Nandrolone Decanoate, Behaviour and Brain:

animal experimental studies

by Ann-Sophie Lindqvist

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Dedicated to Roger

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Abstract: Abuse of anabolic androgenic steroids (AAS) has been linked to psychiatric and physiological complications in humans. Studies have further found a relationship between AAS abuse and abuse of alcohol and other drugs. The main objective of this animal experimental thesis was to examine to what extent the AAS compound nandrolone decanoate (ND; Deca-Durabol® [15 mg/kg/day for 2 weeks]) induces behavioural and physiological changes in sexually mature male rats, when compared to oil-treated control rats. One aim was to investigate if ND stimulates establishment of dominance in a provocative and competitive test situation and if ND enhances reactivity towards physical provocations. Fleeing and freezing behaviours in response to a threatening stimulus were further studied. Another aim was to investigate whether ND stimulates voluntary ethanol consumption and if ND alters behavioural tolerance to ethanol. The results showed that ND stimulated dominance in a competitive and provocative situation, enhanced reactivity to physical provocations and decreased fleeing and freezing responses. ND treatment further increased ethanol consumption and induced behavioural tolerance to ethanol. This thesis also studied if ND-induced reactivity towards physical provocations and ethanol intake were altered when combining ND treatment with physical activity. It was found that physical exercise accentuated the enhancing effects of ND on reactivity and to some degree on ethanol intake. In this thesis, monoaminergic and opioidergic systems were also analysed. It was found that ND altered concentrations of serotonin, dynorphin B and enkephalin in various brain areas. Moreover, during the treatment period, ND-treated animals did not gain as much in body weight as controls. ND treatment also induced thymus atrophy and increased the weight of the adrenal glands. Taken together, the results from this thesis suggest that abuse of ND may constitute a risk factor for induction of behavioural complications, such as increased aggression and enhanced alcohol drinking. ND abuse may further affect physiological parameters like the hypothalamus-pituitary-adrenal axis and neurotransmitter concentrations. These results hopefully bear relevance for further research and in clinical settings when in contact with individuals abusing AAS.

Key words: Aggression; Alcohol intake; Anabolic androgenic steroids; Dominance; Ethanol intake; Fleeing and freezing behaviours; Locomotor activity; Monoamines; Nandrolone decanoate; Opioid; Peptides; Provocation; Reactivity; Serotonin; Wheel-running

Ann-Sophie Lindqvist, Department of Psychology, Göteborg University, Box 500, S-405 30 Göteborg, Sweden. Fax: +46 31 773 4628, e-mail: ann-sophie.lindqvist@psy.gu.se

CONTENTS

PREFACE

This thesis is based on the four research papers listed below, which are referred to in the text by the roman numerals I to IV.

I: Johansson, P., Lindqvist, A-S., Nyberg, F. & Fahlke, C. (2000). Anabolic androgenic steroids affects alcohol intake, defensive behaviors and brain opioid peptides in the rat. *Pharmacology Biochemistry and Behaviour 67(2)*: 271-280.

II: Lindqvist, A-S., Johansson, P., Nyberg, F. & Fahlke, C. (2002). Anabolic androgenic steroid affects competitive behaviour, behavioural response to ethanol and brain serotonin levels. *Behavioural Brain Research 133(1)*: 21-29.

III: Lindqvist, A-S., Jonsdottir, I. H., Nyberg, F. & Fahlke, C. Physical exercise accentuates the enhancing effects of Nandrolone decanoate on reactivity to physical provocations and on voluntary alcohol intake in male rats. *Submitted, 2004*.

IV: Lindqvist, A-S. & Fahlke, C. (in press). Nandrolone decanoate has long-term effects on dominance in a competitive situation in male rats. *Physiology and Behavior*.

ABBREVIATIONS

AAS	Anabolic androgenic steroids
ACTH	Adrenocorticotropic hormone
CNS	Central nervous system
DA	Dopamine
DOPAC	3,4-dihydroxyphenylacetic acid
GABA	γ-aminobutyric acid
HPLC-ED	High-pressure liquid chromatography-electrochemical detection
HPA	Hypothalamic-pituitary-adrenal
HVA	Homovanillic acid
IOC	International Olympic Committee
i.m.	Intramuscular
i.p.	Intraperitoneal
ir	Immunoreactivity
MAD	Median absolute deviation
MEAP	Met-enkephalin-arg^6-phe^7
NE	Norepinephrine
ND	Nandrolone decanoate
PAG	Periaqueductal grey matter
POMC	Proopiomelanocortin
RIA	Radioimmunoassay
s.c.	Subcutaneous
SEM	Standard error of the mean
VTA	Ventral tegmental area
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine (serotonin)

1. INTRODUCTION

1.1 BRIEF HISTORY OF AAS

Anabolic Androgenic Steroids (AAS) have both anabolic and androgenic properties and are synthetic derivates of the endogenous primarily male steroid hormone, testosterone. In males, testosterone is secreted from the Leydig cells in the testes. The anabolic effect of testosterone helps the body retain dietary protein, thereby aiding growth of muscles, bones, and skin. The androgenic properties of testosterone are twofold. Firstly, it has an organizational effect involving the development of male characteristics during the late foetal stage and early postnatal life. Secondly, during puberty testosterone has an activational effect that includes activation of the male reproductive system and secondary sexual characteristics, such as hair distribution, musculoskeletal configuration, genital size, psychic changes and sperm production. Testosterone's activational effect is dependent on the organizational effects earlier in life (Ciccero & O'Connor, 1990; Mottram & George, 2000).

The history of testosterone dates back to the 1840's when the German professor Berthold (1849) conducted a series of experiments on castrated roosters where he implanted the surgically removed testicles in the roosters' abdomen and thereby prevented loss of the comb (secondary sex characteristic in roosters). This led Berthold to conclude that testicles contained a substance that was transported in the bloodstream. Nearly half a century after Berthold's demonstration, Starling named the blood borne factors *hormones*, from the Greek word horma'o meaning "to excite or arouse". Based on Berthold's deduction that the substance existed in the bloodstream, Funk and colleagues in 1929 assumed that the active substance must be cleared in the kidney, thus must appear in the urine. Subsequently, they administered crude extracts of urine to roosters, which led to stimulation of the roosters' capon comb growth (Funk, Harrow, & Lejwa, 1930). Five years later Butenandt and Tschering (1934) succeeded in isolating merely 15 mg of a hormone from 25.000 litres of urine from policemen. In 1935 David managed to isolate ten mg of an active substance out of 100 kg bull testes (David, 1935). Consequently, scientists drew the conclusion that the testicles must contain something more potent than did urine. David named this substance *testosterone*.

Due to the structural similarity between the hormone (androsterone) found in urine and testosterone, Butenandt and Hanisch (1935) assumed that testosterone must be metabolised in the body. It was later discovered that the testosterone molecule has the potential to be

oxidized or reduced to approximately 600 related steroids. They were given the name *androgens*, which derives from the Latin words andros (man) and gennan (to produce) (Kochakian, 1993). For having mapped the structure of testosterone, Butenandt and Ruzicka were awarded the Nobel Prize in 1939 (Butenandt & Hanisch, 1935; Ruzicka & Wettstein, 1935). Today we know that testosterone is the main gonadal steroid in males. Its anabolic effects, in addition to its effects on reproduction, are easily observed in developing boys, and in hypogonadal men receiving testosterone as replacement therapy (Kuhn, 2002).

AAS compounds were originally developed for treatment of hypogonadal dysfunction and commencement of delayed puberty in men and for growth promotion (Basaria, Wahlstrom, & Dobs, 2001). AAS continue to be clinically used for these dysfunctions, but they are also used for other medical conditions, such as anaemia, malignancies, burns and acquired immune deficiency syndrome (Lukas, 1993). AAS have, however, not always been used for pure medical purposes. Due to their anabolic effects (e.g. increase muscle mass, strength, and endurance and fasten recovery from injuries; Lukas, 1993), AAS have become vastly popular among athletes, body builders and power lifters. Although several attempts have been made to diminish the androgenic effect in AAS substances, no pure anabolic steroids exist today. All of the approximately 60 different AAS compounds that are available on the market (Clark & Henderson, 2003) have some androgenic properties, thus the name *anabolic androgenic steroids*. Boje was the first physician to suggest, in 1939, that AAS might enhance athletic performance, but he was also the first to forewarn athletes of potential health effects of steroids (Boje, 1939).

1.2. THREE CLASSES OF AAS COMPOUNDS

All AAS derivates, like the endogenous androgens, are four-ringed structures with 19 carbon atoms. Three main classes of AAS have been described by Clark and Henderson (2003). The first class of AAS, called *testosterone esters*, includes testosterone propionate and testosterone cypionate. These compounds are injectible esterifications of testosterone. The esterification delays degradation and prolongs the action by slowing its release into circulation. These esters hydrolyze into free testosterone and can further be reduced to 5α-dihydrotestosterone or aromatized to estrogens. Molecules that have been 5α-reduced can be metabolized into other androgenic compounds like 3α-androstanediol (3α-diol).

The second class is called *19-nor-testosterone derivates* and embraces, among other, nandrolone decanoate. This class is composed of injectible androgen esters that lack a methyl (CH_3) group at the C_{19} position which lengthens the half-time past that contributed by the

esterification alone. These compounds have reduced androgenic activity compared to dihydrotestosterone. They can also be aromatized to 17β-estradiol but not as efficiently as the testosterone esters.

The third class, *17α-alkyl derivates*, includes compounds that are alkylated at C_{17}, such as oxymetholone and stanozolol. Alkylation diminishes the first passage metabolism in the liver, making these compounds orally active. None of the 17α-alkylated steroids is converted to 5α-dihydrotestosterone or 17β-estradiol, although other active metabolites may be formed.

1.3. THE ANABOLIC ANDROGENIC STEROID, NANDROLONE DECANOATE (DECA-DURABOL®)

Among a vast number of flourishing AAS drugs, the 19-nor-testosterone derivate, nandrolone decanoate (ND), is one of the most commonly abused AAS compound in the world (Eklöf, Thurelius, Garle, Rane, & Sjöqvist, 2003; Perry, Andersen, & Yates, 1990; Verroken, 2001). Thus, ND is the drug used in the experimental animal studies upon which this thesis is based. ND (Deca-Durabol®) is a conjunction of nandrolone and decanoic acid. This structure makes it suitable for intramuscular and subcutaneous injections. After injection, ND is hydrolysed by an esterase to nandrolone (Figure 1). In humans, when administered intramuscularly, ND has a half-life in the muscle of approximately six days. ND is then slowly released from the muscle into the blood where it has a shorter half-life. The duration of the effect is approximately three weeks (FASS, 2002; van der Vies, 1993). The recommended therapeutic dose of ND is 0.4 mg/kg/day (i.m.) in humans (Tamaki et al., 2003). The prescribed dose of ND for uremic anaemia range between 100-200 mg per week, and for osteoporosis between 25-50 mg per three to four weeks (FASS, 2002). The abusers typically administer AAS compounds in suprapharmacological doses that are ten to 100 times the therapeutic dose (Brower, 1993; Clark & Fast, 1996; Fudala, Weinrieb, Calarco, Kampman, & Boardman, 2003). The administration schedule used in this thesis [15 mg/kg/day for 2 weeks] is approximately 40 times the therapeutic dose and was chosen to mimic the self-administered heavy human abuse of AAS (Brower, Blow, Young, & Hill, 1991; Fudala et al., 2003; Williamson & Young, 1992). The treatment schedule used in the present thesis has been shown to affect monoaminergic and opioidergic concentrations in the rat brain (Johansson, Hallberg, Kindlundh, & Nyberg, 1999, 2000; Johansson et al., 1997; Kindlundh et al., 2002; Kindlundh, Lindblom, Bergström, & Nyberg, 2003a; Kindlundh, Lindblom, Bergström, Wikberg, & Nyberg, 2001; Kindlundh, Lindblom, & Nyberg, 2003b; Kindlundh, Rahman, Lindblom, & Nyberg, 2004; Le Greves et al., 1997).

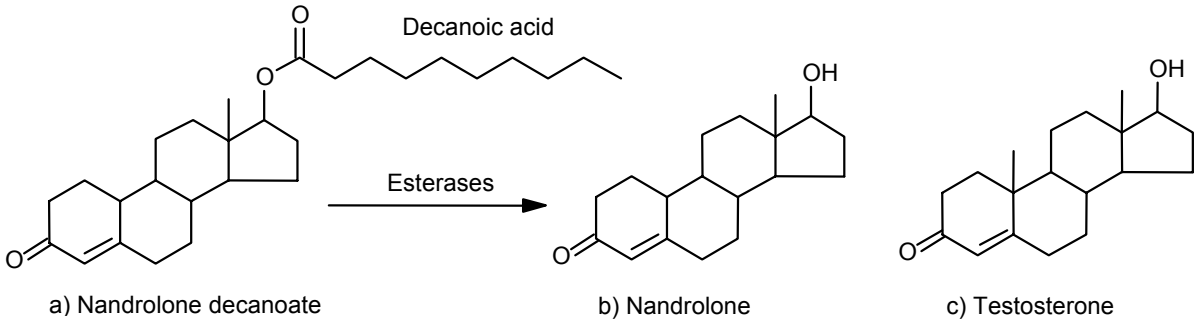

Figure 1. a) Injectible 19-nortestosterone derivate nandrolone decanoate (Deca-Durabol®), and b) converted by estrase to nandrolone. Figure c) shows the structural formula of testosterone. Published with gracious permission of Mathias Hallberg, 2004.

1.4. PREVALENCE OF AAS ABUSE

Russian weightlifters at the 1954 world weight lifting championship were reported to be the first to abuse AAS in order to improve their athletic performance (Strauss, 1987). Not long thereafter, elite American strength athletes began to administer these drugs, and in a short time the use of AAS had spread to endurance sports such as swimming and long-distance running (Yesalis, 1992). In 1972 it was estimated that one third of the Swedish elite track and field athletes abused AAS (Ljungqvist, 1975). In the same year's Olympic Games 68 percent of the participants in the track and field events reported prior steroid abuse (Silvester, 1973). Shortly thereafter, in 1974, the International Olympic Committee (IOC) added AAS compounds to their list of prohibited substances. Nevertheless, in the Winter Games of 1992 when 155 Olympians were asked to estimate the frequency of steroid use in their respective sport, about 40 percent of the respondents estimated that more than ten percent of the participants abused AAS (Pearson, 1990). Statistics published by the IOC show that the percentage of athletes testing positive for AAS has decreased since the mid 1980's (reviewed in Verroken, 2001). Since the introduction of drug control in sport, the frequency of doping tests worldwide has grown to over 100 000 annually, with approximately one to two percent being reported positive by IOC accredited laboratories. The majority of the positive tests showed presence of AAS, although AAS comprise only 15 percent of all drugs banned by the IOC (reviewed in Kicman & Gower, 2003).

Among power sport athletes, the prevalence of AAS abuse is estimated to be higher. Survey studies among male body builders and power lifters report that the lifetime prevalence of AAS abuse ranges between 38 and 55 percent (Blouin & Goldfield, 1995; Curry & Wagman, 1999; Lindström, Nilsson, Katzman, Janzon, & Dymling, 1990; Wichstrøm & Pedersen, 2001; Yesalis et al., 1988). A strong correlation between AAS abuse and practising strength

training was found in a survey study among Swedish high school students (Kindlundh, Isacson, Berglund, & Nyberg, 1999). Surveys indicate that AAS abuse among National Collegiate Athletic Association athletes is approximately between 5 percent and 14 percent (reviewed in Evans, 2004). The highest incidence of reported AAS abuse, among division I athletes in the American National Collegiate Athletic Association, was found among the American football players while track and field athletes use them the least (Yesalis, 1992).

AAS abuse is today not only confined to professional athletes and sporting elites or even to recreational athletes, but also among youths without association to sports. It has been suggested that two thirds of the AAS abusers are non-competitive recreational body builders or non-athelethes, who abuse these drugs for cosmetic purposes rather than to enhance sport performances (Evans, 1997). Recent data imply that AAS abuse has increased over the last decades (Evans, 2004; Yesalis & Bahrke, 1995). The number of AAS abusers in the United States has been estimated to three millions (Evans, 2004). In 1996 the British Crime Survey revealed that steroid abuse was more common among the general British public than was heroin (Ramsay & Spiller, 1997). Recent evidence suggests that AAS were the third most commonly offered drug to children in the United Kingdom, after cannabis and amphetamine (Clark, 1999). Between four and twelve percent of male and up to three percent of female adolescents (12 to 18 years) in the United States report having abused AAS compounds (reviewed in Bahrke, Yesalis, & Brower, 1998; Middleman & DuRant, 1996; Yesalis, Barsukiewicz, Kopstein, & Bahrke, 1997). Prevalence studies performed among high school students in Sweden (16 to 19 yrs) during the late 1990's and early 2000's, showed that approximately three percent of the males and between none to 0.5 percents of the females had abused testosterone or AAS compounds (Kindlundh, Isacson, Berglund, & Nyberg, 1998; Nilsson, Baigi, Marklund, & Fridlund, 2001). The above studies have focused on adolescents (up to 19 years), while survey studies among abusers report that the time of debut is most commonly past 20 years of age (Bahrke, Wright, Strauss, & Catlin, 1992; Copeland, Peters, & Dillon, 2000; Malone, Dimeff, Lombardo, & Sample, 1995; Peters, Copeland, & Dillon, 1999; Silvester, 1995). Thus, it is rather fair to assume that the prevalence figures would be higher if one had asked people in their early 20's. It should be noted that approximately 48 percent of lifetime AAS abusers in US are 25 years of age or younger (Yesalis, Kennedy, Kopstein, & Bahrke, 1993).

1.5. PATTERNS OF AAS ADMINISTRATION

The AAS abusers typically combine, or *stack*, multiple AAS drugs simultaneously, in suprapharmacological doses, *megadoses*, that are ten to 100 times the therapeutic dose

(Brower, 1993; Clark & Fast, 1996). Abusers often administer more than one steroid at a time in order to avoid developing tolerance, *plateauing*, to a particular steroid (Yesalis & Bahrke, 2002). AAS is normally consumed in episodes, or *cycles* of four to twelve weeks (Brower, 1993), although some strength athletes are reported to abuse AAS on a relatively continuous basis with increased doses at certain times, e.g. before competitions (Yesalis, 1992). If the abuser *stacks the pyramid/diamond*, the cycle begins with a few AAS compounds in low dosage followed by an increment of doses and numbers of AAS compounds. The cycle ends by *tapering* which means lowering the dosage and decreasing the number of AAS drugs abused (Yesalis & Bahrke, 2002). Rumour has it that other abusers have a reversed administration pattern; starting with high doses and numbers of compounds followed by lower doses and fewer compounds. After the cycle, an abstention period of four to twelve weeks follows. The reason for having an abstention period is to minimize the side effects and let the body's own hormonal system recuperate, and/or to avoid detection through drug testing (Brower, 1993). Not much is known about the patterns of AAS abuse among individuals not exercising sports.

Builders and athletes often combine their AAS intake with other drugs of abuse or pharmaceutical preparations in order to enhance the desired effects when training. For example, abuse of cocaine has been described by AAS abusers to help prolong time spent training, and to aid when exercising difficult muscles groups (Morrison, 1996). Pharmaceutical preparations, such as e.g. diuretics, gonadotropin, anti-acneiform, anti-inflammatories and oestrogen blockers, are abused to counteract undesirable side effects of the AAS compounds (Brower, 1993; Yesalis, 1992), or to mask AAS metabolites in the urine (Brower, 1993). This polypharmacy is, by the AAS abusers, called *array* (Yesalis, 1992).

