.5171	38.4093	63.1035
31.085	41.006	66.4807	178.015	170.205	138.08	37.1104	134.392
43.2205	70.2282	91.179	154.688	162.659	80.4812	59.934	43.3762
37.2516	125.677	196.792	130.797	1.69237	94.0271	58.9317	35.3871
26.4813	55.4469	74.7946	121.667	81.8356	117.368	31.3139	4.42271
12.1767	57.426	139.067	105.809	84.3963	90.3965	65.9251	36.0392
8.9729	77.4877	131.325	13.8884	108.252	63.857	32.427	86.6614
26.473	40.9499	127.682	69.7401	17.2222	57.316	31.5651	78.3201
32.1969	54.2064	58.8468	184.404	161.835	70.9273	92.0706	76.9428
16.3038	54.1107	2.74006	164.767	129.722	0.767916	54.1067	135.543
59.6181	63.2883	122.967	74.9085	44.0188	83.5334	48.2082	54.6223
94.9456	28.0522	101.546	14.2003	47.2587	1.64126	49.6551	84.6541
39.6091	54.794	154.163	86.2901	105.591	82.4366	39.0723	114.081
45.8345	19.7858	110.516	117.149	92.4093	97.2999	16.7307	125.465
31.5225	43.8548	131.59	102.54	92.3972	125.805	23.7759	63.0844
33.5737	36.4066	99.8452	112.494	104.986	14.2776	44.1169	65.2295
59.1849	51.4991	126.681	116.58	49.8746	111.045	30.2147	31.3967
16.5075	34.7948	94.7598	69.863	112.816	103.525	19.2027	6.4709
72.1583	25.3967	60.7408	162.014	60.8078	108.692	80.6465	74.6737
62.5403	65.2338	134.12	34.3239	113.093	80.0701	39.8306	68.4365
119.77	18.2817	62.4672	123.177	71.2239	103.114	9.31828	0.439631
4.25908	39.3062	73.8188	103.686	90.941	78.7969	38.2426	99.8997
22.3611	12.7163	57.1223	161.201	120.767	56.5677	46.6011	72.6925
62.8351	25.885	34.4248	48.4514	3.20929	159.824	50.357	42.685
94.0597	63.3431	35.8134	168.01	67.4446	104.897	42.8051	44.6908
33.1032	69.8348	151.68	109.71	24.9724	62.0023	43.4908	82.1789
31.9332	44.595	175.385	148.362	71.2025	53.3112	48.9387	75.3589
14.8466	96.2647	65.3989	140.365	80.334	75.1967	68.7349	47.9392
15.1123	151.014	130.963	49.5306	1.85345	62.7118	49.6313	1.13533
22.9879	71.7251	73.8215	97.0142	101.859	47.8095	63.6854	68.749
17.8865	50.6742	114.125	4.64408	80.2951	83.6358	60.3125	35.387
20.6201	49.2189	47.321	13.4495	97.4014	105.939	50.3643	39.858

50.1739	36.1581	55.3883	146.76	156.551	40.2147	83.2833	76.4343
86.4184	64.7534	80.9934	68.2268	49.6366	55.5494	28.7736	74.4509
34.6904	68.8167	49.3765	32.5898	74.9157	43.9705	30.5754	61.9523
27.8414	79.587	102.42	187.632	68.3899	96.5636	12.7124	76.7773
69.9994	70.0793	50.8386	110.918	129.627	81.0536	32.1922	112.321
34.7225	86.9928	66.5939	29.6496	45.7394	92.6557	41.0405	48.8577
29.6093	82.2848	43.5463	127.933	156.403	84.307	101.71	61.551
25.16	118.732	3.28293	126.279	120.15	118.26	38.0693	95.5074
32.7593	13.5538	169.061	20.8808	23.2918	111.89	59.2216	45.8603
4.10254	28.5046	0.228933	124.112	107.75	103.186	19.0458	64.0802
45.8441	82.2801	144.433	29.5721	38.7975	49.019	14.7154	16.5754
32.9125	28.9857	162.2	19.3955	104.213	20.4434	60.0821	18.5758
28.7418	40.8421	90.0502	137.795	33.0876	57.2071	90.4759	57.5124
33.1794	51.7716	56.3549	111.584	118.322	130.412	22.8425	101.91
41.4448	51.5984	78.0469	152.304	69.8968	61.651	28.9764	33.1613
57.1361	46.2253	80.046	141.256	86.4015	58.9073	34.0858	10.79
67.2031	80.073	79.8329	184.278	95.8638	72.0353	62.9584	42.2534
68.1158	82.5067	9.308	52.1643	94.137	1.12128	31.6533	26.7775
43.9952	47.9434	76.6637	114.091	127.036	63.8535	32.4334	30.1444
68.0633	85.0257	38.8247	137.113	57.5179	96.8836	29.8171	108.036
58.556	81.0693	94.8742	179.321	72.5444	101.357	33.6387	23.241
26.4321	49.3591	78.9097	131.21	58.0888	56.9626	30.445	39.7439
60.9979	105.528	87.3609	54.2679	98.1674	74.1552	80.724	72.4661
134.673	52.1037	77.0699	18.9512	87.3657	80.4615	69	19.8683
53.4316	77.2762	126.622	47.5301	80.5176	79.6959	95.0413	23.6073
86.4456	26.9144	187.759	74.003	95.3146	78.7093	109.526	30.3323
20.1831	71.4802	76.784	84.2593	61.2596	69.3525	63.8948	66.8451
22.488	110.693	51.8647	156.766	72.2463	33.1701	50.7122	20.8608
17.0556	49.6301	124.033	37.5151	107.225	82.7046	46.1708	95.7773
50.3076	53.8317	87.1976	52.0393	74.6248	16.2982	25.6556	77.6719
10.8843	61.6252	39.7077	9.995	81.5749	79.5074	46.0102	25.1849
16.3905	79.7176	89.464	79.9721	44.0732	74.6321	29.408	29.3645
30.4513	125.015	61.3788	3.49306	90.3697	83.557	56.9266	57.8599
35.6599	58.8541	102.104	18.4217	49.919	88.4496	44.2359	43.262
10.7139	22.4508	139.609	98.1909	76.0076	68.9941	102.277	94.553
4.19852	65.5745	109.983	45.23	64.1614	62.846	44.5691	35.0048
1.33526	75.2222	108.979	131.36	37.4451	125.773	27.3348	99.0644
44.7479	145.381		77.5616	71.1879	61.7714	47.6733	22.7511
60.0737	48.1772		74.6982	41.2769	80.151	46.2217	34.1015
16.1488	38.9325		56.9008	70.5664	102.439	118.001	36.5301
66.5616	121.651		123.288	2.12183	28.1846	52.2945	51.1662
33.2523	37.7403		61.4572	88.2837	76.7224	77.7077	64.1562
47.1712	79.6283		23.0363	123.155	68.6552	25.7122	63.1214
121.46	43.2696		190.197	95.2258	63.7867	56.5012	68.3758
47.7067	54.4511		142.411	49.5724	101.23	27.1433	56.6253
133.659	52.7805		105.104	107.38	93.4674	7.57752	50.0604
35.0023	45.4589		146.166	91.8875	164.761	21.3905	97.5228
21.9275	67.1772		118.484	152.375	91.2269	38.5432	79.9671

