Behavioural and Neuropharmacological Aspects of Experimental Alcohol Addiction in Rhesus Monkeys

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BEHAVIOURAL AND NEUROPHARMACOLOGICAL ASPECTS OF EXPERIMENTAL ALCOHOL ADDICTION IN RHESUS MONKEYS

Gedrags- en neurofarmacologische aspecten van experimentele alcoholverslaving bij rhesusapen

(Met een samenvatting in het Nederlands)

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PREFACE

In many societies alcohol drinking is a common and socially accepted custom. Alcohol can be consumed for several reasons, e.g. for its taste, in a ceremonial ritual, to grace social events, and because of its behavioural effects. Alcohol can impair the health and social well-being of the consumer and of his/her environment, when ingested too frequently or in too high amounts. The adverse consequences of excessive alcohol drinking are quite well-known and documented, and include medical, psychological and socio-economical problems (National Institute on Alcohol Abuse and Alcoholism, U.S.A. (NIAAA), 1990). Less is known about the behavioural, i.e. psychoactive effect(s) of alcohol and about why some individuals, despite serious risks, are not able to reduce or stop their alcohol consumption (Von Wartburg, 1990). In other words, we know very little about the onset and processes of addiction, that can occur with alcohol drinking. Moreover, successful treatments are not available (Reid and Carpenter, 1990). Monitoring the initial development of alcohol addiction is practically impossible in humans. Therefore, experimental addiction research is for an important part committed to animal studies. In this thesis spontaneous development of alcohol drinking and addiction was studied in rhesus monkeys, because rats were considered less suitable for this purpose. The aim was to gain more insight in the behavioural aspects of alcohol drinking and in addition to explore the possibilities of a neuropharmacological approach in the treatment of alcohol addiction.

Part I of this thesis provides an overview of existing hypotheses on alcohol addiction and of the experimental work performed for this thesis. In addition, the significance of the results for providing an experimental model for alcohol addiction and for a role of neuropharmacological treatment in addiction is discussed.

Part II includes the scientific reports on the subject.

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Part I

Experimental Alcohol Addiction in Rhesus Monkeys

1. HYPOTHESES ON ALCOHOL ADDICTION

1.1. ALCOHOL AND ALCOHOL USE DISORDERS

Public Health

During the last decades the harmful consequences of alcohol have been considered worldwide as a major threat for public health (Radouco-Thomas et al., 1979; Gordis, 1988; NIAAA, 1990). This holds true also for the Netherlands, where the pure alcohol consumption per citizen increased from 2.6 l per year in 1960 to 8.3 l per year in 1988 (de Zwart, 1989). In general, 8 to 10 % of the Dutch population of 15 years of age and older has alcohol-related problems (Projektgroep Alcohol Voorlichtings Plan, 1990). These problems are widespread and concern acute incidents, e.g. uncontrolled behaviour and traffic incidents due to alcohol intoxication, as well as chronic consequences, e.g. liver damage, pancreatitis, gastritis, impotence, heart disease, neuropathy, encephalopathy, memory disorders, psychosis and dementia (NIAAA, 1990). The costs for society are estimated to be at least 2 billion Dfl per year and the extent and variety of alcohol problems demand a lot from the medical and psychosocial service facilities (Projektgroep Alcohol Voorlichtings Plan, 1990). Since 1986 the Dutch government (Ministry of Welfare, Public Health and Cultural Affairs, or WVC) has implemented alcohol discouragement policies aiming at improving awareness among the general public of the numerous risks of alcohol drinking (Staatsuitgeverij, 1986). These policies, which mainly have a preventive and educative character, are not without success in the sense that the average alcohol consumption per citizen per year decreased by 8.3% from 1987 to 1989 (Projektgroep Alcohol Voorlichtings Plan, 1990). However, this success leaves out a number of people (about 60 to 90% of the problem drinkers) who are not able to control or reduce their drinking habit, despite their serious alcohol problems (Projektgroep Alcohol Voorlichtings Plan, 1990), and thus constitute a chronic demand on public service and health care facilities (de Zwart, 1989).

Alcohol Use Disorders

It has been recognized for quite some time that "alcoholics" represent a heterogeneous group, having different socio-economical, educational and cultural backgrounds, and that multiple factors can contribute to the development and maintenance of alcoholism (Jellinek and Jolliffe, 1940; van Dijk, 1979; Marlatt et al., 1988; Mendelson and Mello, 1989, NIAAA, 1990).

Various definitions and diagnoses of "alcoholism" have evolved in the past 20 years, reflecting changing concepts of the basis and the nature of alcohol use disorders (Marlatt et al., 1988). The term "alcohol dependence" has eventually been preferred to the term "alcoholism". (APA, DSM-III, 1980; WHO, ICD-9, 1978; Caetano, 1985).

Signs of physical habituation to alcohol, like tolerance (i.e. when the same amount of alcohol exerts less effect due to repeated use) and withdrawal symptoms (the complex of specific symptoms, when regular or sustained drinking is stopped abruptly), have long been considered important criteria for the diagnosis of alcohol dependence. A more recent view is that physical habituation to alcohol (or other drugs) and drug-taking behaviour represent different aspects of drug-related disorders (Meisch, 1982; Van Ree, 1987; Wise and Bozarth, 1987; Marlatt et al., 1988). In the current edition of the DSM-III (APA, DSM III-R, 1987) more emphasis has been put on behavioural criteria for diagnosis of alcohol dependence, such as a persistent desire for alcohol, one or more efforts to cut down or control substance use, a great deal of time spent in activities necessary to get the substance and continued use despite the related problems.

The different and changing terminologies to identify an "alcoholic" indicate that this is apparently quite a complicated and multi-dimensional problem. Again, revisions of diagnostic criteria in both DSM-III-R (DSM-IV) and ICD-9 (ICD-10) are being prepared (NIAAA, 1990).

Alcohol Addiction

To date the definition of "drug dependence" still leads to different interpretations. For some professionals, dependence definitely includes signs of physical habituation to a drug ("physical dependence"), (APA, 1987; WHO 1978). Some experimental investigators use "dependence" to indicate drug-reinforced behaviour, independent of signs of physical dependence (Meisch and Thompson, 1974a; Kalant et al., 1978; Van Ree, 1979). Occasionally the term "psychic" dependence is used for this sense of dependence (Deneau et al., 1969; Wise and Bozarth, 1987; Sweep et al., 1989).

In this thesis the term "addiction" is chosen to refer to "repeated self-administration of a drug, such that the user will engage in substantial amounts of behaviour leading specifically to further administration of the drug, and will continue to administer this drug even when this requires the sacrifice of other behaviours, or implicates adverse consequences" (Kalant et al., 1978; Van Ree, 1979; Marlatt et al., 1988). Addiction is thus used to describe a behavioural disturbance, and is not considered to be the consequence of some drug-specific physical adaptation (Kalant

et al., 1978; Mendelson and Mello, 1979a; Schuster and Johanson, 1981; Meisch, 1982; Wise and Bozarth, 1987; Marlatt et al., 1988; Samson and Grant, 1990). It is assumed that addiction is a form of learned behaviour in a particular context of personal and environmental factors. Alcohol addiction hence constitutes a problem in which sociological, psychological and biological factors are involved (Zucker and Gomberg, 1986; Marlatt et al., 1988; Reid and Carpenter, 1990). Different stages in addiction have been distinguished: acquisition of the behaviour, maintenance of ongoing use, and attempts at reduction or cessation of the behaviour (Marlatt et al., 1988; Goldberg et al., 1990).

Treatment of Alcohol Addiction

Most people who have difficulties in controlling their drinking behaviour discover this when they have to reduce or abstain from drinking for some extended period (Marlatt et al., 1988). About 10 to 30% succeeds nevertheless without help; the others fail (Projektgroep Alcohol Voorlichtings Plan, 1990). Some people participate in self-help groups, like Alcoholics Anonymous. Professional help is based mainly on detoxification, a period of controlled abstinence and psychosocial/psychiatric assistance. Sometimes a drug like disulfiram (tetraethylthiuramdisulfide) is sup-plied to influence the alcohol metabolism, so that it gives rise to headaches and nausea after alcohol ingestion. However, such "punishing' effects have not proved very successful in abolishing alcohol use for a long term (Kalant et al., 1978; Griffiths et al., 1980). Relapses into the previous habitual pattern of alcohol drinking occur frequently.

Relapsing into a habitual pattern of drug self-administration constitutes a major problem in drug addiction in general (Marlatt and George, 1984; Anokhina et al., 1987; Horwitz et al., 1987; Barnes, 1988) and is an ill-understood phenome-non (Dole, 1986). It shows that addictive behaviour is a very persistent element in the life style of addicts (Kalant et al., 1978), notwithstanding the current intervention techniques (Reid and Carpenter, 1990).

1.2. ANIMAL MODELS OF ALCOHOL ADDICTION

The study of the etiology and initial development of addiction in humans is quite problematic, since problems are generally recognized only when they already have been firmly established and have caused medical and/or environmental complications. On the other hand, exposing healthy persons to addiction-inducing risks is quite unethical.

Research on and evaluation of treatment methods frequently appears to be obstructed by methodological problems: e.g. self-reports of alcoholics are questionable, a variety of treatments are imposed concurrently, there are medical and psychiatric complications and spontaneous recovery cannot be excluded. Furthermore, experimental pharmacological therapies need extensive and controlled testing in animals, before clinical applications can be permitted. Hence it would be valuable if (several aspects of) alcohol addiction could be studied in experimental animal models.

In parallel with historical changes in the definitions and diagnoses of "alcoholism", concepts of and criteria for "valid" animals models of alcoholism (McClearn, 1988) seem to have changed as well. During the last decades a variety of techniques and experimental designs have been developed to construct a valid animal model for alcohol addiction (Cicero, 1980; Holman, 1986; Samson et al., 1988). Most of them have been seriously criticized and some authors have doubted whether it would be possible anyway to reproduce this "typically human" problem in animals (Lester and Freed, 1973, Cicero, 1980; Dole and Gentry, 1984; Kalant, 1988). Nevertheless, these models have provided (and are still doing so) the empirical base of our understanding of factors involved in human condition(s) (Schuster and Johanson, 1981; Samson and Li, 1988; McClearn, 1988; Reid and Carpenter, 1990).

Passive Administration Models

Animals can be made physically dependent on alcohol by repeated nasogastric intubation (Ellis and Pick, 1972; Pieper and Skeen, 1975), intravenous (i.v.) infusion (Winger, 1988), prolonged ethanol vapor inhalation, or by restricting them to a liquid alcohol-containing diet (Hunter et al., 1974; Ho et al., 1978; Allen et al., 1982). Induction of physical dependence on alcohol, measured by with-drawal symptoms when abstaining, appeared however not sufficient to maintain drinking of alcohol (Hunter et al., 1974; Allen et al., 1982; Winger, 1988). Such models therefore lack the most characteristic feature of human alcohol addiction: a sustained wish to consume alcohol (Lester and Freed, 1973; Ho et al., 1978; Dole and Gentry, 1984; Stewart and Grupp, 1989). An important criterion for experi-mental animal models of addiction therefore is that animals have to self-administer alcohol (Lester and Freed, 1973; Meisch, 1977).

Self-Administration Models

Schedule-induced Polydipsia

Schedule-induced polydipsia refers to the excessive water drinking that occurs when food-deprived animals are given small pellets of food at the rate of about one pellet per minute (Tang and Falk, 1983). When an alcohol solution instead of water is given, large amounts of alcohol are consumed (Falk and Tang, 1988). Although this procedure can produce physical dependence in animals, the relevance of schedule-induced alcohol drinking to human addictive behaviour is still a matter of discussion (Meisch, 1982; Grant and Johanson, 1988).

Preference Studies

The 2-bottle water-alcohol preference procedure has been used in numerous studies (Richter and Campbell, 1940; Meisch, 1982; Samson and Grant, 1990), for a free-choice situation was presumed to permit a determination of the animal's motivation for alcohol (Meisch, 1982; Dole and Gentry, 1984). However, rats generally did not prefer alcohol to water when alcohol concentrations were higher than 6 per cent (wt/vol) (Meisch, 1982; Samson et al., 1988; Kiefer and Dopp, 1989) and intoxication and physical dependence were rarely seen (Richter and Campbell, 1940; Mendelson and Mello, 1964; Rick and Wilson, 1966; Veale and Myers, 1969; Dole et al., 1988). The general conclusion was that palatability of alcohol decreased rapidly as the concentration of alcohol increased (Kiefer and Dopp, 1989). In addition, because presence of intoxication and physical dependence were once considered to be quite important criteria (Lester and Freed, 1973; Cicero, 1980; Crowley and al. 1983; Dole and Gentry, 1984), preference studies were seriously criticized (Cicero, 1980; Meisch, 1984; Stewart et al., 1988). Other flaws were related to the low frequency of measurement (once per 24 h), lack of control for individual preferences to drink at a specific location, absence of blood ethanol determination and a lack of distinction between palatability determined and/or ethanol-directed behaviour (Meisch, 1984; Stewart et al., 1988).

Operant Studies

- *Intravenous Injection Studies*

  In the 1960s a line of research developed in which laboratory animals could intravenously inject psychoactive drugs, including alcohol, into themselves (Deneau et al., 1969; Meisch, 1982). Based on these studies it has been concluded that all those drugs known to be addictive in humans (although from distinct pharmacological classes) produced self-administration behaviour in animals, whereas psychoactive drugs which humans do not abuse, like e.g. antidepressants, did not lead to self-administration (Van Ree, 1979; Griffiths et

al., 1980). The hypothesis was formulated that common behavioural and/or biological determinants underly the use of addictive substances across-species (Griffiths et al., 1980). This supposition has strongly stimulated the interest in self-administration animal studies.

- *Drugs as Reinforcers*

The idea was established that drug-taking behaviour could be regarded as a particular case of operant behaviour (Meisch, 1987) that can be defined as behaviour controlled by its consequences (Skinner, 1953). This view placed drug-seeking behaviour in the conceptual framework of the behaviouristic learning theory (Skinner, 1938; Meisch and Thompson, 1974a; Mendelson and Mello, 1979a; Meisch, 1984). A drug was considered to be a positive reinforcer when it increased the probability of the recurrence of the self-administration of that drug (Kalant et al., 1978; Mendelson and Mello, 1979a; Walker, 1987). The (degree of) addiction could thus be defined in terms of the reinforcing strength of a certain drug (Meisch and Thompson, 1974a; Kalant et al., 1978; Van Ree, 1979; Schuster and Johanson, 1981). Other concepts frequently used in operant drug studies are "punishment", "negative reinforcement" and "extinction".

An example of "punishment" of operant behaviour is the delivery of electrical shocks in rats. This however induced an increase in post-shock period ethanol consumption rather than attenuated it (Mello and Mendelson, 1966; Meisch 1977; Volpicelli et al., 1986). An clinical example of a "punishment" therapy is the treatment with disulfiram, by which alcohol causes unpleasant effects. This therapy does not generally lead to long-term success in humans. "Negative reinforcement" is used to describe behaviour that is directed to relieve an aversive state or condition (e.g. relieval of withdrawal symptoms; reduction of anxiety). With respect to withdrawal symptoms in experimental animals, these symptoms caused animals to refrain from alcohol consumption in stead of performing operant behaviour to relieve the unpleasant condition (Myers et al., 1972; Hunter et al., 1974, Falk and Tang, 1988; Winger, 1988).

"Extinction" is a process in which previously reinforced operant behaviour is no longer followed by reinforcement and therefore declines in frequency and/or probability (Griffiths et al., 1980). In operant drug-responding the decline tends to be relatively rapid when no reinforcement is experienced. Furthermore, when the availability of drug reinforcement is reintroduced, drug self-administration rapidly returns. In other words, the removal and reinstatement of drug reinfor-cement can be quickly recognized by both animals and humans and operant behaviour changes rapidly in response to these changing conditions (Sinclair, 1968; Meisch and Thompson 1974a; Meisch, 1977; Schuster and Johanson, 1981). This could be an important factor for the relapse phenome-

non in (alcohol) addiction (Stewart et al., 1984; Goldberg et al., 1990).

- *Alcohol as Oral Reinforcer*

The i.v. route of administration was considered an inadequate model for human alcohol addiction, because this route skips the orogastric effects of alcohol that human drinkers experience (Samson et al., 1988). Oral operant self-administration studies were introduced in which, during daily experimental sessions, animals had to make responses by lever pressing in order to obtain a certain amount of alcohol solution. The rate and frequency of lever pressing and the consumed volumes could thus be compared to similar responses for other reinforcers, like water or sucrose solution. In this way the reinforcing strength of ethanol was determined and could be compared with other reinforcers (Kalant et al., 1978).

In comparison with other psychoactive agents, alcohol's initial reinforcing capacities have been found to be less robust (Meisch, 1977; Crowley and Andrews, 1987; Tabakoff and Hoffman, 1987; Samson et al., 1988; Samson and Grant, 1990). Animal addiction studies revealed that acquisition of oral alcohol consumption was rather problematic (Meisch and Thompson, 1974a; Meisch, 1977; Griffiths et al., 1980; Crowley et al., 1983; Stewart et al., 1988). Particularly in rats, taste and smell aversion seemed to represent major problems in initiating spontaneous alcohol ingestion in meaningful amounts (Meisch, 1977; Myers and Ewing, 1980; Samson et al., 1988). Furthermore, alcohol is assumed to reinforce alcohol consumption mainly by its postingestional intrinsic effects (Meisch and Thompson, 1974a; Meisch, 1977). For a naive animal the delay of onset of postingestional effects also might obstruct acquisition, because of a weak response-reinforcement contingency (Carroll 1987; Kiefer and Dopp, 1989; Samson and Grant, 1990). Therefore, investigators have put effort into enhancing the initiation of oral alcohol consumption in infrahuman subjects (Meisch and Thompson, 1974a) using various induction techniques such as schedule-induced polydipsia, i.e. establishing food-induced drinking and then substituting water for low concentrated alcohol solutions (Meisch, 1984; Stewart et al, 1988); water and food deprivation; weight reduction; restricting drinking episodes to a few hours per day (Meisch and Thompson, 1974a; Meisch, 1984; Marcucella and Munro, 1987); and adding attractive flavours into beverages (Cicero, 1980; Meisch, 1984; Crowley and Andrews, 1987; Samson and Grant, 1990).

Operant oral alcohol self-administration studies have been reviewed extensively (Meisch 1977; Kalant et al., 1978; Griffiths et al., 1980; Meisch 1984; Marlatt et al., 1988), and only those common findings that are most relevant for the subject of this thesis are summarized here.

* Alcohol did function as oral reinforcer in various species of animals by

using a variety of inductions techniques.

* Under conditions of food-deprivation and weight reduction, acquisition occurred quickly and intake levels reached high levels.
* Acquisition was facilitated when procedures started at low alcohol concentrations that gradually increased.
* Intakes varied according to learning-theoretical laws in an orderly way as a function of a number of variables.
* Physical dependence appeared not necessary to generate and maintain drug-induced reinforcement, including that of alcohol.

Food-deprivation and bodyweight reduction was considered requisite for producing acquisition of alcohol drinking, but the underlying mechanism was not so clear (Meisch, 1984; Stewart et al., 1988). Although the initial interpretation was that increases under food-deprivation were due to the caloric property of alcohol, the phenomenon also appeared to exist for other non-caloric drug reinforcers. Nevertheless, a significant decline in drug intake usually is observed when free food access is reinstated (Meisch and Thompson, 1974b; Crowley et al,. 1983; Crowley and Andrews, 1987).

Altogether, the operant conditioned oral self-administration model has provided important knowledge on alcohol consumption and is used in many experimental addiction studies. Still, artificially-induced, conditioned initiation procedures and very strict experimenter-regulated sessions, do not permit the study of a more natural animal-regulated acquisition under more complex environmental circumstances (Goldberg et al., 1990). The conventional operant design might therefore reflect only a part of ethanol's effects, compared to the heterogeneity of human alcohol use (Kalant, 1988; Goldberg et al., 1990).

1.3. FACTORS IN ALCOHOL DRINKING AND ADDICTION

The impact of the operant learning approach was that the research on alcohol addiction has been placed within a theoretical framework, in which interactions between a subject and his environment could be manipulated and systematically investigated (Schuster and Johanson, 1981; Van Ree, 1987; Goldberg et al., 1990; Samson and Grant, 1990). Accordingly, addiction research has been directed to the role of variables within an individual, as well as to the influence of environmental factors on an individual.

Current Environmental Circumstances

The amount and pattern of alcohol consumption can be manipulated by varying e.g. the route of administration, the feeding condition, the alcohol concentration in a beverage, the effort necessary to obtain alcohol, the schedule of reinforcement, the availability of alcohol and duration of access, and the presence of concurrent reinforcers (Kalant et al., 1978; Griffiths et al., 1980; Schuster and Johanson, 1981; Meisch, 1987). Furthermore, present conditioned environmental stimuli of a second order (initially neutral stimuli that have become associated with alcohol drinking) might be important variables in precipitating alcohol consumption (Stewart et al., 1984; Siegel, 1985; Goldberg et al., 1990).

Social Factors

Social influences can be investigated in epidemiological studies, by determining the per capita consumption, trends in drinking habits, types of problems, and influences of the socioeconomic environment on (sub)population(s) in society (Staatsuitgeverij, 1986; de Zwart, 1989; NIAAA, 1990). Developments of multidimensional prevention programs and public health education are based on this kind of information. In animals, the interactions between social status (dominance rank) or social circumstances (single or in group) and alcohol consumption have been studied only occasionally (Cadell and Cressman, 1972; Ellison, 1981; Crowley and Andrews, 1987; Winslow and Miczek, 1988).

Individual Circumstances

Individual life history (e.g. raising conditions, severe loss, disease, psychopathology) and the degree of experience in the consumption of alcohol (or other drugs) can influence a person's drinking habit too. Furthermore, the psychological situation in which an individual can be (e.g. stress, danger, euphoria) can have impact on the probability and the effects of alcohol drinking (Kalant et al., 1978). It has been postulated that exposure to stress and/or anxiety can be a stimulatory factor for alcohol consumption (Volpicelli et al., 1986; Gianoulakis et al., 1990).

Genetic Factors

Quite some interest exists for the role of genetic markers in the etiology of

alcohol addiction (Schuckit, 1986; Gilligan et al., 1987; Holden, 1991). Human adoption and twin studies seem to suggest a genetically transmitted vulnerability to alcohol addiction, at least in some subgroup of the alcoholic population, but mechanisms of genetic transmission are unknown (Kaij and McNeil, 1979; Schuckit, 1987; Marlatt et al, 1988; NIAAA, 1990;). A presumed genetic predisposition (i.e. to be a member of a family with high incidence of alcohol addiction) does however not imply predestination or inevitability, since only some members of such families develop alcohol addiction (Zucker, 1986; Reid and Carpenter, 1990). Although genetic factors may interact with environmental influences in the development of certain patterns of alcohol consumption, the etiological loading of genetic versus environmental influences seems to vary from individual to individual (Cloninger, 1987).

In animal research, selective rodent strains have been bred with either low or high preference for alcohol (Eriksson, 1968; Tabakoff and Ritzmann, 1979; Li et al., 1981; Daoust et al., 1987; Elmer et al., 1988) in order to identify biochemical, physiological and behavioural differences between those strains. The best studied strains are the AA and ANA rats bred at the research laboratories of ALKO in Finland and the P-and NP-rats from the laboratory in Indianapolis, USA. AA- and P-rats consume large and ANA- and NP-rats very small amounts of alcohol (Li and Lumeng, 1984; George, 1987; Suzuki et al., 1988). These findings suggest that genetic components exist in the degree and nature of alcohol drinking behaviour of rodents, although it is unlikely that a complex disorder like alcohol addiction (or even alcohol preference) could ever be fully explained in terms of the action of a single gene (NIAAA, 1990; Holden, 1991). Nevertheless, these studies might contribute to define distinct biological substrates involved in the multiple effects of alcohol (George, 1987; McBride et al., 1990). Hypotheses on the involvement of biological substrates in alcohol addiction will be discussed in the following paragraph.

1.4. NEUROBIOLOGICAL ASPECTS OF (ALCOHOL) ADDICTION

The principal effect of alcohol (and of other psychoactive drugs) in humans is the impingement upon the central nervous system (CNS) (Myers and Ewing, 1980). Currently new perspectives in the field of experimental addiction research are developing in which neurobiological aspects of alcohol drinking and alcohol-related problems are considered important factors in alcohol addiction (Hsu, 1990; Reid and Carpenter, 1990; Samson et al., 1990). Alcohol appears to affect many processes in the CNS, the functional implications of which are not completely understood yet (Hsu, 1990; NIAAA, 1990). It is postulated that (some aspects of)

alcohol and other addictions are mediated by common brain mechanisms that are involved in the reinforcement of behaviour, including drug-taking behaviour (Van Ree, 1986, Wise and Bozarth, 1987; Sweep et al., 1989; Kiyatkin, 1989; Erickson, 1990; Koob and Weiss, 1990).

Brain Reward Mechanisms

The phenomenon of operant electrical brain self-stimulation in non-deprived animals (Olds and Milner, 1954) resulted in the notion of the existence of substrates in the brain (reward or pleasure centers) for reinforcement of behaviour (Kiyatkin, 1989). Observations in humans showed that electrical stimulation of some brain areas (self-stimulation sites in animals) produced sensations of indefinite pleasure, a sense of joy and well-being (Heath, 1963). Olds (1976) wrote: "All pleasures (of food, drinks, sex, play, art, and of other activities) are felt because they somehow activate a specialized reward circuitry in the brain" (Kyatkin, 1989).

The brain self-stimulation studies had important consequences for theories on the motivation for behaviour, because drive states (e.g. hunger, thirst, sex, i.e. negative reinforcement) appeared not requisite to initiate behaviour (Ettenberg, 1989; Reid and Carpenter, 1990).

The notion developed that the reinforcing effects of addictive drugs might be mediated by the same brain reward mechanisms involved in the natural reinforcement of behaviour and in self-stimulation (Crow, 1972; Nichols, 1972). Dysfunction of reward-related systems might then be the cause for impaired ability to experience pleasure or reward (anhedonia) and thus be related to affective illnesses (Fibiger and Phillips, 1987; McCarter and Kokkinidis, 1988; Ettenberg, 1989).

The lateral hypothalamic medial forebrain bundle (MBF) and the ventral tegmental area (VTA) have been considered to be important, perhaps indispensable, structures for reward activity (Bozarth, 1987), although recently also other regions have been supposed to represent reward pathways (Van Ree and Ramsey, 1987; Blander and Wise, 1989).

As neurochemical mediator, the catecholamine system has received most attention (Wise, 1978; Bozarth, 1987). Particularly, mesolimbic dopamine projections, which arise primarily from neurons in the VTA and innervate limbic ("emotion-related") regions, such as the nucleus accumbens, lateral septum, olfactory tubercle and amygdala, and mesocortical dopamine projections (Van Ree, 1987; McBride et al., 1990), were found to be critically related to brain stimulation reward and behaviour reinforcement (Bozarth, 1987; Fibiger and Phillips,

1987; Van Ree, 1987). Subsequent research has pointed out that non-dopaminergic systems can play an important role in brain reward processes as well (Amit and Brown, 1982; Van Ree and Ramsey, 1987). The opioidergic system and neuropeptides have been mentioned particularly in this respect (Van Ree 1987; Schaeffer, 1988; Stein and Belluzi, 1989; Bain and Kornetsky, 1990).

Furthermore, neuroanatomical and neurochemical evidence has been found for the existence of separate mechanisms of physical drug dependence and tolerance (Van Ree, 1987; Bain and Kornetsky, 1990), which appeared to be mediated outside the ventral tegmentum (e.g. in the periventricular gray region) and might be involved in negative reinforcement processes (Liebman, 1985; Wise and Bozarth, 1987).

Ethanol and Brain Reward

The effects of ethanol on brain stimulation reward have been found to be more intricate, in comparison to other addictive agents, such as psychomotor stimulants and opiates (Samson and Li, 1988; Schaefer and Michael, 1987; Bain and Kornetsky, 1990; Lewis and June, 1990). Ethanol's facilitatory effects on brain self-stimulation seem to depend critically on the blood(B) alcohol(A) concentration (C) (only ascending limb of the BAC), the dose ingested (low), the sites of stimulation (lateral hypothalamic), and the method of ethanol administration (only when self-administered) (Erickson, 1990; Kornetsky et al., 1988; Lewis and June, 1990; Moolten and Kornetsky, 1990). Because of the apparently less robust effects of ethanol in brain reward processes, it has been proposed that alcohol addiction, in comparison to other drug addictions, might be relatively more influenced by other factors (Bain and Kornetsky, 1990).

Ethanol and Neurotransmitters

Like in other drug self-administration studies, the effect of pharmacological manipulation of neurotransmitter activities (chemical messenger systems) on alcohol consumption is considered an important issue of research (Koob and Weiss, 1990). Ethanol does not have a specific receptor system in the CNS (Tabakoff and Hoffman, 1987; Reid and Carpenter, 1990), by contrast to other drugs of abuse, such as e.g. dopamine receptors for cocaine, opiate receptors for opiates and GABA receptors for benzodiazepines. Although its way of action is not exactly elucidated, ethanol probably exerts its effects by altering the structure of neuronal cell membranes, thus affecting synaptic transmission functions

(Topel, 1985; Tabakoff and Hoffman, 1987). Depending on the dose ingested, ethanol can exert positive reinforcing effects as well as anxiolytic, depressant and toxic effects (Erickson and Kochar, 1985; Wise and Bozarth, 1987; Prunell et al., 1987). These different aspects are probably mediated through interaction with distinct neurobiological mechanisms (Cloninger, 1987; NIAAA, 1990). With regard to positive reinforcement processes, ethanol has been reported to interact with various monoaminergic neurotransmitters, like dopamine (Wise and Bozarth, 1987; Anokhina et al., 1988; Pfeffer and Samson, 1988), serotonin (5-HT) (Signs and Schechter, 1988; Erickson, 1990; Koob and Weiss, 1990), norepinephrine (Amit and Brown, 1982; Kraemer et al., 1985) and, more recently, with neurohormones and opioid neuropeptides (Topel, 1985; Anokhina et al., 1987; Van Ree, 1987; Erickson, 1990; Gianoulakis et al., 1990). The GABA (gamma-aminobutyric acid)-benzodiazepine receptor complex might play a specific role in the anxiolytic and depressive effects of ethanol (Bowers and Wehner, 1989; Hsu, 1990; NIAAA, 1990) and a very recent finding is the involvement of the NMDA (N-methyl-D-aspartate)-receptor of the glutamate system in ethanol intoxication. Hence, several neurotransmitters, hormones and other factors are possibly involved in mediating initiation, maintenance and cessation of alcohol drinking and addiction (Samson et al., 1990; McBride et al., 1990; Naranjo 1990). Ethanol's positive reinforcing effects have been postulated however to account primarily for the establishment of addiction, and to represent a prime motivational focus in a person's life (Reid and Carpenter, 1990).

Neuroendocrine Variables

Clinical studies in alcoholics have revealed many abnormalities in endocrine functions (Topel, 1985). Disturbances were found not only with respect to reproductive functions in alcoholic men and women (including low testosterone levels and testicular atrophy in men and amenorrhea, and ovary changes in women) (Mello et al., 1985; Mello et al., 1988; Widenius et al., 1988), but also with respect to hypothalamic-pituitary-adrenal (HPA) hormonal functions implying β-endorphin, ACTH, cortisol, vasopressin, growth and thyroid hormones (Marks, 1979; Genazzani et al., 1982; Abou-Saleh et al., 1984; Männistö et al., 1987; Heuser et al., 1988; Müller et al., 1989). Furthermore, abnormal endocrine responses have been found to occur in "high-risk" individuals (being non-alcoholic members of families with high incidence of alcohol addiction) and in abstinent alcoholics, suggesting some relation between endocrine dysfunction and susceptibility to alcohol addiction (Schuckit 1987; Schuckit, 1988; Gianoulakis, 1990). A critical site for endocrine regulation is the hypothalamus, which produces

releasing factors which subsequently signal the pituitary to release various hormones into the circulation (Rivier, 1989). Both in men and animals, ethanol has been reported to significantly affect corticotropin-releasing factor-(CRF) related hormonal responses, that are known to be involved in stress reactions (Schuckit et al. 1988; Patel and Pohorecky, 1989; Pohorecky, 1990; Rivier, 1989). It has been postulated by some authors that the increased susceptibility of some individuals to alcohol addiction, as well as to affective illness, in fact might reflect a decreased ability to cope with stress (Cappel and Herman, 1972; Neff, 1985; Steiger et al., 1985; Kling et al., 1989; Gianoulakis et al., 1990). However, other hypotheses about the function of pituitary-related hormones in alcohol addiction have been formulated as well (Van Ree, 1986; Schuckit, 1988).