1.6. CO-ABUSE OF AAS AND OTHER DRUGS

Whether abuse of AAS constitutes a risk factor for abuse of other drugs in humans, or vice versa, is still unknown. This question is important since several survey studies among teenagers and adults indicate that AAS intake is associated with intake of other drugs of abuse (DuRant, Ashworth, Newman, & Rickert, 1994; DuRant, Escobedo, & Heath, 1995; Korkia & Stimson, 1997; Middleman & DuRant, 1996; Wichstrøm & Pedersen, 2001). For example, survey studies on students in the United States have shown that AAS abuse was associated with abuse of cocaine, injectible drugs, alcohol, marijuana, cigarettes, and smokeless tobacco (DuRant, Rickert, Ashworth, Newman, & Slavens, 1993). Similar results have been found in survey studies on teenagers in Scandinavian countries (Kindlundh, Hagekull, Isacson, & Nyberg, 2001; Kindlundh et al., 1998, 1999; Wichstrøm & Pedersen, 2001). Nilsson and

colleagues (2001) found that 16-17 years old Swedish boys abusing AAS, drank more alcohol more frequently than their AAS non-abusing peers. They also abused home-distilled alcohol and illicit drugs more often than did non-abusers. When comparing AAS abusers and non-abusers recruited from gymnasia, the results showed that AAS abusers also had significantly higher rates of alcohol and illicit substance abuse (Kanayama, Pope, Cohane, & Hudson, 2003; Middleman & DuRant, 1996). Also other types of studies have reported increased intake of alcohol (Conacher & Workman, 1989) and alcohol dependence (Fudala et al., 2003) in AAS abusers. Taken together, clearly there exists an association between abuse of AAS and abuse of other drugs. Nevertheless, it is not yet known whether abuse of other drugs mainly precedes abuse of AAS, or whether AAS intake is a gateway to misuse of other drugs of abuse. While most survey studies report a strong association between abuse of AAS and other illicit substances, some studies suggest that AAS abuse may act as a gateway to abuse of cocaine (Morrison, 1996) and of opioids (Arvary & Pope, 2000; Wines, Gruber, Pope, & Lukas, 1999).

1.7. REWARDING EFFECTS OF AAS COMPOUNDS

Whether AAS compounds possess rewarding potential is not altogether answered in the literature. In animal experimental studies, conditioned place preference paradigm is often used for assessing a drug's positive hedonic effects. The test involves pairing a specific environment (usually coloured compartments) with exposure to a drug. Some studies have failed to show rewarding effects of testosterone by using the test paradigm (Caldarone et al., 1996; Frye, Park, Tanaka, Rosellini, & Svare, 2001). One study observed conditioned place preference in mice only when pairing testosterone with black compartment but not in the white compartment (Arnedo, Salvador, Martinez-Sanchis, & Gonzalez-Bono, 2000). Yet, other studies succeed in showing complete conditioned place preference after administration of testosterone (Alexander, Packard, & Hines, 1994; de Beun, Jansen, Slangen, & Van de Poll, 1992), or after administering the testosterone metabolite 3α-diol (Frye et al., 2001). The results showed that 3α-diol produces positive hedonic effects and that the variable effects of testosterones in the conditioned place preference test might depend on testosterone's metabolism to 3α-diol. Thus, AAS compounds that are easily metabolized to 3α-diol may have higher abuse potential than AAS compounds that are not readily metabolized to 3α-diol (reviewed in Rosellini, Svare, Rhodes, & Frye, 2001).

Studies by Wood and colleagues have shown that testosterone [400 μg/ml] induces oral self administration in male gonad intact hamsters when tested in a 2-bottle choice situation (Johnson & Wood, 2001; Wood, 2002). Results from Wood's studies indicate, however, that

the reinforcing effects of testosterone are not comparable to those of highly addictive drugs like stimulants and opiates, since the preference for the testosterone-containing bottle developed much slower than the preference for other additive drugs. Nonetheless, testosterone has a reinforcing potential. In one study (Johnson & Wood, 2001) cholesterol was not preferred in a two-bottle choice situation, which might indicate that reward is not a general property of all sterols. In another study by the same authors, it was demonstrated that testosterone induces self-administration of testosterone intravenous [50 µg] and intracerebroventricular [50 µg], using an nose-poke operant conditioning chamber in male hamsters (Wood, Johnson, Chu, Schad, & Self, 2004).

The mesolimbic dopamine (DA) pathway, composed of DAergic neurons projecting from the VTA to the nucleus accumbens and to the prefrontal cortex, is an important part of the *brain reward system* (reviewed in Tomkins & Sellers, 2001). It has been demonstrated that AAS compounds induce changes in the DAergic transmission in the mesocorticolimbic system. (Thiblin, Finn, Ross, & Stenfors, 1999). Moreover, Packard et al. (1998) showed that testosterone induced-conditioned place preference is blocked by administering the DA receptor antagonist (α-Flupenthixol) into the nucleus accumbens in the rat brain. Thus, this result suggests that the rewarding properties of testosterone are mediated, at least partially, through interaction with the mesolimbic DA system.

Generally, drugs of abuse increase expression of the immediate-early genes, *c-fos*, especially in the striatum (Harlan & Garcia, 1998). C-Fos is the protein product of the immediate-early gene *c-fos* (Johansson-Steensland, Nyberg, & Chahl, 2002). The basal level of c-Fos expression is usually low or absent (Johansson-Steensland et al., 2002). Induction of immediate-early genes in the striatal projection neurons is a marker for DA receptor response (Steiner & Gerfen, 1998). Acute treatment with an AAS cocktail in rats have shown not to induce *c-fos* (Harlan, Brown, Lynch, D'Souza, & Garcia, 2000), while ND-treated [15 mg/kg/day for 14 days] increased Fos related antigens in guinea pigs (Johansson-Steensland et al., 2002). In addition, chronic treatment with an AAS cocktail blunts the striatal *c-fos* response to morphine (Harlan et al., 2000). ND administration [single dose of 3.75 mg/kg] yielded a denser distribution of c-fos expressing neurons throughout the periventricular regions of the rat brain (Tamaki et al., 2003). A careful interpretation of the *c-fos* results, suggest that AAS may share common mechanisms with other drugs of abuse, and that ND may alter the molecular response to other drugs of abuse. Taken together, although the results from the above-referred studies are not coherent, it would seem as though testosterone and its derivates probably have some reinforcing properties.

1.8. PSYCHIATRIC SIDE EFFECTS OF AAS ABUSE IN HUMANS

There are different types of studies for examining the behavioural effects of AAS abuse in humans, including studies describing behavioural consequences after steroid administration, correlational studies examining the degree of association between AAS abuse and selected behaviours and group comparison studies where groups are selected for the presence or absence of steroid abuse. Furthermore, there is a rather vast amount of case reports in the literature describing different psychiatric symptoms associated with AAS abuse.

Only few randomized controlled studies, using healthy AAS naïve male as subjects, have measured mood changes associated with treatments with supraphysiological testosterone doses. Administration of testosterone enanthate ([600 mg/week for 10 weeks; i.e. ~ 86 mg/ day for 70 days]; Tricker et al., 1996) and of testosterone cypionate ([500 mg/week during 14 weeks; i.e. ~ 71 mg/day for 98 days]; Yates, Perry, MacIndoe, Holman, & Ellingrod, 1999) appears to induce no adverse mood effects in the normal man. Neither administration of 40 mg/day for a week of testosterone produced any measurable mood alterations (Björkqvist, Nygren, Björklund, & Björkqvist, 1994). However, administration of methyltestosterone [240 mg/day] for three days or administration of methyltestosterone [40 mg/day] for three days followed by 240 mg/day for another three days [total of 140 mg/day for 6 days] resulted in mood changes (Su et al., 1993). The results found that the treatment induced positive moods (euphoria, energy, sexual arousal) and negative moods (irritability, mood swings, violent feelings, hostility) but also cognitive impairment (distractibility, forgetfulness, confusion) when compared to the subjects baseline (Su et al., 1993). A study by Daly et al. (2003) observed increased irritability, sexual arousal, energy and distractibility when compared to the subjects' baseline. When rating the effects during six weeks of testosterone cypionate administration [total dose of 2100 mg; 50 mg/day for 42 days] the results showed that mania and ratings of liking the drug were significantly increased after testosterone treatment (Pope, Kouri, & Hudson, 2000). The same treatment regime also yielded increased aggressive responses on the Point Subtraction Aggressive Paradigm (Kouri, Lukas, Pope, & Oliva, 1995; Pope et al., 2000). It has also been found that treatment with testosterone enanthate or ND [100 mg/week or 300 mg/week for 6 weeks; for both drugs] result in increased feelings of hostility, resentment and aggression (Hannan, Friedl, Zold, Kettler, & Plymate, 1991). Limitations concerning these types of studies may be that "normal healthy males" may not be representative of the typical AAS abuser. In addition, subjects with pre-morbid psychiatric disorders and with ongoing substance abuse are carefully excluded from such studies, but it might be that these individuals are more prone to abuse AAS and therefore be the ones that are more susceptible to the psychiatric effects of AAS abuse.

Correlational studies, examining psychiatric and behavioural symptoms in AAS abusers, have found an association or a high incidence (above 50 percent of respondents) of symptoms like; depression (Fudala et al., 2003; Irving, Wall, Neumark-Sztainer, & Story, 2002), anxiety (Fudala et al., 2003), irritability (Bahrke et al., 1992) and hypomania (Malone et al., 1995). Other symptoms that have been observed in AAS abusers are general aggressiveness (Bahrke et al., 1992; Copeland et al., 2000), attempted suicide (Irving et al., 2002), poorer self-esteem (Irving et al., 2002) but also enthusiasm (Bahrke et al., 1992) and increased self-confidence (Olrich & Ewing, 1999). Some of these studies also report a simultaneous abuse of AAS and other drugs. Hence, it is difficult to conclude from these studies whether the reported symptoms derive from the AAS abuse or are results from the co-abuse.

Studies comparing AAS abusers with non-abusers found an increased frequency of symptoms such as verbal aggression (Choi & Pope, 1994; Galligani, Renck, & Hansen, 1996; Yates, Perry, & Murray, 1992), indirect aggression (Galligani et al., 1996; Yates et al., 1992), passive aggression (Cooper, Noakes, Dunne, Lambert, & Rochford, 1996), feelings of hostility (Cooper et al., 1996; Galligani et al., 1996; Yates et al., 1992), feelings of irritability (Galligani et al., 1996), violent acts or assaults (Choi & Pope, 1994; Yates et al., 1992), and high risk sexual behaviours (Middleman & DuRant, 1996). Studies have further reported increased frequency of suicidal thoughts and depressive symptoms (Malone et al., 1995; Middleman & DuRant, 1996; Pope & Katz, 1994), hypomania or symptoms of mania (Pope & Katz, 1994), anxiety (Perry, Yates, & Andersen, 1990), paranoid symptoms (Cooper et al., 1996; Perry et al., 1990) and narcissistic symptoms (Cooper et al., 1996; Porcerelli & Sandler, 1995). It has also been observed that individuals abusing AAS have less feelings of empathy (Porcerelli & Sandler, 1995), less confidence about their body image (Kanayama et al., 2003) and are considered to suffer from eating disorders (Wichstrøm & Pedersen, 2001). However, a few studies have not revealed any differences between AAS abusers and an AAS-naïve control group on attention (Bond, Choi, & Pope, 1995) or other personality characteristics (Bahrke et al., 1992; Malone et al., 1995).

The literature concerning AAS abuse is awash with case reports about psychiatric symptoms associated with AAS abuse, these effects being; increased anxiousness (Perry & Hughes, 1992), depression (Allnutt & Chaimowitz, 1994; Cowan, 1994; Dalby, 1992; Malone & Dimeff, 1992; H. M. Perry & Hughes, 1992; Pope & Katz, 1987; Rashid, 2000), paranoia and hallucinations (Morton, Gleason, & Yates, 2000; Pope & Katz, 1987, 1990; Stanley & Ward, 1994) and increased irritability (Conacher & Workman, 1989; Dalby, 1992; Pope & Katz, 1990; Schulte, Hall, & Boyer, 1993). Others symptoms that have been reported are perpetrated sexual abuse (Driessen, Muessigbrodt, Dilling, & Driessen, 1996), increased verbal aggression (Conacher & Workman, 1989) and committed homicide and violent acts (Conacher & Workman, 1989; Pope & Katz, 1990; Schulte et al., 1993; Stanley & Ward,

1994).

Whether AAS abuse induces psychiatric symptoms or if AAS abuse is a consequence of psychiatric symptoms or even personality disorders, is a question yet to be answered. Results from some clinical studies suggest that AAS abuse may be a function of personality disorders (Porcerelli & Sandler, 1995; Yates et al., 1992), while other studies suggest that AAS abuse rather paves the way for different types of psychiatric symptoms (Cooper et al., 1996; Dalby, 1992; Galligani et al., 1996; H. M. Perry & Hughes, 1992; Perry et al., 2003; Pope & Katz, 1990; Stanley & Ward, 1994; Su et al., 1993). Another possibility is that abuse of AAS and psychiatric symptoms by turns reinforce each other in a negative manner.

1.9. BEHAVIOURAL EFFECTS OF AAS ADMINISTRATION IN ANIMALS

Most of the behavioural changes of AAS abuse in humans still derive from case reports and survey studies. It is almost impossible to compare results from these studies because of the highly individual variation of AAS abuse patterns, including type of AAS, dosage, and frequency of administration. Besides, behavioural and psychological alterations reported by, and observed in AAS abusers, may be a direct result of expectancy, imitation and role modelling (Björkqvist et al., 1994). It is further likely that the AAS abusers also abuse other drugs and this co-abuse may act as a confounding factor when investigating the behavioural and psychological effects of AAS abuse. With the intention of overcoming some of these validity considerations, animal are used as subjects in experimental models.

Animal experimental studies that have investigated the acute behavioural effects of different AAS compounds mainly confirm the observed human behaviours. For example, in human case studies, AAS abuse has been linked to *roid rage* which has been conceptualized as indiscriminate, unprovoked aggression and violence (Pope et al., 2000). However, by using a rat model, Breuer et al. (2001) observed that AAS compounds did not eliminate the ability to discriminate between social and environmental cues, as would have been expected if AAS induce roid rage as manifested by indiscriminate and unprovoked aggression. Instead, it has been suggested that AAS may lower animals' threshold to respond to provocation with aggression (Breuer et al., 2001). Thus, roid rage could be re-defined to represent AAS-induced exaggerated responses to provocative stimuli. For example, McGinnis and colleagues (2002) found that testosterone propionate-treated male rats produced a heightened state of arousal or sensitivity to external stimuli that resulted in increased aggression. In fact, the authors also found that a mildly stressful stimulus (tail pinch) administered to one control

animal, elicited aggression in the testosterone propionate-treated cage mate (McGinnis, Lumia, Breuer et al., 2002).

Apart from aggressive responses to physical provocations, there are other characterizations of *aggression*. In most mammals, agonistic or fighting behaviours often determine access to resources through an intermediate step, which is establishment of dominant hierarchies in group living mammals. Aggression often arises over resources that are; important for survival and reproduction, in limited supply and substantial enough to justify the energy costs necessary to defend them (Blanchard & Blanchard, 2003). Offensive aggression is defined as obtaining/maintaining power, influence or valued prerogatives over a conspecific (Blanchard, Wall, & Blanchard, 2003). One often used test for examining offensive aggression is the resident-intruder paradigm, in which a rodent defends its home area against unfamiliar intruding conspecifics (Koolhaas & Bohus, 1991). Displays of offensive aggression, observed in rats tested in resident-intruder models, are also increased after treatment with testosterone propionate (Breuer et al., 2001; Lumia, Thorner, & McGinnis, 1994), ND (Long, Wilson, Sufka, & Davis, 1996) or different AAS cocktails (Grimes, Ricci, & Melloni, 2003; Melloni, Connor, Hang, Harrison, & Ferris, 1997; Melloni & Ferris, 1996). Success in a provocative and competitive situation, like the resident-intruder model, enhances the probability of establishing a dominant position. For instance, it has been observed that testosterone propionate-treated male rats manifest increased, or even induced dominance in competitive tasks (Bonson, Johnson, Fiorella, Rabin, & Winter, 1994; Bonson & Winter, 1992; Lumia et al., 1994). Also studies on non-human primates (cynomolgus monkeys) have shown that administration of AAS cocktails affects dominance and aggressive behaviours (Rejeski, Brubaker, Herb, Kaplan, & Koritnik, 1988; Rejeski, Gregg, Kaplan, & Manuck, 1990). These studies further report that subordinates display increased submission after treatment with the AAS cocktail. Contrary, 17α-alkyl derivate stanozolol seems, however, to suppress aggression (Breuer et al., 2001; Clark & Barber, 1994; Lumia et al., 1994; Martinez-Sanchis, Brain, Salvador, & Simon, 1996). Other studies have also reported minor or no effects on aggression after administration of other AAS compounds than stanozolol (Breuer et al., 2001; Bronson, 1996).

AAS compounds are thought to possess *anxiolytic* effects. Studies have reported that testosterone propionate (Aikey, Nyby, Anmuth, & James, 2002; Bitran, Kellogg, & Hilvers, 1993; Frye & Seliga, 2001) and its metabolites, androsterone, dihydrotestosterone and 3α-androstanediol (Aikey et al., 2002), have anxiolytic effects in rats measured as an increased exploration of the open arms of the elevated plus maze. Metenolon has also proved to posses anxiolytic effects as measured in a open-field test (Ågren, Thiblin, Tirassa, Lundeberg, & Stenfors, 1999). Testosterone-treated rats, tested in the Vogel's conflict test, displayed increased acceptance for electric shocks, which is considered an indirect reflection of a drug's

anxiolytic effects (Bing et al., 1998; Svensson, Åkesson, Engel, & Söderpalm, 2003). In contrast to the reports about testosterone and AAS anxiolytic effects, Minkin and colleagues (1993) found that rats treated with ND increased their peripheral activity during a locomotor activity test, suggesting an increased state of anxiety in the treated rat.

Previous animal studies examining the effect of AAS on spontaneous *locomotor activity* have not been consistent. ND has in some studies been found to have no effect on locomotor activity (Minkin et al., 1993; Salvador, Moya-Albiol, Martinez-Sanchis, & Simon, 1999). Locomotion activity has further been proved unaffected after administration of other AAS compounds like testosterone propionate (Aikey et al., 2002; Bitran et al., 1993; Clark & Barber, 1994; Clark & Harrold, 1997; Salvador et al., 1999), testosterone (Bing et al., 1998; Martinez-Sanchis, Aragon, & Salvador, 2002), stanozolol (Clark & Barber, 1994; Clark & Harrold, 1997; Martinez-Sanchis et al., 1996) or an AAS cocktail (Bronson, 1996; Salvador et al., 1999). On the other hand, van Zyl and colleagues (1995) found that running endurance in trained nandrolone phenylpropionate-treated rats were markedly increased compared to trained rats receiving saline. Another study has found a positive correlation between self-administered testosterone and voluntary exercise (Wood, 2002). The spontaneous locomotor activity was examined by using several different test methods such as activity boxes, wheel running, treadmill running etc.

Concerning the *sexual behavioural effects* of AAS administration, it is indicated by the literature that a low dosage of AAS generally has no effect. On the other hand, high dosage administered during an extended time periods may induce both increased and decreased frequency of sexual behaviours in gonadally intact male rats (reviewed in Clark & Henderson, 2003).

In summary, these studies indicate that AAS interfere with various behaviours, although the results are not always coherent. The divergent results may depend on different treatment regimes and test methods, but also on different animal species and strains used in the different studies.

1.10. PHYSICAL SIDE EFFECTS OF AAS ABUSE

Case reports and clinical studies have reported that human AAS administration may cause several kinds of physical side effects (reviewed in Creutzberg & Schols, 1999; Kicman & Gower, 2003; Parssinen, Kujala, Vartiainen, Sarna, & Seppala, 2000). AAS abuse may lead to hypercholesterolemia (Cable & Todd, 1996), platelet aggregation (Laroche, 1990;

Rosenblum, el-Sabban, Nelson, & Allison, 1987) and increased blood pressure (Grace, Sculthorpe, Baker, & Davies, 2003), all of which constitute risk factors for heart diseases. Cases of myocardial infarction (Ferenchick, 1990; Halvorsen, Thorsby, & Haug, 2004; Mewis, Spyridopoulos, Kuhlkamp, & Seipel, 1996) and pulmonary embolisation (Dickerman, McConathy, Schaller, & Zachariah, 1996) have been reported among young AAS abusers. Furthermore, it has been shown that strength athletes display a left ventricular hypertrophy several years after discontinuation of AAS abuse, in comparison with AAS-naïve strength athletes (Urhausen, Albers, & Kindermann, 2004).