15.0696	76.2281	158.696	48.1913	76.2978	22.2085	41.6822
36.9467	11.1116	148.362	56.9769	89.6761	59.6136	40.6563
32.3076	60.5558	187.026	27.672	56.8512	33.4091	91.469
21.9027	48.0314	137.64	77.672	77.6613	88.0059	49.5668
14.7426	61.3038	61.449	84.9786	143.653	27.2196	40.9568
36.9309	40.607	89.4126	82.7054	43.8427	15.8533	0.84815
28.7812	27.2374	191.373	150.234	36.6908	26.9624	1.38723
38.5392	54.2731	155.624	85.6336	36.0425	21.6562	47.6826
66.0803	36.4211	95.2132	42.8119	86.5037	18.857	36.4932
24.163	49.9874	174.701	103.285	48.6182	44.587	132.355
31.0115	12.1016	18.278	27.3851	72.0567	18.0352	46.0171
43.4991	36.6407	130.919	56.6024	82.135	24.3093	22.281
27.4477	98.9836	109.18	52.1723	65.9055	71.4102	9.84936

Table D.8. Percentage of DNA in the tail of the striata cells (comets) of the rats in Group 4.

TailDNA%							
Rat 25	**Rat 26**	**Rat 27**	**Rat 28**	**Rat 29**	**Rat 30**	**Rat 31**	**Rat 32**
5.34104	19.8421	2.5437	10.9158	6.04418	5.48511	13.1884	3.66359
6.56106	21.5139	8.05899	12.2213	8.16088	5.60641	15.4909	3.85523
10.0062	21.61	10.0107	20.398	9.26725	6.31252	16.066	5.16059
10.6477	25.4327	10.2592	21.3485	9.43909	10.8673	17.8599	7.70683
10.8467	28.8786	11.8637	25.2352	20.0325	18.9653	19.1495	12.6363
12.3486	29.0185	15.8558	26.2045	20.5412	20.8606	19.9408	14.7066
13.6677	29.531	18.616	26.2968	22.6608	23.9026	20.7073	18.2881
14.1108	31.6208	37.3315	26.8794	30.2491	28.1003	21.1307	20.9561
16.7395	31.9219	42.635	30.311	30.7809	30.5527	21.885	25.1143
19.1351	31.9568	42.7215	32.1207	30.8077	34.6563	22.512	25.4393
19.293	32.1477	44.0151	33.0037	31.2155	35.3403	23.5414	28.3833
19.4158	34.0467	44.2457	33.4406	35.1091	35.4903	23.7004	28.5765
21.0059	34.3094	44.8931	33.6786	35.9648	36.0911	24.1189	28.5782
21.4759	34.9537	47.4862	36.2109	36.8959	36.3633	25.147	28.897
21.5733	35.0652	47.5823	36.6044	37.1311	36.555	25.4779	30.5219
21.7975	35.5961	47.8406	37.9129	37.146	36.8942	25.9574	31.1391
22.2129	35.6307	50.5627	41.0605	38.7614	37.1445	26.3508	31.1658
22.3581	36.6407	50.7263	44.1355	39.4118	37.4551	26.6704	31.3331
22.3945	37.2614	52.1804	45.2882	39.5417	37.5794	26.87	31.4068
22.4948	38.466	52.4167	46.5982	39.6566	38.9133	27.1786	32.7656
22.812	39.5724	52.5282	46.98	40.1222	39.148	27.2294	32.8462
22.9749	39.8169	53.6801	48.5594	40.1807	39.1881	27.326	33.0587
23.2498	41.078	53.7739	49.7033	40.4141	39.857	27.4273	33.3379
23.6129	41.4081	53.7887	51.3909	40.4969	40.1795	28.3815	33.6512
23.6615	41.5316	54.2206	51.524	40.7012	41.5014	28.9486	33.7523
23.7012	41.7486	54.2614	52.0823	40.8063	41.6941	29.0108	34.026
24.1924	41.8854	54.7745	52.763	40.9689	41.993	29.2217	34.1307
24.3674	42.1492	54.8399	52.9265	41.1978	42.3756	29.2691	34.3564