1.5. NEUROPEPTIDES AND ADDICTION

The effect of pituitary hormones on behaviour was discovered in hypophysectomized animals, that displayed behavioural disturbances, which could in turn be reversed by hormonal treatment (De Wied, 1964; De Wied, 1977). Structure-activity studies showed a dissociation between the classical endocrine action and the central action of these hormones (De Wied et al., 1972; De Jong et al., 1985); small parts of the molecule, that were devoid of endocrine effects, appeared to have specific effects on CNS functions (De Wied, 1977). Such peptide molecules that specifically affected CNS functions were indicated as neuropeptides (De Wied et al., 1974).

Many of the hypothalamic releasing factors and pituitary hormones appear to generate neuropeptides, that also are present in brain areas other than hypothalamus and communicate with various transmittter systems (Van Ree, 1983; Topel, 1985; Van Ree, 1986). Hormones (and their fragments) thus can function also as neurohormones and, like neurotransmitters, can affect central synaptic transmission and subsequent behaviour (De Wied, 1978; Koob and Bloom, 1982; Iyengar et al., 1989; Barna et al., 1990).

This knowledge led to the hypothesis that hormonal systems, present in pituitary and the brain, play a critical role in behavioural homeostasis and that disturbances in the (neuro)hormonal systems may lead to psychopathology, including addiction (De Wied, 1978; Van Ree, 1986; Anokhina et al., 1987).

Neuropeptides can be derived from precursor molecules with classical endocrine effects, like e.g. pituitary hormones, but also from inactive storage molecules. The precursor molecules are metabolized by proteolytic enzymes resulting in the generation of neuropeptides; further enzymatic processing of the neuropeptides may yield neuropeptides of the second and third order. In the next paragraphs, the

neurohypophyseal and opioid neuropeptides will be discussed in particular, because they might function as common factors in drug as well as in non-drug addictions (Topel, 1985; Van Ree, 1986; Sweep et al., 1989; Van Ree et al., 1990).

Neurohypophyseal Neuropeptides

Vasopressin and Oxytocin

The neurohypophyseal hormones, vasopressin and oxytocin, are predominantly formed in the hypothalamus and stored in the posterior pituitary. The bloodstream acts as a transport route for these hormones to their target organs in the periphery. The classic endocrine function of vasopressin, or antidiuretic hormone, is the control of body fluid and blood pressure, and oxytocin has a function in milk production and during birth (Sawyer, 1964). In addition, distinct vasopressin and oxytocin neuronal pathways have been demonstrated in the brain, with terminals present e.g. in areas of the limbic system, hypothalamus, brain stem and spinal cord, thus gaining access to brain and behaviour processes (Zimmerman et al., 1977; Buijs et al., 1978; Van Ree, 1986). Initially, the central action of vasopressin-related peptides was found to be a facilitation of learning and memory processes, like consolidation and retrieval (De Wied, 1971; Bohus et al., 1978; Rigter et al., 1974), whereas oxytocin produced amnesic effects (Bohus et al., 1978; Van Ree et al., 1978). Learning and memory processes are likely to be involved in the reinforcement of behaviour, so that optimal adapted responses of an organism can be consolidated and retrieved. Hence, it was hypothesized that neurohypophyseal neuropeptides might also be of relevance in "learning", i.e. acquisition of, drug self-administration (Van Ree, 1986). A vasopressin-related neuropeptide, desglycinamide-(Arginine^8)-vasopressin (DGAVP), was identified, that was practically devoid of vasopressin's endocrine effects (De Wied et al., 1972). Subsequent experiments revealed that daily treatment (either subcutaneously, orally, or intracerebroventricularly) with DGAVP could decrease i.v. heroin and cocaine self-administration behaviour during acquisition in rats (Van Ree and De Wied, 1977a; Van Ree, 1982; De Vry et al., 1988; Van Ree et al., 1988). Moreover, DGAVP decreased the acquisition of ventral tegmental brain self-stimulation and of opiate self-administration directly into the ventral tegmental-substantia nigra area (Dorsa and Van Ree, 1979; Van Ree and De Wied, 1980). The C-terminal oxytocin-fragment PLG had an increasing effect (Van Ree and De Wied, 1980). The effect of DGAVP was attributed to attenuation of the positive reinforcing efficacy of drugs, possibly through interaction with mesolimbic reward pathways (Van Ree et al., 1978; Wise, 1978). The effects of DGAVP

became manifest after some days of treatment and appeared to be of a long-term nature, indicating that the action of DGAVP was physiological, rather than acute pharmacological (Van Ree and De Wied, 1977a; Van Ree and De Wied, 1977b). Thus, the hypothesis was formulated that neuroendocrine brain systems are involved in physiological processes underlying brain reward and as a consequence in the reinforcing effects of addictive drugs (Van Ree, 1987).

Research has been directed also to the influence of neurohypophyseal hormones on the development of tolerance and physical dependence on opiates and alcohol (Van Ree and De Wied, 1980), that can be regarded as adaptive processes more or less resembling learning and memory (Hoffman et al., 1979; Crabbe and Rigter, 1980; Reus, 1980). Since physical adaptation processes are not considered critical factors in positive reinforcement processes and the development of addiction (Meisch, 1982; Van Ree, 1987; Reid and Carpenter, 1990), these studies are not major points of interest in this paper.

Alcohol and DGAVP

The relationship between alcohol and neurohypophyseal neuropeptides, including DGAVP, has been studied primarily with respect to the development and maintenance of tolerance and physical dependence (Crabbe and Rigter, 1980; Hoffman and Tabakoff, 1984). Results appeared to be quite similar to those for opiates (Mucha and Kalant, 1979; Van Ree, 1980; Van Ree, 1986).

With respect to the interaction between DGAVP and initiation of ethanol consumption, animals have been studied exclusively under forced ingestion conditions (Finkelberg, 1978; Mucha and Kalant, 1979). DGAVP thus appeared to enhance acceptance of alcohol, and probably interacted with the tolerance for the aversive effects of high doses of alcohol. The effect on acquisition of alcohol self-administration behaviour, as has been investigated for heroin and cocaine, has not been described.

Opioid Neuropeptides and Addiction

Opioids

The discovery of specific opiate binding sites (Terenius, 1973; Simon et al., 1973) led to the idea that some endogenous substances with opiate-like characteristics might be naturally present as well (Hughes, 1975). Indeed several endogenous substances (opioids) were discovered in brain tissue and cerebrospinal fluid (CSF), that interacted with opiate receptors and exerted opiate-like activities, such as stimulatory, analgetic or narcotic effects, depending of the dose ingested (Hughes et al., 1975; Bradbury et al., 1976; Graf et al., 1976a; Beaumont and

Hughes, 1979). Subsequently, subtypes of opioid receptors have been distinguished (μ, δ, κ, ε), that are probably receptive for specific classes of opioid peptides (Martin et al., 1976; Chang and Cuatrecasas, 1979; Wuster et al., 1980; Cox, 1982). Three different precursors for opioid peptides are known, being the products of three distinct genes (Cox, 1982; Unterwald and Zukin, 1990). Proenkephalin is the precursor of the enkephalins; prodynorphin produces dynorphins and neoendorphins, and proopiomelanocortin (POMC) is the precursor for ACTH and β-lipotropin (β-LPH). The major site of production of POMC is the pituitary (Moon et al., 1973), but in addition POMC is found in peptidergic pathways with terminals in limbic structures and lower brain stem (Watson and Akil, 1980; Kachaturian et al., 1985; Barna et al., 1990). Particularly, POMC opioid peptides, also frequently indicated as endorphins (Cox, 1982; Koob and Bloom, 1982), have been related to reward and to addiction (Van Ree, 1983; Sweep et al., 1988) .

β-Endorphin

β-Endorphin, when administered intracerebroventricularly, appeared to be the most potent opiate-mimicking substance (Graf et al., 1976b). β-Endorphin is derived from the non-opiate-like precursor molecule β-lipotropin (β-LPH) and in turn is a precursor molecule itself for the neuroleptic-like γ-type endorphins and for the psychostimulant-like α-type of endorphins and for a number of non-opioid peptides (Burbach et al., 1980). β-Endorphin has affinity for μ- and δ-opioid receptors (Unterwald and Zukin, 1990).

The question was raised whether β-endorphin also had positive reinforcing effects, in the same way as morphine and heroin, both well-known exogenous opiates (Van Ree, 1983). Experiments on β-endorphin intracerebroventricular self-administration in rats demonstrated that β-endorphin acted as an endogenous positive reinforcer of behaviour, and thus in this way might exert intrinsic control on behaviour, including self-administration of addictive drugs (Van Ree et al., 1979). Additional evidence that an endogenous opioid system is involved in reward processes and in behavioural reinforcement was provided by the effects of opiate agonists and antagonists on brain self-stimulation (Stein, 1984; Van Wolfswinkel and Van Ree, 1985; Schaeffer, 1988; Bain and Kornetsky, 1990) and on non-opiate drug (e.g. cocaine, ethanol) self-administration (Altshuler et al., 1980; Pulvirenti and Kastin, 1988; De Vry et al., 1989). Noteworthy is that intracerebroventricular injections with β-endorphin appeared able to stimulate mesolimbic dopaminergic projections (Koob and Bloom, 1983; Iyengar et al., 1989), which, as was already mentioned in paragraph 4, are regarded as particularly important in brain reward.

It has been speculated that the inherent reinforcing effects of β-endorphin may

lead to the development of addiction to all those behaviours that are associated with a significant release of endorphins, thus including non-drug-associated behaviours, like eating, gambling and jogging (van Ree, 1987).

Alcohol and Opioids

The idea of a common mechanism for alcohol and opiate addiction is quite firmly established, since early clinical observations indicated that addicts frequently used alcohol and morphine interchangeably (Blum et al., 1977; Ho et al., 1977).

In 1970, a biochemical link was proposed between alcohol and opiate addiction (Davish and Walsh, 1970), based on the finding that in the presence of dopamine, epinephrine and norepinephrine, a metabolite of alcohol (acetaldehyde) produced tetrahydroisoquinolines (TIQ's) that seemed to have opioid-like effects and thus could directly affect opioid receptors. The so-called alcohol-addictive metabolite (AAM) hypothesis (Myers, 1980) has not gained much scientific support, because the physical reactions and withdrawal syndromes of alcohol and opiates differ significantly (Saddler et al., 1985), and alcohol withdrawal could not be elicited by opiate antagonists (Goldstein and Judson, 1971; Reid and Carpenter, 1990). It was concluded that alcoholism could not be identified as a TIQ dependence (Goldstein and Judson, 1971).

It was demonstrated that alcohol could interact with endogenous opioid activity, e.g. by stimulating release of β-endorphin from the pituitary (Gianoulakis et al., 1989; Patel and Pohorecky, 1989). It seemed therefore possible that alcohol's addictive effects are mediated by means of an interaction between endogenous opioids and reinforcement substrates (Genazzani et al., 1982; Froehlich and Li, 1990). In chronic alcohol abusers, changes in endorphinergic homeostasis have indeed been reported (Topel, 1985; Olson et al., 1988). β-endorphin levels in the CSF of abstinated alcoholics appeared to be lower compared to human controls (Borg et al., 1982; Genazzani et al., 1982); in addition high-risk persons appeared to have lower plasma content of β-endorphin than low-risk persons (Gianoulakis et al., 1989). Based on these observations, an endorphin-compensation hypothesis has been formulated, in which a predisposed or acquired (Blum, 1983; Erickson, 1990) deficiency of endorphinergic activity is supposed to be compensated by alcohol ingestion (Volpicelli, 1986). A similar hypothesis for cocaine- and heroin addiction was formulated by Sweep et al. (1989), who found that, after a 16 h drug-free period, rats awaiting their daily self-injection session with either cocaine or heroin showed a marked decrease in β-endorphin in the pituitary and the brain limbic system, whereas post-drug session β-endorphin levels were normal. Hence, repeated use of opiates (i.e. heroin), psychostimulants (i.e. cocaine) and of depressants (i.e. alcohol) may cause decreases in endorphinergic activity that seem to be restored by renewed drug intake. This phenomenon

could thus be an important common factor in feelings of craving for a drug, "psychic dependence" and relapse (Sweep et al., 1989; Van Ree et al., 1990).

In the last decade, animal research on the role of opioids in alcohol addiction shifted its interest for physical dependence towards opioid involvement in alcohol self-administration and reinforcement (Altshuler et al., 1980; Pulvirenti and Kastin, 1988; Czirr, 1987a; Hubbell et al., 1987; Milano et al, 1989; Hsu, 1990). Studies with opiate antagonists and opiate agonists revealed that opioid modulation can significantly affect alcohol consumption (Altshuler et al., 1980; Sinclair et al., 1973a; Hubbell et al., 1986; Hubbell and Reid, 1990). Issues of inconsistency however concern the specificity of the effects of opiate antagonists on alcohol consumption, in comparison to other non-drug reinforcers (De Witte, 1984; Samson and Doyle, 1985; Hubbell et al., 1987; Sandi et al., 1988; Koob and Weiss, 1990). Another point of discussion is whether the effects of opiate antagonists (like naloxone or naltrexone) and agonists (like morphine) on alcohol intake should be each others opposite to acknowledge specific opioid receptor involvement (Critcher et al., 1983; Czirr et al., 1987a; Prunell et al., 1987; Hubbell and Reid, 1990; Volpicelli et al., 1990). To date, results have not been consistent (Olson et al., 1988) and the underlying mechanism(s) of action are still under speculation (Koob and Weiss, 1990; Volpicelli et al., 1990). Nevertheless, opioid modulation is being considered as a possible pharmacological tool in the treatment of alcohol addiction (Carpenter and Reid, 1990; Volpicelli et al., 1990).

2. OUTLINE OF INVESTIGATIONS

It can be concluded from the preceding review that alcohol addiction is a complex disorder and that research cannot but be multidisciplinary in order to address the different aspects of the disorder. An important aspect of addiction to alcohol (and to other drugs) seems to be related to the positive reinforcing effects of addictive agents in the brain. These effects seem to be mediated by activation of neurobiological substrates. In the light of these current concepts and new hypotheses on addiction, it was investigated in this thesis whether free-choice alcohol drinking by rhesus monkeys *(Macaca mulatta)* could provide an useful model for the study of experimental alcohol addiction. Subsequently, hypotheses on neuropharmacological aspects of (alcohol) addiction were tested.

2.1. FREE-CHOICE ALCOHOL DRINKING

Different stages in addiction have been distinguished: acquisition of the behaviour, maintenance of ongoing use and attempts at reduction or cessation of the behaviour (Marlatt et al., 1988). Therefore, alcohol drinking behaviour in rhesus monkeys was studied under conditions of spontaneous acquisition, prolonged experience and after periods of interruption of the supply. Questions addressed in the experimental studies, that are contained in Part II of this thesis, were:

Study 1: To what extent do rhesus monkeys initiate alcohol drinking spontaneously if deprivation- or other induction-procedures are not implemented? Do rhesus monkeys develop ethanol-reinforced behaviour?

Study 2: What is the effect of experience with differently concentrated ethanol solutions on fluid preference and on the amount of ethanol ingested?

Study 3: What is the effect of interrupting the alcohol supply for several days on subsequent drinking behaviour?

2.2. NEUROPHARMACOLOGICAL ASPECTS

The hypothesis has been formulated that neurohypophyseal and opioid neuropeptides might be common factors in addictions (Van Ree, 1986; Sweep et al., 1989). Neurohypophyseal neuropeptides, that play a role in learning behaviour, might influence the acquisition of addictive behaviour. Opioid neuropeptides, having opiate-like effects, seem to be involved in the positive reinforcing effects of addictive drugs (or activities) in the brain.

It has been postulated furthermore that disturbances in (neuro)hormonal systems, present in pituitary and brain, may contribute to the development of psychopathology and addiction (Van Ree, 1986; Anokhina, 1987).

These hypotheses were tested for alcohol addiction in the following experimental studies, which are contained in Part II of this thesis:

Study 4: What is the effect of daily treatment with the neurohypophyseal neuropeptide desglycinamide-(Arg^8)-vasopressin (DGAVP) on spontaneous acquisition of alcohol drinking?

Study 5: What is the effect of acquisition of alcohol drinking in naive monkeys on the endocrine profile? Is there a difference between placebo-treated and DGAVP-treated individuals?

Study 6: What is the effect of the opioid receptor antagonist naltrexone on unrestricted alcohol drinking and on relapse after interruption of alcohol supply?

Study 7: What is the effect of the opioid receptor agonist morphine on unrestricted alcohol drinking and on relapse after interruption of alcohol supply?

2.3. METHODOLOGICAL ASPECTS

Various arguments led to preferring a non-human primate as an experimental species for performing the studies here presented. As mentioned in the theoretical introduction, studying spontaneous acquisition of free-choice alcohol drinking when no additional induction procedure is to be used, was considered quite problematic in rodent species. Some reports gave the suggestion that rhesus monkeys might be more apt for this approach (Sinclair, 1971; Myers et al., 1972). The rhesus monkey, a non-human primate, is phylogenetically closer to humans than a rodent species. As alcohol addiction is a rather complex behavioural disturbance, it seemed worthwile to explore the possibilities to do research on alcohol addiction in rhesus monkeys. Studies in non-human primates could function as a bridge between rodent research and applications in men, for example by evaluating new possibilities of addiction therapy using preclinical models.

Performing monkey research asks for specific facilities and demands professional expertise. The Primate Center of TNO could provide such an unique facility. Altogether, twenty eight rhesus monkeys participated in the various experimental studies described in this thesis. The monkeys were (young-) adult male animals, bred at the TNO Primate Center. Each monkey was allowed to have a free acquisition procedure in alcohol drinking, having normal access to

food and drinking water. The animals were housed in one room, in individual cages, but some participated in social groups during several hours per day (Studies 4, 5).

After an initial acquisition experiment (Study 1) eight monkeys were studied under long-term access conditions, in such a way that the effects of experience with alcohol (Study 2), of periods of interruption of the alcohol supply (Study 3) and of experimental pharmacological treatments (Studies 6, 7) could be analyzed. Twenty monkeys participated in an acquisition study of four weeks (Studies 4, 5). Two ethanol/water solutions with different ethanol concentrations were supplied concurrently with drinking water (all Studies), except during alcohol interruption periods (Studies 3, 6, 7). Consumed volumes were measured throughout the day and data samples were further processed by means of computer programs. Overt behaviour was observed several times per day; in some occasions behavioural activities were quantified by means of event recording (e.g. Study 7).

Animals were under regular veterinary control and experiments were performed according to legal standards of animal research. Experimental pharmacological agents were administered in the home cages via intramuscular injections (Studies 4, 5, 6, 7). All experimental drug-administrations were placebo-controlled and double blind. In the group of twenty monkeys half of them received DGAVP-injections, half of them saline daily during fourteen days (Studies 4, 5). In the group of eight monkeys, low doses of naltrexone and morphine were injected not more than once per two weeks (Studies 6, 7).

Blood samples were drawn under light sedation for determination of blood alcohol concentrations (Study 1). For determination of hormone levels in plasma, blood was drawn three times from unsedated monkeys that had been trained in advance for such a procedure (Studies 4, 5).

In the present experimental design, monkeys remained in good physical conditions across a period of about five years. Liver functions remained normal. Overt physical withdrawal reactions during abstinence could not be detected.

3. RESULTS AND DISCUSSION

3.1. FREE-CHOICE DRINKING IN MONKEYS AS A MODEL FOR EXPERIMENTAL ALCOHOL ADDICTION.

Acquisition and Maintenance

By no manipulation other than making two ethanol/water solutions available in addition to drinking water, all rhesus monkeys presently studied initiated alcohol drinking within a few days (Studies 1, 4). Subsequently alcohol drinking was maintained as long as alcohol remained available (Studies 1, 2, 3, 6, 7 and Studies 4, 5).

In the experimental design of 24 hr-per-day free-choice access to alcohol and water, water drinking and ethanol ingestion appeared to be differently motivated behaviours. Water drinking was for a major part determined by the daily feeding pattern, whereas ethanol intake was more evenly distributed across day and night (Studies 1, 2). When ethanol concentration in the two ethanol solutions increased (2%-4%; 4%-8%; 8%-16%; 16%-32%), consumed volumes of ethanol solutions decreased (Studies 1, 2).

During the stage of acquisition (Study 1), the monkeys maintained a rather constant total daily net ethanol intake when drinking solutions of 4% and 8% and higher (up to 16% and 32%). After experience with alcohol (Study 2), total daily net ethanol intake increased progressively as a function of increasing ethanol concentrations. In this study (Study 2), the concentration-effect relationship was quite similar to findings in previously conditioned, operant-responding animals (Griffiths et al., 1980; Meisch, 1984). It was concluded that free-choice alcohol drinking behaviour in rhesus monkeys was under control of reinforcement principles (Studies 1, 2, 3, 4), notwithstanding the fact that palatability factors seemed to play some role as well. Furthermore, experience with alcohol appeared to modify the preference for different concentrations and to enhance the acceptance of a higher ethanol intake level. In experienced monkeys, the reinforcing effects of ethanol thus seemed less inhibited by other regulatory factors, for which tolerance might have been developed (Study 2). Furthermore, it was concluded that fluid preference and pharmacological reinforcement by ethanol represented different aspects of alcohol drinking behaviour in the performed studies.

Occasionally measured BAC's (varying between 3 to 70 $mg.dl^{-1}$) (Study 1) indicated that the consumed amounts of ethanol were sufficient to induce central effects (Vree et al., 1975; Lumeng and Li, 1986; George, 1987). Nevertheless, monkeys did not consume ethanol in clearly intoxicating quantities, which corroborates the assumption that particularly at initiation low doses of ethanol are

positively reinforcing, whereas high doses seem to be aversive (Mendelson and Mello, 1979b; Myers and Ewing, 1980; Prunell et al., 1987; Bain and Kornetsky, 1989). This was demonstrated also by the placebo-treated monkeys in Study 4, that were provided with 4% and 8% ethanol solutions. Although these monkeys started with higher ethanol intakes, they subsequently adapted intakes to levels that were comparable to the intake levels of monkeys provided with 1% and 2% solutions.

From Studies 1, 2 and 4 it can be concluded that free-choice alcohol drinking, like alcohol consumption after induction procedures (Henningfield and Meisch, 1979; Meisch, 1984; Suzuki et al., 1988), is under the influence of the positive reinforcing effects of ethanol. Humans and animals, including the presently studied monkeys, having unrestricted access to ethanol solutions, nevertheless show more day-to-day fluctuation in ethanol intake (Griffiths et al., 1980; Mello and Mendelson, 1980; Hyytiä and Sinclair, 1989; Samson and Grant, 1990) than animals conditioned to ingest alcoholic beverages during daily restricted sessions, which usually ingest constant and relatively large amounts of ethanol (Meisch, 1977, Griffiths et al., 1980; Marcucella and Munro, 1987). Spontaneously initiated and unrestricted alcohol consumption, or conditioned and time-restricted alcohol consumption, in animals might therefore be comparable to different drinking patterns of humans consumers, like regular frequent drinking versus episodic binge-drinking, respectively (Griffiths et al., 1980; Ellison et al., 1981; Samson and Grant, 1990). Hence it should be taken into account that different animal models can represent different forms of human drinking (Griffiths and Bigelow, 1978; Ellison et al., 1981; Kalant, 1988).

Relapse in Alcohol Consumption after Cessation

One, two and seven days of imposed interruption of alcohol supply led to a temporary increase in subsequent ethanol intake, after which the animals continued their pre-interruption drinking habit (Study 3). This phenomenon also occurred after longer interruption periods in monkeys (over 4 weeks; Kornet, unpublished data) and has been reported in free-choice drinking rats (even after 75 days of interruption!) (Sinclair et al., 1973b) and in humans (Burish et al., 1981) as well. The interruption-induced increase could not be interpreted in terms of "relieval (of withdrawal distress) drinking" (Caetano, 1985) since physical withdrawal reactions did not manifest themselves and moreover are known to be transient in monkeys over a few days (Ellis and Pick, 1972; Myers et al., 1972; Winger, 1988). The increased ethanol intake and subsequent relapse in the pre-interruption drinking habit rather seems to reflect a reinstatement of the previously acquired

ethanol-reinforced operant behaviour (Griffiths et al., 1980; Stewart et al., 1984; Cornell et al., 1989), with a temporary increased motivation for ethanol directly after renewed availability of alcohol solutions. This behaviour induced by imposed interruption might be mediated by the same mechanism(s) as the relapses in alcohol, and other drug addicts (Barnes, 1988), after a (sometimes quite prolonged) period of abstinence (Marlatt and George, 1984; Horwitz et al., 1987; Sinclair and Li, 1989). Relapse has been indicated as the major problem in addiction, which is poorly understood (Marlatt et al., 1984; Dole, 1986; Barnes 1988). Current hypotheses on relapse include the influence of incentive-motivation processes (Stewart et al., 1984: Hand et al., 1989; Cornell et al., 1989), so that an incentive can elicit behaviour independent from conditions of deprivation or satiation, and the role of multiple-order conditioned stimuli, so that previously neutral stimuli become associated with drug-taking behaviour and hence can elicit this behaviour (Cornell et al., 1989; Goldberg et al., 1990). Other hypotheses on relapse have been formulated on the basis of drug-opposite conditioned responses, i.e. anticipatory physiological responses before drug ingestion, that are opposite to (some) effects of the drug to be ingested and might produce a subjective feeling of "drug craving" (Siegel, 1985; O'Brien, 1986; Macfarlane and White, 1989). Alternatively, relapse might frequently occur because of some internal deficiency (e.g. endorphin deficiency), developed by frequent drug ingestion (Genazzani et al., 1982; Volpicelli et al., 1990). The presently described experimental model for relapse in monkeys (Study 3) could provide a useful model to investigate the mechanisms of and to test methods for prevention of this major problem of alcohol addiction.

3.2. NEUROPHARMACOLOGICAL ASPECTS

The Effect of DGAVP

The neuropeptide DGAVP reduced the acquisition of alcohol drinking in the majority of the thus-treated monkeys having access either to relatively low (1% and 2%) or relatively high concentrated (4% and 8%) ethanol solutions. The consumption of drinking water was not changed, supporting previous conclusions that DGAVP in such doses does not exert classical endocrine vasopressin effects (De Wied et al., 1972; De Jong et al., 1985; Laczi et al., 1987). The effect of DGAVP manifested itself after several days of treatment and was maintained after treatment was terminated. Since DGAVP has high clearance value (Van Bree et al., 1988), it seems more likely that DGAVP altered ongoing processes rather than exerting an acute pharmacological effect (Van Ree, 1983). These findings are

in agreement with DGAVP-studies on cocaine and heroin self-injection in rats (Van Ree and De Wied, 1977; Van Ree, 1986; De Vry et al., 1988). DGAVP could also attenuate electrical brain self-stimulation in rats (Dorsa and Van Ree, 1979). This, together with similar findings for different classes of addictive drugs, including ethanol, suggests that DGAVP might attenuate the positive reinforcing effects of addictive drugs by interaction with brain reward systems (Van Ree, 1983). Two DGAVP-treated monkeys, having access to 4% and 8% solutions, behaved differently and DGAVP seemed ineffective. These monkeys initiated at and continued with quite high ethanol intake levels. It has been reported that DGAVP was ineffective also during maintenance of heroin and morphine self-administration in rats and monkeys respectively (Mello and Mendelson, 1979; Van Ree, 1986). In addition, electrical brain self-stimulation was attenuated by DGAVP at threshold level and not at high current intensities (Dorsa and Van Ree, 1979). Hence DGAVP may be especially effective in situations in which the reinforcement control over behaviour is still developing (i.e. during gradual acquisition) or is changed (Van Ree, 1986).

Endocrine Profile and Acquisition of Alcohol Drinking

With respect to the effect of acquisition of alcohol drinking on the endocrine profile, no significant differences between DGAVP- and placebo-treated monkeys could be detected (Study 5), suggesting that DGAVP's effect on reinforcement processes was mediated at a central level (Greven and De Wied, 1980; Van Ree, 1980; Barna et al., 1990). Furthermore, no relationship was found between individual basal endocrine profile and subsequent ethanol intake. In general, the acquisition of alcohol drinking in placebo- and DGAVP-treated monkeys led to changes in the endocrine profile in a time-dependent way (4-week period), which could not be simply related to classical stress- and/or acute ethanol-induced responses (Axelrod and Reisine, 1984; Patel and Pohorecky, 1988; Rivier, 1989). Of particular interest is that plasma β-endorphin remained significantly increased over time. Cortisol on the other hand appeared to have decreased after 4 weeks of alcohol drinking. β-Endorphin has been postulated to play a central role in positive reinforcement and addictive behaviour (Sweep et al., 1988; Sandi et al., 1989; Van Ree, 1990). Furthermore, central endorphinergic activity appeared to be significantly reduced in abstinent alcoholics (Genazzani et al., 1982; Borg et al., 1982; Barret et al., 1987, Volpicelli et al., 1990). The observed sustained elevation in plasma β-endorphin of the monkeys might thus be an early stage of ethanol-induced modification in neuroendocrine homeostasis. The decrease of plasma cortisol over time might represent an impaired pituitary function, as has

been reported for human chronic drinkers (Marks, 1979; Reus, 1980).

Interesting was that two placebo-treated subjects that developed high increases in ethanol intake over time seemed to deviate from the others in endocrine reactions. These observations resemble some human studies, which revealed that baseline hormonal levels of persons with a high incidence of alcoholism in their family (which might indicate a higher susceptibility for alcohol addiction) did not differ from controls, but that however such persons showed significantly less intense reactions to ethanol than controls did with respect to ACTH, cortisol and prolactin (Schuckit et al., 1987; Schuckit et al., 1988). Hormonal responses have been conceived as providing a "window" to neurochemical changes in the brain and pituitary (Schuckit et al., 1988). As neuroendocrine disturbances might be related to psychopathology and addiction (Gold, 1980; Van Ree, 1986; Anokhina, 1987; Gianoulakis et al., 1990), experimental animal studies of free alcohol selection and pituitary-related endocrine changes could yield valuable information in this respect.

The Effect of Opioid Modulation on Alcohol Consumption

Chronic alcohol drinking in monkeys was significantly modified, i.e. reduced, by the opioid receptor antagonist naltrexone, as well as by the opioid receptor agonist morphine, during conditions of unrestricted access to alcohol and after a 2-day alcohol interruption period (Studies 6, 7). Effects of acute and single treatments did not extend beyond a 24 h-period, after which the monkeys returned to normal drinking patterns.

During conditions of unrestricted access to water and alcohol (Experiment I of Studies 6 and 7), effects were not completely selective for alcohol consumption. However, effects on water consumption were generally of a shorter duration (Studies 6,7) and were sometimes followed by a rebound effect (Study 6), whereas alcohol consumption remained reduced for longer periods and rebound effects were absent (Studies 6,7). A possible explanation is that opioid modulation specifically affects those behavioural activities (including water drinking) that are a consequence of positive reinforcement, provided that physiological demands (e.g. dehydration) are not critically involved (Hubbell et al., 1986; Milano et al., 1989; Hubbell and Reid, 1990; Yeomans et al., 1990). Apparently, such negative reinforcement mechanisms were not involved with respect to alcohol consumption (Hubbell et al., 1986; Czirr et al., 1987b; Reid and Carpenter, 1990).

After two days of alcohol interruption, effects of opioid modulation were selective for alcohol consumption (Experiment II of Studies 6 and 7). As already mentioned, the animals showed specifically ethanol-motivated behaviour after

interruption of the alcohol supply (Study 3). The effects of opioid modulation might therefore have been more specific because motivation for ethanol was high (Hubbell et al., 1987).

The fact that naltrexone and morphine, being respectively an antagonist and agonist, both produced a reduction in ethanol intake, is not easy to understand, but has been found in other behavioural studies as well (Sinclair et al., 1973; Critcher et al., 1983; Young, 1986; Olson, 1988; Cuthbert et al., 1989). Since monkeys remained alert and active (Study 7), the suppressive effect of morphine could not be attributed to a general sedative effect. Furthermore, the doses administered of naltrexone (Study 6) and morphine (Study 7) were very low (Hubbell and Reid, 1990), so that effects can be considered to be opioid-receptor specific (Frenk and Rogers, 1979; Prunell et al., 1987).