AAS abuse may also result in decreased production of testosterone by the testes and reduced production of sperm (Alen, Rahkila, Reinila, & Vihko, 1987). Furthermore, AAS abuse can cause gynecomastia (Korkia & Stimson, 1997) and atrophy of the testes leading to gonadal dysfunction (Korkia & Stimson, 1997; Palacios, McClure, Campfield, & Swerdloff, 1981). Frequently appearing external signs of AAS abuse are severe acne and striae principally located over biceps- and pectoral muscles (Scott, Scott, & Scott, 1994). Common side effects in women are deepening of the voice, menstrual irregularities, clitoral enlargement, and growth of body hair (Korkia, Lenehan, & McVeigh, 1996; Strauss, Liggett, & Lanese, 1985). When adolescents abuse AAS compounds, the epiphysis can close prematurely and thereby halt bone growth (Blue & Lombardo, 1999).

Steroids exist in both injectible (testosterone esters and 19-nor-testosterone derivates) and oral (17α-alkyl derivates) preparations. With oral preparations, the liver receives a higher concentration of the AAS drug due to the portal vein system. Consequently, oral preparations are thought to be associated with a higher degree of hepatocellular carcinomas (Haupt & Rovere, 1984), peliosis hepatitis (Cabasso, 1994) and cholestasis (Yoshida, Karim, Shaikh, Soos, & Erb, 1994). On the other hand, injectible steroids can put abusers at risk for contracting HIV and viral hepatitis through sharing needles and syringes (Aitken, Delalande, & Stanton, 2002; Midgley et al., 2000; Rich, Dickinson, Feller, Pugatch, & Mylonakis, 1999). Among AAS abusers injecting AAS compounds, 25 percent are reported to share needles (DuRant et al., 1993). There is one study that has observed an increased premature mortality among power lifters suspected to have abused AAS compounds (Parssinen et al., 2000). This suspicion has been supported by a study in which administration of an AAS cocktail in rodents dramatically shortens their life span (Bronson & Matherne, 1997).

1.11. LONG-TERM BEHAVIOURAL EFFECTS OF AAS ABUSE

Whether AAS abuse induces long-term behavioural alterations in humans is little

investigated, since it is difficult to conduct well-controlled studies due to the highly individual variation of AAS abuse patterns (e.g. type of AAS compound, dosage and frequency of administration). In spite of these difficulties, some studies have examined the potential long-term behavioural or psychological effects caused by earlier abuse of AAS. Galligani et al. (1996) found an enhanced verbal aggression in adult men that had been abstinent from AAS for at least six months. Other studies have shown minor or no alterations in aggressive behaviours in past abusers which had been abstinent for a year (Malone et al., 1995; Yates et al., 1992) or for a longer time period than a year (Silvester, 1995). However, one study has found that past (abstinent period not defined) AAS abusers had significantly more psychiatric diagnoses, as diagnosed by DSM-IV, than current abusers (Malone et al., 1995).

Concerning animal studies, long-term effects of AAS on aggressive behaviours have also been poorly investigated. To our knowledge, only one study has investigated this specific relationship in rats. In that study, McGinnis and colleagues (2002) measured aggression three and twelve weeks after the end of treatment with testosterone propionate, ND or stanozolol. They found that testosterone propionate induced an increased aggression at the test occasion three weeks after the end of treatment, but none of the AAS compounds yielded alterations in aggression twelve weeks after the end of the end of the treatment period. In conclusion, more knowledge concerning long-term behavioural effects of AAS is needed since AAS abuse has become an increased health and societal problem.

1.12. AAS AND THE CENTRAL NERVOUS SYSTEM

The alterations in neurobiochemical systems are in this thesis of interest in order to understand the underlying mechanisms behind the observed AAS prompted behaviours. Results from animal experimental studies demonstrate that AAS affect several neurotransmitter systems, of which the GABAergic, serotonergic, dopaminergic and opioidergic are discussed in this thesis.

The *γ-aminobutyric acid* type A ($GABA_A$) receptor is a ligand-gated chloride ion channel, which is the primary mechanism for fast inhibition of neural activity. The $GABA_A$ receptor is a target site for various drugs, like benzodiazepines, barbiturates, neurosteroids, anticonvulsants and ethanol (Mehta & Ticku, 1999). These drugs act on the $GABA_A$ receptor through allosteric modulation that makes the receptor more sensitive to GABA by adjusting the influx of chloride ions (Sieghart, 1995). Bitran and colleagues (1993, 1996) suggested that also the AAS compound, testosterone propionate, possessed the ability to induce allosteric modulation of the $GABA_A$ receptor. This effect was hypothesized to be mediated by the

conversion of the AAS compound to neurosteroids that act on the $GABA_A$ receptor. It was later demonstrated that 17α-methyltestosterone also directly, i.e. not via conversion to neurosteroids, allosterically modulates the $GABA_A$ (Jorge-Rivera, McIntyre, & Henderson, 2000). It has further been demonstrated that stanozolol and 17α-methyltestosterone, can modulate the $GABA_A$ receptor by blocking the binding of the benzodiazepine compound, flunitrazepam (Masonis & McCarthy, 1995, 1996). The $GABA_A$ receptor is a pentamer composed of three different subunits (alpha, beta, gamma) where the subunits exist in several subtypes. Yang and colleagues (2002) have shown that 17α-methyltestosterone alters the $GABA_A$ receptors depending on their subunit compositions. Since different subunits exist in different brain regions, 17α-methyltestosterone has various effects in different brain regions (Yang et al., 2002). Furthermore, 17α-methyltestosterone also affect the $GABA_A$ receptors by decreasing the level of alpha and gamma subunit mRNAs (McIntyre, Porter, & Henderson, 2002). The mechanism behind this decrease is not yet understood, but McIntyre et al. (2002) suggest that, since the $GABA_A$ receptor subunit gene expression is regulated by 17β-estradiol and testosterone, 17α-methyltestosterone can mimic these steroids by direct action at the nuclear hormone receptors.

The *serotonergic* (5-hydroxytryptamine, 5-HT) pathways originate in the midbrain, the raphe nuclei, and innervate both the substantia nigra (Moukhles et al., 1997) and the ventral tegmental area (Herve, Pickel, Joh, & Beaudet, 1987), as well as the striatum and the nucleus accumbens (Azmitia & Segal, 1978). Serotonergic activity is known to regulate sexual behaviours, aggression, fear, anxiety and reward (Bonasera & Tecott, 2000; Leshner & Koob, 1999). Administration of testosterone propionate decreases the concentration of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the rat hippocampus (Bonson et al., 1994). Also Grimes and Melloni (2002) have reported similar results, demonstrating that pre-adolescent male hamsters treated with an AAS cocktail reduced the serotonergic activity in the hypothalamus and the forebrain. In contrast to these studies, Thiblin et al. (1999) reports that administration of testosterone propionate, nandrolone propionate, methandrostenolone or oxymetholone increased the 5-HT metabolism (i.e. 5-HIAA/5-HT ratio) in the hippocampus. The same authors further demonstrated that methandrostenolone administration increased 5-HT metabolism in the hypothalamus and that treatment with oxymetholone and testosterone propionate increased 5-HT metabolism in the frontal cortex (Thiblin et al., 1999). Similar results are reported by Tamaki et al. (2003) who observed a significant increased 5-HIAA in the hypothalamus and a clear trend to an increased 5-HT concentration in cerebral cortex and hypothalamus after ND administration. Concerning the 5-HT receptors, Bonson et al. (1994) found that administration of a $5\text{-}HT_{1A}$ receptor agonist reduced the observed testosterone propionate-induced aggression. The $5\text{-}HT_{1A}$ receptor is known to be involved in regulating anxiety related behaviours (Ramboz et al., 1998) and depressive related behaviours (Parks, Robinson, Sibille, Shenk, & Toth, 1998) in rats. After administration of ND, using the same

treatment regime as used in the present thesis, a down-regulation of the 5-HT_{1B} receptor density was observed in the hippocampus and the medial globus pallidus in rats and a up-regulation of the 5-HT_2 receptor density in the rat nucleus accumbens and the amygdala/hippocampus (Kindlundh, Lindblom, Bergström et al., 2003a). The 5-HT_{1B} receptor density is thought to regulate aggressive behaviours (reviewed in Simon, Cologer-Clifford, Lu, McKenna, & Hu, 1998) and exploratory behaviours (Malleret, Hen, Guillou, Segu, & Buhot, 1999). There are inconclusive results concerning the association between 5-HT_{1B} receptors and self-administration of drugs of abuse (reviewed in Bonasera & Tecott, 2000). Since a 5-HT_2 receptor antagonist have been shown to have positive effects in the treatment of stress, anxiety and psychosis (Feldman, Newman, Gur, & Weidenfeld, 1998; Rosenberg, Rosse, Schwartz, & Deutsch, 2000). Kindlundh et al (2003a) propose that the observed up-regulation of 5-HT_2 receptor density after ND administration may reflect compensatory mechanisms that would relieve these symptoms.

The midbrain and the forebrain have long been hypothesized to be involved in the brain reward system. The components of this system includes the VTA where the mesocorticolimbic *dopamine* (DA) pathway originates and projects into parts of the basal forebrain that includes nucleus accumbens, olfactory tubercle, amygdala and frontal cortex (Koob, 1999). Thiblin and colleagues (1999) demonstrated that administration of different AAS compounds (testosterone propionate, nandrolone propionate, methandrostenolone, oxymetholone) induced increased concentrations of the DA metabolites 3,4-dihydroxyphenylacetic (DOPAC) and homovanillic acid (HVA) in the striatum, and that oxymetholone increased DA concentrations in the same area. The authors suggest that these findings might reflect a stimulatory influence in the mesolimbic DA system by AAS compounds. Kindlundh and colleagues (2001, 2003b) demonstrated that ND reduced amount of D_1-like receptors in the caudatum/putamen and in the core and the shell of the nucleus accumbens. Data concerning D_2-like receptors indicate an increase in the caudatum/putamen, but inconclusive results for the nucleus accumbens and the ventral tegmental area (VTA) (Kindlundh, Lindblom et al., 2001; Kindlundh, Lindblom, & Nyberg, 2003b). Psychostimulants, like *d*-amphetamine and cocaine, elevate extracellular DA concentrations by inhibiting the reuptake by the DA transporters (Giros, Jaber, Jones, Wightman, & Caron, 1996). An up-regulation of the DA transporters in the caudatum/putamen has been observed after administration of ND (Kindlundh et al., 2002; Kindlundh et al., 2004) suggesting to reflect a response to an enhanced DA activity (Jaber, Jones, Giros, & Caron, 1997).

There exist three classical *endogenous opioid* families, namely the enkephalins, endorphins and dynorphins. Their respective precursors and typical peptides are proenkephalin and Met-enkephalin-arg^6-phe^7 (MEAP); proopiomelanocortin (POMC) and β-endorphin; prodynorphin and dynorphin B. The opioid peptides bind to the μ-, δ- and κ-opioid receptors (Tordjman et

al., 2003). The mesolimbic DA neurons in the brain reward system, projecting from the VTA to nucleus accumbens, are under the control of opposing endogenous opioid systems. Inhibition of DA neurons is mediated by dynorphin, acting through the κ-opioid receptor. DA neurons are under tonic inhibition of GABA interneurons, but enkephalins and endorphins can stimulate these DA neurons by binding to the inhibitory μ- and δ-opioid receptors on the GABA interneurons (reviewed in Spanagel, Herz, & Shippenberg, 1992). Menard et al. (1995) reports that administration of an AAS cocktail decreases the density of ir-β-endorphin neurons in the rostral part of the arcuate nucleus in hypothalamus. It has also been demonstrated that the expression of POMC mRNA, the prohormone for β-endorphin, was decreased in the hypothalamus (Lindblom, Kindlundh, Nyberg, Bergström, & Wikberg, 2003a). An in vitro experiment by Pasquariello et al. (2000) showed that when exposing a hypothalamic cell line to ND, reduced levels of δ-opioid receptor mRNA and δ-opioid binding sites were observed. Furthermore, midline thalamic nuclei is an area that receives input from hypothalamic β-endorphin neurons and also projects glutaminergic neurons to striatum (Harlan et al., 2000). Harlan and colleagues (2000) showed that ir-β-endorphin was increased in midline thalamic nuclei after administration of an AAS cocktail, thus suggesting a mechanism for AAS to affect the reward system by modulating these thalamic nuclei. In the first report by Johansson et al. (1997), the μ- and δ-receptor agonist MEAP and the κ-receptor agonist β-endorphin were analysed in amygdala, hippocampus, hypothalamus, nucleus accumbens, pituitary and VTA. All brain regions were unaffected except for a 20-fold increase of the β-endorphin level in VTA. In a subsequent study, the same group reports that both MEAP and dynorphin B levels were increased in hypothalamus, striatum and PAG (Johansson et al., 2000). The variability in the effects of AAS compounds on opioid peptides, might be a result of problem detecting significant alterations within a subpopulation of neurons in given brain regions (Clark & Henderson, 2003).

2. AIM OF THE THESIS

The main objective of this animal experimental thesis was to examine to what extent, the anabolic androgenic steroid (AAS) nandrolone decanoate (ND; Deca-Durabol® [15 mg/kg/day for 2 weeks]) induces behavioural and physiological changes in sexually mature male rats.

Clinical studies and case reports have observed an association between AAS abuse and aggressive behaviours. Animal experimental studies have, to some extent, confirmed the reported human behaviours. One aim of this thesis was to explore if ND stimulates establishment of dominant relationships in a provocative and competitive situation, enhances reactivity to physical provocations and alters anxiety related behaviours. These behaviours were assessed by using a competitive test (papers II, IV), a reactivity test (paper I), a locomotor activity test (paper II) and a flight and freeze test (paper I).

Several survey studies have reported a concurrent abuse of AAS and alcohol as well as other drugs of abuse. However, if abuse of AAS may constitute a risk factor for abuse of other drugs in humans is still unknown. A further aim of this thesis was to investigate whether ND stimulates ethanol intake, and if it alters behavioural tolerance to ethanol. Animals were tested by employing a voluntary ethanol consumption model (papers I, III), and an ethanol-induced locomotor activity test (paper II).

In humans, AAS are often abused by people involved in a variety of sports with the intention of improving physical performance. Little is yet known about how this combination (AAS abuse and physical exercise) affect behaviours. Hence, another aim of this thesis was to study if ND-induced reactivity to physical provocations and voluntary ethanol consumption were altered when combining ND treatment with physical activity (paper III). Physical activity was provided for by using a wheel-running model.

A final aim of the present thesis was to study the effects of ND on the concentrations of brain monoaminergic and opioidergic neurotransmitters, since these systems are suggested to be involved in modulating certain behaviours, and mediating the brain reward mechanisms. The analyses of the concentrations of peptides and monoamines were employed by radioimmunoassay (RIA; paper I) and high-pressure liquid chromatography-electrochemical detection (HPLC-ED) techniques (paper II), respectively.

3. METHODOLOGY

3.1. SUBJECTS

Male Wistar rats purchased from Möllegard Breeding Laboratories (Denmark) served as subjects in all experiments. At the beginning of the experiments, the rats were 80-90 days of age, sexually matured and weighing between 250 and 300 g (Öbrink & Waller, 1996). The animals were housed in an air-conditioned colony room on a reversed 12:12 light dark cycle (lights-off 10:00 a.m. - 10:00 p.m.) at a temperature of 23°C, and a humidity of 50-60 %. They were housed in groups of four rats (paper IV) or five rats (papers I - III) per cage (clear plastic cages; 59 x 38 x 20 cm; Macrolon® IV). The animals were given two weeks to adapt to the new laboratory conditions before the experiments started. According to the routines of the laboratory (Experimental Bio Medicine, Göteborg University), the rats had *ad libitum* access to water and food pellets (Labfor, Lactamin, Vadstena Sweden; R34: paper I, R70: papers II-IV). These conditions were the same throughout the experiments, unless otherwise stated. All experiments presented in this thesis were approved by the local ethical committee of the Swedish National Board for Laboratory Animals (Göteborg ethical committee for laboratory animals; Göteborg administrative court of appeal).

3.2. NANDROLONE DECANOATE

Animals were, in all four studies presented in this thesis, randomly divided into experimental and control groups. The experimental group received daily (10.00 – 11.00 am) subcutaneous (s.c.) injections of the anabolic androgenic steroid nandrolone decanoate (ND; Deca-Durabol®, Organon, Oss, Netherlands [50 mg/ml]) of 15 mg/kg for 14 days. During the corresponding period, the controls were given daily injections of the oil vehicle arachidis oleum (1 ml/kg s.c; Apoteket AB, Sweden).

3.3. BEHAVIOURAL MEASUREMENTS

All behavioural tests were conducted during the dark phase of the light-dark cycle.

Dominance in a provocative and competitive situation

Dominance status was assessed one week (paper II: n = 24) and five, eight, eleven and 14 weeks (paper IV: n = 20) after the end of the two-week ND/oil treatment period. For the test, (modified from Albert, Dyson, & Walsh, 1987), two water deprived rats competed for access to water. For two (paper IV) or three (paper II) days animals were water restricted, except for one hour daily (11.00 - 12.00 am) when they had unlimited access to water. Throughout the restriction period the rats were either housed singly (paper IV), or placed together with its randomly chosen counterpart (paper II). On the third day (paper IV) or on the fourth day (paper II) of water restriction, the rats of the randomly chosen pairs (one ND-treated rat and one control) had to compete for waterspout access. The spout had a suspended cone at the point where the waterspout entered the cage, which gave only one rat the opportunity to drink at a given time. In paper IV, on each of the four repeated test occasions, the ND-treated rat was paired with a new unfamiliar control counterpart. Behaviours, listed in Table 1, were recorded during the first four-minute period of the competitive test by two observers. It was recorded which of the two animals that started to drink and the total drinking time [s] for each of the two rats. After the four-minute competition period, the animals had an additional hour of free access to water but without the suspended cone. Following the competition day, animals were placed in individual cages and kept on water deprivation for an additional day in order to assess the four-minute drinking baseline for each of the rats. The cages used, throughout the whole procedure of the dominance test, were clear plastic cages (42 x 26 x 15 cm; Macrolon® III). The cages used for the test situation and the cage used for baseline drinking, represented a neutral environment for the animals.

Table 1.
Description of the behaviours recorded during the competition test.

Behaviours	**Description**
Pushes	Quick simultaneous hitting movements with the forepaws toward the counterpart occurring in the vicinity of the water spout
Lateral attacks	Lateral move towards the counterpart occurring anywhere in the cage
Piloerection	Erection of the fur occurring anywhere in the cage
Paw strikes	Quick simultaneous hitting movements with the forepaws occurring anywhere in the cage

Reactivity toward physical provocations

Reactivity toward physical provocations was assessed immediately (paper I: n = 40), one week (paper III: n = 40) and six to eight weeks (paper I: n = 40) after the end of the ND/oil treatment period. For the reactivity test, the rat was placed in a Plexiglas® cage (60 x 31 x 41

cm), and allowed to habituate for 30 seconds. The test cage represented a neutral environment to the rats. The rat's reactions to four different physical stimuli were then assessed (modified from Lee, Yamamoto, & Ueki, 1983). For description of the stimuli and scorings, see Table 2.

Table 2.
Description of the four different stimuli that constitute the reactivity test, and scoring points for each type of response.

Stimuli		**Scoring (points)**	
I:	A wooden rod was slowly moved to approach and touch the rat's snoot	0:	No response or sniffs at the rod
		1:	Intermittently bites or attacks the rod and/or adopts an upright posture
		2:	Continuously bites/attacks the rod
II:	Startle to an air puff (air blown from a 50 ml syringe) at the back	0:	No response or some movement
		1:	Jumping response
		2:	Exaggerated jumping response
III:	Poking with wooden rod at the flanks	0:	No response or sniffing at the rod
		1:	Upright posture
		2:	Upright posture together with biting/attack
IV:	Capturing with a gloved hand	0:	Very easy to capture
		1:	Easy to capture but some resistance and/or prolonged vocalization
		2:	Difficult to capture because of escape
		3:	Difficult to capture because of attacking or biting
		4:	Very difficult to capture because of continuous violent attacks/bites

Fleeing and freezing

Immediately after the end of the ND/oil treatment period, the fleeing and freezing test was performed (paper I: $n = 40$). The rat was placed in a circular Plexiglas® cage (∅ 39 cm) with its floor divided by two lines to form four 90° sectors (Hård, Engel, Larsson, & Musi, 1985). The cage was enclosed in a soundproof test chamber, illuminated by a 15 W white light bulb. The rat was allowed a five-minute adaptation period in the test chamber. During that time, different open-field behavioural items were observed (Table 3). Immediately after the five-minute adaptation period, a doorbell (95 dB) was sounded during six seconds. In response to the sound, the rat would attempt to flee. The number of lines crossed by the animal during the flight attempt was recorded and used as a measure of flight distance, i.e. *fleeing response*. Concurrently with, or slightly before, the termination of the sound, the rat immobilized and adopted a motionless rigid posture, i.e. *freezing response*. The first distinct movement of some part of the rat body (usually the head) excluding eye blinks, respiratory, or vibrissae movements indicated the cessation of the freezing response. The freezing response was measured in seconds. The chamber was cleaned between the tests. The test chamber represented a neutral environment for the animals.