24.7758	42.2285	55.0572	53.8767	41.2278	42.3821	29.5593	34.7253
24.8081	42.6433	55.8959	53.9682	41.2501	42.5257	29.6742	35.027
25.4183	42.8012	57.0888	54.0621	41.6938	43.5136	29.751	36.0882
25.5092	42.8698	57.1223	54.4028	41.7396	43.635	30.0701	36.2782
25.6141	42.8852	57.587	54.7123	42.7902	44.287	30.096	36.317
25.625	43.1566	58.0786	54.7275	42.8934	44.7436	30.5445	36.9906
25.793	43.1745	58.2313	54.9097	42.9584	44.7599	30.6296	37.2756
25.982	43.1987	59.1553	55.3141	43.306	44.8678	31.0525	37.3498
26.0655	43.4197	59.2071	55.7801	43.3211	44.9097	31.5609	37.3924
26.6493	43.7444	59.4536	55.9236	43.7466	45.3263	31.6533	37.5553
27.8286	43.9848	59.7327	55.9743	43.8778	45.3794	31.6767	37.6833
28.1746	44.1643	59.9951	57.1241	44.1681	45.839	31.8049	37.6917
28.3052	44.5864	60.2472	57.4624	44.2	45.9469	32.0474	37.8826
28.3158	44.6314	60.2778	57.5941	44.2911	46.6183	32.0695	38.3395
28.3312	45.4438	60.4301	57.6855	44.7629	46.7999	32.3298	38.386
28.7553	45.5005	60.4599	58.7829	44.9228	47.0334	32.7028	38.7203
29.0514	45.547	60.7232	58.9125	45.0784	47.0619	32.7172	38.7663
29.4961	45.8239	61.307	58.9226	45.1381	47.0959	32.761	39.1329
29.5983	45.944	61.4543	59.155	45.1841	47.2508	32.7947	39.2223
29.6318	46.0742	61.5157	60.3455	45.4363	48.1481	32.9084	39.3309
29.7449	46.1443	61.661	60.4101	45.5498	48.463	33.1279	39.7305
29.8362	46.3302	62.0697	61.3905	45.8993	48.6219	33.1377	40.1024
30.1935	46.4115	62.2202	61.76	46.0143	48.8625	33.3924	40.4406
30.3062	46.6937	62.7061	62.3449	46.0343	49.0395	33.4545	40.6265
30.5949	47.3806	63.0273	62.9429	46.0943	49.2118	33.6387	40.9559
30.7039	47.3987	63.4303	63.1988	46.2213	49.4229	33.7381	41.0329
30.9735	47.4969	64.9846	63.2938	46.4202	49.4307	33.7629	41.6757
31.2334	48.0602	65.4781	63.8477	46.6118	49.6325	34.1115	41.9879
31.4501	48.1921	65.73	63.9015	46.9106	49.8393	34.2103	42.1187
31.5898	48.306	65.755	64.0094	47.1218	49.9235	34.244	42.1631
31.9749	48.4598	66.1226	64.088	47.3609	49.9672	34.2441	42.3778
32.0367	48.8061	66.1429	64.3682	47.7046	50.2092	34.4619	42.449
32.2942	48.8456	66.6742	64.487	47.8645	50.2946	34.4938	42.5357
32.3076	49.0065	69.7572	64.7392	48.0044	50.3895	34.6287	42.6095
32.3955	49.1915	69.858	65.2319	48.3592	50.4294	34.6869	43.1161
32.4094	49.3372	69.86	65.5197	48.3706	50.4995	35.0096	43.1749
32.7429	49.5536	70.0821	65.7036	48.394	50.6004	35.0368	43.2216
32.836	49.8091	70.2198	66.0258	48.6316	50.7002	35.2657	43.3503
33.084	49.8764	70.8675	66.1206	48.7203	50.7175	35.4997	43.4864
33.3355	50.3737	71.1254	66.4384	48.8151	50.8683	36.1123	43.5515
33.5145	50.4924	71.3913	66.6303	49.0077	50.9523	36.3828	43.7318
33.5598	50.5018	71.5162	67.0876	49.0917	50.9945	36.3993	44.0328
33.56	50.7128	71.8863	67.2172	49.2306	51.0268	36.6428	44.2457
33.6139	51.1598	72.2165	67.2673	49.2853	51.0518	36.6921	44.2561
33.6737	51.2935	72.4106	67.3247	49.436	51.1068	37.2896	44.7557
33.8057	51.4307	73.9821	67.4374	49.5973	51.1668	37.2906	45.3987
33.9618	51.4539	75.2727	67.5291	50.005	51.1704	37.3578	45.7716
33.9663	51.4873	78.0225	67.634	50.7695	51.6282	37.427	46.4534

34.5381	51.66	78.0952	68.1384	51.0482	52.157	37.4927	46.5179
34.7791	52.5097		68.2156	51.0549	52.3486	37.5383	46.5741
34.7977	52.645		68.6499	51.9536	52.3717	37.7276	46.7566
34.8933	52.8889		68.8566	52.3886	52.444	37.9596	47.1976
35.6079	53.2987		69.1367	52.5498	52.6547	38.025	47.2675
35.7194	53.4418		69.3653	52.63	52.7715	38.0253	47.3412
35.9272	53.4804		69.6027	52.6605	53.0238	38.1495	47.421
36.0599	53.7809		69.7318	52.8655	53.2141	38.1616	47.7906
36.1155	54.1752		69.8099	53.3034	53.3551	38.1779	47.9456
36.1438	54.291		70.1157	53.4227	54.3153	38.8034	47.9723
36.3551	54.3615		70.3209	53.7048	54.5866	39.0168	48.0492
36.899	54.5415		70.8466	53.7943	54.9212	39.0719	48.7921
37.0116	54.8534		70.9865	53.8561	54.9577	39.2587	49.1166
37.0178	54.8553		71.0614	53.8751	55.1364	39.5565	49.6728
37.3465	55.0225		71.9821	54.0387	55.1736	39.6834	49.8481
37.4026	55.9336		73.3436	54.156	55.6287	39.7896	50.1592
37.8439	56.1826		74.0065	54.2196	55.7047	39.8306	50.2858
37.9219	57.5215		74.0134	54.2604	56.0496	40.0659	50.3677
38.0641	57.6527		74.0256	54.4932	56.0627	40.0892	50.5114
38.2703	58.0407		75.7729	54.7712	56.0949	40.2358	50.5762
38.3297	58.9533		76.2442	54.9904	56.1469	40.2808	50.5952
38.5163	58.9807		76.5846	55.3017	56.2427	40.5148	50.7543
39.0462	60.0514		76.9386	55.3977	56.3277	40.7068	50.9291
39.0559	60.5373		78.7513	55.5613	56.9257	40.7397	51.0428
39.0868	60.7133		80.0542	55.8737	56.9923	40.7559	51.2035

GROUP 5

Table D.9. Tailmoments of the striata cells (comets) of the rats in Group 5.