The present findings seem to be in contradiction with the effects of opioid agonists and antagonists on alcohol consumption in rats (Czirr et al., 1987; Hubbell et al., 1987; Reid et al., 1987). In rats naltrexone reduced and morphine stimulated alcohol intake, and these findings led to the "stimulation" hypothesis that: "a surge (e.g. injection with morphine) or a surfeit (e.g. chronic infusion of morphine) of opioidergic activity potentiates alcohol intake, while a functional decrease (e.g. by injection with naltrexone) will attenuate alcohol intake" (Hubbell and Reid, 1990).

A different hypothesis is the so-called "endorphin-compensation" hypothesis that presumes that: "if alcohol drinking is reinforced by increased activity at opioid receptors, the following should be true: 1. alcohol can pharmacologically stimulate activity of opioid receptors, 2. alcohol consumption should decrease during conditions of excess opioid receptor activity and increase during conditions with deficiencies in opioidergic activity, and 3. opioid antagonists should block the reinforcing effects of alcohol and hence decrease alcohol intake" (Volpicelli et al., 1990). The hypothesis on endorphin compensation seems to be supported by the observations in alcoholics (Genazzani et al., 1982) and in experimental heroin- or cocaine-addicted animals, who, after a drug-free interval, show deficits in central endorphinergic activity (Sweep et al., 1988). From these observations it has been hypothesized that a lowered endorphinergic activity might be a common condition in various types of addiction, that can be complemented by a repeated action of the addictive behaviour, or by other ways of endorphinergic stimulation (Genazzani et al., 1982; Sweep et al., 1989; Volpicelli et al., 1990; Van Ree, 1990).

The increase in plasma β-endorphin found in Study 5 and the temporary reduction in alcohol consumption by morphine (Study 7) in the monkeys seem to fit best with the endorphin-compensation hypothesis.

If alcohol is ingested for its opiate-like consequences (e.g. release of β-

endorphin), point 3 of the "endorphin compensation" hypothesis raises the question whether blocking opioid receptors by antagonists should lead to an increase, instead of a decrease in alcohol drinking (Hubbell and Reid, 1987). The hypothesis of reinforcement blockade suggests, however, that this can lead to extinction of the behaviour. Extinction of operant reinforced behaviour has been described as preceded by an initial burst of responding. Although such a phenomenon was not noticed in the monkeys treated with naltrexone, it may have been missed if this occurred within a very short time period.

To date, the precise mechanisms of interaction between opioid receptors and alcohol consumption are still not elucidated (Linseman, 1989; Koob and Weiss, 1990). The present studies showed that apparently opioid modulation of alcohol consumption is not a simple matter of classical agonism and antagonism, which generally produce opposite effects (Koob and Weiss, 1990). One possibility is that ethanol does not produce opioid activity by directly stimulating opiate receptors, but by influencing opioid systems indirectly (Critcher et al., 1983; Linseman, 1989; Koob and Weiss, 1990).

Several speculations can be made from the results reported so far. Morphine (Study 7) could almost abolish (maximal 92%) alcohol consumption, including relapse-like drinking. Morphine is an opioid receptor agonist and, unlike ethanol, binds directly with opioid receptors. Morphine is known to produce euphoric, pleasurable effects. The single injection with morphine in the monkeys could have produced a temporary subjective state of well-being, that was qualitatively as good as the effects of the usual ethanol ingestion. Hence, it could be that morphine merely resembled, but was not identical to, (some) effects of ethanol, via a different pharmacological action. The naltrexone study (Study 6) showed that the highest dose could not definitely abolish the increase in alcohol consumption, and that, compared to unrestricted access and to the morphine study, overall consumption levels remained higher. Hence it seems possible that ethanol reinforcement was also mediated by non-opioid reinforcement, e.g. via dopaminergic or serotonergic (Stewart et al., 1984, Schaefer, 1988; Signs and Schechter, 1989; Koob and Weiss, 1990) pathways, because it was blocked to some extent only by opioid receptor antagonism. Morphine on the other hand could have stimulated other neurotransmitter systems involved in reward as well, thus mimicking non-opioidergic effects of ethanol.

On the other hand, it might have been possible that naltrexone was less potent because of a lowered opioid activity in the monkeys due to chronic alcohol drinking (Young, 1986; Volpicelli et al., 1990). In this respect, the existing level of endogenous opioids (influenced by chronic alcohol consumption or by other factors) might be of significance for which results are to be expected (Volpicelli et al., 1990). Furthermore, it cannot be excluded that species difference (rats versus

monkeys) also might have contributed to inconsistencies in the effect of morphine (Mansour et al., 1986; Cuthbert et al., 1989; Billington et al., 1990). Finally, not so much is known about the functions of other subtypes of endogenous opioids, that may however be involved in the effects of alcohol as well (Critcher et al., 1983).

With respect to the previously mentioned hypotheses on relapse (par. 3.1.), several arguments can be considered. If morphine functioned as a temporary compensation for ethanol, ethanol consumption after interruption of supply apparently could not have been primarily triggered by incentive-induced motivation (Cornell et al., 1989), because in that case renewed presence of ethanol should have led to drinking irrespective of the degree of (opioid) satiation. But morphine could have compensated for some endogenous deficiency that might have been present in the monkeys due to chronic alcohol drinking (e.g. "endorphin compensation" hypothesis; Genazzani et al., 1982) or as a result from an opponent process response preceding (and eliciting) the alcohol consumption (O'Brien, 1986; Macfarlane and White, 1989).

4. FUTURE SCOPE

As can be concluded from the theoretical introduction, alcohol addiction represents a multi-sided disorder with biological, social and psychological aspects (Van Dijk, 1979). In what respect does the experimental work of this thesis contribute to understanding and treating this disorder? A first conclusion is that free selection of alcohol by rhesus monkeys can be used as an experimental animal model for (some aspects of) human alcohol drinking behaviour and alcohol addiction. The findings supported the hypothesis that addiction to alcohol is (partly) mediated by the same behavioural and neurobiological mechanisms that are assumed to be important in other forms of addiction. The role of these mechanisms could be studied in the presented experimental design. In this design, monkeys remained in a good physical condition across a period of about five years. Some may object that the absence of overt physical dependence and/or alcohol-induced medical complications might indicate that this design does not provide an relevant model for human alcohol addiction. Physical dependence, toxicity by excessive alcohol consumption and medical complications however are consequences and not criteria of addiction. Absence of these consequences makes it in fact less complicated to interprete the results in terms of the mechanisms of addiction.

The application of the used free-choice alcohol drinking design in future research seems of particular interest for studying those factors that determine the acquisition of alcohol drinking and the onset of addiction such as the conditions under which alcohol is available (e.g. Studies 1, 2, 3, 4), the psychosocial circumstances and the individual susceptibility (risk factors) for psychopathology and/or addiction (e.g. Study 5).

Furthermore, as relapse is a major problem in (alcohol) addiction, the effect of imposed abstinence on subsequent alcohol consumption reported in this thesis (e.g. Studies 3, 6, 7), provides an important model to investigate the underlying mechanisms of relapse in (alcohol) addiction.

The experimental primate abstinence model seems also valuable as a preclinical model for evaluating treatment methods, e.g. behavioural therapies and/or neuropharmacological interventions (e.g. Studies 6, 7). Developing methods of relapse prevention will not be important only to break the cycle of addiction, but in addition can contribute significantly to prevent development of or progression in alcohol-related diseases. Neuropharmacological intervention in alcohol addiction is a rather new, barely explored approach in alcohol therapeutical strategies (NIAAA, 1990), but scientific attention towards this possibility is increasing fast (Volpicelli et al., 1990; NIAAA, 1990; Holden, 1991). To date it seems quite unlikely that "one pill" could solve all problems of addicts. But by understanding more about the interaction between a drug, the internal factors of an individual and his/or her

environment and by using a multidisciplinary approach, therapies can be developed that are more made-to-measure for the person involved. Neuropharmacological interventions could provide in this respect a helpful adjunct in conventional therapies that in many cases do not score great success.

Based on the work described in this thesis several guidelines are proposed for research on and development of possible therapeutic strategies in alcohol addiction. A conventional procedure to treat alcoholics is to implement complete abstinence from alcohol. Extinction of addictive behaviour does not seem to be accomplished however just by detoxification and forced abstinence in men nor in animals. Behavioural modification and manipulation of the reinforcing effects of alcohol drinking might be a more promising approach. One line of treatment could be based on the effects of the neuropeptide DGAVP, that was shown to suppress the acquisition of alcohol drinking (Study 4) and of heroin and cocaine self-administration in rats (Van Ree et al., 1988) without further adverse side-effects. The hypothesis that DGAVP is most effective under changing conditions of reinforcement led to the idea that DGAVP could also be effective in abstinence procedures. Some positive results have been reported with respect to treatment of heroin and cocaine addicts (Van Beek-Verbeek et al., 1983; Fraenkel et al., 1983). The long-lasting effect observed in Study 4, suggests that medication could be restricted to some limited time period; an attractive prospect in the treatment of addicted persons.

Addressing the opioid system might be a possibility of treatment as well. At first sight, administration of opioid receptor agonists in order to reduce alcohol drinking (e.g. morphine, Study 7), seems an example of helping patients from the frying pan into the fire. Depending on the clinical condition of the patient however, it could be that low doses of particular agonists (e.g. methadone, or specific opioid subtypes agonists) are less harmful than excessive alcohol intake.

A wider application might be possible for treatment with opioid receptor antagonists that are supposed to block the reinforcing effects of alcohol and hence produce behavioural extinction of the addictive behaviour. Some encouraging findings have been reported about the effect of naltrexone (Jonas, 1990) and nalmefene (Yeomans et al., 1990) on human food intake and bulimia, as well as on human heroin intake (Judson and Goldstein, 1984). Attention for possible effects of opioid antagonists on human alcohol drinking behaviour is just developing (NIAAA, 1990). Preliminary data from human alcoholics (Volpicelli et al., 1990) treated with naltrexone for 12 weeks after being detoxified, suggest that naltrexone can decrease craving, the number of drinking days and rates of relapse. However the study was not completely without bias, and has to be supported by future studies as well. Treatment of addiction during which patients are allowed to continue drinking (e.g. under experimental control) is quite

unconventional in current therapeutic programs. Nevertheless, application of opioid antagonists in combination with controlled drinking sessions, might be useful as extinction procedure (Volpicelli et al., 1990). This procedure might be used when the physical health of the person involved is not contra-indicative for alcohol consumption, as can be the case in early stages of addiction. Recently, clinical interest has developed for other possible agents such as serotonin 5-HT2-antagonists (Meert and Janssen, 1990; Linnoila et al., 1990), and calcium-acetylhomotaurinate (AOTA-Ca) (Pelc et al., 1990) for maintaining abstinence in weaned alcoholic patients.

It should be noted that the observed results (Studies 4, 5, 7) did not always match those reported for rodents. This might indicate that assumptions about clinical effectiveness based on rodent findings alone are perhaps too restricted to apply to humans. As non-human primates are phylogenetically closely related to man, the use of non-human primate research in addition to rodent research might help to shorten the distance between basic research and applied therapy. It is therefore relevant that research on alcohol addiction is performed cross-species and also includes different models of alcohol drinking.

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Part II

Experimental Studies

1. ANALYSIS OF SPONTANEOUS ALCOHOL DRINKING IN RHESUS MONKEYS

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Abstract

This analysis aims at determining to what extent spontaneous alcohol drinking in adult male rhesus monkeys *(Macaca mulatta)* represents ethanol-directed behaviour. It is shown that in a condition of free access to an ethanol/water solution (2 per cent v/v) and drinking water, alcohol drinking was initiated in all subjects (n=4) within a few days, without any specific induction procedure. Relationship between drinking behaviour and ethanol concentrations was studied in eight subjects by use of a concurrent 3-bottle-design, two bottles containing ethanol solution (concentrations 2,4; 4,8; 8,16; 16,32 per cent v/v), one bottle contained drinking water. When ethanol concentrations in the solutions increased, consumption of ethanol solutions decreased, of drinking water increased and of total water decreased. Net ethanol intake from a certain solution was influenced by its concentration and the concentration of the concurrently available solution. After an initial increase, total net ethanol intake remained relatively constant. Consumed amounts of ethanol (on the average 2-6 $ml.kg^{-1}$ per day) could lead to notable blood ethanol levels. Drinking from ethanol solution was not just an alternative for ingesting water. The observed alcohol drinking is interpreted as resulting from a central reinfo-rcement of ethanol intake and avoidance of negative, potentially harmful effects of ethanol.

Introduction

Ethanol, a substance which is potentially addictive for humans, is reported to act under certain conditions as a positive reinforcer of self-administration behaviour in animals (8, 15, 18, 31). Under voluntary, free-choice conditions animals did not generally ingest large quantities of ethanol solutions orally (3, 20, 30). Hence, it was assumed that the reinforcement of drinking ethanol solutions under such conditions was too weak to be of relevance (6, 31, 49).

By use of operant conditioning techniques a high oral ethanol intake could be induced in monkeys (6, 19, 32) and in rats (17, 33, 34, 48). Blood ethanol concentrations indicated that ethanol intakes in such a design were sufficient to exert central effects (6, 11, 16). Hence, operant conditioning paradigms are regarded as providing an appropriate conceptual framework for predicting drug dependence potential (23, 31, 51) and for experimental analysis of drug self-administration (31, 34, 48, 52). However, operant conditioning studies also have been recognized to have some limitations (3, 20, 22, 27). Induction of alcohol drinking is achieved by means of food-induced drinking in food-deprived animals which are given dry food and an ethanol solution as the only liquid source (6, 10, 19), or by

means of water deprivation (17, 21). These restrictions make it difficult to accept such models applicable to certain aspects of human drinking behaviour, especially with regard to initiation, and possibly also to factors controlling the maintenance of such behaviour (3, 22, 27, 47).

Animal studies on spontaneously self-initiated drinking in which the subjects could choose between an ethanol solution and drinking water (1, 36, 42, 53) have been seriously criticised (3, 20, 27, 31). Flaws were related to a low frequency of measurement, confounding of liquid preference by a possible position preference of the liquid source, control of equipment, only comparing the daily consumption of water and of ethanol solution, absence of a blood ethanol determination. Moreover, there was a lack of distinction between palatability determined and/or ethanol-determined ingestive behaviour.

This study on spontaneously self-initiated alcohol drinking in rhesus monkeys aimed at determining to what extent alcohol drinking was directed at ingesting certain fluids and/or to obtaining ethanol. To this end we addressed the following issues:

a. To what extent do rhesus monkeys initiate alcohol drinking spontaneously after an ethanol solution is made available without additional conditioning procedures?
b. Can spontaneous alcohol drinking be dissociated from being merely an alternative method to meet daily fluid demands? (3, 9, 27)
c. Is consumption of ethanol solution dependent on just the concentration of the solution? In previous studies preference drinking only concerned a choice between water and one ethanol solution (2-bottle-design) (30, 42, 43, 53). These studies showed a concentration dependent decrease in consumption of solution. In the 2-bottle-design in which there is only one ethanol solution available, one cannot distinguish palatability-determined from ethanol-determined aspects in the drinking behaviour (31). With a simultaneous choice between two ethanol solutions (3-bottle-paradigm), relative preference for different concentrated ethanol solutions can be distinguished from reinforcement of net ethanol intake.
d. Can the ethanol consumption lead to blood ethanol levels sufficient to expect central reinforcing effects (6, 16, 28)?

Materials and Methods

Observation series I

The first observation series aimed at determining whether the monkeys initiated and maintained alcohol drinking. To this end, each subject had attached to its cage

two 500-ml drinking bottles. These drinking bottles were inverted cylinders each closed by a perforated rubber stopper which held a stainless steel nipple; the bottles were attached to the cage so that only the nipple was in reach of the monkey. The monkey could drink only by licking or sucking at the nipple. One bottle contained only water, the other an ethanol-water solution of 2 per cent (v/v). The subjects had continuous access to both bottles. The amount of liquid drunk from each bottle was measured six times a day (at 9.00, 10.30, 12.00, 13.30, 15.00 and 16.30 hours) on working days and three times a day on Saturdays and Sundays (at 9.00, 12.00, 16.30 hr). Measurements were completed within a matter of minutes. After measurements the bottles were always fully refilled so that the liquids were available practically ad lib. Frequent visual inspection showed that possible spillage of fluid did not play a role. Location of the bottles was altered daily so that a preference for either of the liquids was not confounded by a possible preference to drink at a certain location.

Subjects were four male rhesus monkeys *(Macaca mulatta)* (VJ, 2U, TS, LH), weights between 7 and 10 kg, ages between 7 and 9 years, and each housed in a separate cage in the same room. Artificial illumination was on the basis of 12 hr/12 hr light-dark cycle; in addition the room was also accessible to normal daylight. Diet consisted of regular monkey pellets (over 200 g) provided at 9.10 hr in the morning supplemented with fruit at 13.40 hours and a slice of bread at 15.40 hr. The room was temperature (24°C) and humidity (60 percent) controlled.

Observation series II

In this series of observations we studied how the monkeys discriminated between differently concentrated ethanol solutions. To this end we used a 3-bottle preference design. Two of the bottles contained differently concentrated ethanol solutions, the third bottle contained only drinking water. The combinations of concentrations simultaneously presented were: a) 2 and 4, b) 4 and 8, c) 8 and 16, d) 16 and 32 per cent (v/v). Each combination was supplied for one experimental period which lasted fourteen days or more. To prevent that a preference for either of the liquids was confounded by a preference to drink at a certain location, position of the bottles was altered daily. Four rhesus monkeys without experience in alcohol drinking (males between 6 and 9 kg body weight and between 4 and 8 years of age (OI, 1DW, 1DM, QV)), were added to the four reported above. The group of subjects thus consisted of eight monkeys. The housing conditions were the same as those during observation series I. This held true also for the procedure of measuring the consumption and refilling of the bottles.

In order to get some indication of the blood ethanol levels that were reached, blood ethanol levels were determined, by use of an ADH method (13), ten times in each animal (detection limit was 3 $mg.dl^{-1}$). Blood samples were taken under

light sedation (Ketamine HCl (Vetalar) 10 mg.kg^{-1} together with acepromazine base (Vetranquil) 1 mg.kg^{-1}) at 9.00 hr. Sampling started just after the observation series reported here were terminated because it could interfere with the drinking behaviour. The water and alcohol supply (16 and 32 per cent (v/v)) were continued in the same manner and the subjects maintained consumption at the same level. The taking of blood samples occurred with intervals of a few weeks so as to avoid possible interference with drinking behaviour. Throughout the observation series the monkeys were checked for possible signs of intoxication such as drowsiness, unsteady posture, movement or gaze (6, 38).

Statistical analysis

Comparison between consumption of simultaneously offered fluids was performed by means of the Wilcoxon matched pairs test using the Statistic W (40).

Statistical analyses of the variation in the daily consumed volumes as a function of the change in concentrations across observation periods (observation series II) was performed by use of Kruskal-Wallis one-way analysis of variance for each subject separately (44). Obtained p-values for the whole group of subjects were then combined and tested by combining individual probabilities (COP), using the statistic $\chi^2 = -2\Sigma \ln P_i$; $df = 2N$). Because differences in consumption between different observation periods could vary in direction between subjects, the degree of concordance among the subjects was tested by means of Friedman two-way analysis of variance using statistic χ^2_R; $df = 3$ (44). Comparisons between two consecutive observation periods were made by use of the Mann Whitney U-test (44). In the analysis, mean consumptions are given in ml per subject. The net ethanol intake is expressed in ml per subject that corresponds to about 10 times the consumption in g.kg^{-1} body weight since the mean body weight was 8.0 kg and the specific density of ethanol 0.79.

Results

Observation series I

Figure 1 shows the volumes of ethanol solution consumed by the four subjects, during a period of 27 days. All subjects showed a steep rise within the first three days from about 500 ml or less during day 1 to about 1300-1900 ml during day 3. Thereafter, the consumption of ethanol solution remained variable but showing no trend in some subjects (2U, LH); in others (VJ, TS) there was a decreasing trend. Around day 10, all subjects had reached a more or less stable level of consumption. During the subsequent period of 17 days, three subjects (VJ excepted) drank significantly more from the ethanol solution than from the drinking water (mean consumption: VJ 779 ml water, Wilcoxon W = -1, p ns;

2U 322 ml water, W = -5, p<0.0001; TS 201 ml water, W = -5, p<0.0001; LH 421 ml water, W = -4, p<0.0001). Consumed volumes could rather fluctuate from day to day; a pattern which remained present also later on. The above results show that the initiation of drinking was quite rapid. During the subsequent days 4-27 mean net ethanol intake per kg body weight was 1.7 (VJ), 3.1 (TS), 4.1 (LH) and 5.4 (2U) ml.kg^{-1} per day.

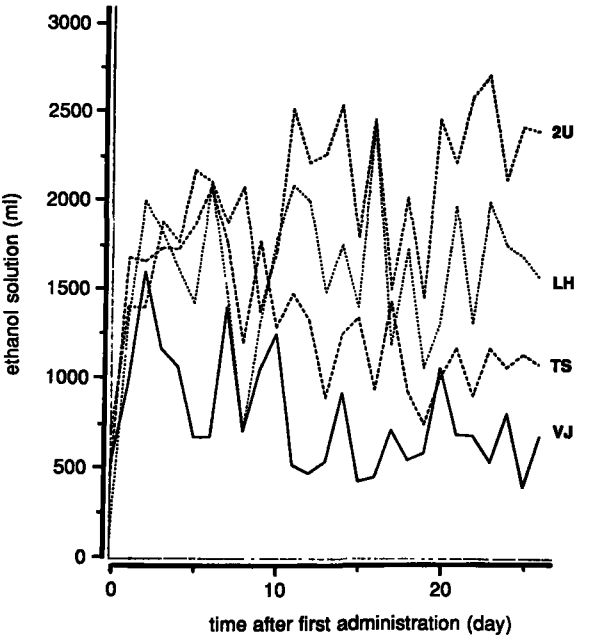

Figure 1. Daily consumption of ethanol solution by four subjects after first access to an ethanol/water solution of two percent v/v, in addition to drinking water.

Observation series II

Initiation of alcohol drinking in the four monkeys that were added to the panel, was fully comparable to that of the first four monkeys reported above. The mean daily consumption of all eight subjects, from the different liquids as a function of the concentrations offered is shown in Figure 2. It shows that when concentrations were both low (2 and 4 per cent), the subjects consumed significantly more from each ethanol solution than from drinking water; in the consecutive experimental periods, consumption from the 4 per cent ethanol solution was not different from drinking water consumption, and from the higher concentrations (8,

16, 32%) consumption was always less than from drinking water. Comparing the two ethanol solutions in each observation period, the figure shows that the subjects consumed significantly more from the least concentrated of the two solutions during the last three observation periods.

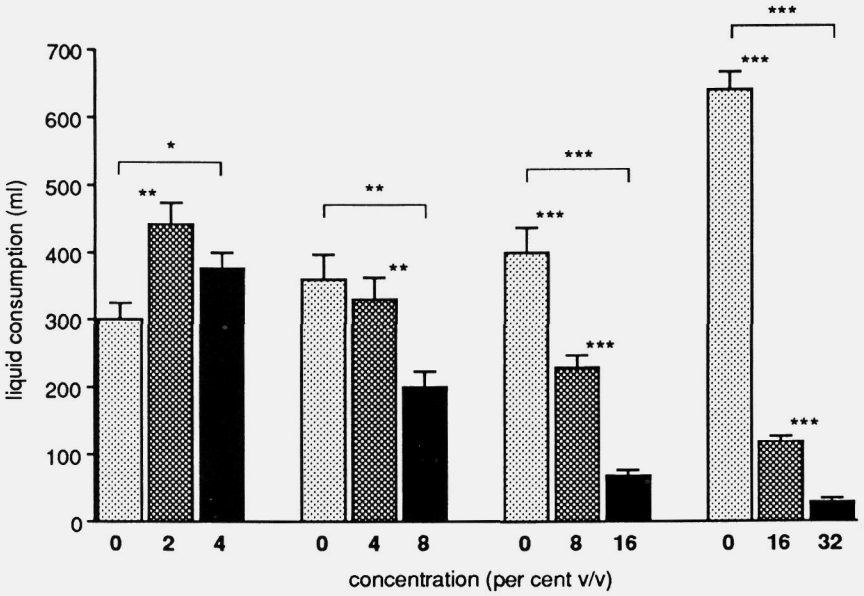

*Figure 2. Mean daily consumption of the different liquids offered simultaneously, as a function of the ethanol concentration (*Wilcoxon matched pairs test, *p<0.05, **p<0.01, ***p<0.001). Vertical bars represent standard errors of the mean.*

The mean daily consumption of total ethanol solution, drinking water, total water and total net ethanol intake of eight subjects when differently concentrated ethanol solutions were given, is shown in Figure 3. As the pairs of ethanol concentrations were higher, the consumption of ethanol solution was less (Kruskall Wallis ANOVA; COP, N = 8, χ^2 = 284, p<0.001) (Friedman ANOVA N=8, χ^2_R = 22.95, p<0.001) and of drinking water was more (Kruskal Wallis ANOVA, COP, N = 8, χ^2 = 124, p<0.001; Friedman ANOVA N = 8, χ^2_R = 10.35, p<0.01).

Figure 3 also illustrates that when the concentrations were low, the consumed volume of drinking water was less, but that the total volume of water ingested was more (Kruskall Wallis ANOVA; COP, N = 8, χ^2 = 126, p<0.001; Friedman ANOVA, N = 8, χ^2_R = 10.05, p<0.05). This shows that the variation in the

consumption of ethanol solution and drinking water did not result in a stable total water intake.

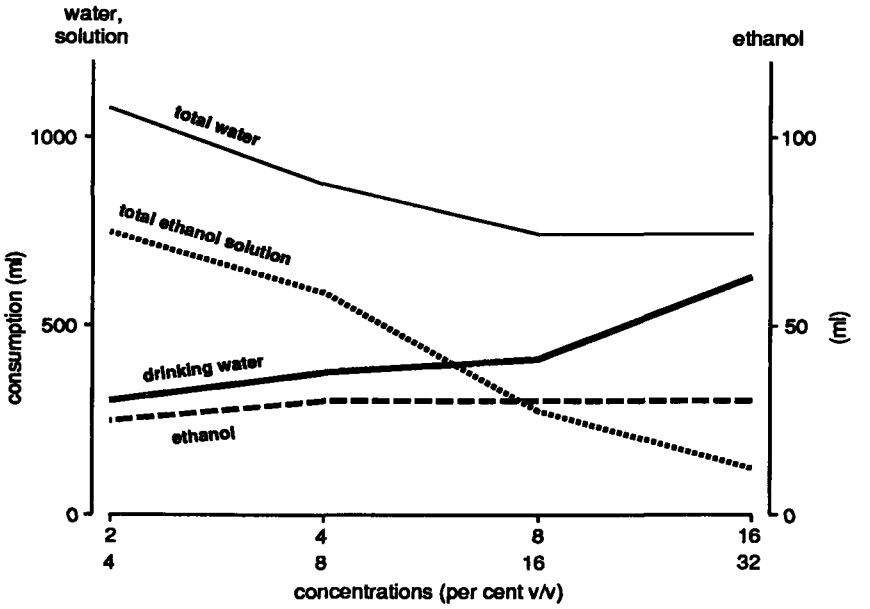

Figure 3. Mean daily consumption of total ethanol solution, drinking water, total water (left vertical axis) and total net ethanol intake (right vertical axis), as a function of the ethanol concentration of the solutions. Statistical test results are given in the text.

Individuals varied total net ethanol intake significantly with the concentration of the solutions (Kruskal-Wallis ANOVA; COP N = 8, χ^2 = 80, p<0.001). When the concentrations were low (2-4 per cent), net individual ethanol intake was low but otherwise there were no consistent differences in ethanol intake between experimental periods; overall the concordance among subjects was just beyond statistical significance (Friedman ANOVA N = 8, χ^2_R = 7.35, p = 0.06).

Figure 4 gives the amount of net ethanol ingested through consumption of each of the different, simultaneously presented solutions. The figure shows that the amount of ethanol ingested was quite distinct for the various alternatives. When the concentrations were low (2-4 per cent) most ethanol was ingested through the more concentrated solution (Wilcoxon matched pairs, N = 183, W = -5, p<0.0001). When the presented concentrations were high, more ethanol was ingested through the less concentrated solution (8-16 per cent: Wilcoxon-matched

pairs N = 110, W = -3, p<0.001; 16-32 per cent: N = 181, W = -5, p<0.0001). In other words, solutions of intermediate concentrations led to a higher net ethanol intake compared to the high and low concentrations offered.

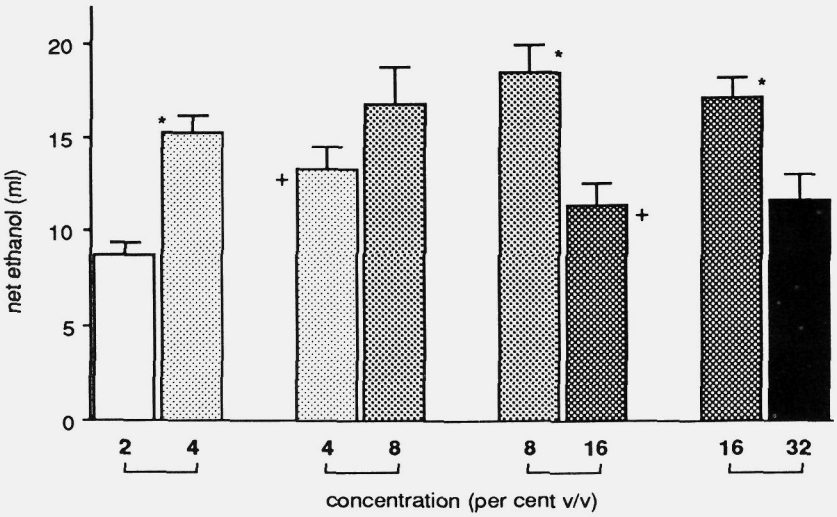

*Figure 4. Mean daily net ethanol intake from the simultaneously offered solutions. Comparison between intakes from simultaneously offered solutions was made by use of Wilcoxon matched pair test (*p<0.001). Comparison between intakes from solutions of the same concentration in a different combination was performed by means of the Mann-Whitney U test (+p<0.05). Vertical bars represent standard errors of the mean.*

However, significantly less ethanol was ingested through 4 per cent solution when it was presented with 8 per cent than with 2 per cent solution (Mann-Whitney U-test, N1 = 183, N2 = 112, U = 8753, p<0.05). And significantly more ethanol was ingested from 16 per cent solution when it was presented with 32 per cent than with 8 per cent solution (Mann-Whitney U-test, N1 = 110, N2 = 181, U = 7196, p<0.0001). This shows that net ethanol intake through a certain solution depended also on the concentration of the alternative solution.

Figure 5 illustrates the relative distribution over the day of the consumption of drinking water and the net ethanol intake as measured at different times of the day during the last observation period (16-32 per cent). The figure shows that the measured drinking water consumption was relatively high across subjects at 10.30 and 16.30 hr (Friedman 2-way ANOVA N = 8, χ^2_R = 25, p<0.001) which represents the time intervals shortly after the two dry daily meals. The distribution of

ethanol intake over the time of the day showed a high value at 9.00 hr (Friedman 2-way ANOVA, N = 8, χ^2_R = 12.93, p<0.05), which represents a long time interval (from 16.30 hr the previous day to 9.00 hr).

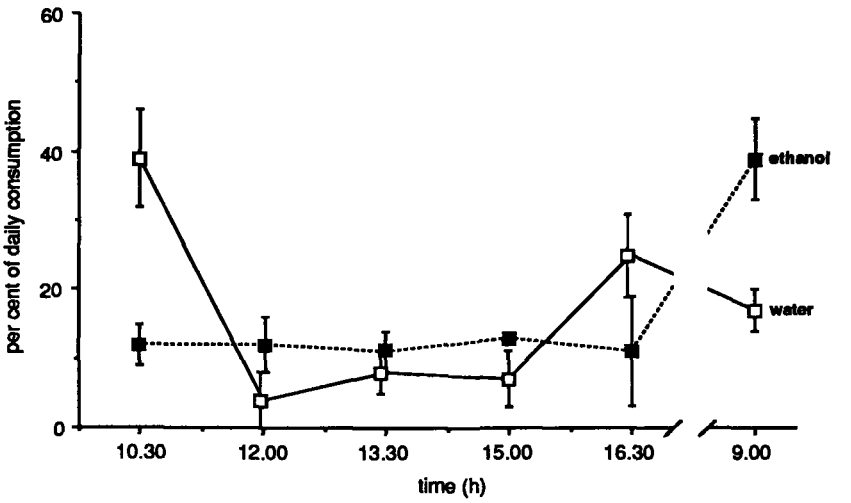

Figure 5. Mean relative distributions over 24 hours of net ethanol intake and drinking water consumption. Registration times represent mean consumption as per cent of total 24 hr water or ethanol intake during the previous inter-measurement interval (1.5 h-during daytime; 16.5 hr-during the night). Points 10.30 hr and 16.30 hr represent consumption after a dry meal. Ethanol concen-trations of the solutions were 16 and 32 per cent v/v. Vertical bars represent standard errors of the mean. Statistical test results are given in the text.