Table 3.
Description of the different open-field behaviours during the five minutes adaptation period in the test chamber before the fleeing and freezing test.

Behaviours	**Measurements**
First crossing	Latency to leave the sector where the animal was first placed
Locomotor activity	Number of lines crossed by the hind legs
Rearing	Number of raisings on hind legs
Grooming	Cumulative time recorded
Defecation	Number of boli deposited

Wheel running activity

In paper III, the rats were individually housed in cages (42 x 26 x 15 cm; Macrolon® III) during the two-week ND/oil treatment period. Half of the ND (n = 10) and half of the controls group (n = 10) had free access to an activity wheel in their home cages (i.e. *ND-wheel* and *control-wheel* groups; n = 10 /group). The wheel (Ø 22.5 cm) was attached to one side of the cage. To turn, the wheel had to be loaded with the weight of the animal. The rats had continuous access to the wheel-running equipment for an additional week after the cessation of the ND/oil treatment period (i.e. a period of three weeks). During the 3-week period of wheel-running, the activity was registered on two occasions (first registration: end of the second week; second registration: end of the last week of the activity period). On each of the two registration occasions, the activity was measured during 48 hours and the activity was recorded as meters per day. Wheel revolutions were automatically registered by a microprocessor and printed out every 30 minutes (detailed description in Shyu, Andersson, & Thorén, 1984). Immediately after the end of the three-week wheel-running activity period, all animals were returned to their original home cages of five rats per cage.

Food intake

In paper III (n = 40) food intake was weighed during the two-week ND/oil treatment period. The intake was registered as g food/kg body weight/day.

Voluntary alcohol intake

One week (paper I: n = 40) and three weeks (paper III: n = 40) after the end of the ND/oil treatment period, the animals' ethanol intake was registered. Animals were given continuous access to a water bottle and a second bottle containing ethanol in gradually increasing concentration (2 – 4 – 6 % v/v) over a two-week period. After the these two weeks, the animals were housed individually in clear plastic cages (42 x 25 x 14 cm; Macrolon® III) for

three consecutive weeks. During this time, animals had continued access to two bottles (plastic 300 ml bottles with ball valve spouts; ALAB, Sweden); one containing tap water and the other a six percent ethanol solution. Fluid consumption was recorded daily at 9.00 a.m. for three consecutive weeks; see Table 4 for recorded measurements. The bottles were cleaned and refilled with fresh beverages twice a week. Throughout the voluntary ethanol intake test, the animals' body weights were recorded weekly. In paper III (n = 40) the rats' voluntary ethanol intake was also registered before the ND/oil treatment period, using the same procedure as described above.

Table 4.
Description of the different measurements for the voluntary ethanol intake test.

Behaviours	**Measurements**
Ethanol intake	Absolute ethanol [g/kg body weight/day]
Ethanol preference	Proportion [%] of ethanol solution intake relative to the total fluid consumption
Water intake	Water [ml/kg body weight/day]
Total intake	Ethanol solution and water [ml/kg body weight/day]

Ethanol-induced locomotor activity

Two weeks after the end of the ND/oil treatment period all rats in paper II (n = 26) were tested for voluntary locomotor activity during an hour. Immediately after this hour, half of the ND-treated animals (n = 7) and half of the controls (n = 6) were randomly chosen to receive intraperitoneal (i.p.) injections of 0.5 g ethanol/kg dissolved in 0.9 % saline solution and injected in a volume of 4 ml/ kg body weight. The remaining ND-treated animals and controls were given a corresponding volume of the saline solution. 15 minutes after the ethanol/saline injections, the animals' locomotor activity were measured for an hour. The test chambers were made of Plexiglas boxes (70 x 70 x 35 cm high; Kungsbacka Mät och Reglerteknik AB).

Table 5.
Description of the different measurements for the locomotor activity test according to Kungsbacka Mät och Reglerteknik AB.

Behaviours	**Measurements**
Locomotion	Successive interruption of the lower grid of infrared beams, i.e. registered the rat's forward movements.
Rearing behaviour	Interruption of the high-level series of infrared beams, i.e. registered every time the rat raised itself onto its haunches.
Peripheral locomotion	Interruption of the lower grid of infrared beams spaced 25 mm from the wall, i.e. registered the animal's activity along the walls.

Each test box was surrounded by two series of invisible infrared photocell beams to measure spontaneous motor behaviours. The two series of infrared beams were situated 14 and four cm from the box floor. The photocells were situated nine cm apart in a grid pattern. See Table 5 for recorded behaviours. The boxes were cleaned between the tests (detailed description in Ericson, Samuelsson, & Ahlenius, 1991). The boxes represented a neutral environment for the rats.

3.4. PHYSIOLOGICAL MEASUREMENTS

Since animals were exposed to voluntary ethanol intake (paper I) or ethanol injections (paper II), an ethanol washout period for a minimum of one week was given before decapitation to minimize the effects on the biological variables. The animals were decapitated away from their cage mates in a room separate from where they were normally housed.

Monoamine concentrations

Four weeks after the end of the two-week ND/oil treatment period, brains were analysed for monoamine concentrations by means of the high-pressure liquid chromatography with electrochemical detection (HPLC-ED) technique (paper II: $n = 26$). The brains were rapidly dissected out and placed on a chilled petri dish. The following parts of the brain were used for analysis: basal forebrain (medial frontal cortex, nucleus accumbens, olfactory tubercle, septum), dorsal striatum (caudate/putamen), hippocampus, amygdala, and hemispheres (remaining cortical tissue). The brains were dissected according to the rat brain atlas of Paxinos and Watson (1986). The tissues were weighed and kept at -80°C until analysed for DA, norepinephrine (NE), 5-HT, HVA, DOPAC, and 5-HIAA concentrations. A detailed description of the HPLC-ED technique can be found in paper II.

Opioid peptide concentrations

Seven weeks after the end of the two-week ND/oil treatment period, radioimmunoassay (RIA) was performed in order to measure brain opioid peptide activity (paper I: $n = 40$). Brains were rapidly dissected and sliced in coronal sections, using a rat brain matrix (Activational System Inc., Mortella Drive Warren, MI, USA). Brain regions (hypothalamus, nucleus accumbens, striatum and periaqueductal grey matter [PAG]) were dissected according to the rat brain atlas of Paxinos and Watson (1986). The tissue parts were put into eppendorf tubes and kept at -80°C until further analysis of the concentrations of opioid peptides dynorphin B and MEAP were assessed. A detailed description of the RIA technique can be found in paper I.

Body weight

The body weight of the animals in all four studies presented in this thesis was recorded regularly, with a minimum of once a week throughout the experiments.

Thymus and adrenal glands

After the decapitation the thymus and adrenal glands were rapidly dissected out, and the wet thymus gland (papers I: n = 40, II: n = 26) and adrenal glands (paper II: n = 26) were weighed. The weight of the thymus was expressed as mg/ 100 g body weight, and the two adrenals as mg/ 100 g body weight. Decapitation occurred seven weeks (paper I) and four weeks (paper II) after the end of the ND/oil treatment period.

3.5. STATISTICAL ANALYSES

In paper I, between-group comparisons (*ND versus oil*) of behaviours and biological measurements were employed by the parametric Student's *t*-test and within-group comparisons were analysed with paired *t*-test. Between- and within-group comparisons of the drinking data were analysed with the non-parametric Mann-Whitney *U*-test and Wilcoxon matched-pairs signed-ranks test, respectively. In paper II, all between-groups comparisons (*ND versus oil*) of behaviours and biological measurements were analysed by help of Mann-Whitney *U*-test. In paper III, overall effects for the four treatment groups (*ND-wheel*, *ND-sedentary*, *control-wheel* and *control-sedentary*) and between-group comparisons were statistically tested by using the Fisher's non-parametric permutation test with Mantels Technique of pooling (Good, 2000; Mantel, 1963). Between- and within-group comparisons of wheel-running activity (*ND-wheel* and *control-wheel*) were employed by the Mann-Whitney *U*-test and the Wilcoxon matched-pair signed-rank test, respectively. In paper IV, Mann-Whitney *U*-test was employed when analysing body weight and individual drinking time (*ND versus oil*). Between-group comparisons of number of lateral attacks and time spent drinking during the four subsequent competition tests were analysed with the Wilcoxon matched-pairs signed rank test. For the nominal data, between-group comparisons were analysed with the Binominal test.

The data are presented as median ± median absolute deviation (MAD; papers I-IV) or as mean ± standard error of the mean (SEM; paper I). Two-tailed levels of significance were used in all statistical analyses.

4. SUMMARY OF RESULTS

4.1. PAPER I

Immediately after the end of the two-week ND/oil treatment, animals were tested for fleeing and freezing responses. During the five-minute adaptation period in the test chamber, open-field behaviours were observed. The ND-treated animals displayed significantly prolonged latency to leave the sector (first crossing) compared to controls ($p < 0.01$). ND-treated rats also showed significantly decreased locomotor activity ($p < 0.001$) and less rearing behaviour ($p < 0.01$) compared to the control group. No group differences were observed in numbers of boli deposited or amount of grooming behaviour. After the adaptation period, the animals were exposed to an auditory stimulus. The flight reaction (number of lines crossed) during the signal was significantly decreased in the ND group compared to the control group (Figure 2, left panels) and the freezing reaction was significantly shortened in the ND group than the control group (Figure 2, right panels).

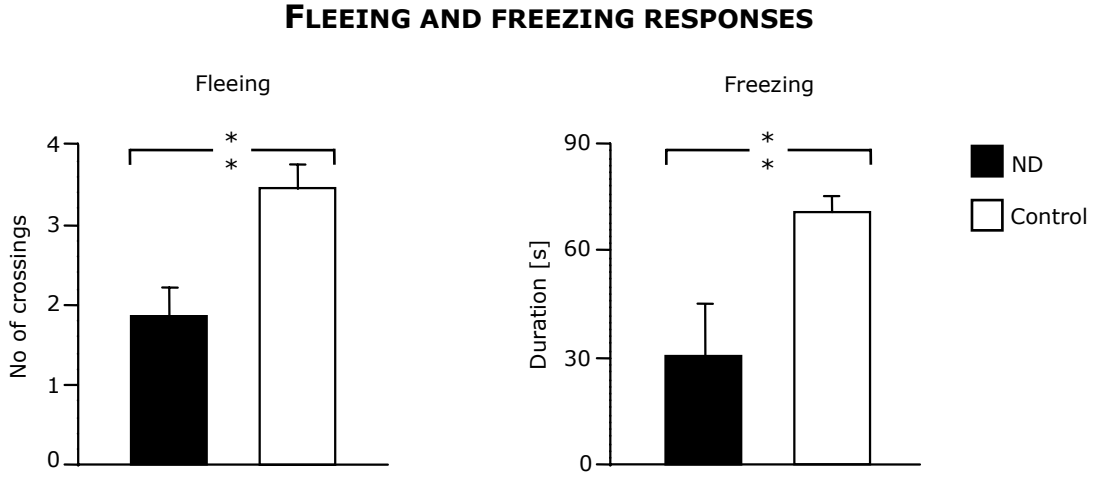

Figure 2. Mean ± SEM of flight reaction (number of lines crossed; left panels) and duration [s] of freezing reaction (right panels) in rats treated with nandrolone decanoate (ND; daily injections of 15 mg/kg for 14 days; $n = 20$) or with oil (controls; $n = 20$). **: $p < 0.001$ (ND *vs*. controls; Student's t -test).

Immediately after the end of the ND/oil treatment period, animals were also tested for reactivity to four different types of innocent physical provocations. The test was reconducted six to eight weeks after the end of the ND/oil period, after the animals had been tested for voluntary ethanol intake. As seen in Table 6, ND-treated animals showed significantly increased reactivity responses toward the *air puff*, *flank prods* and *capturing* at both test

occasions, compared to controls.

Table 6.
Mean ± SEM reactivity in response to an approaching rod, an air puff, flank prods, and capturing with gloved hand in rats treated with nandrolone decanoate (ND; daily injections of 15 mg/kg for 14 days; n = 20) or with oil (control; n = 20). Test I: immediately after the end of the 2-week ND/oil treatment period; Test II: 6-8 weeks after the end of the ND/oil treatment period.

	Test I	**Test II**
Approaching rod		
ND	0.00 ± 0.00	0.00 ± 0.00
Control	0.00 ± 0.00	0.00 ± 0.00
Air puff		
ND	0.30 ± 0.13*	0.20 ± 0.09*
Control	0.00 ± 0.00	0.00 ± 0.00
Flank prods		
ND	0.65 ± 0.11***	0.30 ± 0.10**
Control	0.00 ± 0.00	0.00 ± 0.00
Capturing		
ND	3.20 ± 0.14***	2.85 ± 0.13***
Control	0.35 ± 0.11	0.20 ± 0.09

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ (ND *vs.* control; Student's *t* -test).

Concerning the 3-week period of voluntary ethanol intake, there was no significant difference in *ethanol intake* during the first week of fluid registration between the ND-treated rats and controls (Figure 3). However, ND-treated animals drank significantly more ethanol during the second and the third week compared to the controls. The ND-treated animals significantly increased their *preference* for ethanol the third week of ethanol consumption compared to control animals ($p < 0.05$). No significant alteration in *total fluid* and *water intake* were observed. In comparison to the first week, there was a gradual increase in alcohol consumption within the ND group over the subsequent weeks which was significantly enhanced during the third week (*ethanol intake*: $p < 0.02$; *ethanol preference*: $p < 0.05$; *total fluid intake*: $p < 0.01$). *Water intake* did not change significantly during the corresponding period. There were no within-group differences in fluid intakes over the subsequent weeks for the controls.

There were no differences in body weight between the ND-treated and controls at the start of the experiment. Throughout the experiment period, the ND-treated group did not gain as much weight as the controls. At time for decapitation, the body weights of the ND-treated rats were significantly less than those of the control group ($p < 0.0001$) and a significant reduction in thymus weight was observed in the ND-treated animals compared to the controls ($p < 0.0001$).

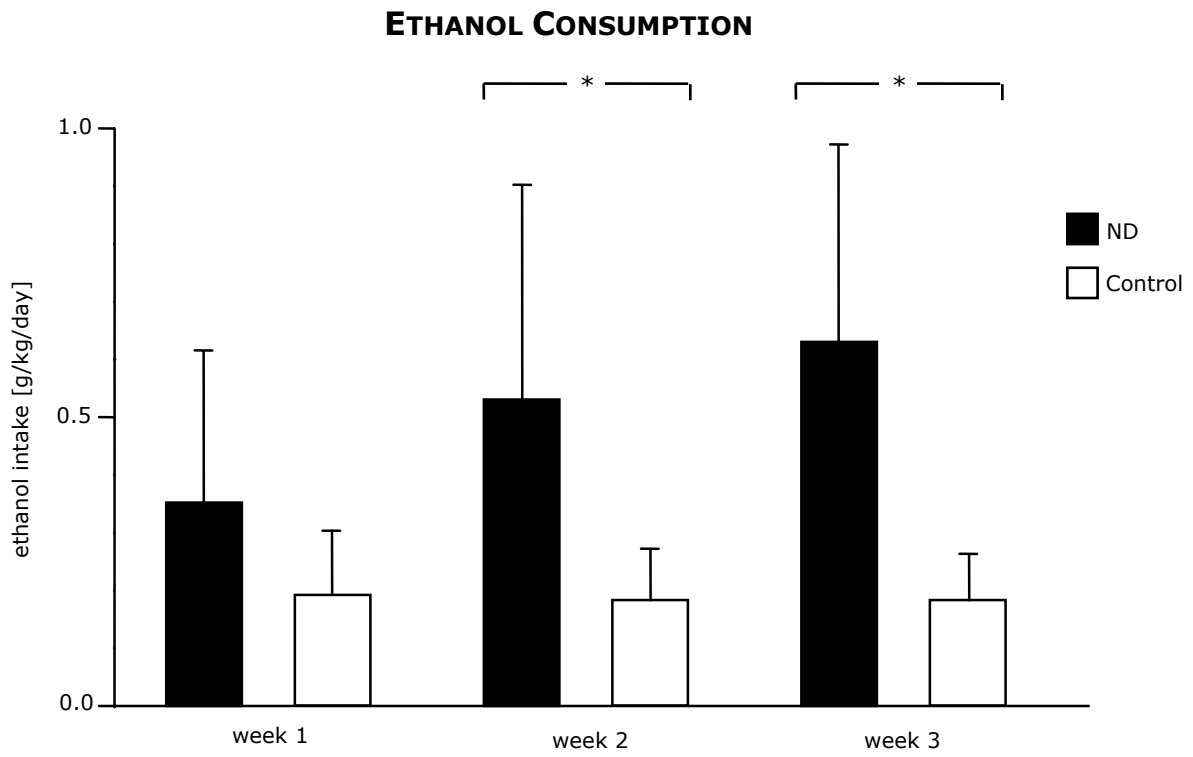

Figure 3. Median ± MAD ethanol intake (expressed as g/ kg per day of absolute ethanol) for 3 weeks in rats treated with nandrolone decanoate (ND; daily injections of 15 mg/kg for 14 days; $n = 20$) or with oil (controls; $n = 20$). *: $p < 0.05$ (ND vs. control; Mann-Whitney *U*- test).

In this paper, concentrations of the opioid peptides ir-dynorphin B and ir-MEAP were examined in various brain regions. The results showed that the ND-treated rats displayed decreased concentrations of ir-dynorphin B in the nucleus accumbens compared to the controls ($p < 0.01$). A decreased concentration of the peptide ir-MEAP was observed in the PAG in the ND-treated animals compared to the controls ($p < 0.05$), whereas ND-treated animals had a higher concentration of ir-MEAP in the hypothalamus compared to the controls ($p < 0.02$).

4.2. PAPER II

In this study, dominance relationships in a provocative and competitive situation were tested one week after the end of the two-week ND/oil treatment period. At the time for the competition test, the ND-treated animals had a significantly lower body weight compared to the controls ($p < 0.01$). As seen in Figure 4 (left panels), the two treatment groups showed no difference in time spent drinking during baseline. When access to water was restricted to only one animal at a time ten ND-treated animals, of the twelve competing pairs, were the ones that first approached the waterspout. The ND-treated animals also maintained access to the waterspout more than two times as long as the controls (Figure 4, right panels). No signs of aggressive rage attacks such as *lunge attacks* and *paw strikes* were observed, but some ND-treated animals showed *piloerection* during the drinking time. This behaviour was never observed among the controls.