Tailmoment							
Rat 33	**Rat 34**	**Rat 35**	**Rat 36**	**Rat 37**	**Rat 38**	**Rat 39**	**Rat 40**
2.6698	4.60204	8.69707	0.967124	2.24553	0.121957	0.018134	0.013465
2.36686	0.049588	34.5649	6.08683	57.3346	0.806903	4.00239	0.338735
1.43997	5.85399	10.7926	10.9846	193.519	2.3221	5.40956	6.39571
0.827222	0.032199	36.9805	0.392617	34.2441	8.01121	5.44695	0.000101
0.916065	19.0777	12.6087	4.08286	33.6776	7.40506	2.16918	1.55052
0.174087	0.587047	2.19314	8.67848	1.3887	0.166381	7.9807	1.1083
1.42671	0.387853	0.000435	0.110438	8.81E-05	0.000318	1.24559	4.97996
6.7465	1.17705	0.053368	15.0901	1.19522	1.17809	16.588	0.111843
5.58482	3.67851	0.00046	1.79436	3.41676	1.4699	7.54636	2.21654
0.30401	4.37735	2.32773	7.74341	0.630613	0.01706	18.6506	4.60068
1.9246	3.97697	9.7792	21.7131	107.432	0.000328	1.25576	3.13812
4.52132	6.10276	9.87482	0.964151	3.52968	0.169859	4.84362	3.17327
1.696	0.000327	8.96084	0.754924	3.48703	0.144263	16.1858	1.57084
0.330874	1.79378	1.36201	0.954646	41.6363	8.42523	0.000128	1.60423
1.15439	28.2057	3.96046	1.6754	2.135	0.751277	7.5029	8.32974
2.4186	2.93692	11.4081	2.5635	0.708991	3.49E-05	8.87171	8.21376
3.86964	0.105707	36.7188	2.48122	1.35255	9.16914	7.41573	2.32194
0.495608	2.92032	37.1759	0.385101	133.892	0.7994	1.04614	2.33776
0.84518	3.02135	3.26676	6.91637	0.433589	0.000181	8.98802	5.59376
0.146712	8.43561	9.62251	10.7653	3.21297	1.39383	2.11842	0.316233
0.673764	0.447588	8.90626	3.55446	1.98983	1.3486	3.86858	25.1105
4.42827	1.7189	1.19873	2.41868	0.601433	0.607268	27.6028	0.045852
1.45294	9.03535	3.60722	36.0069	3.82325	3.03783	0.627563	0.000108
0.001426	3.5696	7.15119	0.202351	17.7276	8.83E-05	7.50746	0.001036
5.67381	0.220094	5.7236	6.67613	2.79303	0.845118	12.3666	0.977855
1.60515	1.11098	12.3774	3.26291	2.2543	0.844786	6.99712	0.000769
0.811103	0.637339	5.61137	2.30734	6.69E-05	0.00038	3.15903	0.013345
0.306419	3.519	4.07417	1.06109	0.098574	0.332448	0.693936	2.19221
0.597441	0.40904	0.645336	1.90372	0.000298	4.07524	12.132	8.36125
0.0635	19.4261	12.2929	28.4185	0.042347	0.130762	4.83568	141.299
4.83968	5.41393	13.1046	0.08998	1.46294	0.266378	4.3345	33.9097
0.004501	2.60144	8.20398	1.06706	0.221512	0.001438	8.36292	2.1807
0.872972	6.69506	9.24232	1.31287	0.283189	0.000468	43.7475	2.18929
1.87414	6.67222	6.7027	21.7878	0.000131	0.215815	1.41499	145.396
1.17989	9.68315	3.26552	0.790377	0.000189	0.775854	24.8859	0.016363
1.9257	5.59283	0.360085	0.507014	4.10112	0.000267	2.41799	2.80217
1.32961	0.315479	18.5496	0.84181	0.000256	63.4757	1.10953	2.59166
0.073575	3.91144	28.2709	0.123825	5.91374	0.166425	9.84015	3.55545
1.64535	8.64291	3.35687	30.6008	0.000675	0.300312	7.20606	2.78004
0.033338	0.016308	13.2379	1.01692	116.004	66.6923	36.4664	0.034948

1.93217	1.46561	5.43919	0.21961	0.00069	0.572752	22.4755	0.000104
0.760641	3.3067	1.0471	14.0344	113.342	6.0445	0.675349	0.001051
0.816305	5.7484	3.08834	30.4674	5.30242	2.85222	13.0967	0.83928
1.15248	11.0544	10.9667	3.58449	7.33233	46.453	1.38213	1.20456
0.00437	3.51444	0.213407	0.012077	0.552642	48.429	7.24732	8.24703
4.22532	6.89813	10.9396	0.247353	4.56E-05	0.012058	7.10733	0.260238
1.38007	0.0003	0.355835	3.40144	0.018777	0.140794	7.26511	0.294365
2.31487	3.89108	11.1573	27.809	0.273068	67.0251	26.2862	25.2007
0.624053	2.10954	26.0667	8.42604	0.000241	6.09205	4.72E-05	
0.050804	2.22942	13.922	11.9436	0.269553	0.981222	3.2318	
5.38267	2.07236	10.1874	17.2798			43.7717	
0.655052	6.29151	31.3779				1.41248	
4.26171	2.64744	4.00397					
1.97832	3.7306	23.0306					
0.791425	7.87046	10.2536					
4.00763	6.00535	45.7524					
0.832943	10.9412	9.25548					
0.470295	1.27524	0.30424					
2.48038	0.699977	0.00011					
0.527483	1.9859	2.04761					
1.09635	2.33603	6.77143					
0.817407	1.97787	51.4264					
2.9933	2.02415	0.528033					
1.19402	6.52784	2.77196					
2.0836	1.92842	0.228099					
1.12354	17.4754						
2.50907	1.94996						
0.008028	0.127144						
0.743056							
1.45805							
0.589849							
1.16362							
1.50483							
1.32756							
2.68429							
1.28371							
1.6052							
2.55059							
4.28972							
1.21558							
0.000216							
0.002101							
0.712955							
2.50611							
3.1334							
2.15974							
2.07544							

Table D.10. Percentage of DNA in the tail of the striata cells (comets) of the rats in Group 5.