However, the variation in the ratio between the net ethanol intake and drinking water consumption was also significantly similar among subjects (Friedman 2-way ANOVA, N = 8, χ^2_R = 19.93, p<0.002). This shows that net ethanol intake and of drinking water consumption were differently distributed over the time of the day. Consumption of drinking water was clearly related to dry food meals, whereas the intake of ethanol was not. The same difference between water and ethanol intake existed throughout the whole series of observations as well as during observation series I (not shown).

The blood samples, which were taken after the night period at 9.00 hr yielded in most cases blood ethanol levels (BEL's) below detection limit but in 16 per cent (N=80) BEL's were detected. Values were above 3 mg.dl^{-1}. Positive values were rather evenly distributed over the subjects; one to three positive samples

were found in seven of the eight subjects. Of these positive samples 46 per cent (N=13) were over 20 mg.dl^{-1} and 23 per cent over 50 mg.dl^{-1}.

In general the monkeys gave no indication of intoxication; only twice we noted a clear sign of intoxication. In both cases the animal appeared unable to support itself and had a very drowsy gaze.

Discussion

In this study the initiation of alcohol drinking was quite rapid, even though no other manipulations than just making ethanol/water solutions available were used. This seems somewhat surprising in view of the variety of conditioning techniques reported necessary for initiating drinking behaviour (3, 6, 20, 31). The general significance of these techniques is presumably to overcome an initial aversion from alcohol drinking and to establish a specific contingency between the consuming behaviour and the effect of ethanol (6, 7, 16, 31). The present results, however, are in agreement with other, sometimes occasional observations that considerable consumption of alcohol can also occur after spontaneous initiation in monkeys (14, 35, 38, 45) as well as in rats (25, 29, 34).

One might presume that the present study inadvertently, represents a specific induction yet, namely through: a) deprivation of food which could have occurred during time intervals between the meals; food-associated drinking as a probable consequence (6, 19, 31); and b) temporary inavailability of drinking water possibly as a consequence of bottle draining, which would leave the ethanol solution as the sole liquid source temporarily. This hypothesis, however, is unlikely. Although food was supplied in meals, consumption took place over a period of time and some food remained available to the monkeys throughout the intervals between feeding times. Bottle draining did not occur during the onset of drinking; the few times it did occur later on it concerned bottles with a low concentrated ethanol solution rather than the drinking water bottle. Moreover, the one and a half hour interval to the next refill is too short to effectuate a degree of water deprivation comparable to that in food-associated drinking induction.

The alcohol drinking presently reported might be interpreted as just an alternative method of consuming water that became less attractive when ethanol concentrations were higher (3, 31). If this holds true, one expects that the consumed volume of drinking water was complementary to the volume of water ingested with the solution; the average total volume of water intake would be stable. Results showed that the total volume of water intake was high when ethanol concentrations of the solutions were low. This indicates that a concentration dependent decrease in ethanol solution consumption was only partly com-

pensated for by the increase in drinking water consumption; i.e., consumption of large quantities of ethanol solution involved an excessive net water intake. If drinking of ethanol solutions were just an alternative method of consuming water, one would also expect that the variation in the consumption during the day, was similar for drinking water and ethanol intake (9). However, results showed that consumption of drinking water was clearly food related whereas the ethanol intake was not. The drinking of ethanol solution therefore, cannot be interpreted as just an alternative for consuming water.

The consumption of ethanol solution clearly depended on the concentration of the solutions. In successive periods when the pairs of concentrations were higher, consumed volume of solution was lower. This relationship could be attributed to a just concentration dependent decrease in palatability (3, 30, 31). However, this interpretation alone is not sufficient. Consumption from the 4 per cent solution was more when the alternative was 2 per cent than when it was 8 per cent, and consumption from 16 percent was more when the alternative was 32 than when it was 8 percent. This demonstrates that consumption was not just concentration determined; it also depended on the concentration of the alternative solution. Consumed volumes of solution decreased with increasing concentrations, but the total net ethanol intake was fairly constant (only when ethanol concentrations were lowest, the intake was low). Interpreting the present results in terms of positive reinforcement, the data suggest that net ethanol intake was reinforced, up to a certain limit, rather than the drinking of ethanol solutions.

Blood samples were taken after the observation series were completed. As alcohol supply was continued and the monkeys maintained their level of consumption, results are likely to apply also to the observation period reported. Blood level determinations showed a number of positive values, some of which were between 50 and 100 mg/dl. This indicates that the consumption could lead to blood ethanol levels at which central effects can be expected (16, 28, 54). The occurrence of undetectable levels probably related to the fact that samples were taken at 9.00 hr. Within the interval between 16.30 to 9.00 hr, the drinking could have occurred shortly after 16.30 as well as shortly before 9.00 hr. The frequency of undetectable levels together with the fact that the rate of ethanol elimination in monkeys is comparable to that in humans (6, 24, 54), suggests that consumption was more often in the late afternoon rather than in the early morning.

In studies of operant self-administration of drugs, including ethanol, net total drug intake has been reported to increase with increasing concentrations (11, 23, 32, 48, 50). This concentration dependent relationship has been regarded as characteristic for positive reinforcement in drug self-administration (2, 11, 26). In free-choice studies involving unrestricted or long-term ethanol supply, net daily

ethanol intake is reported to be fairly constant, independent of the concentration available (5, 12, 19, 34; and this study across the last three experimental periods).

The present 3-bottle-preference design, however, showed a dose-dependent increase in the lowest part of the range of concentrations (intake increased when concentrations changed from 2-4 into 4-8 per cent). Moreover, within the simultaneous choice, a higher ethanol intake was derived from the stronger concentration in the 2-4 per cent combination; for some monkeys (5 subjects) this held also true in the 4-8 per cent combination (although this was below statistical significance for the group as a whole, p: = 0.13). These phenomena suggest a similarity with findings in operant drug self-administration studies (18, 26, 31, 51) at least for the range of lower concentrations. Furthermore, close examination of the reported data on operant drinking, shows that ethanol intake does not increase linearly with concentration; the increase diminishes or levels off in the range of higher concentrations (11, 17, 19, 34). In other words, a horizontal or constant level (like in free-choice drinking studies) probably does occur also in the operant paradigms, at higher concentrations (18). These observations suggest that differences in free-choice and operant controlled drinking are influenced by the same variables; in free-choice drinking ethanol intake, however, levels off at lower concentrations than in operant controlled drinking. This difference could relate to a difference in the way in which alcohol drinking was initiated. However, it is also possible that the difference relates to whether alcohol is supplied only during relatively brief sessions or periods throughout the day (29).

In current concepts on self-administration of potentially addictive drugs, it is assumed that a drug (including ethanol) will positively reinforce this behaviour by exertion of central effects (4, 49, 52, 55). It has been reported that the centrally mediated effects of ethanol, as well as of opiates, are experienced as pleasurable and stimulating in low doses but negative and depressive in high doses (4, 18, 37, 39, 41). Empirical evidence indicates that the pleasurable positive actions and the negative, potential harmful action of certain drugs are represented by different neurobiological systems of positive reinforcement (reward) and of behavioural inhibition i.e. harm avoidance (4, 46, 49, 55). The levelling off of the intake at higher concentrations could indicate that at higher concentrations an increase in positive reinforcement was counterbalanced by an increased avoidance of negative effects (39, 49).

The present results show that when meeting the criticisms to earlier studies, spontaneously initiated alcohol drinking under free-choice conditions in rhesus monkeys appears to be controlled by ethanol reinforcement as well as by other regulatory factors. Since spontaneously initiated drinking lacks some of the limitations of operant controlled drinking, it is likely to represent a valuable additional model of human drinking. As free choice drinking and operant controlled

drinking differ quantitatively, they could represent different forms of human drinking.

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2. ALCOHOL CONSUMPTION AFTER EXPERIENCE IN FREE-CHOICE DRINKING RHESUS MONKEYS; THE EFFECT OF DIFFERENT ETHANOL CONCENTRATIONS

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Abstract

Experience with alcohol drinking can modify alcohol drinking behaviour in men and animals. The present study reports on the effect of ethanol concentration on drinking behaviour in 8 free-fed non-conditioned rhesus monkeys who had experience with alcohol drinking for 6 months. Fluid preference and net ethanol intake were compared with findings of a previous study in which these monkeys had been provided with differently concentrated ethanol solutions for the first time. Drinking water and 2 ethanol/water solutions were concurrently available, 24 h per day. Concentrations of the solutions were respectively 2% and 4%, 4% and 8%, 8% and 16%, and 16% and 32%. Each pair of concentrations was provided for at least 2 weeks. Consumed volume of total ethanol solution decreased as concentrations increased and was generally less than consumed volume of drinking water. Water drinking appeared to follow a prandial pattern, whereas ethanol intake did not. Total daily net ethanol intake progressively increased as a function of increasing concentrations. Compared to a previous acquisition study, preference for 8% was increased, but for 2% solution decreased. Furthermore, total daily net ethanol intake was decreased in the 2%-4% period, but increased in the 16%-32% period. Unlike under naive conditions, the reinforcement strength of separate ethanol solutions (i.e. yielded amount of net ethanol) in experienced monkeys revealed an orderly concentration-dependent relationship. It is concluded that in the presented 24 h-access design, water drinking and ethanol intake were differently motivated behaviours and that fluid preference and pharmacological reinforcement by ethanol represented different aspects of alcohol drinking behaviour. By experience with alcohol, free-choice alcohol drinking appeared to have become more under control of reinforcement principles and less inhibited by an aversion factor, although (in)palatability of high concentrations still may play some role. Tolerance for the effects of ethanol might have led to the enhanced ethanol intake from high concentrations. It has been postulated that tolerance does not develop for central reinforcing stimuli. Whether tolerance also could have been responsible for less reinforcement by the low concentrations, leading to less ethanol intake, seems therefore discutable. Another hypothesis to explain the reduced reinforcement by low ethanol concentrations, could be an altered central bioavailability of ethanol.

Introduction

Alcoholic beverages are consumed for reasons of palatability as well as for the effects ethanol exerts upon the central nervous system (Myers and Ewing, 1980;

Kiefer and Dopp, 1988). Individual preferences for the kind of beverage and for a certain intake level in general get established after some period of experimentation with alcohol, having explored the various available beverages and the different effects of ethanol (Van Dijk, 1979; Samson and Grant, 1990). Thereafter, most consumers appear to continue alcohol consumption in a stabilized drinking pattern; in some cases the drinking behaviour evolves into recurrent alcohol abuse and alcohol dependence (Van Dijk 1979; APA, 1987).

Alcohol consumption by experimental animals and humans seems to be (at least partly) controlled by common mechanisms (Griffiths et al., 1980; Meisch, 1982; Samson and Grant, 1990). A general observation is that initially beverages with a high content of ethanol are disliked by men and animals (Cicero, 1980; Myers and Ewing, 1980; Meisch, 1982). By use of operant conditioning procedures however, ethanol could positively reinforce alcohol drinking in experimental animals in a wide range of ethanol concentrations (Meisch and Thompson, 1974; Henningfield and Meisch, 1979; Elmer et al., 1987). These studies indicated a relationship between the ethanol concentration and the amount of net ethanol ingested; ethanol intake was more when ethanol concentration was higher (Griffiths et al., 1980; Meisch, 1984). Ethanol-naive, non-conditioned animals, which averted consuming large amounts or high concentrations of ethanol initially (Lester and Freed, 1973; Cicero, 1980; Crowley et al., 1983), developed an enhanced acceptance of high concentrations and increased intake levels after experience with alcohol (Rick and Wilson, 1966; Veale and Myers, 1969; Myers et al., 1972; Samson et al., 1991). The various studies thus indicate that alcohol drinking behaviour of animals changed by specific conditioning procedures and/or by prolonged experience with alcohol.

It has been suggested that experience with alcohol drinking might reduce the aversion for alcohol, because of the development of tolerance for the effects of ethanol (Rick and Wilson, 1966; Holloway et al., 1989; Vogel-Sprott and Sdao-Jarvie, 1989). Tolerance has been postulated to develop mainly for the aversive effects of alcohol, rather than to the positive reinforcing effects (Cicero, 1980; Marlatt et al., 1988; Kiefer and Dopp, 1989). If these assumptions are correct, they lead to the hypothesis that alcohol drinking in experienced animals will be less determined by aversion factors than in naive animals and hence will be more under control of reinforcement laws (Cicero, 1980; Meisch, 1984).

Data collected in a previous study (Kornet et al., 1990) indicated that non-conditioned free-choice alcohol drinking by alcohol-naive rhesus monkeys was directed to ingest a certain amount of net ethanol per day, but also was influenced by a concentration-dependent aversion. In general the monkeys preferred to drink the lowest available concentration in a concurrent choice between water and two differently concentrated ethanol/water solutions. Nevertheless, the average daily

intake of total net ethanol, after an initial increase, remained rather constant (individual intake levels: 2-6 ml.kg^{-1}), when concentrations were raised from 2% to 32% (v/v). The present study investigated the effect of reintroduction of concurrent choices between water and differently concentrated ethanol solutions on fluid preference and on daily net ethanol intake. The results were compared to those obtained for the same monkeys under the same experimental conditions, but collected during a period of initial acquisition of alcohol drinking (Kornet et al., 1990).

Materials and Methods

Subjects

The subjects were eight male adult rhesus monkeys *(Macaca mulatta)*, who were 5 to 9 years of age and weighed 7 to 10 kg of bodyweight. At the onset of the present study the subjects had been drinking differently concentrated (2% to 32% v/v) ethanol/water solutions for about half a year. No other induction procedure had been used than making ethanol/water solutions, in addition to drinking water freely available (Kornet et al., 1990). The subjects were housed in separate cages in one room, that was temperature (24^{0}C) and humidity (60%) controlled and illuminated from 08.00 to 18.00 hr, in addition to the natural daylight through a window. Diet was composed of monkey chow (200 g per day, at 09.10 hr), fruit and vegetables (at 13.40 hr) and bread (at 15.40 hr).

Drinking equipment

Three inverted cylinders with a scale graduation (per 5 ml) and closed by a rubber stop holding a stainless steel nipple, had been attached at the side of each cage, so that only the nipples were in reach of the monkeys. Liquids were consumed by licking or sucking at the nipple. Spillage was negligible as could be concluded from the frequent observation of the animals and control of the equipment.

During four experimental periods of at least two weeks each, monkeys had concurrent access to the three drinking cylinders for 24 h per day, one containing drinking water, the other two containing differently concentrated ethanol/water solutions. In the consecutive experimental periods the following fluids were thus available: 1) 0%-2%-4%, 2) 0%-4%-8%, 3) 0%-8%-16%, 4) 0%-16%-32%. Consumed volumes were measured 6 times per day (at 09.00; 10.30; 12.00; 13.30; 15.00; 16.30 hr). After each measurement, the drinking cylinders were refilled up to 500 ml, so that the need for fluid between the times of measurement was amply met.

Data analysis

Preferences for concurrently available fluids within each experimental period, were defined by comparing the daily consumed volumes of the presented fluids. Whenever a day of measurement was interrupted by technical or other unforeseen disturbances, such a day was skipped in the data analysis. Comparison of preference data were made within subjects and analyzed by means of t-tests for related samples (Friedman, 1988).

The consumption pattern across the day (24 h) of net ethanol intake and of drinking water was analysed, for each of the four experimental periods, by transforming the mean consumption at the six times of registration, into per cent of total 24-h net ethanol and water consumption, respectively.

Daily net (100%) ethanol intake was determined by calculating the ethanol content of the consumed volumes of the two ethanol solutions and by transforming it into $ml.kg^{-1}$ bodyweight (bwt).

Relative reinforcement by the two concurrently available ethanol solutions was based on the amount of net ethanol obtained from each solution, which was subsequently transformed into the percentage an ethanol solution had contributed to the total daily net ethanol intake (i.e. the amount of net ethanol obtained from the lower (ES_L), respectively. higher concentrated ethanol solution (ES_H) divided by the total amount of daily net ethanol obtained from the lower and the higher ethanol solution ($ES_L + ES_H$).

Comparisons across the experimental periods between daily patterns of drinking water and net ethanol intake, and between total net ethanol intake during acquisition and after experience, were statistically analysed by means of a 2-way analysis of variance for related measurements (Friedman, 1988).

Results

Fluid Preference

Figure 1 shows the mean consumed volume of drinking water and the mean consumed total volume of ethanol solution (sum of both solutions) for the different pairs of concentrations, that were available during the consecutive experimental periods. When the concentrations were low (2%-4%), water and ethanol solution were daily consumed in comparable amounts, but as the concentrations increased, progressively more water than ethanol solution was consumed. The total (i.e. from all fluids available) daily water intake did not significantly vary across the consecutive experimental periods.

The mean consumed volume of each ethanol solution is also indicated in Figure 1. A significant preference for the higher over the lower concentrated

ethanol solution occurred only for the pair of 2% and 4% solutions. The opposite was found for the two pairs of higher concentrations, i.e. the lower was significantly preferred over the higher concentrated solution. As can be inferred from Figure 1, the consumption of 16% solution increased significantly as the concentration of the alternative changed from 8% to 32% (N=8, $p<0.01$).

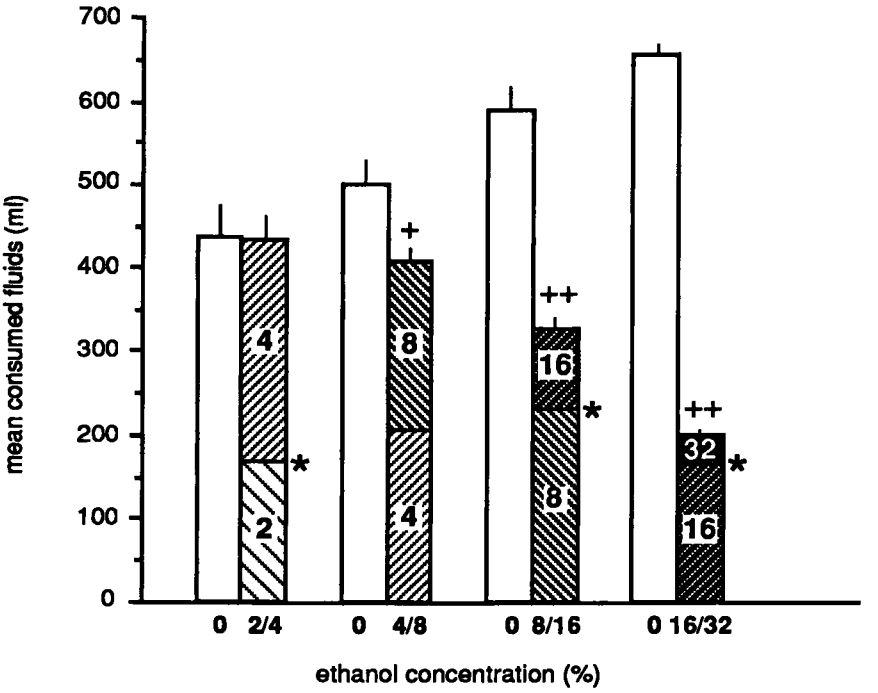

Figure 1. Fluid Preference. Mean (+sem) daily consumed volume of water and total ethanol solution (sum of two solutions), and mean consumed volume of single ethanol solutions, as a function of ethanol concentration(s). + $p<0.05$, ++ $p<0.01$ significant difference between consumed volume of water and total ethanol solution;
**$p<0.01$ significant difference between consumed volumes of each ethanol solution, paired t-tests.*

Consumption Patterns

The consumption pattern across 24 hours for drinking water and total net ethanol intake was analysed by determining for each individual to what extent (in per cent) a consumption measured at one of the six daily registration times, contributed to the total amount (across 24 hr = 100%) of drinking water or net ethanol. Figure 2

shows the relative distribution of net ethanol and drinking water during the *2%-4%* (a), *4%-8%* (b), *8%-16%* (c) and *16%-32%* (d) period. It should be noticed that each registration point in Figure 2 represents the consumption (in per cent) during the previous intermeasurement interval (1.5 h-periods during day time; a 16.5 hr-period during the night); the points 10.30 hr and 16.30 hr represent consumption during intervals following a meal.

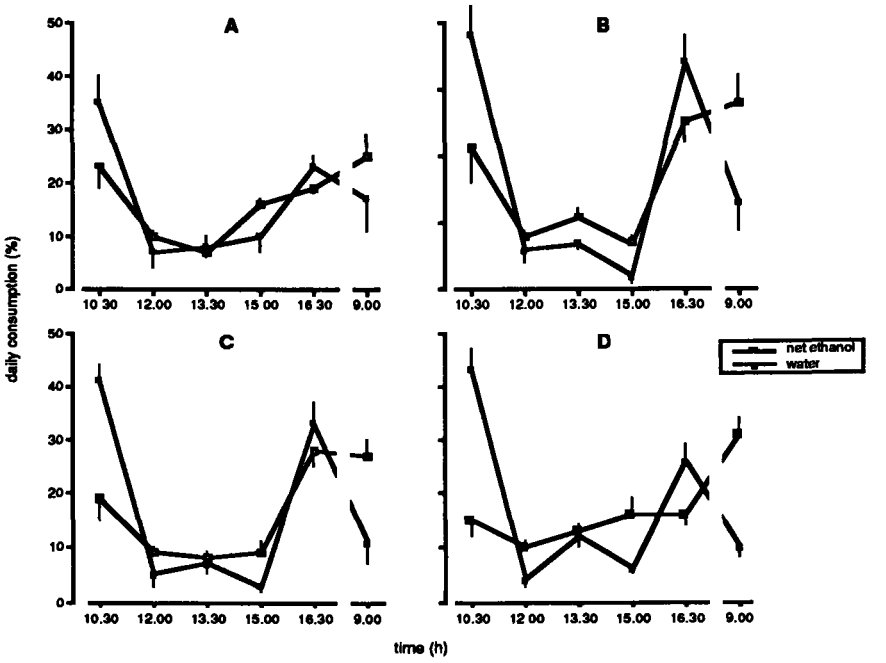

Figure 2. Consumption Pattern. Consumption of net ethanol intake (ethanol) and drinking water (water) as the mean (+sem) per cent of the total (24 hr) net ethanol and total (24 hr) drinking water consumption, measured at the six daily registration times during the 2%-4% period (a), the 4%-8% period (b), the 8%-26% period (c) and the 16%-32% period (d). Points 10.30 hr and 16.30 hr represent consumption after a dry meal. Statistical results are given in the text.

Comparison of the pattern of water drinking and ethanol intake revealed significant differences in all experimental periods. For the *2%-4% period* (a), a significant distinction existed between the relative distributions of ethanol and water intake (ANOVA Per Cent Consumption $F(1,7)= 6.99$, $p<0.05$; Time of

Registration F(5,7)= 9.59, p<0.001; Per Cent Consumption x Time of Registration F(2.44) p=0.05). During the 16.5 hr-interval water drinking was relatively low and ethanol intake relatively high; after the dry meal in the morning the water drinking was relatively high. Results for the other experimental periods were quite similar, the interaction effects being even stronger. Statistical (ANOVA) outcomes were: *4%-8% period* (b): Per Cent Consumption F(1,7)=0.64, p=ns, Time of Registration F(5,7)=25.65, p<0.01, Per Cent Consumption x Time of Registration F(1,5)=4.70, p<0.01; *8%-16% period* (c): Per Cent Consumption F(1,7)=0, p=ns, Time of Registration F(5,7)=23.56, p<0.001, Per Cent Consumption x Time of Registration F(1,5)=8.61, p<0.001; *16%-32% period* (d): Per Cent Consumption F(1,7)=0.37, p=ns, Time of Registration F(5,7)=25.73, p<0.001, Per Cent Consumption x Time of Registration F(1,5)=15.85, p<0.001. Although to some lesser extent than after the meal in the morning (9.10 hr), in general water drinking also was relatively high after the slice of bread in the afternoon (16.30 hr).

Net Ethanol Intake

Figure 3A shows the mean daily total net ethanol intake ($ml.kg^{-1}$ bwt) during the different experimental periods when the monkeys had been provided with differently concentrated ethanol solution for the first time (acquisition; Kornet et al., 1990), and when these solutions were provided again after some period of alcohol drinking (experienced; present study). During the acquisition period total net ethanol intake as a function of increasing ethanol concentrations represented a different concentration-effect relationship, than total net ethanol intake when the monkeys were experienced drinkers (ANOVA Condition F(1,7)=0.56, p=ns; Increasing Concentrations F(3,7)=11.11, p<0.001; Condition x Increasing Concentrations F(1,3)=15.19, p<0.001). As Figure 3A illustrates, total net ethanol intake during acquisition initially increased and subsequently remained at a rather constant level, whereas in the experienced condition total net ethanol intake increased progressively. The within-subject reliability coefficient for individual total net ethanol intakes during acquisition was R=0.82, during experienced drinking R=0.86. Compared to the acquisition condition, significantly less total net ethanol was ingested in the experienced condition when the 2%-4% pair of ethanol concentrations was available, but significantly more was ingested in this condition during access to the 16%-32% pair. Figure 3B shows the mean relative (in per cent) reinforcement (defined by the quantity of net ethanol in a consumed volume of a solution) by the highest concentrated alternative during acquisition and in experienced subjects. Figure 3B suggests a partial shift to the right in the concentration - reinforcement relation-ship; up to the 8%-16% pair of concentrations a higher concentrated alternative seems to have become more reinforcing in

comparison to a lower alternative. The 32%-solution however seems to have become less reinforcing.

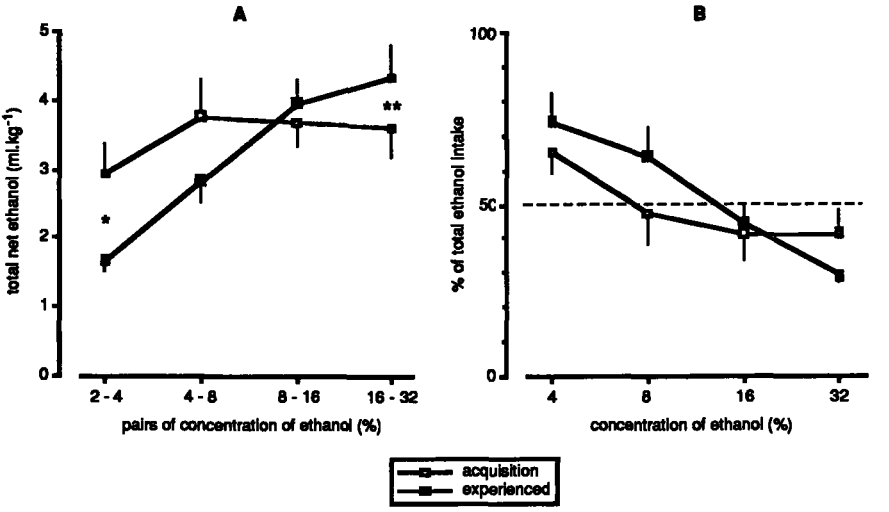

*Figure 3. Net ethanol intake. a: Mean (+sem) total (sum of both solutions) net ethanol intake per day as a function of the ethanol concentrations under conditions of acquisition of and experience with alcohol drinking. *p<0.05, **p<0.01 significant difference between total net ethanol intake during acquisition and after experience, paired t-tests. Further statistical results are given in the text.*

b: Mean (+sem) amount in per cent of net ethanol (reinforcement) of the higher concentrated ethanol solution compared to the concurrent lower alternative as a function of ethanol concentration, under conditions of acquisition of and experience with alcohol drinking. Statistical results are given in the text.

This suggestion from the figure could not be confirmed by statistically; analysis indicated only a general effect of increasing concentrations (ANOVA Condition $F(1,7)= 0.76$, p=ns; Increasing Concentrations $F(3,7)=8.92$, $p<0.001$; Condition x Increasing Concentrations $F(1,3)=1.74$, p=ns). Figure 4 basically shows the same data as Figure 3B, but now displays the individual data on relative reinforcement, during acquisition (A) and during the experienced condition (B). The figures demonstrate that experienced individuals (B) showed a more consistent and orderly concentration-effect relationship compared to the first time they had

access to these ethanol solutions (A). From the Figure 4B it is furthermore obvious that when experienced, all individuals unanimously obtained the most ethanol from the 16% solution when the concurrent alternative was a 32% solution.

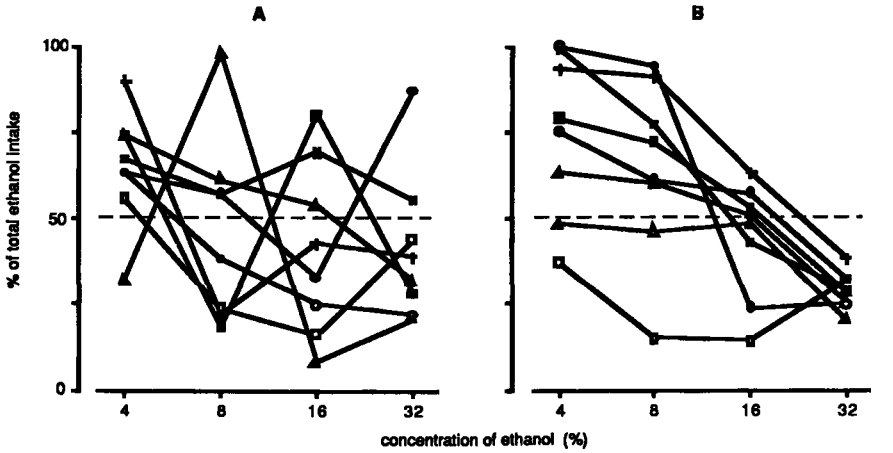

Figure 4. Individual concentration-reinforcement relationships. Mean amount in per cent of ethanol (reinforcement) of the higher concentrated ethanol solution compared to the concurrent lower alternative for each individual monkey (n=8) as a function of ethanol concentration a. during acquisition (a) and b. after experience with alcohol drinking (b).

Discussion

The different ethanol concentrations of the solutions significantly influenced the consumed volumes of ethanol solution and drinking water as well as the total daily net ethanol intake. Like in operant responding monkeys, that had been habituated to alcohol in advance (Henningfield and Meisch, 1979), when ethanol concentrations increased total consumed volume of ethanol solution progressively decreased and of drinking water increased. Unlike these monkeys (Henningfield and Meisch, 1979), the consumption of drinking water of the presently studied monkeys was more than consumption of ethanol solution, except for the first experimental period.

A preference for water over alcohol has been regarded as an indication that alcohol is disliked (Kiefer and Dopp, 1989), and might suggest that the drinking

behaviour is not ethanol-reinforced (Crowley et al., 1983; Elmer et al., 1987; Ritz et al., 1989). The present data which were sampled within a 24-h access paradigm, support however the assumption that preference (i.e. consumed volumes) for water versus ethanol solutions and pharmacological reinforcement by ethanol represent different aspects of alcohol drinking behaviour (Cicero, 1980; Dole and Gentry, 1984).

The major part of water drinking occurred during eating of dry food supplies. Ethanol intake was not apparently dependent on feeding times, but was more evenly distributed across day and night, especially when ethanol concentrations were higher. This non-prandial pattern of ethanol ingestion suggests that, unlike drinking water, drinking of ethanol solutions did not primarily function as an alternative way to meet fluid demands. The discrepancy in patterns therefore suggests that water drinking and ethanol ingestion were differently motivated behaviours (Dole and Gentry, 1984). In rats this has been found only occasionally (Samson et al., 1988a).