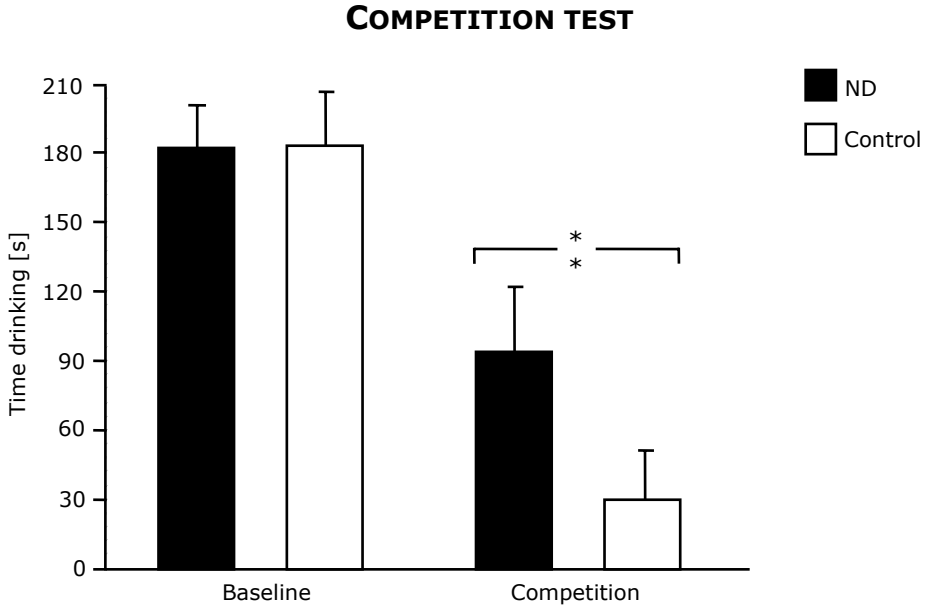

Figure 4. Time spent drinking [s] during baseline (left panels) and competition test (when water drinking was restricted to one animal; right panels). The competitive pairs ($n = 12$) were one rat pre-treated with nandrolone decanoate (ND; daily s.c. injections of 15 mg/kg for 14 days) and one oil-treated control. Values are expressed as median ± MAD. **: $p < 0.01$ (ND *vs.* control; Mann-Whitney *U*-test).

Two weeks after the last ND/oil injection, ND and controls were tested for spontaneous locomotor activity. Immediately thereafter, the animals were tested for locomotor activity in response to ethanol treatment [0.5g/kg; i.p.]. ND-treated and control animals, that received a saline injection, showed similar locomotor activity (Figure 5, left panels). There were no differences in locomotor activity between the ND-treated rats receiving ethanol compared to the ND-treated group receiving saline. Nor were there any differences in locomotor activity

when comparing the ND-treated rats receiving ethanol with ethanol-treated controls (Figure 5, right panels). However, controls receiving ethanol showed a significant reduced locomotor activity compared to the controls injected with saline. Regardless of group or treatments, all animals showed the same activity of rearing behaviour and peripheral locomotion.

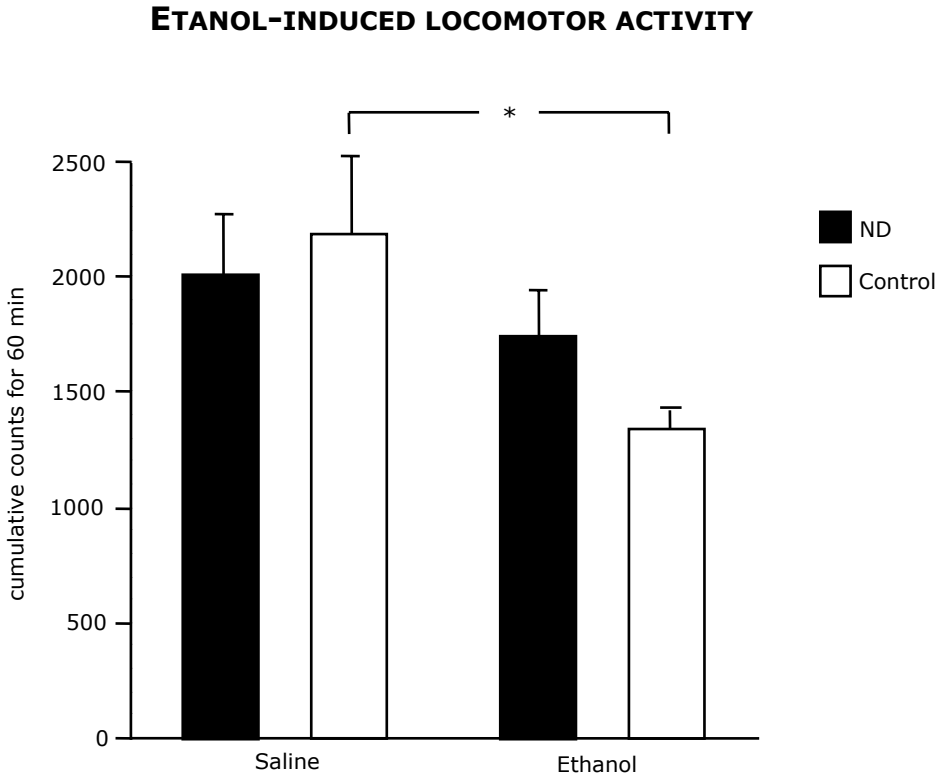

Figure 5. Median ± MAD locomotor activity (cumulative counts for 60 min) in animals treated with nandrolone decanoate (ND; daily s.c. injections of 15 mg/kg for 14 days) or with oil (control). At the time for the locomotor activity test animals received an injection (i.p.) of 0.5 g ethanol/kg or saline 15 min before the test. ND-ethanol: n = 7; ND-saline: $n = 7$; control-ethanol: $n = 6$; control-saline: $n = 6$. *: $p < 0.05$ (control-saline *vs*. control-ethanol; Mann-Whitney *U*-test).

Although there were no differences in body weight between the ND-treated group and controls at the start of the ND/oil treatment period, the ND-treated group did not gain as much weight as the controls during the two-week treatment period ($p < 0.05$). This group difference in body weight had disappeared at the time of decapitation. The ND-treated animals had a significant reduction in thymus weight ($p < 0.001$), as well as a significantly increased adrenal weight ($p < 0.01$), compared to the controls.

Biochemical analyses of the monoaminergic neurotransmitter concentrations showed that ND-treated rats, compared to controls, had a significantly lower concentration of 5-HT in the basal forebrain ($p < 0.05$), and in the dorsal striatum ($p < 0.05$). There was a trend toward a

lowered concentration of 5-HT in the hippocampus and in the amygdala ($p = 0.06$ for both brain areas). The concentrations of NA and DA were not significantly altered in any of the brain regions. Concerning the monoaminergic metabolite concentrations, the ND group had lower concentration of 5-HIAA in the dorsal striatum, compared to controls ($p < 0.05$), whereas the other metabolites, DOPAC and HVA, were not altered.

4.3. PAPER III

In this paper, rats were randomly divided into four groups, two groups were subjected to ND treatment, and the other two groups were treated with the oil vehicle and served as controls. Half of the ND-treated rats and half of the controls had daily access to an activity wheel in their home-cage from the start of the ND/oil treatment period and for three consecutive weeks. When comparing the two groups with access to activity wheels, the ND-treated rats exhibited significantly lower activity at the end of the two-week ND/oil treatment period (first registration) compared to controls (Figure 6). One week later (second registration), there was no difference in wheel running activity between the two groups. There were no within-group differences between the two registration occasions for the ND-treated animals or for the controls.

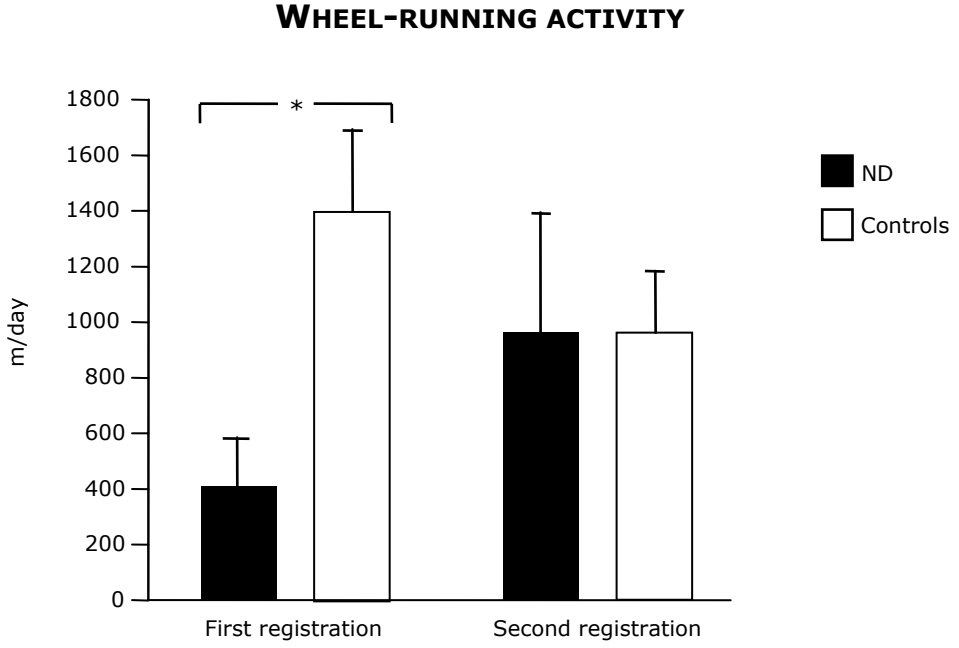

Figure 6. Median ± MAD values for wheel-running activity [m/day]. Animals were treated with either nandrolone decanoate (ND; 15 mg/day for 14 days) or with oil. During the 2-week treatment period and one week thereafter, they had daily access to the wheels in their home cages. The rats' activity was registered at two occasions; first registration: end of the second week of wheel-running and second registration: end of the third week of wheel-running. *$p < 0.05$ (ND *vs.* controls; Mann-Whitney *U*-test).

Overall effects for the four treatment groups (*ND-wheel*, *ND-sedentary*, *control-wheel* and *control-sedentary*) were statistically tested by using the Fisher's non-parametric permutation test with Mantels Technique of pooling (Good, 2000; Mantel, 1963). For the analyses of overall effects, steroid treatment (ND/oil) was entered as the main variable and accessibility

to wheels (wheel/sedentary) was entered as the background variable. Thereafter the variables changed order, i.e. accessibility to wheels (wheel-running/sedentary) was entered as the main variable and steroid treatment (ND/oil) as the background variable. Between-group comparisons of the ND-treated groups (i.e. *ND-wheel* versus *ND-sedentary*) and of the controls (i.e. *control-wheel* versus *control -sedentary*) were also tested by using the Fisher's non-parametric permutation test. Concerning the rats' body weight, there were no overall differences between the four groups at the start of the experiment. Regarding the body weight at the end of the two-week ND/oil treatment period, the result showed a significant overall difference with steroid treatment (ND/oil) entered as the main variable and with accessibility to wheels (wheel-running/sedentary) entered as the background variable ($p < 0.001$); the *ND-wheel* group had the lowest body weight and the *control-sedentary* group had the heaviest body weight. No significant overall effect was observed, when entering accessibility to wheels (wheel-running/sedentary) as the main variable, and with steroid treatment (ND/oil) as the background variable. There was no between-group difference in body weight between the *ND-wheel* and *ND-sedentary* groups, or was there any significant difference between the *control-wheel* and *control-sedentary* groups.

A significant overall effect was found when analysing daily food intake during the two-week ND/oil treatment period when entering accessibility to wheels (wheel-running/sedentary) as the main variable and steroid treatment (ND/oil) as the background variable ($p < 0.05$); the *control-sedentary* group had the highest food intake and the *ND-wheel* group had the lowest food intake. There was no significant overall effect on food-intake when the variables were entered in the reversed order. Between-group comparisons showed a significant difference between the *control-wheel* and *control-sedentary* groups ($p < 0.01$), where *oil-wheel* had a lowered food intake. There was no difference in food intake between the *ND-wheel* and *ND-sedentary* groups.

One week after the end of the ND/oil treatment period (i.e. immediately at the end of the three-week wheel-running/sedentary period) all animals were tested for reactivity towards four different physical provocations. Of these provocations, reactivity to an air puff and the reactivity to captivity by a gloved hand resulted in significant overall effects. For startle response to air blowing, the significant overall effect was found with steroid treatment (ND/oil) as the main variable and accessibility to wheels (wheel-running/sedentary) as the background variable ($p < 0.05$), but also when entering the accessibility to wheels as the main variable and steroid treatment as background variable ($p < 0.05$). Notably, only the *ND-wheel* group showed reactivity to air puff (Table 7). Regarding reactivity to captivity, a significant overall effect was found with steroid treatment (ND/oil) as the main variable and accessibility to wheels (wheel-running/sedentary) as the background variable ($p < 0.01$). The overall effect was also significant when the variables changed order entering the accessibility to wheels as

the main variable and steroid treatment as background variable ($p < 0.05$). The group that scored highest reactivity to captivity was *ND-wheel*. No differences were found between *ND-wheel* and *ND-sedentary* groups, or between *control-wheel* and *control-sedentary* groups (Table 7). Concerning reactivity scores towards the other two physical provocations (response to an approaching rod or to flank prods), none of the four treatment groups exhibited any reactivity (i.e. all rats scored 0).

Table 7.

Median ± MAD and range (within brackets) values for reactivity scores in response to an air puff and to captivity with gloved hand for nandrolone decanoate- (ND) and oil treated animals with or without access to wheels (ND-wheel, ND-sedentary, control-wheel and control-sedentary; n = 10/group).

	ND-wheel	**ND-sedentary**	**control-wheel**	**control-sedentary**
Air puff	0.5 ± 0.5* (0-1)	0.0 ± 0.0 (0-0)	0.0 ± 0.0 (0-0)	0.0 ± 0.0 (0-0)
Captivity	3.0 ± 0.0 (1-3)	1.0 ± 0.5 (0-3)	0.0 ± 0.0 (0-3)	0.0 ± 0.0 (0-1)

A significant overall difference were found with steroid treatment entered as the main variable and accessibility to wheels entered as the background variable for reactivity to air puff ($p < 0.05$) and for reactivity to captivity ($p < 0.01$). The overall difference remained significant for the two reactivity parameters when the variables changed order (i.e. when entering the accessibility to wheels as the main variable and steroid treatments entered as background variable) $p < 0.05$ for both (Fisher's non-parametric permutation test). Concerning reactivity scores to air puff a significant difference was found between the *ND-wheel* and *ND-sedentary* groups, *$p < 0.05$ (Fisher's non-parametric permutation test).

In this study, a two-week period of voluntary ethanol intake was assessed before (baseline), as well as three weeks after the ND/oil treatment period. There were no significant overall effects concerning the baseline fluid consumption. No between-group differences were found concerning the four measured fluid parameters between *ND-wheel* and *ND-sedentary* groups, or between *control-wheel* and *control-sedentary* groups. Three weeks after the end of the ND/oil treatment period (i.e. two weeks after the end of wheel-running/sedentary period), fluid intake was registered for another two-week period. There were significant overall differences with steroid treatment (ND/oil) as the main variable and accessibility to wheels (wheel-running/sedentary) as the background variable for ethanol preference (Figure 7a), ethanol intake (Figure 7b) and water intake ($p < 0.01$). The *ND-wheel* group showed the highest intake of, and preference for, ethanol, whereas the *control-sedentary* group exhibited the lowest ethanol intake and ethanol preference among the four groups. The *control-wheel* group registered the highest water intake, whereas the *ND-wheel* group had the lowest water intake. For total fluid intake there was a trend for an overall effect with steroid treatment (ND/oil) as the main variable and accessibility to wheels (wheel-running/sedentary) as the background variable ($p = 0.052$; highest intake: *control-wheel*, lowest intake: *ND-wheel*). When accessibility to wheels (wheel-running/sedentary) was entered as the main variable and steroid treatment (ND/oil) as the background variable, no overall effect was found in any of

the four measured fluid parameters. Neither were there significant differences found between the *ND-wheel* and *ND-sedentary* groups, or between the *control-wheel* and *control-sedentary* groups.

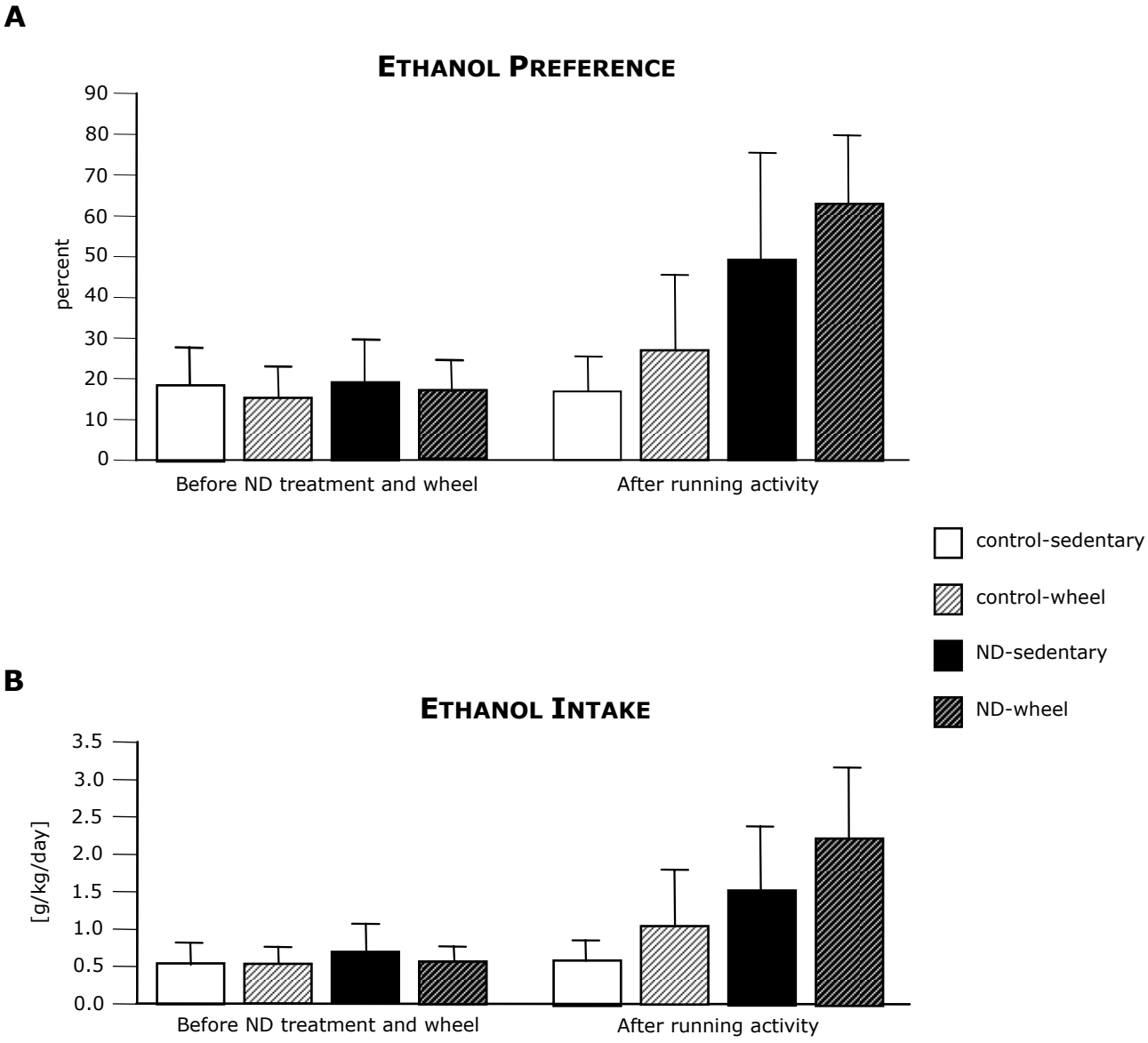

Figure 7. Median ± MAD values for ethanol preference (proportion ethanol [%] of total fluid intake: fig. A) and ethanol intake ([g/kg/day] of absolute ethanol; fig. B). The measurements of fluid intake were performed on two occasions: two weeks before the period of nandrolone decanoate (ND)/oil treatment and accessibility to wheels (baseline), and for another 2-week period three weeks after the end of the treatment period. The animals were divided into four groups: ND-wheel (n = 10), ND-sedentary (n = 10), control-wheel (n = 10) and control-sedentary (n = 10). There was a significant overall difference between the ND-treated rats controls with respect to accessibility to wheels in ethanol intake ($p < 0.02$) and ethanol preference ($p < 0.01$; Fisher's non-parametric permutation test).

4.4. PAPER IV

In this study, dominance in a provocative and competitive situation was assessed five, eight, eleven and 14 weeks after the end of the two-week ND/oil treatment period. Three ND-treated animals and seven oil-treated animals started to approach and drink from the water spout at the first test occasion (week 5; *ns*). At the three subsequent test occasions, the ND-treated animals were the ones who started to drink significantly more often than the oil-treated group: week 8: $p = 0.04$, week 11: $p = 0.02$ and week 14: $p = 0.02$.