TailDNA%							
Rat 33	**Rat 34**	**Rat 35**	**Rat 36**	**Rat 37**	**Rat 38**	**Rat 39**	**Rat 40**
10.2684	18.4081	21.2124	8.79204	13.209	1.74225	0.604469	0.448838
11.2708	1.23971	46.7094	18.4449	46.2376	6.72419	17.4017	3.38735
5.75987	16.2611	22.963	15.4712	77.7186	10.555	17.4502	23.6878
7.5202	0.804965	33.6186	3.92617	46.2758	18.2073	17.5708	0.003352
7.04665	35.3292	28.0193	11.6653	24.4041	25.5347	9.85992	8.61402
2.48696	5.33679	10.9657	22.8381	7.71498	1.84868	18.138	7.91641
9.51141	3.52593	0.014496	1.84063	0.002936	0.010583	7.78492	16.0644
17.754	7.847	1.77894	32.8046	7.96815	11.7809	15.7981	2.23687
14.3201	13.1375	0.015347	9.96865	12.2027	9.1869	22.1952	13.0384
3.37789	17.5094	7.75911	21.5095	5.2551	0.568658	25.9036	14.8409
10.1295	15.296	21.2591	36.1885	54.2587	0.010947	8.96973	14.2642
13.701	15.2569	27.4301	6.02595	11.7656	2.42656	13.4545	11.3331
9.97649	0.010906	21.8557	6.29104	11.6234	1.80329	27.4336	8.7269
4.13592	8.15352	7.16846	8.6786	43.3712	21.6031	0.004257	8.91236
8.87994	29.3809	15.8418	8.81791	6.67189	8.34752	22.7361	21.3583
14.2271	10.489	25.3512	12.8175	5.45378	0.001162	20.163	20.0336
14.332	2.64268	31.3836	9.18971	5.63561	25.4698	18.5393	11.0569
4.95608	11.6813	31.7743	3.50092	56.257	5.71	7.47243	11.1322
6.037	10.0712	13.6115	13.8327	3.94172	0.00603	20.4273	15.5382
2.09589	16.8712	27.4929	19.5733	10.7099	10.7218	8.82676	3.95292
2.69506	4.06898	24.7396	13.1647	22.1093	10.3738	12.8953	38.6315
10.2983	7.1621	5.99366	10.516	5.46757	8.67526	30.3327	1.5284
10.3781	21.0125	10.3063	28.3519	10.9236	12.6576	5.2297	0.003592
0.047528	13.2207	12.77	2.52939	31.6565	0.002943	19.7565	0.034545
15.7606	3.1442	12.7191	15.5259	13.9651	6.03656	23.3332	6.11159
7.64357	6.94359	17.9382	11.2514	10.2468	6.03419	16.6598	0.025649
7.37366	4.90261	17.0041	8.54569	0.002231	0.012652	10.8932	0.444839
3.40465	15.3	15.0895	7.07397	1.97148	2.5573	4.95668	12.8954
5.97441	4.54489	4.60954	8.27705	0.009947	12.3492	15.5538	17.4193
1.27	31.3325	25.0876	35.0845	1.05868	1.86803	28.4452	64.816
16.6885	12.8903	27.3013	1.7996	8.12745	2.95975	17.338	38.5337
0.150032	13.0072	16.408	5.9281	3.69187	0.04793	21.4434	9.08623
8.72972	16.7377	26.4066	5.96758	2.83189	0.015587	39.0603	9.12203
11.7134	16.6806	19.1506	36.3131	0.004373	2.39794	8.32346	66.6955
6.94052	21.5181	13.6063	8.78197	0.00629	4.84909	26.759	0.545421
11.3276	17.4776	3.60085	4.22511	14.6468	0.008904	12.09	10.0078
9.49721	3.15479	30.916	5.61206	0.008526	33.4083	6.93455	9.25594
1.47149	11.8528	36.2447	1.76893	15.1634	1.84916	18.5663	10.1584
10.969	17.2858	8.39218	27.8189	0.022497	10.0104	18.4771	7.72235
1.11126	0.543597	19.1854	11.2991	48.9469	34.9174	28.2685	1.16492
9.2008	8.14229	18.7558	3.13728	0.023011	4.77294	26.1343	0.003454

6.33868	11.4024	9.51908	24.6217	47.8237	24.178	6.13953	0.035048
7.42095	15.5362	13.4276	31.0892	13.9537	23.7685	21.47	5.5952
8.8652	19.74	23.3334	11.2015	36.6616	44.2409	8.13021	8.03039
0.145661	11.7148	2.66759	0.402561	5.02401	46.1229	18.5829	17.1813
16.9013	14.9959	27.349	2.47353	0.00152	0.401921	14.8069	3.25297
10.6159	0.00999	3.95372	10.6295	0.625895	1.75993	18.6285	3.67956
14.4679	9.49043	21.4564	41.506	2.73068	35.0917	27.9641	38.7704
6.93392	9.58882	31.4056	22.1738	0.008024	24.3682	0.001572	
1.69345	11.1471	19.8886	32.28	2.69553	5.77189	13.4658	
17.3635	9.01028	25.4686	32.6034			39.0819	
5.95502	18.5044	28.016				8.3087	
13.7475	10.1825	13.8068					
11.6372	11.6581	33.8685					
7.91425	16.7457	16.5381					
12.5238	13.9659	47.6587					
6.94119	22.7941	21.0352					
3.91912	6.71177	3.38045					
10.3349	5.83314	0.003674					
4.7953	11.0328	7.3129					
7.30899	11.1239	17.8196					
6.81172	8.99033	46.7513					
13.0143	5.62263	4.06179					
9.95014	19.1995	9.89984					
11.5756	8.76553	3.25855					
8.6426	29.1256						
10.4544	6.96414						
0.267586	1.81634						
6.19213							
10.4146							
6.55388							
7.75747							
11.5756							
10.212							
14.9127							
9.16936							
10.0325							
15.0035							
14.2991							
6.07789							
0.007188							
0.052528							
7.12955							
10.0244							
14.9209							
10.2845							
10.3772							

GROUP 6

Table D.11. Tailmoments of the striata cells (comets) of the rats in Group 6.