Alcohol drinking when in combination with feeding has been shown in monkeys to lead to lower (by about 42%) and delayed peak plasma ethanol concentrations than when not in combination with feeding, due to a difference in rate of absorption (Kalhorn et al., 1986). Delay in peak plasma ethanol concentration could imply a post-ingestion delay of pharmacological effects of ethanol. Such a delay has been regarded as obstructing the acquisition of alcohol drinking in animals, because it affects establishment of a response-reinforcement contingency (Carroll, 1987; Meisch, 1987; Samson et al., 1988b). The present non-prandial ethanol intake, observed in the acquisition and in the present study, might have contributed therefore to the response-reinforcement association.

Daily total net ethanol intake increased as a function of the concentrations. It can be inferred from the presented data (Fig. 1) that the consumed volumes of ethanol solutions (i.e. drink response) described an inverted U-shaped function as a function of the ethanol concentration (dose). Such a relationship between dose-response together with an increase in drug intake, are well-known characteristics in operant conditioned drug (including ethanol) self-administration studies and are regarded as an indication that the drug (i.e. ethanol) has pharmacological positive reinforcing effects (Meisch and Thompson, 1974; Griffiths et al., 1980; Elmer et al., 1987). Hence, it is assumed that in the monkeys ethanol functioned as a positive reinforcing drug under the presented experimental conditions.

In comparison to the acquisition study (Kornet et al., 1990), drinking behaviour of the rhesus monkeys had changed after experience, with respect to their preference for ethanol solutions as well as to net ethanol intake.

Relative preference for one of the two concurrently available ethanol solutions had changed particularly in the lower range of concentrations (2% to 8%). After

experience with alcohol drinking, the preference for the 8% solution was increased, but the preference for the 2% solution was strongly decreased.

In the higher range of concentrations (8%-16%; 16%-32%) relative preferences were rather similar in experienced and naive animals. More volume was consumed from the lower alternatives. However, consumption from the less preferred 16% solution increased unanimously when the alternative changed from an 8% to a 32% solution. Apparently, the inference of inpalatability of a certain fluid on the consumption, depended on the alternative available fluids.

In contrast to the orderly concentration-dependent increase of total net ethanol intake in the present study, total net ethanol intake in the acquisition study only increased with concentration in the lower range of concentrations, but subsequently was sustained at one level. After experience, free-choice drinking behaviour seemed therefore to be more under control of reinforcement principles.

Compared to the acquisition study, total net ethanol intake in experienced monkeys was significantly less when 2% and 4% solutions were available, but significantly more when 16% and 32% solutions were available. This shows that the monkeys apparently were not striving to titrate some pleasurable intake level, as might be inferred from the rather constant mean intake level observed in our acquisition study and in other unrestricted drug self-administration studies (Griffiths et al., 1980; Grant and Johanson, 1987; Samson et al., 1988b).

The opposite changes in total net ethanol intake might be explained by two different mechanisms in alcohol drinking: i.e. by tolerance for ethanol's effects and by principles of reinforcement. It could be that at initial contact the taste of high concentrations and/or the pharmacological effects of high (i.e. toxic, depressive) intake levels were aversive to the subjects and set some maximal threshold to the ingestion (Cicero, 1980; Myers and Ewing, 1980; Kiefer and Dopp, 1989). Due to the development of habituation to the solutions and/or of tolerance to ethanol's adverse effects, aversion might have become less (Gatto et al., 1987; Kiefer and Dopp, 1989). This could explain why experienced monkeys ingested more total net ethanol intake in the high concentration period (16%-32%) than under naive conditions.

According to the principles of reinforcement, weak positive reinforcement will lead to less operant behaviour than strong reinforcement (Van Ree, 1979; Meisch, 1984). If the monkeys had become less sensitive not only for the adverse, but also for the pleasurable effects of ethanol, low concentrations of ethanol could have become less reinforcing than before, thus leading to less operant behaviour (free-choice drinking) (Meisch, 1982). The decrease in the relative reinforcement by the lower concentrated alternatives (Fig. 3B) seems to support such an interpretation. Empirical evidence however, indicates that central systems involved in positive reinforcement do not develop tolerance (Broekkamp et al.,

1976; Colpaert, 1978; Wise and Bozarth, 1987), a phenomenon which seems specific for the toxic and depressant effects of certain addictive drugs, incl. ethanol (Cicero, 1980; Marlatt et al. 1988; Kiefer and Dopp, 1989). Therefore, it seems likely that the reduced reinforcement by lower ethanol concentrations in the experienced subjects could have been due to a lower central bioavailability of ethanol, rather than to a reduced receptor sensitivity for the positive reinforcing effects of ethanol (Van Ree, 1979). Pharmacokinetic studies on the effects of ethanol have revealed that chronic alcohol drinking enhances the metabolism of ethanol in humans and nonhuman primates (Kater et al., 1969; Pieper and Skeen, 1973). The low concentrated solutions might thus have failed in the experienced monkeys to exert significant central effects, leading to less activation of positive reinforcement systems.

Although relative reinforcement of ethanol in a concurrent choice has been investigated relatively little (Samson and Grant, 1990), it has been assumed that a higher concentrated drug dose (or concentration) will be more reinforcing than a lower alternative (Johanson and Schuster, 1975; Lemaire and Meisch, 1984; Carroll, 1987). For the higher range of ethanol concentrations this was not always the case (Fig. 4B). The present study on free-choice alcohol drinking suggests that although behaviour was under control of reinforcement principles, it could still be influenced by the degree of (in)palatability of a solution.

An interesting finding was that within-individual relationships between ethanol concentration and relative reinforcement were quite variable during acquisition, but quite orderly after the same rang of concentrations were reintroduced again.

Not unlike reports on humans (Van Dijk, 1979), it seemed as if the initially alcohol-naive monkeys had used the previous acquisition period, to experience and to experiment with the aversive as well as the reinforcing effects of alcohol drinking. Thereafter their behaviour showed quite some similarities with the ethanol-reinforced behaviour of operant conditioned animals (Hyytiä and Sinclair, 1989), which usually also are habituated to different ethanol concentrations in advance (Griffiths et al., 1980; Meisch, 1984).

Free-choice and unrestricted availability in the present study did not lead to aberrant alcohol drinking; intoxication was rarely observed and blood alcohol levels never were extremely high at similar intake levels (maximal value was 0.7 $^0/_{00}$; Kornet et al., 1990). Probably due to the development of tolerance (Vogel-Sprott and Sdao-Jarvie, 1989) the maximum thresholds of intake level and acceptance of high concentrations nevertheless appeared to have shifted in an upwards direction. Interestingly reintroduction of low concentrations led to less ethanol ingestion. A conclusion could be that the monkeys after half a year of alcohol drinking were not addicted to alcohol yet, for they were not striving to adapt consumed volumes of low concentrated beverage to sustain some net

ethanol profit (Lester and Freed, 1973). On the other hand, it is far from certain that human alcoholics would consume large volumes if only low concentrated beverages were available. Another point worth of consideration is whether for regular social drinkers low concentrated alcohol solutions (e.g. currently available "light" alcoholic beverages) might prove to be a helpful strategy to prevent or reduce an upwards shift of ethanol ingestion (Samson et al., 1988b).

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3. THE EFFECT OF INTERRUPTED ALCOHOL SUPPLY ON SPONTANEOUS ALCOHOL CONSUMPTION BY RHESUS MONKEYS

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Abstract

The alcohol supply (a 16% and a 32%, v/v, ethanol-in-water solution) for eight male rhesus monkeys, who already have had free access to water and ethanol solutions concurrently for about one year, was interrupted for one, two or seven days. The previously acquired ethanol consuming behaviour appeared very resistant to extinction, since ethanol consumption was immediately resumed after renewed access, even at a temporarily increased level. Since physical withdrawal distress was not observed and the increase was higher when interruption lasted longer, the observed behaviour could be attributed to the reinforcing effects of ethanol, leading to specific ethanol-directed behaviour.

Introduction

It has been demonstrated in operant drug self-administration models that, under certain conditions, animals will self-administer specifically those substances, including ethanol (Meisch, 1982, 1984) that are known to cause dependence in humans (Kalant et al., 1978; Griffiths et al., 1980; Van Ree, 1979, 1987; Meisch, 1987). A possible explanation is that drug-directed behaviour in general is due to interaction with central reinforcement mechanisms that positively reinforce drug self-administration behaviour (Van Ree, 1979; Stewart et al., 1984; Dole, 1986; Wise and Bozarth, 1987). In alcohol-dependent humans, after periods of interruption of the self-administration (alcohol drinking), even when detoxification is achieved, remission of the alcohol-directed behaviour (i.e. dependence) is a commonly observed phenomenon (Jellinek, 1955; Nichols, 1972; Mendelson and Mello, 1979; Dole, 1986). Such a phenomenon suggests a strong resistance for extinction of ethanol-reinforced behaviour (Kalant et al., 1978; Griffiths et al., 1980; Cloninger, 1987). A matter of debate is to what extent experimental animals will consume ethanol primarily for its reinforcing effects under conditions of free-choice and unrestricted access (Cicero, 1980; Dole and Gentry, 1984; Meisch, 1984; Falk and Tang, 1988) and whether alcohol drinking by animals under such conditions bears any resemblance or relevance to human alcohol-induced behaviour (Lester and Freed, 1973; Marfaing-Jallat, 1979; Dole, 1986; Samson and Li, 1988). In a previous study, spontaneous ethanol drinking rhesus monkeys were shown to maintain ethanol intake also when only previously adversive solutions were available. This was interpreted as an indication that the behaviour, at least in part, represented ethanol-reinforced behaviour (Kornet et al., 1990). The present study was performed to investigate whether or not interruption of ethanol supply affected the subsequent drinking behaviour of

these rhesus monkeys. The study was meant in particular to examine the following hypotheses: a) If the absence of ethanol solutions leads to a diminished consumption of ethanol (extinction) afterwards, ethanol probably is a weak reinforcer for spontaneously drinking rhesus monkeys (Cicero, 1980; Meisch, 1984; Crowley and Andrews, 1987); b) If ethanol intake is continued at the pre-interruption, or at an even higher level, the reinforcing potential of ethanol is shown to be resistant to extinction (Elmer et al., 1986, 1987; George, 1987; Suzuki et al., 1988).

Methods

Subjects and materials

The subjects were eight male, adult rhesus monkeys *(Macaca mulatta)* (body weights 8-12 kg) housed individually in cages in one room. The room was temperature- and humidity-controlled. Pelleted food was supplied in the morning, whereas bread and fruits or vegetables were provided early in the afternoon. The subjects had continuous access to three drinking bottles, one of which contained water whereas the other two contained ethanol-in-water solutions (16 and 32% v/v). Supply of two solutions allowed the monkeys to ingest ethanol from different solutions and therefore it permitted some distinction between reinforcement of ethanol intake and preference for a solution concentration.

Consumed volumes were measured and bottles were refilled 6 times per day; all liquids were therefore available almost ad lib. At the onset of the study the monkeys had been given access to drinking water, 16% and 32% solutions concurrently for 4 months, ingesting between 2-6 $ml.kg^{-1}$ net ethanol per day.

Procedure

In five experiments interruption of alcohol supply lasted 2, 2, 2, 1 and 7 days, respectively; time intervals between successive experiments were respectively 2 weeks, 6, 2 and 3 month(s). In each interruption experiment, ethanol and water consumption were measured every 30 min at two pre-interruption days between 16.00 - 18.00 hr. In addition, consumption after 18.00 hr and up until 09.00 hr the following morning was also measured. At day three, at 16.00 hr, the two ethanol solutions were replaced by drinking water. Ethanol solutions were supplied again after the interruption, at 16.00 hr and consumption was measured again for the next two hours, every 30 min, and once for the interval between 18.00 hr and 09.00 hr. During the period of interruption, we checked for symptoms (such as tremor, vomiting, hyperactivity, irritability, convulsions) which have been reported to occur as withdrawal reactions in monkeys (ingesting 6 $g.kg^{-1}$ ethanol/day)

within 48 hr after forced abstinence (Ellis and Pick, 1972; Myers et al., 1972).

Data analysis

Paired comparison of the mean consumed volume in the two pre-interruption periods and the consumed volume in post-interruption periods was performed for each experiment by means of Wilcoxon matched pair signed rank test (Siegel, 1956). Concordance among the subjects in drinking behaviour after the various interruption intervals and on days during interruption was tested for each experiment by means of Friedman two-way analysis of variance (ANOVA) (Siegel, 1956). Because subjects behaved significantly concordant and each experiment showed a post-interruption increase, data for the three two-day interruption experiments are pooled in the illustration.

Results

Before as well as after interruption, the monkeys consumed significantly more 16% than 32% ethanol solution (before interruption average consumption ± SEM between 16.00 and 18.00 hr of 16%: 54.0 ± 9.5 ml and of 32%: 9.8 ± 2.1 ml, Wilcoxon $p<0.05$; after interruption, average consumption of 16%: 131.7 ± 20.6 ml and of 32%: 17.4 ± 5.7 ml, $p<0.05$). Thus, the subjects maintained a preference for the 16% solution after interruption. Figure 1A gives the mean total net ethanol intake between 16.00 and 18.00 hr before and after interruption. Statistical data for the different experiments are given in the legends. The figure illustrates that after interruption of the alcohol supply for 1, 2 or 7 days, total ethanol intake was increased during the first 2 hr. Moreover, the data also suggest that the increase was greater as the duration of the interruption lasted longer (Friedman two-way ANOVA, $\chi^2_R = 5.25$, $p=0.07$). After interruption for 1 or 2 days, ethanol intake through the 16% and 32% solution increased proportionately. But after 7 days, relative preference appeared to have changed; ethanol intake through 16% increased on the average, with a factor 11 ± 6, whereas intake through 32% decreased with a factor 0.6 ± 0.4. After interruption of 1 or 2 days, increase an intake occurred only shortly after the renewed access to alcohol; during the subsequent night the level of ethanol intake was not significantly different from that during pre-interruption nights. After an interruption of 7 days, however, net ethanol intake during the subsequent night between 18.00 hr. and 9.00 hr was also increased significantly (before: 9.4 ± 3.1 ml and after interruption: 19.0 ± 4.0 ml; $p<0.01$).

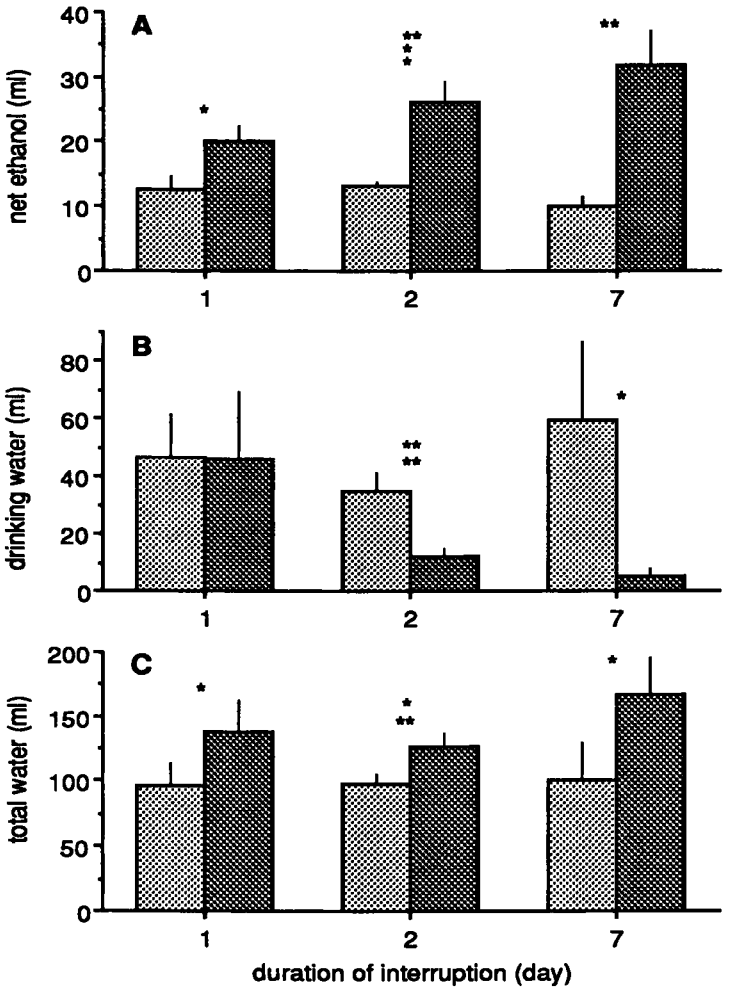

Figure 1. *Mean total net ethanol intake (±SEM) (fig. 1A); mean consumption of drinking water (±SEM) (Fig. 1B) and mean total water intake (±SEM) (Fig. 1C) by 8 rhesus monkeys between 16.00 and 18.00 h for pre-(light bars) and post- (dark bars) interruption days after an interruption period of 1 (one experiment), 2 (three experiments) and 7 (one experiment) days. *$p<0.05$, **$p<0.01$, Wilcoxon matched pairs test.*

1A. After interruption, ethanol intake is higher than the mean ethanol intake on pre-interruption days.

1B. After interruption, the consumption of drinking water is lower than consumption before interruption.

1C. After interruption, the consumption of total water is higher than before interruption.

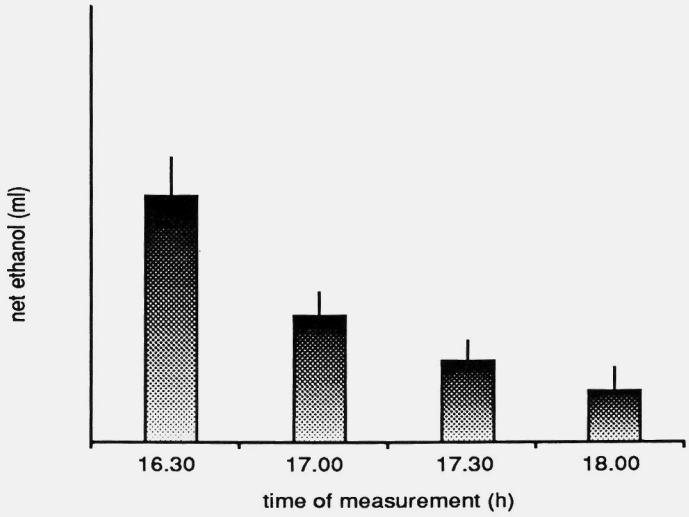

Figure 2. Mean total net ethanol intake (±SEM) for all experiments during four successive half-hourly periods after interruption. Concordant variation in ethanol intake of the subjects was found after interruption of one day (Friedman two-way ANOVA, $p<0.01$), two days ($p<0.01$) and seven days ($p<0.001$).

Figure 1B illustrates that the mean consumption of drinking water between 16.00 and 18.00 hr was significantly decreased after an interruption for 2 days and even more strongly after an interruption for 7 days. Between 18.00 and 9.00 hr the drinking water consumption after a two-day interruption significantly decreased, as compared to that on pre-interruption days in two experiments (from 70.7 ± 25.6 ml to 23.1 ± 9.4 ml, and from 58.8 ±14.9 ml to 21.3 ± 10.3 ml, $p<0.05$).

Figure 1C shows that after interruption, total water ingestion through all three liquids between 16.00 and 18.00 hr was significantly higher in most experiments. Between 18.00 and 9.00 hr total water ingestion did not significantly differ between pre- and post-interruption periods. The increase in ethanol consumption after interruption showed a consistent time pattern in all experiments (Fig. 2); it was largest in the first half hour after the interruption ended and from then on gradually decreased to control levels. As is expected from the data given in Figure 1A, the first half hour peak was highest after the 7-day interruption period. Drinking water consumption after renewed access did not show a consistent time pattern in the different experiments.

The daily total water intake during the days of interruption did not differ

significantly from that during pre- and post-interruption days (Friedman 2-way ANOVA: duration of interruption: one day $\chi^2_R = 1.75$ ns, two days, $\chi^2_R = 5.40$ n.s., seven days $\chi^2_R = 11.42$ ns). Mean daily total water consumption (ml) before interruption was 742 ± 101; during the first 2 days of interruption 785 ± 113 ml and at the last interruption day (day 7) 764 ± 126 ml. After the longest interruption period (7 days) consumption was 892 ± 133 ml. During interruption days we did not observe any signs of physical withdrawal reactions as described by Ellis and Pick (1972) and Myers et al. (1972). The animals did not lose weight in any of the experiments.

Discussion

The results show that interruption of alcohol supply affected the spontaneous drinking behaviour of the monkeys. After interruption net ethanol intake was resumed immediately at a higher level and then gradually returned to pre-interruption intake level (see Fig. 2). Consumption of drinking water was reduced and did not show a distinct time course.

The pre-interruption preference for 16% over 32% solution was maintained after interruption. The preference for drinking water compared to ethanol solutions was diminished shortly after renewed alcohol supply. If extinction of reinforcement by ethanol was involved (Griffiths et al., 1980; Crowley and Andrews, 1987; Meisch, 1977), net ethanol ingestion was expected to diminish as interruption lasted longer. The present results show that as the interruption period lasted longer, the subsequent net ethanol intake showed a larger increase which also tended to last longer. In operant studies rats and mice also resumed responding to ethanol solution (8% v/v) after a number of non-reinforced sessions (Elmer et al., 1986, 1987; George, 1987; Suzuki et al., 1988).

This indicates that ethanol reinforcement is highly resistant to interruption: previously acquired ethanol consuming behaviour is immediately re-established (Griffiths et al., 1980). The additional increase after interruption perceived in our monkeys, was not mentioned for operant-responding rats (Elmer et al., 1986, 1987; George, 1987; Suzuki et al., 1988), but it occurred in spontaneous drinking rats and monkeys if they had experience in alcohol drinking (Sinclair and Senter, 1968; Sinclair, 1972; Sinclair et al., 1973). Comparison of the consumptions of the two concurrently presented solutions showed that consumption of both solutions increased proportionately after 1 or 2 days of interruption, but that after the 7 day interruption, preference had changed strongly in favour of the 16% solution; the consumption of the latter was strongly increased, whereas that of the 32% solution was decreased. Apparently, as the interruption lasted longer, the

32% solution became more aversive notwithstanding that ethanol intake was more.

Increased consumption is known to occur also after temporary deprivation from more common reinforcers, such as water and food (Sinclair, 1972; Hilgard et al., 1971). Therefore, one may postulate that in our monkeys interruption of ethanol supply induced a certain degree of deprivation. One hypothesis could be that the abrupt abstinence caused physical withdrawal distress, the relief of which involved an increased ethanol intake (Jellinek, 1955; Caetano, 1985). This explanation is, however, unlikely as no physical withdrawal symptoms were observed (Friedman, 1980; Ellis and Pick, 1972; Myers et al., 1972). Also, withdrawal distress, if it occurs, is transient, lasting up to 48 hr after onset of abstinence. In our monkeys, the increase in ethanol intake became stronger as the interruption lasted longer. Sinclair et al. (1973) reported an increased intake even after 75 days of interruption in rats. Moreover, animals exhibiting clear withdrawal symptoms did not spontaneously consume alcohol to relieve it (Hunter et al., 1974; Myers et al., 1972; Meisch, 1984), nor do humans do so consistently (Mendelson and Mello, 1979). The data rather support the view that physical dependence and ethanol-motivated behaviour are separate phenomena (Van Ree, 1979; Cicero, 1980; Meisch, 1984; Stewart et al., 1984; Wise and Bozarth, 1987).

It could be argued that ethanol was a source of calories and that during the interruption the monkeys became partly (food) (energy)-deprived, which can lead to subsequent enhanced ethanol drinking (De la Garza and Johanson, 1987; Meisch, 1987). This explanation, however, seems unlikely because food supply was generous and the animals showed no indication of weight loss. Because during abstinence, total fluid intake did not change compared to pre-abstinence level, and water drinking after interruption was even reduced, it is also unlikely that an increased ethanol intake is attributable to some form of water deprivation.

The specific effect of interruption on the consumption of ethanol solutions therefore seems to be an indication of a strongly ethanol-motivated behaviour, resembling an increased drive after deprivation, as reported for natural reinforcers (such as food and water) (Hilgard et al., 1971; Nichols, 1972; Sinclair, 1972). Recent findings indicate that natural reinforcers and other reinforcers (such as addictive drugs) act on common central reward mechanisms involved in the regulation and reinforcement of behaviour (Stewart et al., 1984; Stein, 1985; Wise and Bozarth, 1987; Van Ree, 1987; Pfeffer and Samson, 1987). It remains to be investigated to what extent ethanol-motivated behaviour after a period of interruption is attributable to negative reinforcement processes, such as drive-reduction or restoring a state of deficiency (Nichols, 1972) or to reward-dependent behaviour that is very resistant to extinction (Cloninger, 1987; Wise and Bozarth, 1987; Stewart et al., 1984). It seems possible that the problem of frequent relapse in

human alcoholics is related to mechanisms of central reward. The specifically ethanol-directed remissions demonstrated by the alcohol-interrupted monkeys might be mediated by such mechanisms too. The phenomenon described in this study seems an interesting experimental animal model to study such mechanisms and to explore how they can be modulated.

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4. THE EFFECT OF DESGLYCINAMIDE-(ARG^8)-VASOPRESSIN (DGAVP) ON THE ACQUISITION OF FREE-CHOICE ALCOHOL DRINKING IN RHESUS MONKEYS

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Abstract

The vasopressin analog desglycinamide-(Arg^8)-vasopressin (DGAVP) has been reported to reduce the acquisition of heroin and cocaine self-injection behaviour in rats. This led to the hypothesis that DGAVP can reduce the self-administration of psycho-active drugs (including ethanol) by attenuating central reinforcement processes. Under forced ingestion conditions, DGAVP has been reported, however, to enhance alcohol drinking in rats. We studied the effect of DGAVP on the acquisition of voluntary, free-choice alcohol drinking in naive rhesus monkeys, that had concurrent access to either 1% and 2% (n=12) or to 4% and 8% (n=8) ethanol/water solutions in addition to drinking water. Half of the monkeys were injected twice per day with 50 $\mu g.kg^{-1}$ of DGAVP for 14 successive days, the other half received placebo. Subsequently, all subjects had access to the same solutions for another 14 days without treatment. DGAVP did not significantly affect concentration preference behaviour. With regard to net ethanol ingestion in animals drinking 1% and 2% solutions, DGAVP decreased net ethanol intakes, having a time-dependent and long lasting effect; placebo-treated animals gradually increased net ethanol intakes over time. The placebo-treated animals in the 4% and 8% group, showed a different acquisition pattern; DGAVP reduced net ethanol intake in two animals in a similar way as above. Two animals behaved differently. It is concluded that in a free-choice condition DGAVP did not enhance the acquisition of alcohol drinking in monkeys, but rather inhibited ethanol self-administration in the majority of the subjects.

Introduction

A current view is that psycho-active drugs from various pharmacological classes, are self-administered by humans and animals, because they activate central reinforcement systems (1, 2, 3, 4). It has been proposed that central reinforcement processes can be modulated by vasopressin derived neuropeptides (5, 6, 7, 8). The neuropeptide desglycinamide-(Arg^8)-vasopressin (DGAVP), which lacks the classical endocrine actions of vasopressin and is only centrally active (9, 10, 11), was found to reduce the acquisition of heroin (12, 13) and of cocaine intravenous self-administration (7, 14) in rats. Furthermore, DGAVP appeared to attenuate the electrical brain self-stimulation behaviour (ICSS), when reward associated brain areas were involved (5). On the basis of these findings it is postulated that by attenuating central reinforcement, DGAVP could diminish the acquisition of self-administration of drugs from quite distinct classes (4).

Ethanol, considered also a psycho-active drug, has been demonstrated to func-

tion, under certain conditions, as a reinforcer in experimental animals (15, 16, 17) although its reinforcing effects are considered to be weaker or more indirect in comparison with opiates and psychomotor stimulants (18, 19 20). Relationships between vasopressin derived neuropeptides and ethanol have been studied predominantly with respect to the development and maintenance of tolerance and physical dependence, because of their potency to affect learning and memory processes (21, 22, 23). These neuropeptides appear to act similarly for morphine and ethanol with regard to the development of tolerance and physical dependence (24, 25, 26, 27). However, tolerance and physical dependence on reinforcing substances are attributed to other neural systems than are central reinforcement processes (28, 4, 3).

With regard to ethanol self-administration in rats, DGLVP (which acts similar as DGAVP), has been reported to enhance acceptance of ethanol solutions in concentrations that are generally aversive for naive rats (29, 30, 31) and thus to increase net ethanol intake (32, 24). Because the rats in these studies had to initiate alcohol drinking by forced procedure, it could not be determined whether DGLVP interacted with the central reinforcing effects of ethanol, or rather with a process of habituation to the aversiveness of the ethanol solution offered (24, 21). Rhesus monkeys have been shown to initiate alcohol drinking spontaneously in a free-choice procedure and to maintain individual net ethanol intakes at a quite constant level (2-6 $ml.kg^{-1}$) across ethanol concentrations between 2% and 32% per cent (v/v) (33). In the present study we investigated the effect of DGAVP on ethanol self-administration (i.e., alcohol drinking) in naive rhesus monkeys when alcohol drinking could be spontaneously initiated (i.e., voluntary acquisition) by providing the animals with a free choice between water and two ethanol/water solutions. Ethanol concentrations in the solutions offered were either relatively low, or relatively high in order to be able to distinguish between a possible effect of DGAVP on ethanol's reinforcing effect (by measuring net ethanol intake) and a possible alteration in the aversiveness of high concentrations (by comparing the relative preference for the available fluids).

Methods

Animals

Twenty free-fed male rhesus monkeys (*Macaca mulatta*) (weights: 3-8 kg; age: 2-13 years), which had no experience with alcohol nor with DGAVP application, were housed (four monkeys at a time) in the experimental room in which they had the opportunity for at least two weeks to get acquainted with the new environment and the drinking equipment in their home cages. The monkeys were together

daily between 10.00 and 12.30 hr in a large play cage; during the rest of the day they remained singly in their home cages. Daily food supply was unchanged, consisting of a generous supply of monkey chow in the morning (at 8.30 hr) and fruit and bread in the afternoon (at 13.30 hr).

Drinking equipment

Each monkey had access to three identical drinking cylinders attached at the side of the home cage behind an opaque board, with only the three drinking nipples protru-ding into its cage. During the daily group sessions (from 10.00 to 12.30 hr) animals had no access to the drinking equipment nor to other drinking devices. During the acquaintance period only tap water was available. During the experimental period one cylinder provided tapwater, the other two provided ethanol/water solutions in concentrations of either 1% and 2%, or 4% and 8% (v/v). Position of the drinking cylinders was changed daily. Consumed volumes were measured by electronic counters that registered every 10 ml of volume consumed. Cylinders were refilled automatically. The equipment was checked several times daily.

Treatment

DGAVP was donated by Organon International BV, Oss, The Netherlands. Half of the subjects (2 per group) were injected intramuscularly with DGAVP 50 $\mu g.kg^{-1}$ body weight (dissolved in saline) twice per day, for 14 successive days; the other subjects were injected intramuscularly with placebo (saline) twice per day. Injections were administered at 8.00 and 13.00 hr in the home cage of the subject.

Ethanol Concentrations

Eight subjects had concurrent access to 4% and 8% ethanol/water solutions, besides drinking water (0%). Twelve subjects had access to water (0%), 1% and 2% ethanol/water solutions. Thus, combined with the administered treatments, there were four different experimental groups: four subjects received placebo and four DGAVP, while drinking 4% and 8% ethanol solutions (i.e., a placebo and a DGAVP high concentration group); six subjects received placebo, six subjects DGAVP, while drinking 1% and 2% ethanol solutions (i.e., a placebo and a DGAVP low concentration group).

Procedure

At the start of the experiment, the first injection was given at 8.00 hr, 30 min before the monkeys were given access to ethanol solutions for the first time. During the treatment period there always was a 30-min time-out, in which there was no access to ethanol and water solutions, following the injections (at 8.00 and 13.00 hr). Otherwise access was free except during the group session (from

10.00 to 12.30 hr) as mentioned. The experiment comprised two successive periods. During the first period (14 days) animals received either DGAVP or placebo (treatment period). During the subsequent second period (14 days) the monkeys still had access to ethanol solutions and water, but did not receive injections (post-treatment period). After the experiment, animals were given no longer access to ethanol solutions, and were observed for signs of physical withdrawal (34, 35).

Data Analysis

Relative preference for each of the three fluids available, is expressed as proportion (per cent) of the daily total fluid intake. Individual daily net(100%) ethanol intake was determined from the consumed volumes of both ethanol solutions and expressed as ml net ethanol per kg ($ml.kg^{-1}$). Comparison between two experimental groups was performed by means of a Mann Whitney U-test; comparison within each group by use of a Wilcoxon matched pair test (36). Analysis of a time-related effect during treatment and during post-treatment was performed for each experimental group by means of a linear regression tested for goodness of fit by one-way ANOVA (37). Whenever daily data recorded from a subject were incomplete or lacking due to technical or practical circumstances, this day was skipped in the analysis. Significance levels are accepted at 5% level.