As seen in Figure 8 (left panels), the ND-treated animals and controls showed no difference in individual time spent drinking water during the baseline. During the competitive test, i.e. when two animals had to compete for access to the spout which was restricted to only one animal at a time, the ND-treated animals tended to drink from the water spout significantly longer time compared to controls at week 5 (Figure 8). The difference between the groups was significant also in week 8 and week 11, whereas there was no significant difference in time spent drinking between the two groups on the last test occasion (week 14).

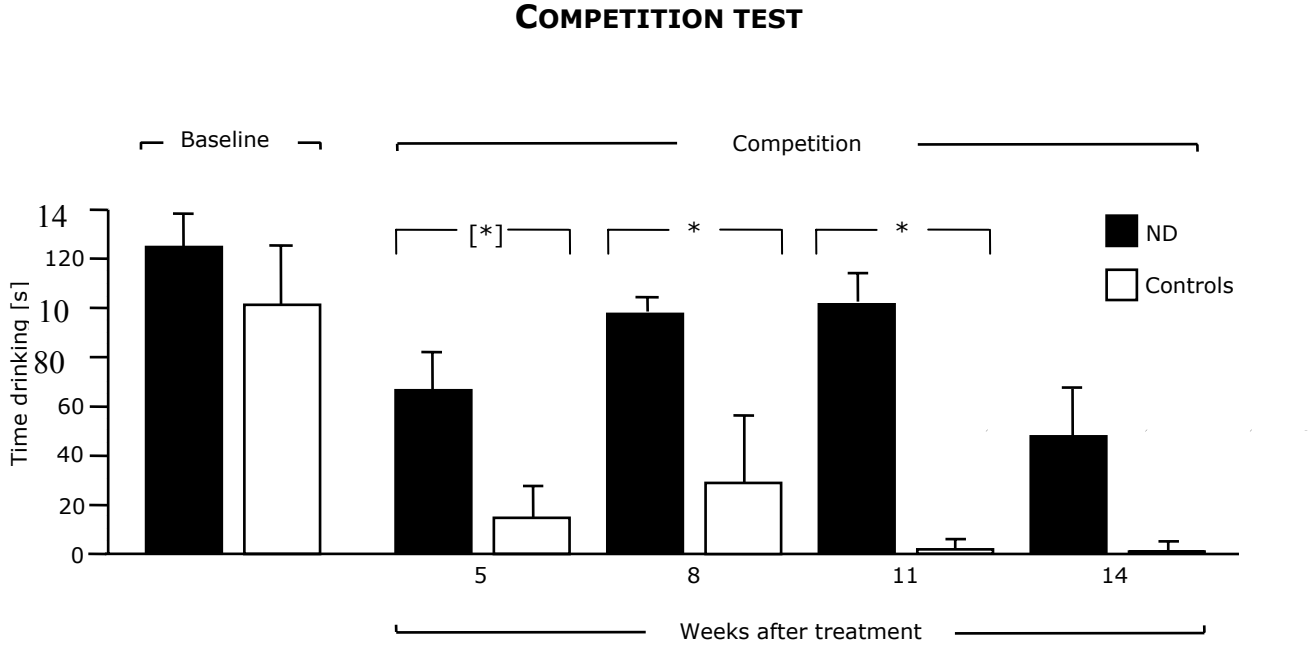

Figure 8. Median ± MAD values for individual drinking time [s] (i.e. baseline; left panels) and for the four subsequent competition tests (week 5, 8, 11 and 14). During the competition tests, access to the waterspout was only available for one animal at a time. One nandrolone decanoate (ND; daily s.c. injections of 15 mg/kg for 14 days) and one oil-treated rat had to compete for the access to the spout. Total pairs were $n = 10$. [*]: $p = 0.05$; *: $p < 0.05$ (ND *vs.* control; Wilcoxon signed ranked test).

During the competitive tests, presence of *piloerection* was more frequently observed in the ND-treated animals on all test occasions than in the control group, although the difference was only significant in week 8 ($p = 0.04$) and week 11 ($p = 0.05$). Regarding *lateral attacks*, this behaviour occurred more seldom. Out of the ND-treated rats, three to four animals showed this behaviour constantly during all four competitive tests, whereas only one control showed this behaviour at week 8.

Concerning the body weight development, the ND-treated animals weighed significantly more than the control group at the start of the two-week treatment period ($p < 0.01$). During the treatment period the body weight of the ND-treated rats decreased and remained lower at each time point of the four test occasions compared to controls (week 5: $p < 0.001$; week 8: $p < 0.01$; week 11: $p < 0.01$; week 14: $p < 0.01$).

5. DISCUSSION

Can animal behaviours reflect a human subjectively experienced emotional state? No, subjective experiences are not measurable in animals. The only way to study subjective emotion is to find means to assess the emotional expressions, i.e. emotional reactivity. The emotional reactivity is behavioural and physiological response patterns, elicited by the state of emotion experienced by the animal. Thus, the measurable outputs, involving behavioural, neuroendocrine and physiological manifestations, have been preceded by an external input, which in turn yields an emotional state. This reasoning requires that the expression, behavioural as well as physiological, of a given emotion should be similar across species (reviewed in Belzung & Griebel, 2001; Ramos & Mormede, 1998).

5.1. ND AND AGGRESSIVE BEHAVIOURS

There exists not one unanimous definition of human aggression. One definition states that "behaviour directed towards the goal of harming or injuring another living being who is motivated to avoid such treatment" (Baron, 1977). This definition does not take the aspect of intent into consideration. The definition does not either differ between aggression and violence, which some authors argue as being two different classifications of aggression. A difference between anger and aggression has further been proposed, in which aggression refers to overt behaviours whereas anger concern a subjective experience that is accessible to the investigator only by the verbal description of those with that experience (Blanchard & Blanchard, 2003). The relationship between anger and subjective experience and the lack of a consensus concerning definition of aggression are major problems in the integration of animal data with human aggression.

In animals, seven categories of aggression have been described, categorized by the type of stimuli and situation that precedes the aggressive behaviours: predatory, intermale, fear induced, irritable, territorial, maternal and instrumental (Moyer, 1968). More explicitly, intermale aggression is also referred to as intermale social aggression and is characterized as attacking a conspecific male to whom the male attacker has not become habituated. The most potent releaser of this aggressive response in several species is the presence of a male conspecific. Intermale social aggressive behaviours does not appear in male, and female, rodents until after sexual maturity (Moyer, 1968). Yet another distinction of animal and human aggression was made by Blanchard and colleagues (1977) and by Lehman and Adams (1977). These authors argued that animal aggression could be classified as offensive and

defensive aggression. Briefly, offensive aggression involves responses to challenge over adaptively important resources, whereas defensive aggression is attack in defence of the subject's own bodily integrity (Blanchard et al., 2003). Aggressive behaviours can be observed in a great variety of test models. The models mainly vary in the environmental conditions used to induce the aggressive behaviours (Koolhaas & Bohus, 1991). Increased frequency of aggressive behaviours can also be induced by performing specific manipulations of the brain, such as depletion of 5-HT neurons and lesioning certain brain areas (Vergnes, Depaulis, Boehrer, & Kempf, 1988).

Dominance in a provocative and competitive situation (papers II and IV)

In most mammals, agonistic or fighting behaviours often determine access to resources through an intermediate step, which is establishment of dominant hierarchies in group living mammals. A rat is said to act dominantly if its apparent intent is to achieve or maintain high status, that is to obtain power, influence or valued prerogatives, over a conspecific (Mazur & Booth, 1998). High status also enhances the probability of maintenance of territories which leads to increased access to resources, including food, shelter and mates (Gammie, Hasen, Rhodes, Girard, & Garland, 2003). In this thesis, we have focused on establishment of dominance in a provocative and competitive situation as one measurement of aggression. Success in a such situation is related to the so called intermale social aggression (Albert et al., 1987), which is mainly distinguished by the presence of behaviours like piloerection and lateral attacks (Albert & Walsh, 1982; Blanchard & Blanchard, 1977). Challenge is a fundamental aspect of the elicitation of aggression in both animals and humans. In humans, verbal and gestural insults are a form of challenge, although the insults are very culture specific (Blanchard, Hebert, & Blanchard, 1999). Thus, the mechanisms that elicit offensive aggression are suggested to be the same both in humans and non-humans, i.e. a challenge to the angry individual's authority, independence, image or control (Blanchard & Blanchard, 2003).

The effect of ND on dominance was examined by using a competitive and provocative situation where two highly motivated animals (i.e. water deprived) compete for the same goal (i.e. water). The animals were deprived during three days, and their drinking behaviour was thereafter tested for two subsequent days when exposed to a competitive situation (Albert et al., 1987). This test situation evokes intermale social aggression, where males fight each other in order to establish dominance relationships (Albert, Walsh, & Jonik, 1993; Koolhaas, Schuurman, & Wiepkema, 1980). When analysing the examined test parameters in this thesis, the results showed that the ND-treated rats and controls displayed few lateral attacks when subjected to the competitive test situations. In paper II, lateral attacks were not observed in any of the two groups. In paper IV, lateral attacks were only seen in three to four ND-treated

rats and the same rats showed this behaviour on all four competitive tests, whereas only one control showed this behaviour at one occasion (week 8). This finding is in accordance with Breuer et al. (2001), who found no difference in attack/fight behaviour, including lateral attacks, between ND-treated rats and controls. Animals in the present thesis, and in the study by Breuer et al. (2001), were tested in a neutral environment. Studies have shown that non-treated gonadally intact male rats, but also testosterone propionate treated rats, exhibit more aggression toward an opponent in their home environment than in a neutral environment (Barfield, Busch, & Wallen, 1972; Breuer et al., 2001). Moreover, it has been found that ND has less effect on attack/fights in comparison to threat (which includes piloerection) in a neutral environment (McGinnis, Lumia, & Possidente, 2002). Thus, the reason for the low frequency of lateral attacks in this thesis may be due to the fact that animals were tested in a neutral environment.

In paper II, only some a few ND-treated animals displayed piloerection, a behaviour never seen in the controls. In paper IV, all ND-treated animals displayed piloerection on the first test occasion (week 5), whereas only three controls exhibited this behaviour. At the subsequent test occasions (weeks 8, 11 and 14), no presence of piloerection was observed among the controls, and the number of ND-treated rats showing piloerection declined over these test occasions. It should be noted that, in paper IV, the decreased presence of piloerection over the subsequent tests could not be due to a habituation of a specific dominant-submissive pair constellation, since the ND-treated rats were always paired with a new and unfamiliar control on each test occasion. It is recognized that rats show an inhibition of offensive aggressive behaviours in the presence of a conspecific that has previously defeated them (Blanchard et al., 2003). Thus, one possible explanation for the seldomly observed intermale social aggressive behaviours during the test situation in paper II, might be due to the fact that animals were housed with their counterpart for a period prior to the test situation. It is hence possible that the competitive pair already had established their relationship before the test situation. When the dominance status is well established, there is no need to exhibit intermale social aggressive behaviours unless the dominance has to be re-established.

The results further showed that in paper II, ten ND-treated animals out of twelve, were the first to approach the waterspout to start to drink. Also in paper IV, the ND-treated animals first approached and started to drink from the spout on each test occasion, except for the first occasion (week 5). On that occasion, seven controls out of ten first approached the spout and started to drink. It is possible that the controls' behaviour (first approaching the spout to drink, i.e. obtaining control over a valuable resource) provoked the ND-treated rats, this, in turn, might have resulted in the increased intermale social aggressive behaviours (i.e. piloerection) displayed by the ND-treated group on the first test occasion (week 5) in order to

obtain dominance over the controls. The controls' subsequent subordinate behaviour was obvious on the two last test occasions (week 11 and 14), when the majority of the controls never even made an effort to approach the waterspout. Instead, the water deprived controls stayed on the side of the cage opposite to where the waterspout was placed. According to Blanchard et al. (2001) subordinate rats almost never fight in the presence of a dominant conspecific. Considering the declining display of intermale social aggressive behaviours throughout the test series, the ND-treated rats' dominance over the controls may hence have been achieved by other non-directly observable cues. Non-observed cues that may have been of importance are, for example, ultrasonic vocalization and odours (Blanchard & Blanchard, 1977; Rawleigh, Kemble, & Ostrem, 1993).

Concerning the total drinking time, the ND-treated animals in paper II drank water (i.e. took control over valuable resources) for significantly longer time than controls. The results from paper IV showed that the group of ND-treated animals also exhibited a longer drinking time, compared to controls, on the first three test occasions (week 5, 8, 11 after the end of treatment period). Interestingly, results from paper IV further showed that the control group's drinking time, increased when they were subjected to the competitive situation with other controls as counterparts. It can therefore be assumed that the ND-treated rats' success in obtaining and maintaining access to water was not due to a predisposed submissive behavioural profile in the control group.

Our results are in line with other studies showing that dominance is enhanced, or even induced, in rats after testosterone propionate treatment when subjected to competitive situations (Bonson et al., 1994; Bonson & Winter, 1992). Further, frequency of dominant postures used by the rat in order to establish dominance, is increased in rats pre-treated with testosterone propionate (Lumia et al., 1994). Albert and colleagues (1986, 1987) have shown that animals with testosterone implants achieve success in a competitive situation and that the aggressive behaviours that the animals display are mainly piloerection and lateral attacks. In addition, castration results in decreased intermale aggression and loss of social dominance in the competition situation (Albert et al., 1987; Albert et al., 1986). Moreover, testosterone improves the success rate of rats tested in competition tasks, such as food reward (Bonson & Winter, 1992) and copulation with a sexually receptive female (Lumia et al., 1994). Studies including non-human primates (cynomolgus monkeys) have also shown that an AAS cocktail affect dominance and aggressive behaviours (Rejeski et al., 1988; Rejeski et al., 1990). There are, however, contradictory findings reporting a decreased frequency of dominant postures after ND administration [10 mg/kg 5 days/week for 12 weeks] in rodents (Breuer et al., 2001). The divergent results may depend on the different administration schedule and on the different behavioural test procedures.

Data from the present thesis indicate that ND treatment induces a relatively long-term (i.e. up to three months) alteration of dominance in male rats. To our knowledge, only one other study (McGinnis, Lumia, & Possidente, 2002) has investigated long-term behavioural effects after steroid administration. It would, however, not be relevant to compare our results with those of McGinnis et al., since the dose and treatment schedules were quite different. Interestingly, McGinnis and colleagues suggest a potential reversibility of AAS-induced aggression following withdrawal from the steroid treatment. This possibility is of concern, since one of the most common psychiatric side effect of AAS abuse in humans is aggression (Thiblin & Parlklo, 2002). Thus, it cannot be excluded that the observed ND-induced competitive behaviours in the present study would eventually return to the rat's baseline behaviours. On the other hand, regardless of ND withdrawal or not, there is a possibility that experiences of success influence the forthcoming responses to repeated test situations. One way to avoid any possible influences of habituation would be to test a new batch of animals on each test occasion.

Concerning the subjects' body weight, the ND-treated animals had significantly lower body weight compared to their control rivals at all competitive test occasions. Nevertheless, they were more successful in terms of time of accessing the water bottle. There is no consistence in the literature concerning offensive aggression and body weight in AAS pre-treated rodents. Some studies have found an increased offensive aggression in AAS-treated animals compared to controls but no difference in body weight (Breuer et al., 2001; Lumia et al., 1994; Melloni et al., 1997), while another study is in agreement with our results, finding smaller but more offensive aggressive AAS-treated animals compared to controls (Grimes et al., 2003). Robitaille and Bovet (1976) found in their work with a free living population of wild rats, that smaller rats tended to avoid aggressive confrontation with heavier rats, and, if confronted them, the smaller rats always lost in fights with larger rats. Since the ND-treated rats in this thesis were smaller compared to control rivals in the competitive situations, it can be concluded that the increased dominance seen in the ND-treated rats was merely due to ND.

It is well known that an inverse relationship exists between different forms of aggressive behaviours and 5-HT. Pharmacological strategies of increasing 5-HT concentrations, like the administration of 5-HT precursors, 5-HT reuptake inhibitors and 5-HT_{1A} and 5-HT_{1B} receptor agonists are able to reduce aggressive behaviours in rodents (reviewed in Nelson & Chiavegatto, 2001). There is an extensive literature implicating a dysfunction of 5-HT in disorders involving aggressive and violent behaviours both in humans (Higley & Bennett, 1999; Linnoila, De Jong, & Virkkunen, 1989; Miczek, DeBold, & van Erp, 1994; Tuinier, Verhoeven, & van Praag, 1995; Virkkunen, De Jong, Bartko, & Linnoila, 1989) and animals (reviewed in Nelson & Chiavegatto, 2001). In experiments by Bonson and colleagues it was found that a treatment regime of 30 mg/kg for 14 days of testosterone propionate resulted in

an androgen-induced aggression (Bonson et al., 1994), but administration of the 5-HT_{1A} receptor agonists quipazin reduced the observed testosterone propionate-induced aggression (Bonson & Winter, 1992). In accordance with this finding, Grimes and Melloni (2002) observed that treatment with an AAS-cocktail diminished the development of 5-HT innervations in the hypothalamus and the forebrain of the male hamster, leading to enhanced aggressive behaviours. They also showed that this effect could be blocked by concomitant administration of the selective 5-HT reuptake inhibitor, fluoxetine (Grimes & Melloni, 2002). Thus, it is possible that the enhanced dominance, seen in this thesis, was partly an effect of ND-induced alteration of the 5-HT systems. Furthermore, a negative correlation has been observed between enkephalins and aggression in animals (reviewed in Tordjman et al., 2003). Studies have, for example, found that administration of an enkephalin peptide might suppress aggression through its action at the PAG brain region in cats (Shaikh, Lu, & Siegel, 1991; Shaikh, Shaikh, & Siegel, 1988; Weiner, Shaikh, Shaikh, & Siegel, 1991). Hence, the ND-treated rats' success in the competitive test observed in this thesis may further be a result of the ND-induced decreased enkephalin peptide MEAP activity in PAG, also observed in this thesis.

Reactivity towards physical provocations (papers I and III)

Defensive aggression, or defensive reactivity, has been conceptualized as an act that occurs in response to a provocation (Albert et al., 1987) or as an attack in defence of the subject's own bodily integrity (Blanchard et al., 2003). It is well known that laboratory rats show lower levels of defensive aggression, due to domestication, than wild rats (Blanchard & Blanchard, 2003). Nevertheless, excessive reactivity can be induced by lesioning certain parts of the brain, such as the septum, the medial accumbens, the medial hypothalamus or the raphe nuclei (reviewed in Albert & Walsh, 1982). These lesioned animals have also been described as hyperemotional, hyperirritable or hyperreactive. When provoked, a non-treated rat will try to avoid the stimulus by fleeing. If this proves unsuccessful, the rodent tries to scare off the stimuli by vocalization (threat) and by biting (attack) (Blanchard & Blanchard, 2003). A very reactive rat may respond strongly to a neutral stimulus that a non-treated rat would ignore. Thus, reactivity to neutral stimuli seems to occur because a threat was perceived when, in fact, no "threat" was present. Reactivity can consequently be associated with perception, which makes the origin of the reactivity very complicated (Albert & Walsh, 1982). In non-treated animals, as well as in humans, risk assessment (defined as obtaining information that helps to avoid/reduce a damaging outcome from a potential danger), is thought to be the underlying mechanism behind the behavioural outcome following provocation. This information processing, together with fight, flight and freezing behaviours, are normal human actions in response to potential threatening events or situations (Blanchard & Blanchard, 2003). The levels of reactivity are thought to be pathologic when the level, or form, of

behavioural outcome is not appropriate as a response to the provocation (Blanchard & Blanchard, 2003). In humans, the distinction between what is appropriate and what is not, is culture and gender specific. In the animal experimental models we judge what is an appropriate response based on the behaviours of untreated control rats.