Tailmoment							
Rat 41	**Rat 42**	**Rat 43**	**Rat 44**	**Rat 45**	**Rat 46**	**Rat 47**	**Rat 48**
24.4611	5.32146	4.92554	1.45059	0.000127	1.39275	6.45013	2.14552
5.86E-05	0.907489	29.2574	1.34452	1.72306	0.592124	0.807977	0.416029
7.71E-05	0.000255	0.015237	0.094569	1.68296	0.16293	1.5451	0.407367
35.5541	0.04639	4.95029	0.169589	0.174118	0.797327	4.16011	0.935132
1.62452	1.08298	12.6111	1.48877	1.3536	0.735143	7.64984	0.367523
34.8178	1.00743	2.00821	0.76873	5.30E-05	0.743304	0.949565	0.36231
0.624232	6.65408	12.5749	2.57768	0.050658	12.7555	6.46328	0.231825
6.54508	2.6136	6.17057	3.41345	4.08426	162.699	1.45124	0.100058
0.000113	0.000455	1.96839	1.10642	0.000575	39.3589	2.48878	7.81E-05
53.4286	1.06772	2.91829	12.4183	2.27555	7.83446	11.6257	3.13418
0.290345	0.57728	1.06204	3.84563	0.96299	169.178	6.07184	9.47E-05
9.73385	0.809187	0.000113	1.10088	0.444	1.19505	0.293997	0.063494
8.57234	0.030514	12.4239	2.38483	0.000395	125.796	2.28388	4.76417
37.699	4.68509	3.86471	0.209006	0.596465	0.882578	1.92203	6.67377
1.57622	0.02701	4.89953	1.97515	0.660833	30.7144	3.25187	7.21794
2.10979	5.59862	8.47353	1.59673	0.572599	0.517188	0.424723	1.5215
2.22559	7.79463	1.32E-05	7.94187	0.162767	0.490339	3.5498	32.7765
35.7449	49.8948	0.178682	1.90012	0.000152	15.0896	1.66564	1.09677
4.95591	13.0967	1.00897	0.508299	0.000111	7.42	1.2642	1.48073
4.14158	10.7128	0.067133	0.009686	0.000172	2.41185	3.00046	0.809791
13.8782	1.01615	1.47437	0.218436	0.263	0.527686	1.30467	0.001105
25.3522	3.1047	0.696217	7.48641	0.000152	8.88028	1.31865	0.050881
0.000103	4.24184	0.243816	17.9632	0.070401	6.37596	2.7213	0.096329
9.05982	7.73632	5.52235	0.711336	10.5994	1.62092	3.28521	1.04944
5.63549	0.997394	0.116416	0.010478	0.699268	1.24972	2.28109	0.000182
41.5056	3.08257	0.000102	6.60E-05	3.55814	5.24639	2.79866	0.439078
0.000179	0.81283	4.83461	0.046029	3.05942	6.2312	2.06033	0.517114
9.23094	3.2201	0.745491	1.0124	0.308991	0.009239	2.09451	3.27074
0.103798	2.38938	1.42784	0.121059	0.131542	0.275943	7.55258	0.000262
5.01947	0.01659	0.355473	6.91E-05	1.59168	4.19324	4.11287	2.12001
0.162063	10.9212	0.169001	0.000215	0.515444	2.69877	7.01584	0.000134
8.03247	20.3341	2.38572	1.27332	0.518998	1.314	0.729777	23.072
6.98062	47.0494	1.59172	0.477222	0.87811	0.035406	1.44596	5.5162
39.1365	9.65695	5.15488	0.000213	0.438325	12.6395	0.154327	7.0837
8.68019	8.00744	7.19948	5.02E-05	0.779735	1.4774	1.04727	0.452741
0.385466	3.13866	0.895605	0.045803	0.500634	0.059337	2.83079	5.14E-05
5.98102	11.6882	4.54077	6.60E-05	1.12031	0.000462	0.958073	1.03129
6.54527	5.42764	0.539377	16.9827	0.035913	0.140061	0.370221	7.75355
6.96E-05	1.95862	0.109506	0.000159	0.611516	1.66323	2.53329	0.036297
9.20138	11.6531	4.97003	2.98E-05	0.278419	0.075317	1.00625	0.000148

38.5338	3.09363	1.09745	0.0002	2.41317	0.067132	0.579973	4.21141
7.48902	0.980815	0.506573	1.93546	0.018458	0.196282	0.424911	0.091891
9.70593	0.000237	0.37274	0.006469	1.06697	0.013695	0.023485	2.73733
7.78073	10.5351	0.069123	7.96638	7.02467	1.53338	0.032233	1.46E-05
8.38262	2.33992	2.16968	0.305018	4.48188	2.01698	1.94476	0.182826
3.85325	0.071619	0.557376	0.094569	0.039071	14.1551	0.028632	4.74047
20.9598	0.350514	7.1982	1.47086	0.001588	2.59476	0.870744	2.73153
0.168992	0.130955	0.157587	1.32454	0.719762	28.9101	3.27904	0.404078
9.22983	1.3754	1.19262	0.090239	1.3748		0.20285	3.30986
0.119562	0.330042	4.42772	0.490665	3.53366		5.46959	
8.87853		0.360327	1.60713	25.4055		0.480072	
8.12382		0.368622		29.4734		0.592329	
7.89E-05		0.266443		0.000164		0.583462	
5.53488						0.969839	
						3.48464	
						0.882449	
						0.89824	
						0.492785	
						0.092549	
						0.939307	
						2.86915	
						1.19534	

Table D.12. Percentage of DNA in the tail of the striata cells (comets) of the rats in Group 6.