Results

Concentration Preference

Figure 1 gives the individual relative preferences (in per cent) for the available fluids (water and two ethanol solutions) during treatment and during post-treatment for placebo- and DGAVP-treated subjects, in the low concentration (Fig. 1a and 1b, respectively) and in the high concentration groups (Fig. 1c and 1d, respectively).

Water Versus Ethanol Solutions:

During treatment, the placebo-treated subjects in the low concentration groups (Fig. 1a) generally drank more from the drinking water than from each ethanol solution. Tested for the group of subjects this difference was significant for the 1% solution (Wilcoxon $p<0.05$), but not for the 2% ethanol solution. From the Figure 1a, it can be noted that one subject (□) preferred to drink mainly from the 2% ethanol solution. During post-treatment, water was significantly (Wilcoxon, $p<0.05$) preferred over the 1% solution, but four out of six subjects now drank more 2% solution than drinking water.

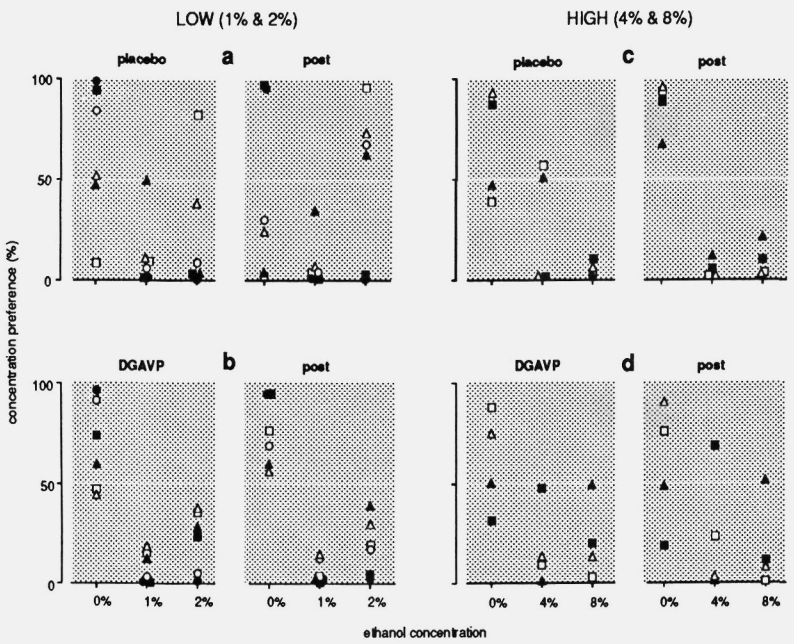

Figure 1. Mean individual relative preference for the three fluids, concurrently offered (concentration preference), expressed as proportion (%) of the daily total fluid ingested, during treatment (placebo or DGAVP) and during post-treatment (post), in the placebo low (a), the placebo high (c), the DGAVP low (b) and the DGAVP high (d) concentration group. Statistical analyses are given in the text.

DGAVP-treated subjects in the low concentration groups (Fig. 1b) always drank significantly more water than 1%- (Wilcoxon, $p<0.01$) and 2% ethanol solution (Wilcoxon, $p<0.01$) in both periods.

In the high concentration groups, during treatment, placebo- (Fig. 1c) as well as DGAVP-treated (Fig. 1d) subjects diverged in their preference for water or 4% solution, but all subjects preferred water over 8% solution (Wilcoxon $p<0.01$). During post-treatment placebo-treated subjects significantly preferred water over 4% (Wilcoxon, $p<0.05$) and over 8% solution (Wilcoxon, $p<.05$). DGAVP-treated subjects were divergent in their preferences: 2 subjects, monkey DAB (□) and DCY (Δ) preferred water over 4% and over 8% solution; monkey DAJ (■) drank mainly 4% solution and monkey DGT (▲) drank water and 8% solution in comparable amounts.

Consumed volumes (ml) of drinking water were never significantly different between placebo- and DGAVP-treated groups. After treatment the consumed

volume of drinking water did not significantly change within placebo-(Wilcoxon ns) and within DGAVP-treated (Wilcoxon ns) groups.

Relative Preference Among the Two, Concurrently Available, Ethanol Solutions: For each experimental group, it was analyzed to what extent one ethanol solution was preferred over the other. In the low concentration group, during treatment (Fig. 1a), four placebo-treated subjects preferred 2% over 1% solution; one preferred 1% (▲). During post-treatment a preference for 2% over the 1% solution was manifest in five of the six subjects (Wilcoxon, $p<0.05$). DGAVP-treated subjects (Fig. 1b) preferred the 2% above the 1% solution during treatment (Wilcoxon, $p<0.05$) and during post-treatment (Wilcoxon, $p<0.05$). In the high concentration group, during treatment, preference for either the 4% or the 8% solution was rather divergent in placebo- (Fig. 1c) as well as in DGAVP-treated subjects (Fig. 1d). During post-treatment placebo-treated subjects significantly preferred 8% over 4% (Wilcoxon, $p<0.05$); the group of DGAVP-treated subjects remained divided: 2 subjects, monkey DAB (□) and monkey DAJ (■) further increased a preference for 4%; the monkeys DCY (Δ) and DGT (▲) increased their preference for 8%.

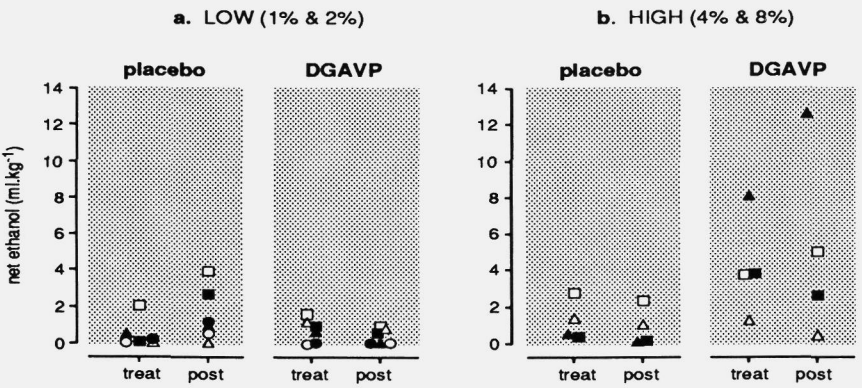

Figure 2. Mean individual daily net(100%) ethanol intake (ml.kg^{-1}) during treatment (treat) and during post-treatment (post) by the placebo- and DGAVP-treated subjects in the low concentration groups (Fig. 2a) and in the high concentration groups (b). Statistical analyses are given in the text.

Net Ethanol Intake

Figure 2 shows the mean daily individual net ethanol intake (ml.kg^{-1}), through consumption of the ethanol solutions, during treatment and during post-treatment for the low concentration (a) and for the high concentration (b) groups.

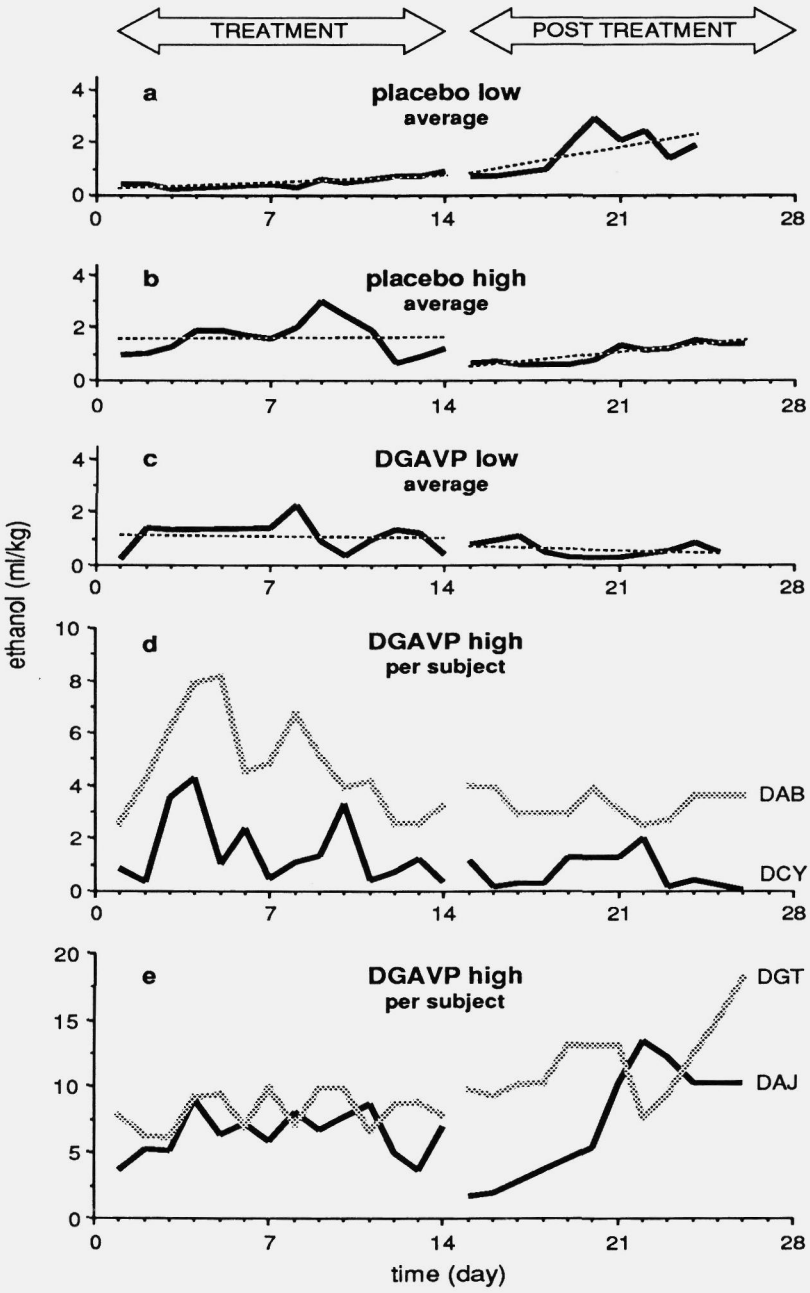
TREATMENT
POST TREATMENT
a
placebo low
average
b
placebo high
average
c
DGAVP low
average
d
DGAVP high
per subject
DAB
DCY
e
DGAVP high
per subject
DGT
DAJ
ethanol (ml/kg)
time (day)

Figure 3. Mean net ethanol (100%) intake (ml.kg^{-1}) per treatment day (days 1-14) and per post-treatment day (days 15-28) by placebo- (a) and DGAVP-treated animals (c) in the low concentration group, and by the placebo-treated animals in the high concentration group (b). The daily net ethanol intake by the DGAVP-treated animals in the high concentration group are shown per individual (d, e), because individuals did not behave concordantly throughout the experiment. Dotted lines in Fig. 3a - 3c represent the lineair regression lines. Statistical analysis of acquisition rate in net ethanol intake: PLACEBO LOW (Fig. 3a): slope during treatment 0.034, ANOVA $F(1,9) = 15.43$, $p<0.01$; slope during post-treatment 0.167, ANOVA $F(1,8) = 5.8$, $p<0.05$. PLACEBO HIGH (Fig. 3b): slope during treatment 0.0001, ANOVA $F(1,12)$ = ns; slope during post-treatment 0.088, ANOVA $F(1,10) = 35.97$, $p<0.001$. DGAVP LOW (Fig. 3c): slope during treatment -0.009, ANOVA $F(1.9) = 0.04$, ns; slope during post-treatment -0.025, ANOVA $F(1,7) = 0.86$, ns.

Comparison Between Groups

Comparison between placebo- and DGAVP-treated subjects, within the low as well as within the high concentration group, did not reveal a significant difference in ethanol intake during treatment or during post-treatment. Comparison between placebo-treated subjects, drinking either low (Fig. 2a) or high (Fig. 2b) concentrated solutions, did not reveal a significant difference in net ethanol intake during treatment nor during post-treatment. Comparison between DGAVP-treated subjects, in both the low and high concentration groups, revealed that the subjects of the high concentration groups had a higher net ethanol intake during treatment (Mann-Whitney U-test $p<0.05$) than the subjects of the low concentration groups (Mann-Whitney U-test during post-treatment $p=0.06$).

Comparison Within Groups

Comparison between net ethanol intakes during treatment and posttreatment revealed that placebo-treated animals drinking 1% and 2% solutions (Fig. 2a), had increased the net ethanol intake (Wilcoxon, $p<0.05$). DGAVP-treated subjects (Fig. 2a), drinking 1% and 2%, had decreased net ethanol intake (Wilcoxon, $p<0.05$). This change was significantly different from the change in the placebo-treated subjects (Mann-Whitney U-test, $p<0.01$). In the high concentration groups, placebo-treated subjects (Fig. 2b) had decreased net ethanol intake (Wilcoxon, $p<0.05$) and DGAVP-treated subjects diverged: two subjects, monkey DAJ (□) and monkey DGT (▲), ingested more and two monkeys, DAB (■) and DCY (Δ), ingested less net ethanol in the post-treatment than in the treatment period.

Rate of Acquisition

The time course of the acquisition of ethanol intake across the successive days (shown in Fig. 3) was analyzed during the treatment (days 1-14) and during the post-treatment period (days 15-28) by means of a regression analysis and tested with one-way ANOVA (for statistical results, see legends Fig. 3). The average net ethanol intake across the days (solid lines) and the regression (dotted lines) are illustrated for the placebo-treated low in Figure 3a, and for the placebo-treated high concentration group in Figure 3b.

Placebo-treated subjects of the low concentration group showed a significant gradual increase over time, during and after treatment. Placebo-treated subjects of the high concentration group showed a variable pattern initially. At the end of treatment, intake levels were relatively low and then gradually increased thereafter. At the end of the post-treatment period the levels of net ethanol intake were comparable for the low and high concentration placebo groups. Figure 3c shows that in the low concentration group, the rate of acquisition in the DGAVP-treated subjects was different from the placebo-treated subjects (Fig. 3a). Initial intakes in the DGAVP-treated subjects were rather variable and then continued at very low levels during post-treatment period. No significant trend over time was present in either period.

Because in the high concentration group, DGAVP-treated subjects behaved divergent, net ethanol intake across days is given per individual in Figures 3d and 3e. Two subjects (DAB, DCY) showed fluctuating intake levels, which remained comparatively low from day 10 onward, throughout the post-treatment period (Fig. 3d). The other two monkeys, DGT and DAJ (Fig. 3e), had high initial intake levels during treatment (note the difference in scale). During post-treatment DGT showed a further increase, DAJ showed a sharp decline after DGAVP treatment was stopped, after which the intake level gradually rose again.

After the experiment, when the ethanol solutions were not longer available, no clear signs of physical withdrawal were observed in any of the subjects.

Discussion

DGAVP had no significant effect on the consumption of drinking water, which seems in agreement with previous conclusions that DGAVP does not exert classical vasopressin-related endocrine functions (4, 11, 38, 39). Differences in the drinking behaviour between placebo- and DGAVP-treated groups were specifically found with regard to the ethanol solutions. During treatment, the individual relative preferences for water (0%) versus ethanol solution were not clearly different for the placebo- and DGAVP-treated groups. During post-treatment,

however, the majority of the placebo-treated subjects in the low concentration group showed a relative preference for the 2% solution over water, whereas, on the other hand, the DGAVP-treated group developed a clear preference for water over ethanol solution. Most animals in both treatment groups preferred 2% over 1% solution, notwithstanding that during post-treatment real consumed volumes by the DGAVP-treated animals were less than in the placebo group. In the high concentration group, all placebo-treated subjects eventually developed a preference for water over ethanol solution, but the DGAVP-treated subjects remained divergent in preferring water over ethanol solution, or vice versa. The placebo group unanimously preferred 8% over 4% solution during post-treatment, whereas the DGAVP-treated monkeys again diverged in preference for either the 4% or the 8% solution.

If DGAVP would have altered the habituation to the aversiveness of ethanol solutions (24), it could have been expected that DGAVP-treated animals would have consumed a larger proportion of their daily total fluid intake from ethanol solution than placebo-treated animals. This appeared not to be so; in the low concentration group, the reverse seemed to hold true during post-treatment. With regard to the relative preference among the two ethanol solutions, there also was no evidence that DGAVP, compared to placebo, had significantly enhanced the animals to consume more from the higher concentrated solution. Therefore, it can be concluded that DGAVP did not enhance the habituation to drink ethanol solutions, nor did it enhance the acceptance of relative high, possibly aversive, ethanol solutions.

Although mean individual net ethanol intakes in placebo-treated subjects, drinking either low (Fig. 2a) or high (Fig. 2b) concentrations, were not statistically different, day-to-day analysis revealed a different acquisition pattern in both placebo groups. Placebo-treated monkeys, drinking 1% and 2% solutions, initiated net ethanol intake at a low level (less than 0.5 $ml.kg^{-1}$/day) that subsequently increased over time (up to about 2 $ml.kg^{-1}$/day on the average). Placebo-treated monkeys drinking 4% and 8% solutions, on the other hand, initiated net ethanol intake at a relatively higher level (1 to 2 $ml.kg^{-1}$), that subsequently dropped to levels below 0.5 $ml.kg^{-1}$ and from there gradually increased, in a similar way as the low concentration group did. The occurrence of a decline in net ethanol intake during acquisition has been reported also for placebo-treated rats that initiated ethanol intake at rather high (i.e., 3 $g.kg^{-1}$ per day) levels (32, 40). These observations can be explained by the findings that relatively low doses of ethanol are reinforcing due to stimulatory effects (3, 28, 41); and that high doses, by contrast, lead to depressive and aversive reactions in men and animals (29, 42, 43). In addition, there are findings that low doses of ethanol can facilitate, but high doses decrease electrical brain self-stimulation in

reward areas (44, 45, 20). Hence, it could be that the initial high intake levels, as a result of drinking relative highly concentrated solutions, produced some aversive effects in the monkeys, that eventually led to a drop in net ethanol intake levels. The observation that the two placebo groups tended to converge to comparable net ethanol intake levels, suggests that both groups needed an acquisition period (adapting behaviour in opposite ways) to achieve an intake level that produced the optimal positive reinforcement with minimal aversive effects.

Effects of DGAVP on daily net ethanol intake, compared to placebo, became manifest after some time of treatment. Although the initial intake levels in the placebo- and DGAVP-treated groups, drinking 1% and 2% solutions were not significantly different, the acquisition curve of the DGAVP-treated group (Fig. 3c) showed a decline in ethanol intake around day 10, and intake remained low during post-treatment. This contrasts with the placebo-treated low concentration group, that increased daily ethanol intake over time, and indicates that DGAVP seems to have inhibited the acquisition of ethanol self-administration rather than to have reinforced it. A counter-argument to explain the low level of self-administration under DGAVP-treatment, discussed also by other authors (7, 46), is that DGAVP might alter the reinforcing efficacy of a substance (e.g., ethanol), hence producing more reinforcement by the same dose. However, experimental drug self-administration studies have shown that a stronger reinforcement will lead to more self-administration rather than to less (18, 47, 48).

The results found for the DGAVP high concentration group seem to be puzzling. Although not statistically different from the placebo-group, the mean net ethanol intake during treatment (Fig. 2b) seemed quite high (significantly different from the low concentration DGAVP group), suggesting that DGAVP might have been enhancing ethanol intake in (some animals of) this group. However, a number of observations make such explanation not very likely.

The effect of DGAVP has been reported in several studies to manifest itself after repeated days of administration and to be of a long lasting nature (22, 40, 49, 50, 51). Also the increase in net ethanol intake in DGLVP-treated rats, compared to placebo controls, during forced acquisition started after 6 to 7 days of treatment and this effect then persisted after treatment was stopped (24, 32). If DGAVP would have stimulated likewise ethanol intake in monkeys DAB and DCY (Fig. 3d), an increase rather than a decrease was to be expected in the second week of treatment and thereafter. Monkeys DAB and DCY showed rather a reversed pattern, suggesting a time-dependent inhibition of ethanol intake by DGAVP.

Monkeys DAJ and DGT (Fig. 3e) started immediately at high intakes. After about 10 days, DAJ also started to decrease intake, with a sharp drop after treatment was stopped. Since the time-related pattern in DAJ quite resembled that of the placebo high concentration group (the decrease is followed by a gradual

increase again; Fig. 3b), it is possible that DGAVP had had no effect at all in this monkey, nor in monkey DGT. This could be in agreement with the observation by others (5, 27, 52) that when reinforcement control over behaviour is too strong (in our case immediately established high ethanol intakes), DGAVP is ineffective. The conclusion from our results is that DGAVP did not enhance the acquisition of ethanol self-administration as has been suggested by others (21, 32, 53); it seems even more likely that DGAVP inhibited net ethanol intake after some time of treatment in eight of the ten DGAVP-treated subjects.

This primate study on the effect of DGAVP on the acquisition in alcohol drinking differed from rat studies (21, 24, 32) in providing: free access to drinking water (versus no drinking water for rats), and a free choice between two ethanol solutions (instead of one ethanol solution, that gradually increased in concentration). In this free choice situation monkeys did not ingest 80% of their daily fluid intake by drinking ethanol solution, as the rats had to in order to meet their water demand. The difference in results could be explained by the hypothesis that the consumption of large amounts of ethanol solutions was aversive for the rats (31, 54) that, nevertheless, had to accept them in order to satisfy fluid demands. DGLVP could have enhanced the habituation to the aversive effects (i.e., tolerance) of ethanol under this condition (23, 55, 56). The alcohol drinking of the monkeys, who could regulate their intake levels without risking water deprivation, was probably more determined by the positive reinforcing effects of ethanol (33). The observed decline in ethanol intake seems more similar to the effect of DGAVP on the acquisition pattern of cocaine (7) and heroin self-administration in rats (12, 14, 46). Therefore, our data seem to fit in with the hypotheses that DGAVP can attenuate the reinforcing effects of different psychoactive drugs (13), and that ethanol has reinforcing effects by interaction with brain reward systems (20, 44). The difference in results between our study and those using forced acquisition procedures probably reflect interactions of the peptide with the different effects of ethanol on the central nervous system (19, 20, 57). A similar dissociation in effects of neuropeptides on central reinforcement and tolerance has been mentioned with respect to heroin and morphine, suggesting that different mechanisms are involved for both facets of drug ingestion (27, 49).

However, the present study includes only small numbers of subjects, that showed quite some interindividual variability in the amounts of alcohol drinking; a phenomenon also reported in other animal alcohol drinking studies (24, 35). The specific conditions (e.g. ethanol concentration, administration procedure, duration of treatment and individual variables) under which DGAVP can cause ethanol intake to decrease rather than to increase, requires further study before a possible therapeutic use in alcohol dependence, like e.g., in heroin detoxification treatment (4, 58, 59, 60) can be considered. The present study demonstrates that a

primate model of volitional alcohol drinking could be a valuable tool in performing such research.

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5. ENDOCRINE PROFILE DURING ACQUISITION OF FREE-CHOICE ALCOHOL DRINKING IN RHESUS MONKEYS; TREATMENT WITH DESGLYCINAMIDE (ARG^8) VASOPRESSIN

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Abstract

The significance of the relationship between alcoholism and hormonal processes is still unknown. Hormones are involved in the homeostasis in the brain, and hormonal dysfunction thus might relate to psychopathology and addiction. This paper reports on the effect of spontaneous acquisition of alcohol drinking on endocrine profile in 12 naive male adult rhesus monkeys, and explores the relationship between individual profile and alcohol intake. Monkeys were free-fed and had a concurrent free-choice, day and night, between tap water, a 1% and a 2% ethanol/water solution during four weeks. Half of the monkeys were daily injected (i.m.) during the first two weeks with 0.50 $\mu g.kg^{-1}$ desglycinamide (Arg^8) vasopressin (DGAVP); a neuropeptide, that has been postulated to interfere with positive reinforcement processes. Half of them was treated with placebo. Blood samples were drawn from unsedated monkeys three times: after two weeks of water drinking (BASELINE), after the first two weeks of alcohol drinking and daily injections (TREATMENT) and after the last two weeks of alcohol drinking (POST). Plasma levels of β-endorphin, ACTH, prolactin, cortisol and testosterone were determined. The placebo-treated subjects significantly increased net ethanol intake over time, whereas the DGAVP-treated subjects decreased net ethanol intake significantly over time. After two weeks significant increases were found in β-endorphin and ACTH. After four weeks prolactin was increased, cortisol decreased and particularly β-endorphin remained significantly increased. No significant differences in endocrine responses existed between DGAVP- and placebo-treated subjects, although the increase in prolactin and testosterone was less pronounced in DGAVP-treated monkeys. Probably, DGAVP's effect on ethanol's reinforcing effect was mediated at a central level. No relationship was found between basal hormonal levels and subsequent ethanol intake. However, two placebo-treated subjects that showed the highest increase in ethanol intake over time, reacted differently, by reducing β-endorphin and ACTH levels over time, showing the largest decreases in cortisol and hardly any prolactin reaction. It is concluded that alcohol drinking by naive subjects disturbes hormonal processes and that two animals deviated with respect to the acquisition in alcohol drinking and endocrine responsivity. It thus seems very interesting to test in spontaneous alcohol-selecting animals current hypotheses on interactions between addiction and neuroendocrine variables.

Introduction

Endocrine disturbances have been reported in active as well as in abstinated

alcoholics (Schuckit et al., 1987; Gianoulakis et al., 1989; Müller et al., 1989) and are frequently associated with patterns found in affective disorders (De Soto et al., 1985; Heuser et al., 1988; Müller et al., 1989). Furthermore, it has been suggested that endocrine responses might indicate some genetic vulnerability to alcoholism: persons with a family history of alcoholism, but not alcoholic themselves, show different endocrine responses to alcohol compared to non-alcoholic persons with no family risks (Schuckit et al., 1987; Gianoulakis et al., 1989).

But to date, it is still not understood which endogenous conditions precede alcoholism, which conditions are related to the expression of alcoholism and which conditions are merely the consequences of alcohol abuse (Kraemer et al., 1985; Von Wartburg, 1990; Holden, 1991).

Evidence is accumulating that neurobiological systems, involved in the regulation of behaviour, play a critical role in addiction to psychoactive substances, including alcohol (Cloninger, 1987; Van Ree, 1987; Holden, 1991). Besides their classical peripheral function, hormones and their fragments have specific interactions with brain processes and behaviour, (De Wied, 1980; Le Moal et al., 1984; Anokhina et al., 1987). Disturbances in hormonal homeostasis in the brain and pituitary thus might be related to psychopathology and to the development and maintenance of addictive behaviours, including alcoholism (Gold, 1980; Van Ree, 1986; Anokhina, 1987). Hormones related to the hypothalamus-pituitary-adrenal axis (HPAA) and the endorphinergic systems are mentioned particularly in this respect (Reus, 1980; Genazzani et al., 1982; Kreek et al., 1984; Van Ree, 1986; Vescovi et al., 1990; Patel and Pohorecky, 1989).

Experimental studies concerning relationships between (changes in) endocrine responses and the genesis of alcohol addiction in humans are rather complicated, because alcoholics generally come to medical attention when the disorder is already well established and consequently includes a variety of factors due to chronic abuse (Watson et al., 1985). In naive experimental animals it has been demonstrated that acute as well as chronic administration of ethanol significantly changes levels of HPA-related hormones and β-endorphin in plasma, hypothalamus and pituitary (Guaza and Borrell, 1985; Patel and Pohorecky, 1989; Rivier, 1989; Thiagarajan et al., 1989). Experimental animal studies on the initiation of self-selection of alcohol and hormonal processes are however rare.

In a previous study we reported that free-fed rhesus monkeys were quite willing to spontaneously initiate and maintain alcohol drinking under non-deprivation conditions (Kornet et al., 1990).

In the present study we investigated the effect of alcohol drinking on the endocrine profile in naive rhesus monkeys during an acquisition period of 4 weeks. In addition the relationship between individual endocrine profile and

alcohol intake was explored. It has been postulated that the neurohypophyseal hormonal fragment desglycinamide-(Arg^8)-vasopressin (DGAVP) can interfere with the positive reinforcing, i.e. addictive, effects of various psychoactive agents, including alcohol (Van Ree, 1977; Van Ree, 1986; Kornet et al., 1991). Therefore, half of the monkeys was treated with the vasopressin fragment (DGAVP) during the first 2 weeks of acquisition of alcohol drinking, half of them with placebo. In a previous paper the alcohol drinking behaviour of these monkeys has been described in detail (Kornet et al., 1991). The present paper focusses on the endocrine profile of these animals.

Methods and Materials

Subjects

The subjects were 12 healthy free-fed male rhesus monkeys *(Macaca mulatta)*, which had no experience with alcohol, nor with DGAVP. Half of them were selected to receive DGAVP, half of them placebo (saline), in such a way that individual ages in both treatment groups were matched:

DGAVP			PLACEBO		
Id.	age	kg	Id.	age	kg
XD	2	3.0	XF	2	4.0
UR	3	4.5	PM	4	5.3
MA	5	4.8	MG	5	5.5
GR	6	7.9	AQ	6	5.0
MR	12	7.4	NL	11	7.1
NZ	13	7.5	TD	13	7.5

Each monkey was trained in advance of the study: a. to leave the home cage, leashed on at a monkey collar, and then b. to be restrained in a monkey chair for about 15 minutes, while they were handled by the experimentator. The monkeys were together in a group of four in a large play cage from 10.00 hr to 12.30 hr; during the rest of the day they remained single in their home cages. Daily monkey chow was supplied at 8.30 hr, and bread and fruit at 13.30 hr. The monkeys participated in the experiment in groups of four at a time (i.e. 3 groups all together).

Acquisition of alcohol drinking

Each monkey had access to three identical drinking cylinders attached at the side of the home cage, only the nipples protruding into the cage. During the daily group

session in the play cage, drinking was not possible. In the first two weeks of the experiment (BASELINE period) only tap water was available. Thereafter one cylinder provided tapwater, the other two provided ethanol/water solutions in concentrations of 1% and 2% (v/v). Positions of the cylinders were daily changed. For more details on the drinking equipment, we refer to our previous paper (Kornet et al., 1990). The monkeys were given for four weeks concurrent access to drinking water (0%) and to 1% and 2% ethanol solutions.

Treatment

The monkeys were treated daily during the first two weeks of alcohol drinking with either placebo (saline) or desglycinamide (Arg^8) vasopressin (DGAVP) (TREATMENT period). DGAVP was donated by Organon International BV, Oss, The Netherlands. In each group of four monkeys, half the subjects were injected i.m. with 50 $\mu g.kg^{-1}$ of DGAVP (dissolved in saline) twice per day in their home cages (n=6) at 8.00 hr and 13.00 hr; the other half (n=6) were injected with placebo twice per day at 8.00 hr and 13.00 hr. During the last two weeks alcohol supply was continued, but no treatment was given (POST period).

Hormone determinations

Blood was drawn by venipuncture in the arm from unsedated monkeys, that were restrained in a monkey chair; a procedure they had been trained for in advance. Blood samples were taken three times, following each 14-day period, at the end of the BASELINE period (water only), at the end of the TREATMENT period (2 weeks alcohol and treatment with either DGAVP or placebo) and at the end of the POST period (alcohol only) around 9.00 hr. Heparin blood was sampled for determination of testosterone, prolactine, and cortisol. An EDTA synthetic tube was used for sampling blood for ACTH and β-endorphin. For the latter trasylol was added. After centrifugation both tubes were stored at -80^0. Measurements of hormonal parameters were done with techniques described previously for β-endorphin (Gispen-de Wied et a., 1987), ACTH (Arts et al., 1985), cortisol (Thijssen et al., 1980) and testosterone (Landeghem et al., 1981). Interassay variations were calculated for these determinations at 6.2 per cent for concentrations of 15 pmol/l β-endorphin (n=13); 11.9 per cent at 66 ng/l ACTH (n=25); 7.0 per cent at 0.46 µmol/l cortisol (n=29) and 10.9 per cent at 2.9 nmol/l testosterone (n=26). For prolactin a commercial immunoenzymetric assay (Boehringer, Mannheim, FRG), cross-reacting with monkey prolactin was used. Results have been expressed in international units of human prolactin, code nr WHO 75/504. Interassay variation was 5.3 per cent at 0.49 0.49 IU/l prolactin (n=50).