There are different tests to determine to what extent physical provocations evoke an exaggerated aggressive response in rodents. The experimenter can provoke the rats with a rather harmless stimulus, such a mild tail pinch. In papers I and III, reactivity to physical provocation was elicited by a test situation where the animals were challenged by "innocuous" physical stimuli (e.g. a rod and the experimenters' gloved hand) and the behavioural responses were observed (Albert et al., 1993). In paper I, ND-treated animals and controls were tested for reactivity to such physical stimuli immediately after the end of the ND/oil treatment period and six to eight weeks thereafter. It was found that ND-treated animals displayed enhanced reactivity towards flank prods, air puff and capturing, on both test occasions. Our study is in accordance with that of McGinnis et al. (2002) in the respect that both studies observed enhanced reactivity few weeks after the cessation of the steroid treatment. However, McGinnis and colleagues (2002) further tested the behavioural responses to tail pinches twelve weeks after the end of the steroid treatment. At this time point, they found no enhanced aggressive behaviours. In paper I, we observed enhanced reactivity in the ND-treated rats eight weeks after cessation of the treatment period. It is possible that McGinnis et al. (2002) also would have observed enhanced aggression six to eight weeks after the end of the treatment period if they had tested the rats at this time point. Furthermore, the differing results from that study and ours might be due to treatment schedule discrepancies. In our study, ND was administered during two weeks [15 mg/kg/day], whereas in McGinnis et al's study more or less the same total amount was administered, but dispersed over a period of twelve weeks.

The enhanced reactivity observed in paper I, six to eight weeks after treatment, might not only be due to the ND treatment alone, since the animals had had access to ethanol during the period between the reactivity tests. In this ethanol consumption test, we found that ND-treated animals drank more ethanol compared to controls. It may, therefore, be argued that the enhanced reactivity scores, seen in the ND-treated animals after the ethanol intake period, were due to the increased alcohol consumption. However, Bergvall and colleagues (1996) have shown that there is no relationship between high alcohol intake and reactivity to physical provocations in untreated Wistar rats. Hence, the observed increased reactivity on the second test occasion was probably caused by ND alone, or by a possible interaction between ND and ethanol. In fact, increased anger has been observed in AAS abusers with concurrent intake of alcohol (Lukas, 1996) and there are several case reports and anecdotal stories describing "roid rage" after parallel abuse of AAS and alcohol (Conacher & Workman, 1989).

In paper III, after the end of the 3-week wheel-running period (i.e. one week after the end of the ND/oil treatment period), the four treatment groups were tested for reactivity toward different physical provocations. Regarding the provocations air puff and captivity, overall effects were found with steroid treatment (ND/oil) and access to wheel-running (wheel-running/sedentary) as both main variables and background variables. This suggests that the two treatments (i.e. steroid treatment and access to wheel-running) are both of significance for the observed enhanced reactivity outcome. Concerning the captivity provocation, the group that scored highest was *ND-wheel*, followed by *ND-sedentary*, *oil-wheel* and *oil-sedentary*. Since the ND-treated groups exhibited greater reactivity than did the control groups, it may be suggested that ND treatment may be more the prominent in order to evoke enhanced reactivity toward captivity. To further strengthen the argument, separate analyses showed that there were significant differences between the *ND-wheel* and *oil-wheel* groups ($p = 0.001$; Mann Whitney *U*-test) and between the *ND-sedentary* and *oil-sedentary* groups ($p = 0.02$; Mann Whitney *U*-test). Concerning reactivity towards the air puff provocation, it is impossible to decide which of the two treatments (i.e. ND or wheel-running) had the stronger effect on reactivity, since the only group that showed any reactivity was the *ND-wheel* group. The only possible conclusion that can be drawn is that only a combination of ND treatment and access to wheel-running produced enhanced reactivity towards the air puff provocation.

Concerning the group of *ND-sedative* rats, there was no observed reactivity towards the air puff provocation. Neither did any of the two ND-treated groups exhibit enhanced reactivity towards the wooden rod (approach and touch the rat's snout and poke at its flanks). The absence of ND-induced reactivity in paper III is somewhat surprising, since the results in paper I indicated ND-enhanced reactivity towards air puff, as well as towards flank pokes. The absence of reactivity towards these provocations in paper III may be due to the fact that the animals had been more handled by the experimenters than the animals in paper I. It may be possible that these provocations (air puff and wooden rod) are milder provocations than is the captivity provocation, and therefore do not generate the same reactivity in animals that have been extensively handled.

Hence, the results indicate that a combination of ND treatment and physical exercise may enhance reactivity to certain physical provocations. The results also suggest that steroid treatment is a more prominent factor than is access to wheel-running in order to evoke reactivity to physical provocation, although physical exercise seems to accentuate the enhancing effects of ND. It is today well known that androgens can act as modulators of aggressive behaviours in rodents (Rubinow & Schmidt, 1996). Whether or not physical exercise stimulates aggressive behaviours is, however, not widely investigated, although a positive relationship has been observed between high wheel-running activity and high levels

of another form of aggressive behaviours, namely predatory aggression, in mice (Gammie et al., 2003). A cautious interpretation of our data may suggest that physical exercise has a moderate effect on reactivity towards physical stimuli, whereas ND treatment seems to be more powerful in terms of stimulating this behaviour. However, physical exercise seems to accentuate the enhancing effect of ND on reactivity toward physical provocations. It is has been shown that both ND treatment and physical exercise give rise to neurobiological alterations of the endogenous opioidergic systems (reviewed in Clark & Henderson, 2003; Jonsdottir, 2000). Thus, alterations in these systems may contribute to the observed accentuated effect on reactivity toward physical provocation. Clearly, further research is necessary in order to understand the relationship between the abuse of AAS and exercise and their combined effect on behaviours.

5.2. ND, FEAR AND ANXIOUS BEHAVIOURS

In humans, anxiety is defined as a vague, unpleasant emotional state with qualities of apprehension, dread, distress and uneasiness. Anxiety is often distinguished from fear in that an anxiety state is often objectless, whereas fear assumes a feared specific object, person or event (Reber, 1985). In animals, as well as in humans, fear and anxiety are related emotions, although they are not considered identical. Fear is generally thought to be adaptive, since it is usually produced by potentially harmful stimuli, and may lead to appropriate responses such as avoidance or escape. Anxiety is a term used to describe both a normal emotional state, associated with stressful or psychologically difficult events, and a psychological condition. Pathologic anxiety is defined as a chronic state that is not clearly linked to well defined events (Sanger, 1991). In rodents, the emotional state of anxiety reflects the behaviours induced by the perception and appraisal of potential threats, whereas behavioural responses to direct threats are thought to represent fear or even panic.

In animals there are various models for testing if a drug possesses anxiolytic properties. The most extensively used unconditioned models include exploratory behaviours in unfamiliar environment. Punishment procedures, i.e. conflict test, are also widely used in order to reflect anxiolytic effects (Sanger, 1991). In Vogel's drinking conflict model, where an electric shock is administered to a water deprived animal when attempting to drink water, it was shown that administration of testosterone increased the number of shocks accepted (Bing et al., 1998; Svensson et al., 2003). In another punishment study (Frye et al., 2001), testosterone administration to androgen depleted rats increased the rats' latency to withdraw their tail from hot water. Frye and colleagues (2001) suggest that the results provide evidence for anxiolytic, as well as analgetic, properties of testosterone. The anxiolytic effect of a drug can also be

studied by use of a locomotor activity test and by different forms of exploration tests (Sanger, 1991). Peripheral activity, or wall-hugging behaviour, is another version of the idea behind the elevated maze, and it is assumed to indicate anxiety (Sanberg et al., 1987). Studies on behaviours in a novel environment is of interest because of the hypothesis stating that exploratory behaviours is under the control of two competing drives, namely the drive to approach and investigate novel objects and places and the contrasting drive that induces fear which tends to suppress exploration and produce active avoidance of the novel situation (Montgomery, 1955). Exploratory behaviours can be tested by using an elevated maze with open and closed arms and recording the number of entries and amount of time spent in the open and closed arms. Treatment with testosterone has been shown to cause a decrease in the number of entries into the closed arms and an increased time spent in the open arms (Aikey et al., 2002; Bitran et al., 1993; Frye & Seliga, 2001). Aikey and colleagues (2002) further showed that when a $GABA_A$ receptor antagonist was administered to mice pre-treated with testosterone, the observed testosterone-induced anxiolytic effect was blocked.

Fleeing and freezing behaviours (paper I)

Animals were exposed to the fleeing and freezing test directly after the end of the two-week ND/oil treatment period. The fleeing and freezing test consists of two parts. During the first part of the test, the animals are allowed to adapt to the new environment and open-field behaviours are observed. The results from this part of the test showed that ND-treated animals displayed a prolonged *latency to leave* the sector, decreased *locomotor activity* and less *rearing* behaviour, than did the control group. Increased latency to leave, decreased locomotor activity and decreased rearing behaviour are considered measures of fear/anxiety (reviewed in Ramos & Mormede, 1998). Seemingly, the results may reflect an increased state of anxiety in the ND-treated rats compared to controls.

Two other behaviours were also observed during the open-field phase of the fleeing and freezing test, namely *grooming* and number of defecated *boli*. Grooming is a behaviour that may have several functions, such as displacement activity, self-calming behaviour, or body care (Spruijt, van Hooff, & Gispen, 1992). Grooming behaviour elicited by mildly stressful stimuli can, thus, signify distress (Colbern, Isaacson, Green, & Gispen, 1978). Some authors suggest that also high defecation rate is a response to fear, caused by an activation of the autonomic sympathic nervous system (reviewed in Ramos & Mormede, 1998). Results from the open-field observation (paper I) showed, however, no group difference in the amount of grooming behaviour or in the number of deposited boli. Thus, it is difficult to conclude from the open-field behavioural measurements if ND induces enhanced state of anxiety or not.

During the second part of the fleeing and freezing test (paper I), animals were exposed to a

threatening auditory stimulus designed to induce fear. In response to threatening stimuli, the rats at first attempt to flee if an escape route is available. If this behaviour is not applicable, the animals immobilize, or freeze, in an effort to escape. In rodents, fleeing and freezing responses are innate responses to threatening situations (Boissy, 1995; Bolles, 1970). Decreased fleeing response and shortened freezing response are behaviours thought to reflect anxiolytic properties of a drug. The results showed that the flight distance induced by the audiogenic stimulus, was significantly shorter in the group of ND-treated rats. The duration of the freezing reaction of the ND-treated rats was also significantly decreased compared to controls. Thus, it is possible to assume, judging from the open-field observations (part I) and from the decreased fleeing and freezing reactions (part II), that ND, to some extent, elicits an anxiolytic effect rather than induce an anxiety state in rats.

Neonatal administration of 5-HT neurotoxin (5,7-dihydroxytryptamine), resulting in a decreased concentration of 5-HT, exerts a markedly reduced duration of the immobility reaction in rats (Hård, Ahlenius, & Engel, 1983). Furthermore, treatment with the anxiolytic drug diazepam reduces the duration of freezing (Hård et al., 1985). Diazepam binds selectively to the $GABA_A$ receptor thereby, enhancing the response produced by activity in GABAergic neurones. Hård and colleagues (1985) have further shown that administration of the DA agonist apomorphine reduces the freezing response, while the DA receptor antagonist, haloperidol, increased the freezing duration. Concerning serotonergic receptors, $5\text{-}HT_{1A}$ receptor mutant knock-out mice line display increased anxiety related behaviours, as tested in open-fields models and elevated plus maze models (Ramboz et al., 1998). It is thus fair to suggest that the 5-HT-, DA- and the GABAergic systems are involved in monitoring the responses to threatening stimuli. The same neurotransmitter systems have shown to be altered after administration of AAS. For instance, results from this thesis and other studies have demonstrate decreased concentrations of 5-HT and 5-HIAA after AAS treatment (Bonson et al., 1994; Grimes & Melloni, 2002). Administration of several AAS compounds have further been shown to alter $GABA_A$ receptors (Jorge-Rivera et al., 2000; McIntyre et al., 2002) and to increase DA concentrations in the striatum of the rat brain (Thiblin et al., 1999).

Taken together, the observed open-field behaviours during the fleeing and freezing test did not fully support the notion that ND possesses anxiolytic effects. Nonetheless, the decreased duration of the fleeing and freezing responses in the ND-treated rats suggest a lower potential for fear and anxiety in threatening situations. This conclusion is in accordance with studies showing that testosterone, its metabolites and Metenolon hold anxiolytic properties (Aikey et al., 2002; Bing et al., 1998; Bitran et al., 1993; Frye & Seliga, 2001; Svensson et al., 2003; Ågren et al., 1999). In contrast to the positive reports about the anxiolytic effects of testosterone and AAS, Minkin and colleagues (1993) found that a rat treated with ND [50 mg per week], increased peripheral activity during a locomotor activity test (Minkin et al., 1993).

This effect was not apparent in rats injected (i.m.) with a dose of 10 mg per week. This result may suggest that the anxiolytic effect of ND is dose-dependent. Consequently, dose, type of AAS and administration might be the underlying reason why some human AAS abusers claim to administer AAS to become braver (Kindlundh et al., 1998), although other studies have reported an increased anxiety in AAS abusers (Fudala et al., 2003; Perry & Hughes, 1992).

5.3. ND AND PHYSICAL ACTIVITY

Measurement of voluntary physical activity is perhaps the most classical and commonly used procedure when evaluating the effects of drugs on the central nervous system in rodents. Physical activity can be tested through a variety of test models. For example, one method employs activity boxes equipped with infrared beams (invisible to the rodent) across the cage, which when broken activates a counter that records one movement. Another technique involves placing the animals in a prescribed area and an observer record the movements of the rat. These activity tests allow for measurements of forward locomotion of the animals; which reflects their level of physical activity. States of fear and anxiety may be indicated by rearing and peripheral activity of the rats, and their impulsivity may be indicated by their central activity. Physical activity can further be measured by equipping the home cage with activity wheels that automatically register wheel revolutions.

Wheel-running activity (paper III)

Results from the wheel-running test, performed at the end of the two-week ND/oil treatment period, showed that the ND-treated rats had a decreased activity. One week after the end of the ND/oil treatment period, wheel-running activity was again registered. At this point in time, there was no difference in running activity between the ND-treated animals and controls. The results from the wheel-running test indicate that administration of ND may suppress physical activity for up to one week after the end of the treatment period. This assumption is in line with results from paper I, where it was found that ND-treated rats had decreased activity during the open-field observation executed immediately after ND/oil treatment. In an pilot study from our laboratory (unpublished data by Lindqvist and Fahlke), locomotor activity was registered in activity boxes three days after ND/oil treatment and the result also indicated that ND-treated rats had lowered activity compared to controls. However, when observing locomotor activity two weeks after the end of the ND/oil treatment period (paper II), no difference in activity was found between the two treatment groups. In conclusion, the results from these studies indicate that administration of supraphysiological dosage of ND suppresses physical activity for up to one week after the end of the treatment

period. This suppression seems, thus, to be transient.

It is difficult to conclude whether our results are in accordance with other studies, since the majority of these studies give no exact time-point for the activity test in relation to the end of the steroid treatment period. Most of these studies have, however, observed no physical activity effect after administration of AAS compounds, like ND (Minkin et al., 1993; Salvador et al., 1999), testosterone propionate (Aikey et al., 2002; Bitran et al., 1993; Clark & Barber, 1994; Clark & Harrold, 1997; Salvador et al., 1999), testosterone (Bing et al., 1998; Martinez-Sanchis et al., 2002), stanozolol (Clark & Barber, 1994; Clark & Harrold, 1997; Martinez-Sanchis et al., 1996) or an AAS cocktail (Bronson, 1996; Salvador et al., 1999). On the other hand, van Zyl and colleagues (1995) found that running endurance in trained nandrolone phenylpropionate-treated rats were markedly increased compared to trained rats receiving saline. Another study has found a positive correlation between self-administered testosterone and voluntary exercise (Wood, 2002). In female rats, the use of running wheels is suggested to be regulated by oestrogen (Bronson, Nguyen, & De La Rosa, 1996; Gerall, Napoli, & Cooper, 1973). Results have further showed that when administering an AAS cocktail to female rats their wheel running activity decreased (Bronson, 1996; Bronson et al., 1996). The authors suggest that the depression of activity observed in female rats might reflect a decrease in circulating oestrogen due to a negative feedback action of androgens on gonadotropin secretion. The observed decreased activity in male rats treated with steroids could, thus, indicate a direct central action of the androgens (Bronson, 1996).

A possible explanation for the lowered wheel-running activity (paper III) observed in the ND-treated animals at the end of the ND/oil treatment period, might be sought in the endogenous opioidergic systems. These systems are suggested to be involved in the expression of several behaviours, including exercise, feeding behaviour, alcohol intake and reward (Mansour, Fox, Akil, & Watson, 1995; Yeomans & Gray, 2002). For example, it has previously been shown that voluntary exercise stimulates levels of β-endorphin (Hoffmann, Terenius, & Thorén, 1990) and dynorphin-converting enzyme activity (Persson et al., 1993) in the rat brain. ND treatment [15 mg/day for 2 weeks] has also been shown to increase β-endorphin levels in the ventral tegmental area (Johansson et al., 1997) and dynorphin B in the nucleus accumbens (paper I). Thus, it is possible that a ND-induced stimulation of the opioid peptidergic systems results in a temporary decreased interest, or need, for other reward stimuli, like running in wheels.

The results from paper III also suggest that physical activity can act as a confounding variable when studying the behavioural effects of ND. As mentioned earlier in this thesis, the ND-induced increased reactivity to physical provocations, as well as alcohol consumption, were accentuated by exercise training. As also stated by Bahrke and Yesalis (1994), most studies

examining the psychiatric effects in human AAS abusers overlook the fact that they are often involved in different types of exercise training. This notion is of importance since the behavioural outcome may be even more pronounced if AAS abuse is combined with physical exercise, as observed in paper III.

5.4. ND AND BEHAVIOURAL RESPONSES TO ALCOHOL

Survey studies have demonstrated that male teenagers abusing AAS drink higher quantities of alcohol more frequently (DuRant et al., 1994; DuRant et al., 1993; Kindlundh, Hagekull et al., 2001; Kindlundh et al., 1999) and abuse home-distilled alcohol more often (Nilsson et al., 2001) than non-AAS abusers. When investigating individuals recruited from gymnasia, if was found that those who abused AAS also had a higher frequency of alcohol abuse than the non-AAS abusers (Kanayama et al., 2003; Malone et al., 1995; Middleman & DuRant, 1996). Case reports have also reported increased intake of alcohol (Conacher & Workman, 1989) and alcohol dependence (Fudala et al., 2003) in AAS abusers. However, it remains to be solved whether AAS abuse can act as a gateway for alcohol abuse.

The model used for voluntary ethanol intake in this thesis (papers I and III) was introduced by Richter (1926), and remains today in various forms the most common method for studying ethanol preference and intake in rodents. In this model, animals are presented with a free choice between two bottles in the home cage, one containing water and the other containing a six percent ethanol solution. This particular ethanol concentration stimulates peak levels of consumption in the present strain of rats (Hansen et al., 1994).

Voluntary ethanol intake (papers I and III)

In paper I, all animals were tested for voluntary ethanol intake during a three-week test period, two weeks after the ND/oil treatment period. There was no significant difference in ethanol intake during the first week of the test period between ND-treated rats and controls. During the test period's second and third week, ND-treated animals drank more ethanol, and significantly increased their preference for ethanol during the third week, compared to controls. There was no significant alteration in total fluid intake over the three weeks between the two groups. In this study, the animals' voluntary ethanol intake was not investigated before the start of the two-week ND/oil treatment period (i.e. baseline). Thus, it is possible that the ND-treated rats had an initial higher preference for ethanol. On the other hand, in paper III voluntary ethanol intake was measured before, as well as after the two-week ND/oil treatment period. The results from paper III indicated no difference in baseline ethanol

consumption between the two pre-destined treatment groups. Therefore, it is likely that the observed difference in ethanol intake between the ND-treated rats and controls in paper I was not due to a pre-disposed ethanol preference.