TailDNA%							
Rat 41	**Rat 42**	**Rat 43**	**Rat 44**	**Rat 45**	**Rat 46**	**Rat 47**	**Rat 48**
35.9723	17.166	16.9846	9.06617	0.004249	6.96374	18.4289	11.2922
0.001954	7.56241	35.2499	9.6037	10.7691	5.38295	6.21521	4.16029
0.002571	0.0085	0.507897	2.36421	8.85769	3.25859	11.0364	4.07367
46.1742	1.54634	19.0396	2.82648	2.4874	6.64439	15.4078	5.19518
9.55601	8.33062	23.7946	16.5419	9.66856	8.16826	19.1246	4.08359
34.1351	7.74943	12.5513	6.40608	0.001765	8.25893	6.78261	4.02567
7.8029	20.1639	23.7263	12.2746	1.26644	25.0107	18.4665	3.31178
20.4534	10.4544	18.6987	14.2227	11.6693	72.9592	10.366	1.66764
0.003757	0.015172	11.5788	7.90303	0.019151	41.8712	14.6399	0.002604
41.4175	6.28071	12.6882	30.2885	11.9766	23.0425	22.3572	13.6269
4.14779	5.248	7.58603	15.3825	8.02492	66.0852	16.4104	0.003156
19.086	5.39458	0.003751	7.86345	4.93333	8.53608	3.67497	1.58736
19.0496	1.01712	30.3022	18.3448	0.01318	70.6718	9.13554	15.3683
38.865	17.3522	15.4588	2.61258	6.62739	8.82578	10.1159	16.2775
11.2587	0.90034	17.4983	12.3447	6.60833	29.2518	14.7812	17.1856
13.1862	15.996	24.2101	10.6449	5.20544	4.70171	4.71915	6.33959
10.1163	19.4866	0.000439	20.3638	2.71278	4.45763	15.4339	37.2461
38.0265	35.3864	1.98536	10.0006	0.005051	29.0185	8.32822	6.45161

17.6997	27.2848	8.40806	4.23582	0.003684	28.5385	8.42802	6.73057
17.2566	25.5066	2.23775	0.322862	0.005745	10.0494	15.0023	5.06119
30.17	5.97737	8.67278	3.12052	3.75714	7.53838	7.67451	0.036846
37.8391	9.70219	9.94596	23.395	0.005061	18.5006	9.41893	1.69602
0.003444	13.6834	2.70907	26.4165	1.76004	30.3617	16.0077	1.92658
22.0971	15.7884	15.7781	5.08097	42.3976	7.71866	18.2512	6.99627
18.179	4.7495	1.94026	0.349263	5.37899	6.2486	11.4054	0.006082
31.2072	9.06637	0.00341	0.0022	11.8605	14.1794	15.5481	3.99162
0.005966	5.41887	14.6503	1.53429	10.5497	20.1007	9.81108	4.30928
18.4619	11.9263	5.73455	8.43666	3.86239	0.307972	9.97386	11.2784
1.72996	6.82679	9.51894	2.01764	2.19237	3.44929	15.4134	0.008731
13.943	0.331797	2.7344	0.002304	9.36284	9.98391	14.1823	8.15387
1.8007	23.7418	1.87779	0.007158	5.15444	13.4938	14.318	0.004476
22.9499	27.1121	10.8442	7.95823	5.76665	10.95	5.21269	28.84
19.9446	42.7722	9.94825	5.96528	6.27221	0.88516	8.03312	14.9086
48.3166	20.9934	20.6195	0.007103	3.13089	17.5549	2.20467	15.0717
24.1116	19.5303	17.5597	0.001674	6.49779	6.15585	7.48051	5.03046
3.21221	12.5546	7.46337	1.52676	3.57596	1.18673	12.8672	0.001714
16.1649	24.8686	13.7599	0.0022	8.00218	0.015408	7.98395	6.06642
17.2244	14.2833	4.90342	26.1273	1.1971	1.40061	3.36565	16.8555
0.00232	9.32677	2.19013	0.005307	5.55924	6.93012	11.0143	1.20989
24.8686	21.5798	18.4075	0.000993	3.09354	1.88294	7.18749	0.004918
34.7151	13.4506	7.83894	0.00665	12.7009	1.6783	5.79973	12.3865
20.8028	8.9165	3.89671	10.1866	0.61528	2.80403	4.24911	1.83783
30.331	0.007911	3.7274	0.215645	8.89145	0.228249	0.587118	15.2074
23.578	29.264	0.987465	20.4266	29.2694	6.66686	1.07444	0.000488
19.9586	9.35969	9.8622	6.10036	14.0059	8.76948	7.77903	3.0471
15.413	2.38728	4.28751	2.36421	0.976782	27.2213	0.954412	13.5442
30.3765	2.69626	23.994	9.19288	0.052936	17.2984	6.2196	15.1752
1.87768	2.18258	2.62645	9.46097	8.99702	27.7982	16.3952	3.67344
18.4597	8.09061	7.95083	1.80477	10.5754		2.89786	11.4133
1.9927	6.60084	13.8366	4.46059	13.591		14.7827	
18.1194		3.2757	10.7142	33.874		4.80072	
18.8926		4.0958		33.1161		5.38481	
0.00263		3.33053		0.005451		6.48291	
17.8545						6.92742	
						17.4232	
						8.02226	
						7.48533	
						4.92785	
						1.54247	
						9.39307	
						11.9548	
						8.53812	

APPENDIX E – MONOAMINE RAW DATA

GROUP 1

Table E.1. Monoamine concentrations in the nucleus accumbens of the rats in Group 1. The values indicated in blue were not included in the calculation of the average because the rats did not receive the correct volume of smoke extract.