Data analysis

Data samples met the assumptions for parametric statistics. Daily total net ethanol intake was determined by transforming daily consumed volumes of both ethanol solutions into amounts of $ml.kg^{-1}$ net ethanol. For net ethanol intake and each hormone, two-way analysis of variance (ANOVA) was performed with repeated measures over time (Kirk, 1968; Friedman, 1988). Because one sample for β-endorphin determination (POST period) missed, the analysis of the group with placebo treatment included five instead of six samples.

Results

Net ethanol intake

Figure 1a shows the mean daily net ethanol intake of the placebo- and DGAVP-treated monkeys during the TREATMENT (T) and POST period (P). A significant interaction effect existed between DGAVP and time; placebo-treated subjects increased, but DGAVP-treated subjects decreased the ethanol intake over time (ANOVA TREATMENT $F(1,11) = .83$ ns; Time $F(1,11) = 3.2$ ns; TREATMENT x Time $F(1,1) = 11.46$ $P<0.01$).

β-endorphin

Figure 1b shows the mean plasma β-endorphin level of the placebo- and DGAVP-treated monkeys after the BASELINE (B), TREATMENT (T) and POST period (P). ANOVA revealed no treatment nor interaction effect between treatment and time (TREATMENT $F(1,9) = 0.46$ ns; TREATMENT x Time $F(1,2) = 0.36$ ns). But the overall significant time effect (Time $F(2,10) = 6.68$ $p<0.01$) indicated that in both groups the plasma β-endorphin level increased when ethanol solutions had been introduced. The increase was significant from BASELINE to TREATMENT ($F(1,9) = 8.63$ $p<0.05$) and from BASELINE to POST ($F1,9) = 12.45$ $p<0.01$). No significant change occurred from TREATMENT to POST ($F(1,9)=1.17$ ns).

ACTH

Figure 1c shows the mean plasma ACTH level in placebo- and DGAVP-treated monkeys. No significant difference between DGAVP and placebo treatment was found. Results of ANOVA were: TREATMENT $F(1,11)=0.19$ ns; Time $F(2,11) = 3.23$ $p=0.06$; TREATMENT x Time $F(1,2)=0.29$ ns. ACTH levels predominantly had increased from BASELINE to TREATMENT period ($F(1,11)=21.0$ $p<0.001$).

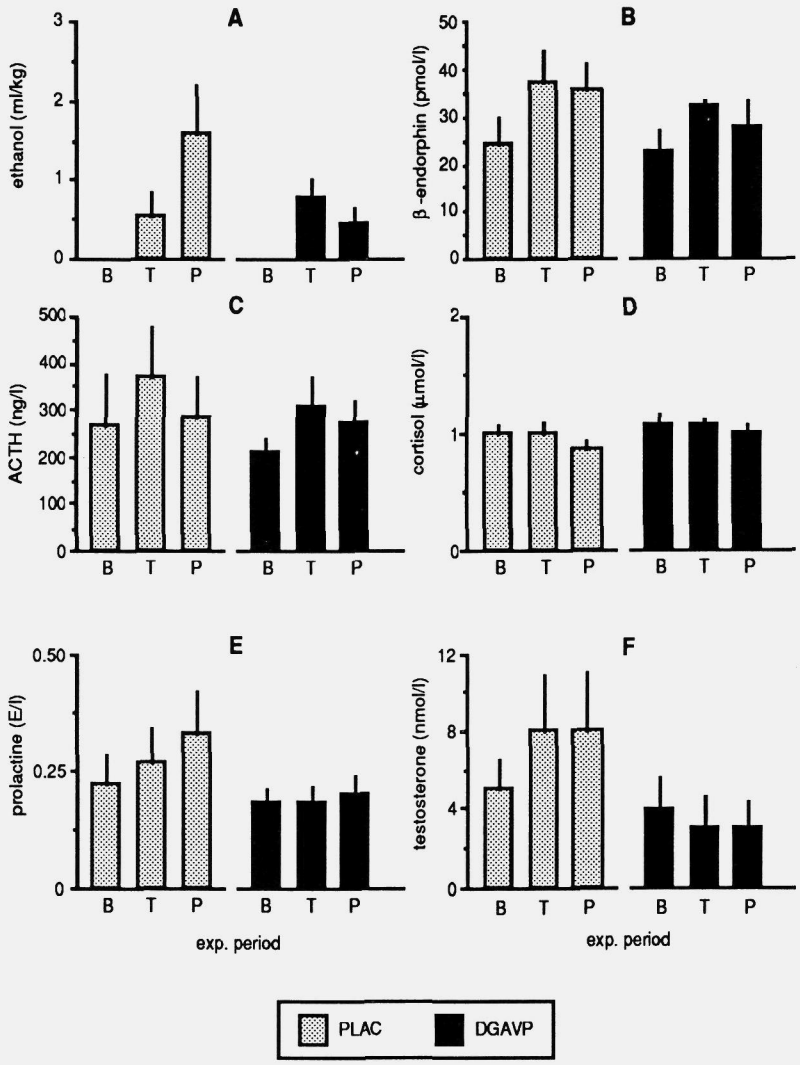

Figure 1: 1a: Mean (+SEM) daily net ethanol intake (ml/kg) during the TREATMENT (T) and during the POST (P) period by the placebo-treated group (left side) and the DGAVP-treated group (right side) of monkeys.
1b-1f: Mean (+SEM) plasma levels after the BASELINE (B), the TREATMENT (T) and the POST (P) period of the placebo-treated group (left side) and the DGAVP-treated group (right side) of monkeys, of 1b: β-endorphin, 1c: ACTH, 1d: cortisol, 1e: prolactin, 1f: testosterone. See text for statistical results.

Prolactin

Figure 1d illustrates the mean plasma prolactin level of both treatment groups for the different periods. No significant difference between DGAVP and placebo treatment was found (TREATMENT $F(1,11) = 1.0$ ns; TREATMENT x Time $F(1,2)=1.78$ ns). The prolactin level increased over time in both groups after the supply of ethanol solutions (Time $F(2,11)=4.13$ $p<0.05$). The increase was significant for BASELINE versus POST period ($F(1,11)=5.94$ $p<0.05$). A covariance analysis with baseline value as covariate indicated a possible difference in time-related change between placebo and DGAVP ($F(1,11)=3.86$ $p=0.08$). t-Tests between placebo and DGAVP-treated animals with respect to the change in prolactin from BASELINE to TREATMENT period and from BASELINE to POST period revealed no significant differences between both treatments.

Cortisol

Figure 1e shows the mean plasma cortisol levels across the three periods for the placebo- and DGAVP-treated monkeys. Overall a time dependent decrease was found for cortisol (Time $F(2,11)=3.3$ $p<0.05$). Further results of ANOVA were: TREATMENT $F(1,11)=1.0$ ns; Treatment x Time $F(1,2) = 0.65$ ns. A covariance analysis, with baseline value as covariate, revealed a difference in time effect for TREATMENT and POST period ($F(1,11)=4.96$ $p=0.05$), indicating that the decrease had occurred predominantly after POST period.

Testosterone

Figure 1F shows the mean testosterone plasma level across the three periods for placebo- and DGAVP-treated monkeys. Main effects were not significant (TREATMENT $F(1,11)=1.75$ ns; Time $F(2,11)=0.86$ ns), but ANOVA suggested a TREATMENT x Time effect ($F(1,2)=3.07$ $p=0.07$). DGAVP-treated animals tended to decrease and placebo animals tended to increase the testosterone level after alcohol introduction. Covariance analysis (with baseline values as covariate) indicated a difference in the direction of change in testosterone level over time for placebo- and DGAVP-treated animals ($F(1,11)=3.98$ $p=0.07$). The result of a t-test between the placebo and DGAVP group, for the change from BASELINE to TREATMENT period was $t(df10)=-1.81$ $p=0.09$ and for the change from BASELINE to POST period was $t(df10)=-2.0$ $p=0.07$.

Relation with net ethanol intake

The relationship between the amount of net ethanol ingested and hormonal levels was explored by analysing the individual hormonal profile. An overview of individual baseline levels and mean net ethanol intake during the TREATMENT period is given in Table I.

Table I. Individual plasma levels of β-endorphin (β-end), ACTH, prolactin (PROL), cortisol (CORT), testosterone (TEST) after the BASELINE period and mean net ethanol intake during the TREATMENT period (ETHA) for the placebo-treated and for the DGAVP-treated subjects.

Placebo	*β-End pmol/l*	*ACTH ng/l*	*PROL E/l*	*CORT µmol/l*	*TEST nmol/l*	*ETHA ml/kg*
2D	24.9	140	0.06	0.89	9.8	0.17
AQ	27.0	135	0.32	1.23	7.5	2.05
PM	44.8	780	0.47	1.10	3.6	0.25
XF	13.4	160	0.08	1.16	1.5	0.04
NL	18.0	275	0.15	0.87	0.9	0.53
MG	18.0	130	0.22	0.66	7.0	0.06
DGAVP						
NZ	17.9	119	0.04	1.0	4.0	1.0
GR	11.4	170	0.27	1.01	12.1	1.18
UR	21.6	215	0.19	0.78	1.5	0.02
XD	26.2	255	0.17	1.19	1.3	0.09
MR	38.2	320	0.21	1.34	1.4	1.61
MA	15.4	190	0.20	1.16	3.1	0.73

Table II. Individual changes for placebo-treated and DGAVP-treated subjects in β-endorphin (β-END), ACTH, prolactin (PROL), cortisol (CORT), testosterone (TEST) 1. after 2 weeks of alcohol drinking and treatment (from BASELINE to TREATMENT period, i.e. T-B) and 2. after 2 additional weeks of drinking without further treatment (from BASELINE to POST period, i.e. P-B); the change in net ethanol intake (ml/kg) is determined by the difference in mean net ethanol intake during TREATMENT and POST period.

	ETHA ml/kg	*β-END pmol/l*		*ACTH ng/l*		*PROL E/l*		*CORT µmol/l*		*TEST nmol/l*	
	P-T	*T-B*	*P-B*	*T-B*	*P-B*	*T-B*	*P-B*	*T-B*	*P-B*	*T-B*	*P-B*
Placebo											
2D	+2.52	-3.5	-5.9	+35	-10	-0.01	0	-0.07	-0.16	+8.5	+10.7
AQ	+1.90	-9.6	-4.8	+55	-45	+0.09	-0.01	0	-0.56	-5.2	-1.0
PM	+0.96	+10.0	+11.7	+101	-300	+0.05	+0.25	+0.03	-0.21	+2.7	+2.4
XF	+0.53	+10.0	+17.8	+101	0	+0.05	+0.10	+0.04	+0.11	+3.0	-0.2
NL	+0.47	+27.0	+13.2	+105	+310	+0.05	+0.09	+0.01	-0.01	+0.6	+0.3
MG	-0.01	+26.3	+13.6	+210	+125	+0.08	+0.26	+0.19	0	+8.3	+5.2
DGAVP											
NZ	-0.37	+0.7	+7.2	+46	+46	+0.05	+0.06	+0.11	+0.08	-2.6	-2.9
GR	-0.36	+18.6	+5.4	+80	+15	-0.02	+0.09	+0.03	-0.08	-1.5	-2.4
UR	+0.04	+0.04	-1.6	+89	-35	0	-0.05	-0.02	-0.04	-0.5	+0.2
XD	-0.01	+8.0	+4.7	+97	+175	0	+0.10	-0.02	+0.01	-0.3	0
MR	-0.61	-1.9	+10.3	+260	+45	+0.08	+0.02	-0.19	-0.47	-0.9	-1.0
MA	-0.65	+14.8	+6.6	+5	+135	-0.11	-0.08	-0.01	+0.05	+0.4	+0.6

Changes in hormonal levels over time and in mean net ethanol intake from TREATMENT to POST period are given in Table II. No consistent relationship existed between BASELINE hormonal levels and net ethanol intake during the TREATMENT period (Table I). In the placebo-treated animals (Table II), it can be noted that the monkeys that demonstrated the highest increase in net ethanol intake (2D and AQ), responded in a different way over time compared to the other monkeys with respect to β-endorphin, ACTH, cortisol and prolactin. Compared to BASELINE period levels, AQ and 2D decreased β-endorphin after TREATMENT period, where as the other monkeys showed increases. Furthermore, 2D and AQ showed no or negative changes in cortisol levels already after TREATMENT period and only their ACTH levels were lower after POST than after BASELINE period. Finally by contrast to the other subjects, AQ and 2D showed no positive change in prolactin after POST.

Monkey UR was the only one in the DGAVP-treated group who did not show a decrease in ethanol intake and also responded differently with respect to β-endorphin (decrease) and ACTH (decrease).

Discussion

Placebo-treated animals

Spontaneously initiated alcohol drinking in placebo-treated monkeys generally started at a low-dose intake level (5 of the 6 animals less than 1 $ml.kg^{-1}$ per day) which then gradually increased over time (see Kornet et al., 1991; for a day-to-day time analysis). The present data made clear that this was accompanied with time-related changes in plasma hormonal levels.

In the majority of the placebo-treated animals, the 2 weeks of alcohol drinking (TREATMENT period) induced an increase in the plasma β-endorphin and ACTH levels; compared to BASELINE period, only β-endorphin still remained significantly higher after the 4 week-period (POST period). It took a period of 4 weeks for plasma levels of cortisol and prolactin to alter significantly; cortisol levels decreased and prolactin levels increased compared to BASELINE period levels. Plasma testosterone tended to increase over time.

An interesting observation was that two monkeys (2D and AQ) with quite high increases in ethanol intake, showed the lowest levels after the TREATMENT and POST period with respect to β-endorphin and ACTH, the highest decrease in cortisol and no increase in prolactin. These animals thus seem to have different hormonal responses than the other subjects. This appeared to be independent of their pedigree, or dominance rank.

Ethanol is known to initially stimulate hypothalamus-pituitary-adrenal (HPA)

activity resulting in the release of β-endorphin, ACTH and corticoids (Rivier, 1989). β-endorphin and ACTH stem from the same precursor molucule POMC (Petraglia, 1988) and the by ethanol stimulated release of ACTH from the pituitary subsequently increases the production of corticosterone (Patel and Pohorecky, 1989; Gianoulakis et al., 1989; Rivier, 1989).

It must be kept in mind that, in comparison with other animal studies (Guaza and Borrell, 1985; Patel and Pohorecky, 1989; Rivier, 1989) we did not measure endocrine responses to a direct test dose of ethanol, but rather we determined the influence of daily alcohol drinking on spontaneous hormonal responses.

In our study, the absence of increases in cortisol, especially after the POST period, in combination with increases in ACTH and β-endorphin seems peculiar. A possible explanation might be that the increase in ACTH and β-endorphin after TREATMENT period reflected an altered HPA-responsivity, causing a higher response in ACTH and β-endorphin to the restraint and venipuncture procedure (Axelrod and Reisine, 1984; Herndorn et al., 1984; Rivier, 1989). Because cortisol responses are known to occur some time after stress-induced ACTH responses, we may have missed a delayed elevation in cortisol response. The decreased cortisol level after the POST period would then reflect a lowered basal level due to daily alcohol consumption, rather than a response to the sampling procedure.

Chronic exposure to alcohol has been reported to cause impaired pituitary responsiveness, blunting ACTH and corticosteroid secretion in men and animals (Marks, 1979; Reus, 1980; Guaza and Borrell, 1985; Heuser et al., 1988; Rivier, 1989). A lowered pituitary responsivity (comprising β-endorphin, ACTH, cortisol and prolactin) seemed particularly present in the monkeys 2D and AQ, which had increased their alcohol intake quite strongly over time.

Although plasma testosterone usually is reported to decrease after acute stress (Patel and Pohorecky, 1988; Parrot and Thorntorn, 1989; Rivier, 1989) and after chronic alcohol consumption in various species (Mello et al., 1985; Widenius et al., 1989), effects in animals also appeared to depend on ethanol dose, dominance rank, baselines and season (Mello et al., 1985; Winslow and Miczek, 1988; Cicero et al., 1990). In the placebo-treated monkeys, plasma testosterone was somewhat increased after the first 2 weeks and 4 weeks of alcohol drinking. No consistent relationship with dominance rank could be detected. Hence, no classical stress response, nor pituitary-gonadal dysfunction due to chronic alcohol, seemed present with respect to testosterone.

The sustained elevated plasma levels of β-endorphin in the monkeys might have reflected a decreased content of β-endorphin in the pituitary due to increased release by the daily alcohol ingestion, as has been demonstrated by Patel and Pohorecky (1989) in rats and also have been found for heroin and cocaine self-

administration in rats (Sweep et al., 1989). This could be the pre-stage for the effects of chronic alcohol ingestion that like chronic use of opiates and cocaine (Sweep et al., 1988; Vescovi et al., 1990) appears to cause a decrease in central endorphin levels (Borg et al., 1982: Genazzani et al., 1982). The presumed ability of addictive drugs in reinstating normal β-endorphin levels again, could then explain the vicious circle of the repeated use and the frequent relapses after abstinence periods (Genazzani et al., 1982; Sweep et al., 1988; Volpicelli et al., 1990). Interesting is that heroin addicts have impaired anterior pituitary function as well (Kreek et al., 1984; Vescovi et al., 1990). The congruent findings with different classes of drugs (alcohol, opiates, cocaine) suggest that the interaction with β-endorphin-related systems plays a central role in addictive behaviour in general (Van Ree et al., 1990).

DGAVP-treated animals

The net ethanol intake in the DGAVP-treated monkeys declined over time, indicating that ethanol had little positive reinforcing effects on alcohol drinking behaviour in thus treated animals (Van Ree, 1979; Meisch, 1984; Kornet et al., 1991). Noteworthy is that this effect remained present after DGAVP-treatment had been terminated, indicating a lasting effect. Similar results have been demonstrated for the acquisition of intravenous self-administration of heroin in rats (Van Ree, 1987). Neurohypophyseal neuropeptides have been postulated to particularly interfere with adaptational behaviour to novel situations and this seems to include the initiation and initial maintenance of drug-taking behaviour (Van Ree, 1986; Cloninger, 1987). Basal hormonal levels were not significantly different between the placebo- and DGAVP-treated group. Neither did time-dependent hormonal changes in plasma β-endorphin, ACTH and cortisol significantly deviate from the placebo-treated group. Although individual data might suggest that changes (either positive or negative) in β-endorphin and prolactine levels were less marked under DGAVP, definite conclusions could not be drawn in this respect. Only testosterone did not notably increase in DGAVP-treated animals, which might be correlated to the general lower alcohol intake by these animals rather than to a direct effect of DGAVP. Thus, although ethanol appeared to be less reinforcing under DGAVP-treatment, pituitary-related hormonal responses measured during acquisition of alcohol drinking were more or less comparable to the responses in placebo-treated animals. Neuropeptides related to hypothalamic and neurohypophyseal hormones that are practically devoid of peripheral hormonal effects, affect behaviour specifically by interaction with the central nervous system (Greven and De Wied, 1980; Van Ree, 1980; Jolkkonen et al., 1987; Le Moal et al., 1984). This suggests that DGAVP's effect on reinforcement processes may be mediated at a central level (Barret et al., 1987; Barna et al., 1990) without significantly

modulating hormonal activities of the pituitary-adrenal axis (Finkelberg et al., 1978; Barna et al., 1990).

Individual difference in endocrine profile

Two individuals (2D and AQ) seemed to have a different endocrine profile after alcohol drinking. In monkeys, different responses in CSF norepinephrine have been postulated to indicate individual biological susceptibility for stress and alcohol addiction (Kraemer et al., 1985). The present data suggest that individual reactions in other neurobiological parameters may provide such an indication too. In human research quite some interest in neuroendocrine predisposition and the motivation for alcohol exists (Holden, 1991). So far, reported observations in humans are not always in agreement with each other. Basal plasma levels of β-endorphin measured in abstinent alcoholics as well as in high-risk family members, have been reported to be lower than in controls (Gianoulakis et al., 1990), but others found such a difference exclusively in the CSF (Borg et al., 1982; Genazzani et al., 1982). Some investigators found lower basal cortisol and ACTH levels in abstinent alcoholics and high-risk persons (Gianoulakis et al., 1989); others detected no difference from controls (Schuckit et al., 1988). Furthermore, it has been reported that lower doses of ethanol are needed in abstinent alcoholic (or high risk) persons to induce increased releases of plasma cortisol and immunoreactive β-endorphin (Gianoulakis et al., 1990), which might be experienced as reinforcing by these persons (Gianoulakis et al., 1990). On the other hand a decreased intensity in ACTH, cortisol and prolactin responses after ethanol was attributed to alcoholics and high risk persons (Schuckit et al., 1988; Holden, 1991).

The findings for monkeys 2D and AQ in the present study suggest a relation between the development of high ethanol-motivated behaviour (high increase during acquisition) and a corresponding reduction in normal (i.e. no acute ethanol challenge) levels of β-endorphin, ACTH, cortisol and prolactin. It would be interesting to further investigate in these monkeys the reponses to acute ethanol challenge.

It must be kept in mind that alcoholics may have divergent reasons to initiate and maintain the use of alcohol, which might correlate with different individual neurobiological characteristics (Cloninger, 1987). Furthermore, peripheral hormonal responses do not have to correlate exactly with central processes (Barret et al., 1987; Barna et al., 1990). However, hormonal responses might provide a "window" to neurochemical changes in the brain and the pituitary gland (Schuckit et al., 1988). The present data show that spontaneous acquisition of alcohol drinking disturbed the hormonal balance in monkeys. It would be very interesting

if current hypotheses on spontaneous motivation for alcohol could be further investigated in free-choice drinking monkeys.

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6. THE EFFECT OF NALTREXONE ON ALCOHOL CONSUMPTION DURING CHRONIC ALCOHOL DRINKING AND AFTER A PERIOD OF IMPOSED ABSTINENCE IN FREE-CHOICE DRINKING RHESUS MONKEYS

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Abstract

Relapse into problematic alcohol drinking is a serious problem in the treatment of alcoholism. Free-choice drinking rhesus monkeys show relapse-like behaviour after imposed abstinence of alcohol, by immediately reinitiating ethanol intake at an increased level. The relapse-like behaviour of the monkeys seems not induced by physical withdrawal, but rather argues for a resistance to extinction of ethanol-reinforced behaviour. It has been suggested that endogenous opioids play a role in the positive reinforcing effect of ethanol. In this study, the effect of the opiate antagonist naltrexone was investigated in eight adult male rhesus monkeys *(Macaca mulatta)* who had about one year experience with alcohol drinking, under two conditions: 1. (Exp I) during continuous and concurrent supply of drinking water and two ethanol/water solutions (16% and 32% (v/v)), and 2. (Exp II) after two days of alcohol abstinence. In both experiments, each monkey received six doses of naltrexone (0.02, 0.06, 0.17, 0.5, 1.0, 1.5 $mg.kg^{-1}$); each dose was paired with a placebo injection (i.m.) in a cross-over design. Consumption was measured from 16.00 hr in the afternoon (30 min after injection) to 9.00 hr the next morning. In Experiment I naltrexone reduced total net ethanol intake in a graded dose-dependent manner. The effect of naltrexone was apparent shortly after injection, and lasted untill the following day. Consumption of drinking water was reduced only shortly after injection. In Experiment II, reduction of net ethanol intake was largely restricted to the first few hours of reinitiation of alcohol drinking, i.e. the period in which the abstinence-induced increase was manifest. Consumption of drinking water was not affected by naltrexone. Naltrexone hardly influenced consumption of the non-preferred ethanol solution of 32%. It is postulated that the opioid modulation specifically interacted with positively reinforced behaviour. In Experiment II naltrexone reduced ethanol intake at a lower dose (0.17 $mg.kg^{-1}$) compared to Experiment I (0.50 $mg.kg^{-1}$), but net ethanol intakes however remained higher. It might be that alcohol abstinence resulted in altered opioid activity, leading to increased ethanol-seeking behaviour. The renewed presentation of ethanol solutions (also) might have stimulated reinitiation of alcohol drinking, representing conditioned incentive stimuli. The reported monkey model of relapse in alcohol drinking could be a useful tool to evaluate new hypotheses and experimental treatments with respect to human alcoholism.

Introduction

The main feature of human alcoholism is the recurrent desire to consume alcohol. Even after withdrawal and after having been alcohol free for some time, relapses

into problematic alcohol drinking frequently occur (Jellinek, 1955; Marlatt and George, 1984; Horwitz et al., 1987) and form a well known and serious problem in the treatment of alcoholism (Barnes, 1988).

Alcohol consumption by rhesus monkeys has been found to show similarities to human alcohol drinking patterns (Woods 1971; Griffiths and Bigelow, 1978, Mello and Mendelson, 1980; Winger 1988). In previous work we reported that after a period of imposed abstinence, experienced free-choice alcohol drinking rhesus monkeys immediately resumed alcohol consumption at a temporary increased intake level compared to pre-abstinence consumption (Kornet et al., 1990a). This effect appeared to become more pronounced as the period of abstinence lasted longer, indicating that it was not mediated by a state of physical alcohol withdrawal (Ellis and Pick, 1972; Myers et al., 1972). We suggested that this observed relapse-like phenomenon represented specific ethanol-directed behaviour, due to the previously experienced positive reinforcing effects of ethanol (Kornet et al., 1990a). Study of this behaviour might be valuable in gaining more insight in recurrence of ethanol-directed behaviour in humans.

A current view is that neurobiological systems that are involved in reinforcement and motivation of behaviour might also function as neurobiological substrates for the positive reinforcing effects of addictive substances (Cloninger, 1987; Stein and Belluzi, 1987; Wise and Bozarth, 1987; Van Ree, 1987). Experimental studies suggested that drug-unsatisfied rats in expectation of their daily self-administration session of heroin (an opioid drug) or cocaine (a non-opioid drug), showed lower levels of central β-endorphin compared to drug-satisfied rats, that had just finished their session (Sweep et al., 1988). These findings were interpreted as an indication that drug-reinforced behaviour is related to central opioid, i.e. endorphinergic activity.

Ethanol (a non-opioid drug) has been found to modulate endorphinergic activity in the brain and pituitary (Topel, 1985; Tabakoff and Hoffman, 1987; Linseman, 1989; Barret et al., 1987; Patel and Pohorecky, 1989). It might therefore be that central opioid activity is also related to ethanol-reinforced behaviour (Altshuler et al., 1980; Prunell et al., 1987). Moreover, if central opioids are important for the persistence of ethanol-reinforced behaviour, it can be expected that they are also involved in the relapse of the behaviour after a period of abstinence.

In the present study we investigated the effect of the opiate antagonist naltrexone on free-choice alcohol and water drinking in monkeys which had been drinking alcohol for more than one year and showed reliable relapse-like drinking after imposed abstinence. Naltrexone was administered under two conditions: in Experiment I (Exp I) in which the monkeys had continuous access to alcohol and water; in Experiment II (Exp II) after abstinence, that was imposed by

interrupting the alcohol supply for 2 days; only water remained continuously available.

Materials and Methods

Subjects

Subjects were eight free-fed, male rhesus monkeys (ages 7-10 years, body weights 8-12 kg), which had spontaneously initiated alcohol drinking under conditions of unrestricted food and water access (for details see Kornet et al, 1990b). The monkeys were housed in single cages together in one room. Before the present study they had been drinking ethanol/water solutions in different ethanol concentrations for more than one year without interruption, except for a few alcohol interruption experiments (Kornet et al., 1990a). The individual with the lowest average net ethanol intake, ingested 2.4 (SE = 0.23) and the subject with the highest average intake 6.2 (SE = 1.1) $ml.kg^{-1}$ per day; the average group net ethanol intake was 4.0 (SE = 1.8) $ml.kg^{-1}$.

Alcohol Supply

Each cage was provided with three graded drinking bottles, attached outside the cage behind an opaque board; only the drinking nipples protruded into the cage. At the start of the present study the subjects had been drinking a 16 and 32% ethanol/water (v/v) solution in addition to drinking water. In the present study the concurrent supply of ethanol solutions with ethanol concentrations of 16 and 32% was continued, in addition to drinking water. The availability of two ethanol solutions provided a possibility to determine the relative preference for water, a lower and a higher ethanol concentration.

Drug

Doses of naltrexone (a gift from Dupont, U.S.A.) tested were 0.02, 0.06, 0.17, 0.5, 1.0 and 1.5 $mg.kg^{-1}$. Each monkey received a single injection, intramuscularly, with each dose in Experiment I as well as in Experiment II. Each injection was placebo controlled. Monkeys were weighed before each trial.

Naltrexone solution was prepared on the injection day, by dissolving the total amount of naltrexone in 5 ml saline, needed for treating the four monkeys that were to receive the drug; each monkey received the amount of the solution to achieve the appropriate dose per kg body weight. Saline was used as placebo and administered to each monkey in the same volume as naltrexone solution.

Experimental Procedure

The execution of the study took more than one year. To control for possible fluctuations in drinking behaviour over time, we paired each naltrexone injection with a placebo injection. The order of doses over time for Experiments I and II is given in Table 1. Figure 1 illustrates the experimental procedure for Experiment I (Fig. 1a) and Experiment II (Fig. 1b).

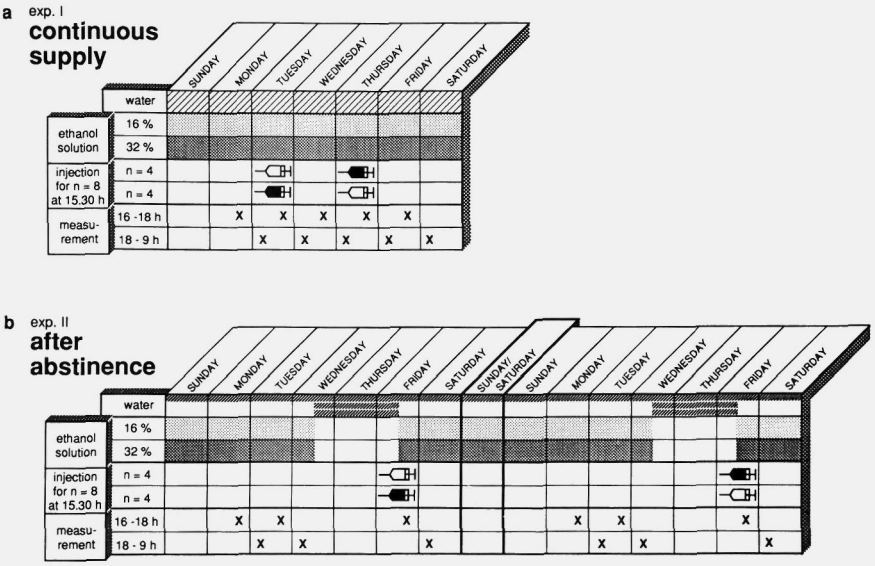

Figure 1. Experimental procedure used in Experiment I (a: continuous alcohol supply) and in Experiment II (b: 2-day imposed alcohol abstinence) for each dose of naltrexone. In Experiment I (a) supply of alcohol and water was continuously available (shaded area); at Tuesday four monkeys received naltrexone (filled syringe), four placebo (empty syringe) at 15.30 hr; at Thursday contents of the syringes were reversed. Consumption between 16.00 and 18.00 hr (X) and between 18.00 and 09.00 hr (X) was measured. In Experiment II (b) alcohol abstinence was enforced by substituting drinking water for ethanol solution from Wednesday 16.00 hr untill Friday 16.00 hr (white area). At Friday 15.30 hr four monkeys received naltrexone (filled syringe), four placebo (empty syringe); at 16.00 hr ethanol solutions were replaced again. After a week of uninterrupted supply (Sunday to Saturday) the procedure was repeated, but the contents of the syringes were reversed. Consumption was measured at Monday and Tuesday (pre-abstinence days) and at Friday (post-abstinence days) between 16.00-18.00 and 18.00-09.00 hr.

In Experiment I (Fig. 1a) the monkeys always had uninterrupted access to alcohol and water 24 hr per day (shaded area). Each dose of naltrexone was tested as follows: at Tuesday at 15.30 hr, half of the monkeys (n=4) received one dose of naltrexone (Fig. 1a: black syringe) and the other half received placebo (Fig. 1a: white syringe); at Thursday naltrexone and placebo administrations were reversed. In this way each dose of naltrexone was paired with a placebo injection in a cross-over design. Measurement of consumption was carried out by registration of the number of milliliters fluid in the bottles; these were immediately refilled. Consumption was measured for the time intervals (Fig. 1a: X) from 16.00 to 18.00 hr (i.e., after the first two hours of measurement following injection) and from 18.00 to 09.00 hr the next morning (i.e., after an additional 15 hours period, including the night).