The hypothesis that ND stimulates ethanol intake was re-tested in paper III. Besides, investigating voluntary ethanol intake before, as well as after the ND/oil treatment period in paper III, half of the ND-treated rats and half of the controls had access to running wheels in their home cages during the two-week ND/oil treatment period. The results showed significant overall effects concerning ethanol intake and preference, where the ND-treated rats with access to wheel-running equipment had the highest voluntary intake and preference, followed by *ND-sedentary*, *oil-wheel* and *oil-sedentary*. The overall effects were, however, only significant when steroid treatment (ND/oil) was entered as the main variable, but not when access to wheels (wheel-running/sedentary) was entered as the main variable. The results indicated that it was mainly ND treatment that affected ethanol intake and ethanol preference, while wheel-running seemed to play a lesser role. The data suggest, although, that wheel-running might accentuate ND treatment's stimulating effect, since the ND-treated rats with access to wheels had the highest ethanol consumption. Taken together, these results indicate that ND administration stimulates ethanol intake in rats. Furthermore, physical exercise seems to accentuate the enhancing effects of ND on ethanol intake, although it has a subordinate role on ethanol intake in comparison to ND. A cautious interpretation of the data suggest that AAS abuse might act as a gateway for the induction of abuse of alcohol and of other drugs, as also suggested by Arvary and Pope (2000).

The mechanism through which ND stimulates ethanol intake is not yet fully understood. One explanation for the observed ND-induced ethanol intake may be related to the function of the brain 5-HT systems. Several studies have found a negative relation between 5-HT concentrations and excessive ethanol intake in rodent. For example, selectively bred alcohol-prone rat strains with reduced brain 5-HT activity, and pharmacological manipulationed rats (e.g. 5-HT neurotoxins and 5-HT antagonists) display enhanced ethanol intake (reviewed in Koob, 1999; LeMarquand, Pihl, & Benkelfat, 1994b). Noteworthy, in paper II, ND treatment was observed to induce a reduction of brain 5-HT concentrations, a finding also reported by Grimes and Melloni (2002). Thus, it is possible that ND has an inhibitory effect on the 5-HT system, and thereby stimulates ethanol intake. Another explanation for the enhanced ND-induced ethanol intake might be that ND reinforces the behavioural response to other drugs of abuse by affecting the brain reward system. For example, low concentrations of dynorphin B in the nucleus accumbens has been suggested to be associated with an increase of DA activity (Das, Rogers, & Michael-Titus, 1994; Koob, Sanna, & Bloom, 1998; Spanagel et al., 1992), and the DA system is considered to mediate positive reinforcement and reward (Wise & Bozarth, 1987). In paper I, it was found that ND treatment induced a decreased concentration

of ir-dynorphin B in the nucleus accumbens. This suggests that ND, by altering dynorphin B concentrations, may affects voluntary ethanol intake via the DA system. Findings from other studies further strengthen the suggestion that dynorphin B, probably in interaction with DA activity, may promote the rewarding effects of ethanol. It has, for example, been found that administration of a kappa opioid agonist (which increases dynorphin B activity) attenuates preference for ethanol, an effect that can be reversed by treatment with a kappa antagonist (Sandi, Borrell, & Guaza, 1988). Moreover, rats genetically predisposed to drink more alcohol have lower basal concentrations of dynorphin B in the nucleus accumbens (Nylander, Hyytia, Forsander, & Terenius, 1994).

The effects of ethanol on locomotor activity (paper II)

In paper II, half of the ND-treated rats and half of the controls received injections of 0.5 g ethanol/kg 15 minutes before the locomotor activity test. This dose was chosen based on preliminary observations, indicating that an untreated Wistar rat given this dose of ethanol would respond with significantly decreased locomotor activity (unpublished data by Lindqvist and Fahlke). The remaining animals were injected with saline. No differences in locomotor activity were observed between the ethanol-treated ND group and the ND-treated animals receiving saline, whereas controls injected with a sedative dose of ethanol exhibited a significantly reduced locomotor activity compared to the saline-treated controls. This finding suggests that the locomotor activity, after a sedative dose of ethanol, was not affected in the ND-treated rats. The observed lack of sedation in the ND-treated rats after ethanol administration, may be due to a tolerance effect (Tabakoff, Cornell, & Hoffman, 1986) between ND and ethanol (i.e. increased tolerance to ethanol due to pre-treatment of ND).

The mechanism behind the development of ethanol tolerance is suggested to be partly mediated by $GABA_A$ receptor function (reviewed in Morrow, VanDoren, Penland, & Matthews, 2001). For example, some behavioural effects are enhanced by $GABA_A$ receptor agonists and attenuated by antagonists (reviewed in Lister & Linnoila, 1991). Furthermore, antagonists reduce signs of ethanol intoxication in rodents (reviewed in Morrow et al., 2001). Administration of AAS compounds has also been shown to alter GABAergic activity in rodents (Bitran et al., 1996; Bitran et al., 1993; Jorge-Rivera et al., 2000; Masonis & McCarthy, 1995, 1996; McIntyre et al., 2002; Yang et al., 2002). Thus, it is possible that ND, through modulation of the $GABA_A$ receptors, might increases ethanol tolerance, resulting in a lesser sedative effect on locomotion, as observed in the ND pre-treated rats. Interestingly, it has been reported that individuals abusing AAS have a delayed response in detecting effects of ethanol (Lukas, 1996).

5.5. ND AND PHYSIOLOGICAL MEASUREMENTS

Body weight (papers I to IV) and food intake (paper III)

In all four papers in this thesis, there were no differences in body weight at the start of the experiments between the sexually matured rats, randomly chosen to receive treatment with ND or oil. During the two-week ND/oil treatment period, the ND-treated groups did not gain as much in body weight as the controls. In all papers, the ND-treated group exhibited a lesser body weight compared to control at the end of the two-week ND/oil treatment period. This difference was statistically significantly in papers II to IV. At the termination of the studies, occurring four (paper II), six (paper III), seven (paper I) and 16 (paper IV) weeks after the end of the ND/oil treatment period, the ND-treated animals' lesser body weight were still evident and significantly differed in papers I, III and IV. Our results reporting a lesser body weight gain in ND-treated rats during the two-week treatment period compared to the oil-treated controls, are similar to the results in the studies by Johansson and colleagues (1997, 2000) who used the same ND treatment schedule as practised in this thesis. Other studies administering ND have also observed decreased body weight after treatment (Ferrandez, de la Fuente, Fernandez, & Manso, 1996; Minkin et al., 1993). For example, Minkin et al. (1993) observed a growth inhibition during treatment when administering ND at doses of ten or 50 once a week for eight weeks. Also, administration of testosterone in rats (implanted tubings containing 200 mg dissolved during 90 days) was demonstrated to induce a reduced body weight compared to controls (Bing et al., 1998).

The lowered body weight gain of the ND-treated rats might be caused by reduced food intake. In paper III, food intake was measured during the two-week ND/oil treatment period. The results showed a significant difference in food intake, where the ND-treated group ingested a lower amount of food. Lindblom et al. (2003) have shown that when using the same treatment regime as in the present study and when the same amount of food was given to controls as the comparable ND-treated animals consumed the previous day, there was no difference in body weight gain between the ND-treated rats and controls. They also found that ND treatment reduced the mRNA levels of the β-endorphin precursor POMC in hypothalamus (Lindblom et al., 2003). Since POMC is the prohormone for α-melanocyte stimulating hormone, known to participate in the central control of body weight homeostasis (Elmquist, Elias, & Saper, 1999), the authors suggest that some of the metabolic consequences of ND may be the result of alteration in the melanocortin system. Hence, the reduced body weights of the ND-treated rats were probably due to a lesser food intake, although the mechanism behind the decreased food intake is not investigated in this thesis.

Since humans abusing AAS normally increase their body weight, it could therefore be assumed that administration of ND would rather induced an increase in the rats' body weight than the observed decrease. In randomized controlled studies, administration of ND ([200 mg/week for 8 weeks]; van Marken Lichtenbelt, Hartgens, Vollaard, Ebbing, & Kuipers, 2004) or with testosterone enanthate ([600 mg/week for 10 weeks]; Bhasin et al., 1996) to AAS naive male volunteers, yielded an significant increase in body mass [kg] at the end of treatment period, compared to baseline values. The results showed further that at the end of the treatment period, men receiving testosterone treatment without having exercised significantly increased in body weight, although the combination of strength training and testosterone produced the greatest increase in body weight than were achieved with either intervention alone (Bhasin et al., 1996). However, in both these studies dietary intake was standardized. Studies indicate that increase in muscle mass and strength during AAS administration is only observed in athletes who are already weight-trained and who continue intensive training while maintaining high-protein, high-calorie diets (Kibble & Ross, 1987). It has further been reported that AAS did not substantially change body composition in a randomized controlled study in men, when the dietary intake was accounted for (Crist, Stackpole, & Peake, 1983). Another study showed that the improvement of athletic performance induced by AAS is effective only in combination with exercise and a high-protein diet (Freed, Banks, Longson, & Burley, 1975).

Thymus gland (papers I and II) and adrenal glands (paper II)

The systemic effect of the nandrolone decanoate was investigated by weighing the wet thymus gland four weeks (paper II) and seven weeks (paper I) after the end of the ND/oil treatment period. A significant reduction in thymus weight was observed in the ND-treated animals compared to controls. The results of lowered thymus weight after ND treatment are in line with other studies administering ND (Ferrandez et al., 1996; Johansson et al., 2000), where Johansson and colleagues used the same treatment schedule as practised in this thesis. It is known that the size of the thymus is reciprocal to the circulating corticosterone levels (Akana, Cascio, Shinsako, & Dallman, 1985), since steroids bind to glucocorticoid receptors in thymus and by a negative feedback mechanism induce a thymus atrophy (Bell & Munck, 1973). Exogenous administration of supraphysiological doses of glucocorticoids has been shown to induce thymus involution (Madden & Felten, 1995). Considering this, the thymus atrophy observed in this thesis could be due to ND-induced high levels of endogenous glucocorticoids. However, the thymus atrophy could also be due to a direct action of AAS, since it has been shown that AAS bind to glucocorticoid receptors in skeletal muscles (Mayer & Rosen, 1975), receptors also present in thymus (Bell & Munck, 1973)

To further investigate if ND affects the glucocorticoid levels, the weights of the adrenal

glands were studied four weeks after the end of the ND/oil treatment period (paper II). A discrepancy was found in the adrenal gland weights between the two groups, where the ND-treated animals had enlarged adrenals compared to the controls. Also in a study of ours (unpublished data by Lindqvist and Fahlke), results showed that seven weeks after the end of the ND/oil treatment period the ND-treated group's adrenal glands weighed significantly more compared to the controls'. The increased adrenal gland weights in the ND-treated animals may suggest a stimulation of the hypothalamic-pituitary-adrenal (HPA) axis, where the adrenal gland growth is mediated by increased concentration of the adrenocorticotropic hormone (ACTH). For example, Schlussman and colleagues (2000) found that administration of ND [15 mg/kg/day for three days] increased concentrations of both ACTH and the glucocorticoid hormone corticosterone in male rats.

5.6. ND AND NEUROTRANSMITTERS

The neurobiochemical mechanisms behind the observed AAS-induced behavioural changes are yet little understood. Neurobiological studies indicate that administration of supraphamacological doses of AAS compounds, affects important neurotransmitter systems that is associated with observed behaviours promoted by AAS administration. In order to further investigate this association, monoaminergic concentrations and the opioid peptides, MEAP and dynorphin-B, were analysed.

Brain monoamine concentrations (paper II)

Four weeks after the end of the two-week ND/oil treatment period, biochemical analyses of DA, NE, 5-HT, HVA, DOPAC and 5-HIAA were performed on the following brain areas; dorsal striatum (caudate/putamen), basal forebrain (medial frontal cortex, nucleus accumbens, olfactory tubercle, septum), hippocampus, amygdala and hemispheres (remaining cortical tissues). The results indicated only the serotonergic system was significantly affected by the ND treatment. ND-treated rats, compared to controls, displayed significantly lower concentrations of 5-HT concentrations in the basal forebrain (medial frontal cortex, nucleus accumbens, olfactory tubercle, septum) and dorsal striatum (caudate/putamen). There was also a trend toward a lowered concentration of 5-HT in hippocampus and amygdala, although not statistically significant ($p = 0.06$ for both brain areas). The ND-treated animals exhibited lowered concentrations of the 5-HT metabolite, 5-HIAA, in the dorsal striatum (caudate/putamen). The ratio of 5-HIAA/5-HT, was unaltered in the ND-treated animals, compared to controls.

Our results are in accordance with other studies demonstrating a decreased 5-HT concentration after AAS administration (Bonson et al., 1994; Grimes & Melloni, 2002). Grimes and Melloni (2002) observed decreased anterior hypothalamic serotonin concentrations in hamsters treated with an AAS cocktail. Serotonin and 5-HIAA concentrations are also significantly decreased in the hippocampus after testosterone propionate treatment in rats (Bonson et al., 1994). Our results, as well as the studies referred to above, are, however, not in agreement with the findings by Thiblin and colleagues (1999) and by Tamaki et al. (2003). Thiblin et al., (1999) reports that administration of testosterone propionate, nandrolone propionate, methandrostenolone or oxymetholone [5 mg/kg/day 6 times for 1 week] increased the 5-HT metabolism (i.e. 5-HIAA/5-HT ratio) in the hippocampus. The same authors further demonstrated that methandrostenolone administration increased 5-HT metabolism in the hypothalamus and that treatment with oxymetholone and testosterone propionate increased 5-HT metabolism in the frontal cortex (Thiblin et al., 1999). Tamaki et al. (2003) also observed a significantly increased levels of 5-HIAA in the hypothalamus and a clear trend towards an increased 5-HT concentration in cerebral cortex and hypothalamus after one injection of ND [3.75 mg/kg] one week before decapitation. Compared to our ND treatment regime, animals in the work of Thiblin et al. (1999) received one injection of AAS per week for six weeks. Thus, the dose was much lower and other AAS compounds than ND were used. Another possible explanation for the conflicting results may be the time interval between AAS treatment and decapitation. In this thesis, the animals' brains were removed four weeks after the last ND injection, whereas Thiblin et al. (1999) decapitated their animals two days after the final ND injection. It is thus possible that the AAS-induced changes of monoaminergic activity vary over time depending on when the brain is taken for analysis in relation to the last AAS injection. By reason of the divergent result concerning the effect of AAS on monoamine concentrations in the rodent brain, it is of great importance to further explore the effects of AAS on the monoaminergic system, especially with focus on the effect of various AAS compounds, doses, treatment schedules, and time intervals between treatment and decapitation. Results from such studies may have important implications for understanding and clarifying to what extent the monoaminergic system can be related to the observed AAS-induced behaviours. The reason why the monoaminergic system is of interest is that animal experimental studies, but also clinical studies, have shown that a dysfunction of the 5-HT system may be associated with disinhibitory behaviours. Firstly, an extensive literature implicates a dysfunction of 5-HT in disorders involving aggressive and violent behaviours both in humans (Higley & Bennett, 1999; Linnoila et al., 1989; Miczek et al., 1994; Tuinier et al., 1995; Virkkunen et al., 1989) and animals (Linnoila et al., 1989; Virkkunen et al., 1989). Secondly, studies have demonstrated a relationship between 5-HT dysfunction and drug abuse, especially excessive alcohol consumption, both in humans and animals (Berggren, Eriksson, Fahlke, & Balldin, 2002; Higley & Bennett, 1999; Koob, Roberts et al., 1998; LeMarquand, Pihl, & Benkelfat, 1994a). Thus, it is possible that

the ND-treated rats' behavioural alterations seen in this thesis, could be explained by the ND-induced decreased serotonergic concentrations.

Results from this thesis found no alterations in the NA, DA, HVA and DOPAC concentrations, nor in the ratios of DOPAC+HVA/DA, between the ND-treated rats and controls. However, the work by Thiblin and colleagues (1999; using the same treatment regime for all AAS compounds [5 mg/kg/day 6 times for 1 week]) demonstrated that DA concentration was increased in the striatum after oxymetholone administration. Results from this study further showed that concentrations of its metabolites, DOPAC and HVA, were increased in the striatum after administration of testosterone propionate, nandrolone propionate, methandrostenolone and oxymetholone and that the dopamine metabolism (i.e. DOPAC+HVA/DA ratio) were increased in the striatum after testosterone propionate, nandrolone propionate, methandrostenolone treatment (Thiblin et al., 1999). Although our data did not differed significantly concerning DA concentrations in the dorsal striatum, the ND-treated group exhibited a higher DA concentration compared to controls.

Brain opioid peptide concentrations (paper I)

In this thesis, radioimmunoassay analyses of the opioid peptides, dynorphin B and MEAP, in the hypothalamus, nucleus accumbens, striatum and PAG were performed. The results showed that seven weeks after the end of the two-week ND/oil treatment period, the ND-treated rats had decreased concentrations of ir-dynorphin B in nucleus accumbens compared to the controls. Results from other studies, using the same treatment regime as in this thesis, have shown increased or unaltered levels of dynorphin B. For example, when measuring ir-dynorphin B in the amygdala, the cortex, the hippocampus, the hypothalamus, the nucleus accumbens, the pituitary and the VTA, only an increased concentration in the VTA was found (Johansson et al., 1997). Increased concentrations of ir-dynorphin B were later shown in the hypothalamus, the striatum and the PAG, but not in the VTA (Johansson et al., 2000). Results from studies using another AAS treatment regime, have shown decreased levels of ir-dynorphin B in the hypothalamus (Menard et al., 1995) and increased levels in the thalamus (Harlan et al., 2000). Results from this thesis concerning ir-MEAP further demonstrate that ND produces decreased levels in the PAG and increased levels in hypothalamus. However, other studies (also administration of 15 mg ND/kg/day for 2 weeks) have reported increased ir-MEAP activity in hypothalamus, striatum and PAG (Johansson et al., 2000) or no effect at all (Johansson et al., 1997). Taken together, these results indicate that ND may alter the opioid peptidergic system, although the results are inconclusive. Thus, further studies are needed in order to clarify the ND's effect on the opioid peptidergic system, since this system is suggested to mediate the brain reward system and various behaviours (reviewed in e.g. Mansour et al., 1995; Tordjman et al., 2003).

6. CONCLUSIONS

This thesis investigated behavioural and physiological effects of the anabolic androgenic steroid nandrolone decanoate (ND; Deca-Durabol®). Sexually matured male rats were treated with suprapharmacological doses of ND [15 mg/kg/day for 2 weeks, s.c.], in a treatment schedule developed to serve as a model for heavy human abuse of AAS. The following main results were observed:

- ND stimulated the establishment of dominant relationships in a provocative and competitive situation, an effect that was prominent for eleven weeks after the cessation of the ND treatment. ND also increased reactivity to physical provocations, an effect that was enhanced for two months after the end of the ND treatment. ND-induced decreased fleeing and freezing responses to a threatening stimulus were further observed.
- ND stimulated voluntary ethanol consumption and induced behavioural tolerance to ethanol.
- ND treatment's enhancing effect on reactivity to physical provocations and, to some degree, on voluntary ethanol consumption, was accentuated by voluntary physical exercise.
- ND decreased body weight gain, induced thymus atrophy and increased the weight of the adrenal glands.
- ND altered concentrations of the monoamine acid serotonin and of the brain opioid peptides dynorphin B and MEAP, in various brain areas.

The results from this thesis suggest that abuse of ND may constitute a risk factor for induction of an array of behavioural complications, such as increased aggression and enhanced alcohol drinking. ND further affected physiological parameters like the HPA-axis and neurotransmitters concentrations. These results hopefully bear relevance for further research and in clinical settings when in contact with individuals abusing AAS.

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APPENDIX

Papers I, II and IV are reproduced with kind permission by Elsevier Science Inc.

I: Johansson, P., Lindqvist, A-S., Nyberg, F. & Fahlke, C. (2000). Anabolic androgenic steroids affects alcohol intake, defensive behaviors and brain opioid peptides in the rat. *Pharmacology Biochemistry and Behaviour 67(2)*: 271-280.

II: Lindqvist, A-S., Johansson, P., Nyberg, F. & Fahlke, C. (2002). Anabolic androgenic steroid affects competitive behaviour, behavioural response to ethanol and brain serotonin levels. *Behavioural Brain Research 133(1)*: 21-29.

III: Lindqvist, A-S., Jonsdottir, I. H., Nyberg, F. & Fahlke, C. Physical exercise accentuates the enhancing effects of Nandrolone decanoate on reactivity to physical provocations and on voluntary alcohol intake in male rats. *Submitted, 2004*.

IV: Lindqvist, A-S. & Fahlke, C. (in press). Nandrolone decanoate has long-term effects on dominance in a competitive situation in male rats. *Physiology and Behavior*.