Rat nr.	Mass (g) of tissue	Concentration in ng/g					
		NA	DOPAC	5HIAA	DA	HVA	5HT
1	0.00805	1754.475	6041.281	6999.339	2314.858	987.656	1486.729
2	0.04570	469.586	921.040	2304.760	474.218	95.532	569.862
3	0.05036	379.461	1297.654	1892.371	438.684	222.911	307.098
4	0.06172	301.583	1062.187	1928.651	496.468	277.800	507.437
5	0.05059	266.811	1044.933	3720.527	400.077	322.427	515.955
6	0.03917	765.555	1486.519	2884.255	570.928	509.287	484.504
7	0.04578	487.698	1012.966	3179.528	534.850	313.053	505.306
8	0.08678	731.642	743.868	3148.404	387.709	212.650	479.795
AVERAGE		**562.927**	**1072.072**	**3233.179**	**473.391**	**339.354**	**496.390**

GROUP 2

Table E.2. Monoamine concentrations in the nucleus accumbens of the rats in Group 2.

Rat nr.	Mass (g) of tissue	Concentration in ng/g					
		NA	DOPAC	5HIAA	DA	HVA	5HT
9	0.02119	260.881	1915.241	1389.603	564.291	296.885	442.109
10	0.04434	403.911	1135.442	1927.074	523.895	337.445	376.745
11	0.03979	312.365	1057.172	3176.984	411.281	260.554	449.538
12	0.06926	470.659	812.604	3476.253	393.897	173.938	429.266
13	0.04551	268.072	1135.590	3700.354	430.500	257.700	626.568
14	0.08601	323.174	683.637	2738.266	288.059	212.831	531.594
15	0.03904	248.645	810.272	2467.638	333.275	235.340	470.817
16	0.05414	825.036	1607.244	6714.695	626.741	489.487	762.618
AVERAGE		**416.232**	**1059.186**	**3905.238**	**419.644**	**298.840**	**597.899**

GROUP 3

Table E.3. Monoamine concentrations in the nucleus accumbens of the rats in Group 3 (treated with NAD).

Rat nr.	Mass (g) of tissue	Concentration in ng/g					
		NA	DOPAC	5HIAA	DA	HVA	5HT
17	0.06496	414.153	973.293	3190.162	452.786	249.340	470.124
18	0.06764	562.272	1146.023	3538.280	473.991	306.147	455.031
19	0.08395	474.771	580.359	2254.609	319.209	232.569	350.046
20	0.11208	208.478	595.569	1576.129	270.682	191.070	256.876
21	0.04928	286.040	1269.649	2927.804	474.879	234.425	319.650
22	0.05419	425.359	1446.285	2874.434	1412.139	389.502	478.394
23	0.07067	280.492	1165.408	2716.527	304.822	262.475	204.406
24	0.09082	351.710	1099.271	2200.396	608.572	260.670	383.422
AVERAGE		**414.919**	**823.811**	**2639.795**	**379.167**	**244.782**	**383.019**

GROUP 4

Table E.4. Monoamine concentrations in the nucleus accumbens of the rats in Group 4 (treated with NAD).

Rat nr.	Mass (g) of tissue	Concentration in ng/g					
		NA	DOPAC	5HIAA	DA	HVA	5HT
25	0.06118	585.258	2433.856	5206.495	1155.958	616.772	657.596
26	0.06498	260.219	1458.996	1418.509	574.904	230.798	265.043
27	0.09758	333.545	1042.033	1998.490	464.984	259.431	400.913
28	0.07470	217.901	1182.781	1760.817	425.446	206.498	198.915
29	0.06598	308.804	1529.927	2469.119	675.816	366.820	446.029
30	0.06297	405.895	1765.774	2001.256	450.888	399.334	284.715
31	0.04910	142.453	2448.088	777.933	645.930	354.684	122.570
32	0.06389	144.239	1747.597	1272.164	503.244	372.205	192.655
AVERAGE		**349.231**	**1529.417**	**2596.078**	**655.323**	**328.375**	**380.617**

GROUP 5

Table E.5. Monoamine concentrations in the nucleus accumbens of the rats in Group 5. The values indicated in blue were not included in the calculation of the average because the rats did not receive the correct volume of smoke extract.

Rat nr.	Mass (g) of tissue	Concentration in ng/g					
		NA	DOPAC	5HIAA	DA	HVA	5HT
33	0.04324	307.051	1852.338	951.649	465.681	296.036	167.807
34	0.06527	248.840	1394.997	1055.946	343.326	327.499	235.968
35	0.07346	188.793	1975.697	1146.246	286.384	225.693	121.713
36	0.06214	98.729	1083.766	67.738	192.562	153.892	-
37	0.04685	110.457	2696.321	818.416	430.924	374.403	135.857
38	0.05743	148.959	1575.885	1303.384	294.377	288.088	206.044
39	0.04771	137.909	2476.528	905.724	417.742	366.735	72.925
40	0.06633	238.551	1459.807	1373.093	457.165	229.885	186.654
AVERAGE		**184.911**	**1814.417**	**952.774**	**361.020**	**282.779**	**160.995**

GROUP 6

Table E.6. Monoamine concentrations in the nucleus accumbens of the rats in Group 6. The values indicated in blue were not included in the calculation of the average because the rats did not receive the correct volume of smoke extract.

Rat nr.	Mass (g) of tissue	Concentration in ng/g					
		NA	DOPAC	5HIAA	DA	HVA	5HT
41	0.07047	190.393	1393.918	654.590	300.969	203.155	109.217
42	0.06150	247.021	1681.673	1092.889	489.560	283.824	129.522
43	0.02356	168.124	3241.693	1185.085	727.898	647.961	323.387
44	0.05040	296.037	1959.342	694.543	360.619	323.692	118.536
45	0.02850	101.798	2799.543	171.252	370.736	452.907	-
46	0.04668	98.388	1469.951	668.202	384.761	298.234	43.931
47	0.06664	111.162	1486.835	457.207	280.840	321.465	62.889
48	0.05227	326.468	1561.405	711.391	336.933	265.721	114.295
AVERAGE		**192.424**	**1949.295**	**704.395**	**406.540**	**349.620**	**128.825**