In Experiment II (Fig. 1b) from Wednesday 16.00 hr until Friday 16.00 hr (white area) abstinence was imposed by refilling all three bottles with normal drinking water (shaded area). At Friday 16.00 hr, the ethanol solutions were made available again. Each dose was tested as follows: half of the monkeys received one dose of naltrexone (Fig. 1b: black syringe) Friday at 15.30 hr (30 min. before renewed alcohol supply), half of the monkeys received placebo (Fig. 1b: white syringe). Then one week of uninterrupted supply (Sunday to Saturday) followed. After this interval we repeated the imposed abstinence procedure, but at Friday naltrexone and placebo administrations were reversed. In this way each dose of naltrexone was paired with a placebo injection in a cross-over design. Consumption was measured as in Experiment I.

During the days of imposed abstinence, the monkeys were checked six times per day for possible physical withdrawal reactions, like hyperactivity, tremor, sickness, irritability and convulsions (Ellis and Pick, 1972; Myers et al., 1972; Friedman, 1980).

Data analysis

Total net ethanol and drinking water

Daily consumed volumes of the two ethanol solutions were transformed into net ethanol intake ($ml.kg^{-1}$), and then added to determine the total amount of net ethanol intake. The consumed volume from the bottle with drinking water was also transformed into consumed $ml.kg^{-1}$. Intake of total net ethanol and of drinking water following naltrexone injection, between 16.00 and 09.00 hr (i.e., a 17 hr period), was compared with the intake following a paired placebo injection.

Effect of imposed abstinence (Exp. II only)
To determine the effect of the imposed abstinence from alcohol on subsequent total net ethanol intake and consumption of drinking water, the averaged individual intakes at Monday and Tuesday prior to abstinence (pre-abstinence days), were compared to the matched intakes after re-introduction of alcohol supply (post-abstinence day).

Time course of the effect of naltrexone
To study the time course of the effect of naltrexone, we compared effects of naltrexone (expressed in difference scores, obtained by subtracting individual intakes following placebo from paired intakes following naltrexone) during the first two drinking hours after injection (from 16.00 to 18.00 hr) with the effects during the subsequent night (from 18.00 to 09.00 hr). The possibility that effects lasted longer than 24 hr was investigated by comparing intakes, between 16.00 and 09.00 hr, at no-injection days that followed placebo, with no-injections days that followed naltrexone treatment days (see Fig. 1a Exp. I).

Concentration preference
Relative preference for the three available fluids (water, 16% and 32% ethanol solution) following placebo and naltrexone injection was determined on basis of consumed amounts of the fluids. Since our data analysis made clear that naltrexone mainly was effective at the three highest doses, the data for concentration preference were summarized a. for low dose (0.02, 0.06 and 0.17 $mg.kg^{-1}$) trials and b. for high dose (0.50, 1.0 and 1.5 $mg.kg^{-1}$) trials.

Statistics
Prior to the study we compared individual intakes with the total group sample by use of a Kolmogorov Smirnov two sample test (Siegel 1956). Although the group data followed a normal distribution, the variance in ethanol intake between subjects was larger than within subjects. Hence we used statistics for related measures within subjects, comparing each monkey with itself under the different experimental conditions.

Comparisons between paired naltrexone and placebo treatments were performed by means of paired Student's t-tests (Friedman, 1988).

Overall analyses (analysis of variance (ANOVA) for completely repeated measures within subjects (Kirk, 1968; Friedman, 1988) were performed in Experiment I and in Experiment II, in order to evaluate dose-effect relationships and time course-dependent effects. Reliability analyses were performed to check within individual consistency (Kirk, 1968; Friedman, 1988).

Variation in net ethanol intake and bodyweight over the whole study was analysed by use of a linear regression analysis.

Results

INSPECTION OF THE DATA

The total data sample of net ethanol intake as well as of water consumption had a normal distribution (e.g. at placebo (Exp I) and pre-abstinence (Exp II) days net ethanol intake: N=96, d=0.11<0.14, p>0.05; drinking water: N=96, d=0.12<0.14, p>0.05). Variance between subjects (BS) in general was larger than within subjects (WS) with respect to ethanol (WS:S^2 = 0.83, BS: S^2 = 1.87). Individual distributions sometimes were significantly different from the total distribution as determined by use of the Kolmogorov-Smirnov test. One animal (VJ) had consistently lower ethanol intakes (p<0.01) and one monkey (DW) consistently higher ones (p<0.001).

Table 1: Order of trials, in which a dose (mg.kg^{-1}) of naltrexone was administered, in Experiments I and II. The corresponding mean total net ethanol intake (ml.kg^{-1}) is shown, measured (from 16.00-09.00 hr) at the paired placebo days (Exp. I) and at pre-abstinence days (Exp. II) as a function of time.

TRIALS	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
DOSE												
EXP I			0.50		0.17	1.5			1.0	0.06		0.02
EXP II	0.50	1.5		0.17			0.06	1.0			0.02	
ETHANOL												
	1.2	1.2	1.8	2.4	1.9	2.4	2.6	2.1	2.5	2.7	2.1	2.0

Table 1 shows the mean total net ethanol intake over time, based on the measurements at days of placebo injection (Exp I) or at pre-abstinence days (Exp II) between 16.00 and 09.00 hr. A linear regression analysis revealed that net ethanol intake had increased with time (F(1,94)=4.24, p<0.05). Body weights had not significantly changed over time (linear regression analysis F(1,111)=1.21 ns).

Total Net Ethanol

Measurement period 16.00 - 9.00 hr: Figure 2 (upper left panel) shows the mean total net ethanol intake following a naltrexone (black circle) and its paired placebo injection (open circle), as a function of the dose. Paired comparisons revealed a significant decrease in total net ethanol intake after the three highest doses of naltrexone: 0.5 mg.kg^{-1} (t(df7)=2.5, p<0.05), 1.0 mg.kg^{-1} (t(df7)=3.9, p<0.01) and 1.5 mg.kg^{-1} (t(df7)=4.9 p<0.01).

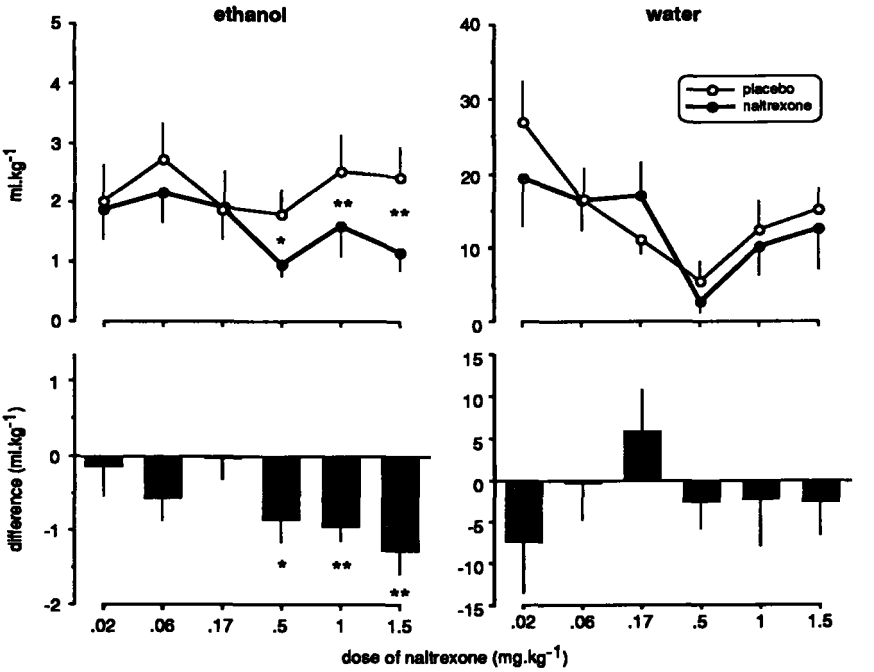

Figure 2. Upper panel, shows the mean (±SE) intake (ml.kg^{-1}) of total net ethanol (left) and of drinking water (rigrht) in Experiment I, during continuous supply (measurement 16.00-09.00 h), for paired injections with placebo and naltrexone (doses: 0.02, 0.06, 0.17, 0.50, 1.0, 1.50 mg.kg^{-1}).
Lower panel, shows the mean (±SE) difference between intakes (ml.kg^{-1}) during continuous supply, following paired naltrexone and placebo injections, for total net ethanol (left) and for drinking water (right), as a function of the dose.
**significant difference between naltrexone and paired placebo injection; paired t-test p<0.05.*
***p<0.01. Further statistical results are given in the text.*

In addition, the mean amount of reduction in total net ethanol intake, i.e. the mean difference in effect between paired naltrexone and placebo injections as a function of the dose is shown in the lower left panel of Figure 2 (bars). Overall analysis (two-way ANOVA) pointed out that net ethanol intake following naltrexone was significantly different from that following placebo (Treatment $F(1,7)=15.5$, $p<0.01$). There was no general effect of the trials (Trial $F(5,7)=2.0$ ns), but there was a significant interaction between treatment and trial (Treatment x Trial $F(5,35)=3.2$, $p<0.01$), indicating a dose-dependent effect of naltrexone. Although there was a significant variation between subjects (Subjects $F(7)=26.8$, $p<0.001$), reliability analysis showed a high correlation coefficient ($R=0.96$) indicating that each subject responded in a very consistent way.

Time course: Figure 3 (upper panel) illustrates that the reduction in total net ethanol intake (determined by the difference score between naltrexone and placebo data) took place during the first two hours of measurement (from 16.00 to 18.00 hr) as well as during the subsequent night period (from 18.00 to 09.00 hr) (Time of day $F(1,7)=0.40$ ns). The effect of dose was similar in both periods (Dose $F(5,7)=3.21$, $p<0.05$), and there was no interaction between the time of the day and the dose (Time of day x Dose $F(1,7)=0.93$ ns, Subjects $F(7)=1.61$ ns).

When the total net ethanol intake levels (between 16.00 hr and 09.00 hr) 24 hours after placebo and naltrexone injections were compared (not shown), no differences were found (Treatment $F(1,7)=0.63$ ns; Trial $F(1,4)=0.69$ ns; Treatment x Trial $F(1,4)=0.40$ ns), indicating that 24 hours later an effect of naltrexone was no longer present.

Drinking Water

Measurement period 16.00-9.00 hr: the mean intake of drinking water following each dose of naltrexone (black circle) and after the paired placebo injection (open circle) is illustrated in Figure 2 (upper right panel). Paired comparisons did not yield significant results. The mean difference in effect between paired naltrexone and placebo treatments, is shown in the lower right panel of Figure 2 (bars). In general there was a significant variation across the trials (Trial $F(5,7)=4.94$, $p<0.01$; Treatment $F(1,7)=0.68$ ns) and between subjects (Subjects $F(7)=4.45$, $p<0.001$). Reliability coefficient (R) was 0.78. There was no interaction between treatment and trial ($F(5,35)=0.79$ ns).

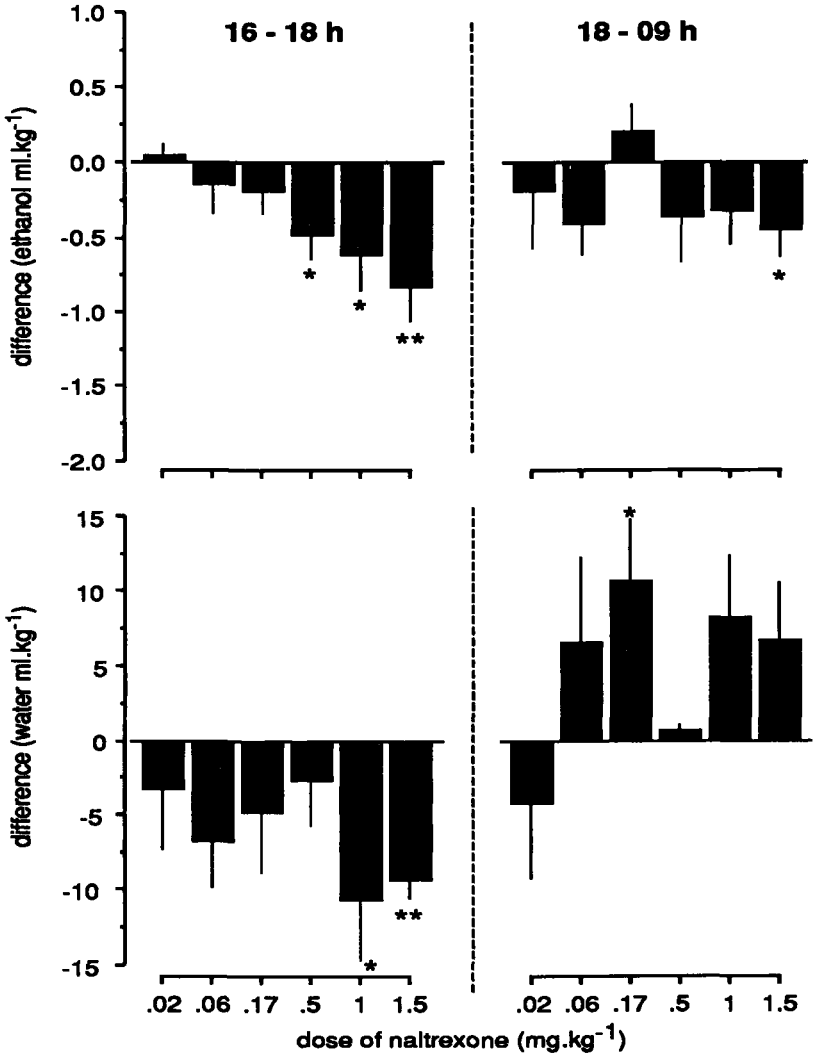

Figure 3. Time course of the effect of naltrexone on total net ethanol intake (upper panel) and on consumption of drinking water (lower panel) in Experiment I (continuous supply). Shown are the mean (±SE) differences between intakes after naltrexone and placebo during the measurement period shortly after injection (left: 16.00-18.00 hr) and during the subsequent measurement period, including the night (right: 18.00-09.00 hr).
**significant effect of naltrexone compared to placebo; paired Student's t-test $p<0.05$;*
***$p<0.01$. Further statistical results are given in the text.*

Table 2: Concentration preference

I. Exp. I. (continuous supply) solution	0%	16%	32%	0-16	0-32	16-32
a. *low dose*						
Placebo	194.2 (±32.1)	94.3 (±19.3)	19.4 (±3.1)	**	***	***
Naltrexone	187.3 (±29.6)	85.3 (±17.2)	21.4 (±3.2)	*	***	***
b. *high dose*						
Placebo	100.7 (±19.0)	98.0 (±16.2)	21.0 (±3.1)	ns	***	***
Naltrexone	88.7 (±25.0)	42.2 (±10.1)	17.5 (±2.1)	ns	**	**

II. Exp. II (imposed alcohol abstinence) solution	0%	16%	32%	0-16	0-32	16-32
a. *low dose*						
Placebo	104.0 (±27.5)	189.5 (±27.8)	29.4 (±7.4)	*	*	***
Naltrexone	116.7 (±25.8)	116.7 (±20.3)	26.2 (±16.9)	ns	**	***
b. *high dose*						
Placebo	82.0 (±20.7)	102.2 (±16.9)	27.4 (±8.1)	ns	*	**
Naltrexone	67.1 (±17.8)	12.4 (±9.9)	25.0 (±6.7)	ns	*	**

Mean (±SE) consumed volume (ml) of solutions, concurrently available, in Exp. I (I) and in Exp. II (II) after placebo and naltrexone a. in trials with low doses of naltrexone administered (0.02, 0.06, 0.17 mg.kg^{-1}) and b. in trials with high doses administered (.5, 1.0, 1.5 mg.kg^{-1}).
** P<0.05; ** P<0.01; *** P<0.001; paired Student's t-test*

Time Course: although no significant effects of naltrexone were found on consumption of drinking water for the total measurement period (from 16.00 to 09.00 hr), a time course analysis (Fig. 3: lower panel) revealed that after naltrexone injections, drinking water appeared to have been reduced during the first two hours (from 16.00 to 18.00 hr), and then to have been increased in the subsequent night period (from 18.00 to 09.00 hr), (Time of day $F(1,7)=21.37$, $p<0.01$; Dose $F(5,7)=0.78$ ns; Time of day x Dose $F(1,5)=1.8$ ns, Subjects $F(7)=0.67$ ns).

Concentration Preference

Table 2.I compares the average volume consumed from each of the three concurrently available solutions in a: trials in which the three lower doses (0.02, 0.06, 0.17 $mg.kg^{-1}$) were administered, and b: trials in which the three higher doses (0.5, 1.0, 1.5 $mg.kg^{-1}$) were administered.

In the low dose trials, water always was preferred over either ethanol solution and 16 per cent was preferred over 32% after placebo as well as after naltrexone (Table 2.Ia). In the high dose trials water preference over 16% was less outspoken (Table 2.Ib). Besides, reduction in alcohol drinking was found to be significant only for the 16%-solution (t-test $t(df22)=2.9$, $p<0.01$).

EXPERIMENT II: AFTER IMPOSED ABSTINENCE

Total Net Ethanol

In Figure 4 (upper left panel) the mean total net ethanol intake after the imposed alcohol abstinence is given for naltrexone (black circle) and its paired placebo injection (open circle). Paired comparisons revealed significant reductions after the doses of 0.17 $mg.kg^{-1}$ ($t(df7)=2.6$, $p<0.05$), 1.0 $mg.kg^{-1}$ ($t(df7)=4.4$, $p<.001$) and 1.5 $mg.kg^{-1}$ ($t(df7)=2.5$, $p<0.05$). The mean differences between the paired naltrexone and placebo injections as a function of the dose are shown in the lower left panel of Figure 4 (bars). Overall analysis (two-way ANOVA) pointed out that total net ethanol intake following naltrexone was significantly different from that following placebo (Treatment $F(1,7)=9.6$, $p<0.01$). There was a significant variation across trials (Trial $F(5,7)=3.6$, $p<0.01$), but no significant interaction between treatment and trial (Treatment x Trial $F(1,5)=1.33$ ns). Although a significant difference existed between subjects (Subjects $F(7)=12.4$, $p<0.001$), the reliability coefficient of $R=0.91$ indicated that each subject itself responded in a quite consistent way.

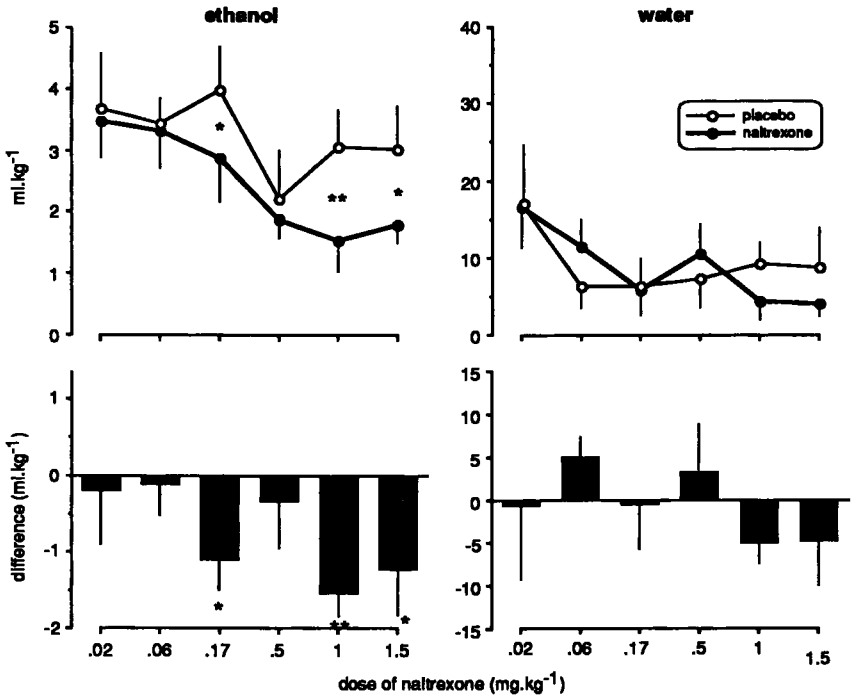

Figure 4. Upper panel, shows the mean (±SE) intake (ml.kg^{-1}) of total net ethanol (left) and of drinking water (right) after imposed alcohol abstinence in Exp. II (measurement period 16.00-09.00 h), for paired injections of placebo and naltrexone (doses: 0.02, 0.06, 0.17, 0.50, 1.0, 1.50 mg.kg^{-1}).
Lower panel, shows the mean (±SE) difference between intakes (ml.kg^{-1}) after abstinence, following paired naltrexone and placebo injections, for total net ethanol (left) and for drinking water (right), as a function of the dose.
**significant difference between naltrexone and paired placebo injection; paired t-test P<0.05, ** P<0.01. Further statistical analyses are given in the text.*

Drinking Water

Figure 4 (upper right panel) shows the effect of naltrexone on the consumption of drinking water after abstinence. Paired comparisons did not yield significant results. The mean differences between the paired naltrexone and placebo injections are shown in Figure 4 (lower right panel) as a function of the doses. ANOVA also did not yield significant results (Treatment F(1,7)=0.03 ns; Trial F(5,7)=1.9 ns; Treatment x Trial F(1,5)=0.56 ns; Subjects F(7)=1.7 ns).

Effect of Imposed Abstinence

Total Net Ethanol

Measurement period 16.00 - 9.00 hr: before abstinence, the total net ethanol intake on pre-abstinence days previous to naltrexone injections was not different from that previous to the paired placebo injections. Overall, ANOVA indicated a significant fluctuation across the trials (Pair $F(1,7)=0$ ns; Trial $F(5,7)=5.48$, $p<0.001$; Subjects $F(7)=19.8$, $p<0.001$; Pair x Trial $F(1,5)=0$ ns). The reliability coefficient (R) within subjects was 0.94.

During abstinence days there were no overt signs of physical withdrawal reactions, like hyperactivity, vomitting, tremor or convulsions.

After the imposed abstinence with placebo injection, significantly more ethanol (post-abstinence: mean intake was 3.23 (SE = ±1.9) $ml.kg^{-1}$) was ingested in comparison to pre-abstinence intake level (pre-abstinence: mean intake was 1.92 (SE=±1.1) $ml.kg^{-1}$). (ANOVA: Abstinence $F(1,7)=17.82$, $p<0.01$; Trial $F(5,7)=2.68$, $p<0.05$; Abstinence x Trial $F(1,5)=0.97$ ns; Subjects $F(7)=12.69$, $p<0.001$). Reliability coefficient (R) was 0.92. The resumed alcohol drinking at reintroduction of alcohol did not lead to signs of overt intoxication. With naltrexone, paired comparisons between pre-and post abstinence intakes revealed a significant increase in ethanol intake after abstinence only in two cases: following a dose of 0.02 $mg.kg^{-1}$ ($t(df7)=3.28$, $p<0.05$), and 1.5 $mg.kg^{-1}$ ($t(df7)=2.5$, $p<0.05$) of naltrexone. ANOVA indicated that overall there was no significant effect of abstinence on net ethanol intake (Abstinence $F(1,7)=3.5$ ns; Trial $F(5,7)=7.23$, $p<0.001$; Subjects $F(7)=10.47$, $p<0.001$, reliability coefficient $R=0.90$). The interaction between abstinence and dose did not quite reach significance (Abstinence x Trial $F(1,5)=2.35$, $p = 0.06$).

Time Course: the increase in total net ethanol intake after abstinence, in the placebo condition, was primarily present during the first two hours of renewed alcohol supply (16.00-18.00 hr). The increase was significant in five out of six trials (paired t-tests (df7) t's>2.7, p's<0.05). Only in the trial of 0.5 $mg.kg^{-1}$, the increase was not significant after placebo ($t(df7)=1.5$ ns). On average the increase was +0.94 (±.26) $ml.kg^{-1}$ of ethanol, i.e. 200 per cent, compared to pre-abstinence level. During the subsequent night (18.00-09.00 hr) no significant differences in the placebo condition were observed between pre- and post-abstinence levels in any of the trials.

Figure 5 illustrates that the reduction (expressed in difference scores) by naltrexone in ethanol intake (upper panel) primarily took place during the first two hours of renewed alcohol supply (from 16.00 to 18.00 hr).

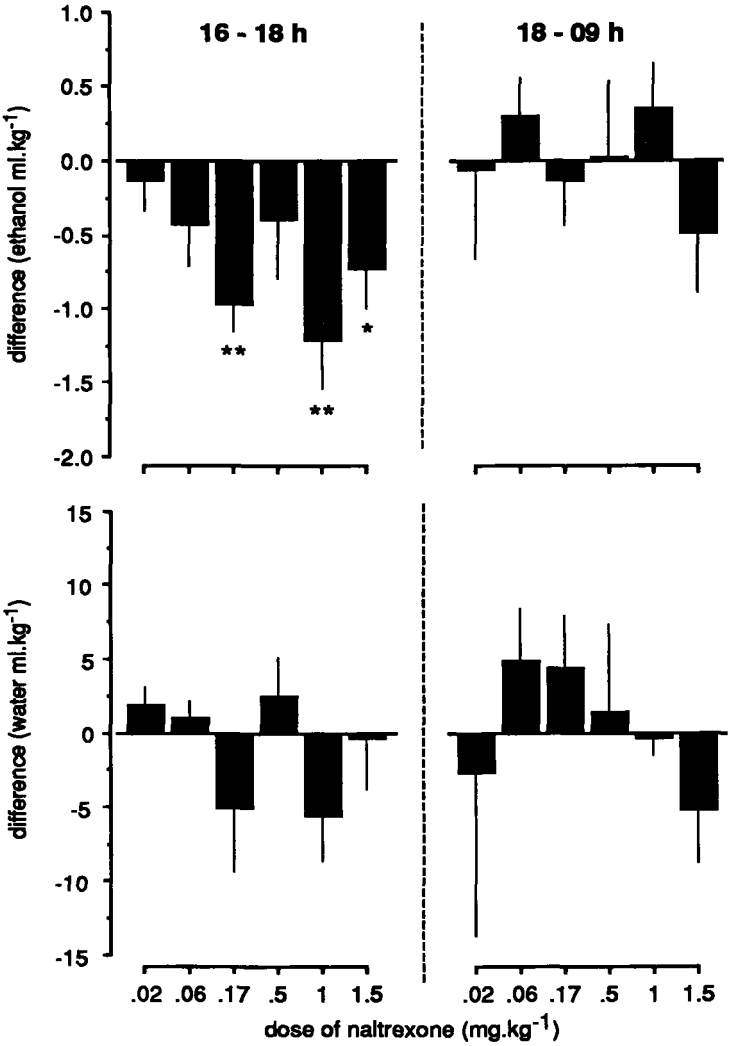

Figure 5. Time course of the effect of naltrexone on total net ethanol intake (upper panel) and on consumption of drinking water (lower panel) after abstinence (Exp. II). Shown are the mean (±SE) differences between intakes after naltrexone and placebo during the measurement period shortly after injection (left: 16.00-18.00 h) and during the subsequent measurement period, including the night (right: 18.00-09.00 h).
**significant effect of naltrexone compared to placebo; paired Student's t-test P<0.05; ** P<0.01 . Further statistical results are given in the text.*

This led to a significant difference in effect compared to the subsequent night, in which no clear reduction was observed (Time of day F (1,7)=12.44, $p<0.001$, Dose F(5,7)=0.82 ns, Time of day x Dose F(1,5)=1.14 ns, Subjects F(7)=1.36 ns).

Drinking Water

Measurement period 16.00 - 9.00 hr: the consumption of drinking water on pre-abstinence days previous to paired naltrexone and placebo injections was not different (Pair F(1,7)=0.08 ns; Trial F(5,7)=7.48 $p<0.001$; Pair x trial F(1,5)=0.23 ns; Subjects F(7)=3.5, $p<0.01$; reliability coefficient R=0.71).

After abstinence, neither after placebo injections nor after naltrexone injections, abstinence had an effect on water drinking (ANOVA Placebo: Abstinence F(1,7)=1.34 ns; Trial F(1,5)=2.16 ns; Abstinence x Trial F(1,5)=0.47 ns; Subjects F(7)=2.10, $p<0.05$, reliability coefficient R=0.52; Naltrexone: Abstinence F(1,7)=2.17 ns, Trial F(5,7)=6.38, $p<0.001$, Abstinence x Trial F(1,5)=0.87 ns, Subjects F(7)=4.0, $p<0.001$, reliability coefficient R=0.75).

Time Course: Figure 5 (lower panel) illustrates that no difference in effect of naltrexone (expressed as difference score) existed for water drinking in the first two hours and during the subsequent night (Time of day F(1,7) = 0.268 ns, Dose F(5,7)=0.56 ns; Time of day x Dose F(1,5)=0.84 ns, Subjects F(7)=0.94 ns).

Concentration Preference

Table 2.II compares the average volume consumed after imposed abstinence from each of the three available solutions in a: low dose (0.02, 0.06, 0.17 $mg.kg^{-1}$) trials and b: high dose (0.5, 1.0, 1.5 $mg.kg^{-1}$) trials. In the low dose trials with placebo injection, the 16% solution was significantly preferred over water and over 32%; water was preferred over the 32% solution. With naltrexone, water and 16% solution was equally preferred; both were preferred over the 32% solution. In the high dose trials placebo and naltrexone administration resulted in comparable preference patterns: preference for 16% over water was not significant; water and 16 per cent were preferred over 32%. Besides, the reduction in alcohol drinking after naltrexone was significant for 16%-solution only, in the low dose trials (t(df22)=2.9 $p<0.01$) and in the high dose trials (t(df22)=2.3, $p<0.05$).

Discussion

Although monkeys differed in intake level, resulting in significant between-subject effects in some of the statistical analyses, intake levels within each

individual was quite consistent. The fluctuation in the ethanol intake levels across the trials appeared to be, at least partly, caused by an increase in ethanol intake over time. The fact that no significant subject variation existed across the difference scores, obtained from the paired observations, indicated that comparisons based on paired observations were quite reliable.

Experiment I. Continuous Supply

Naltrexone appeared to have a different effect on total net ethanol intake and on drinking water consumption. Total net ethanol intake was reduced in a graded, dose-dependent manner, the effects of the three highest doses reaching statistical significance. The maximal reduction was 1.27 $ml.kg^{-1}$ ethanol, i.e. to about 50% of pre-treatment intake, at a dose of 1.5 $mg.kg^{-1}$. The reduction started directly following injection and lasted untill the following day.

Water drinking appeared to be reduced during the first two hours of measurement following injection, but to be increased afterwards. Therefore, the average amount of waterdrinking in the total measurement period (from 16.00 to 09.00 hr) was not different from that in the placebo condition. As the pharmacological activity of naltrexone lasts about three hours, the increase in water drinking in the night period could be a compensation for the temporary suppression by the antagonist. Since net ethanol intake, however, remained suppressed in the night period, it might be that the effect on ethanol intake persisted beyond naltrexone's pharmacological activity.

The relative preference for water, the 16%- and the 32%-solution did not significantly change after low or high doses of naltrexone. The 32%-solution always was the least preferred fluid. With respect to the separate ethanol solutions, reduction was most pronounced for the 16% solution.

Experiment II. After Imposed Alcohol Abstinence

As in previous alcohol abstinence studies (Kornet et al, 1990a) the increase in net ethanol intake occurred primarily during the first two hours of renewed alcohol supply. Interestingly, the reduction by naltrexone also occurred primarily during this first period of renewed supply. The consequence of the administration of naltrexone was that in four out of six trials post-abstinence intake levels could no longer be distinguished from pre-abstinence levels. The effect of naltrexone was specific for net ethanol intake, since water drinking was not affected in the two hours after injection, nor during the subsequent night. An increased net ethanol intake still existed after the lowest dose of 0.02 $mg.kg^{-1}$, indicating this dose had no or little effect. Surprisingly, an abstinence-induced increase also occurred after the highest dose of 1.5 $mg.kg^{-1}$, although it was significantly less than after placebo.

The effect of naltrexone did not increase with the dose. The data suggest therefore, that at a dose of 0.17 $mg.kg^{-1}$ reduction was already maximal within the given range of doses. The dose of 0.5 $mg.kg^{-1}$ does not seem to fit into this pattern, but this probably relates to the fact that intake levels also were low after placebo and abstinence induced no increase in ethanol intake. Since this was the first trial of the whole study, perhaps some other variables might have interfered.

Alcohol abstinence led in general to a higher relative preference for the ethanol solutions versus water. The 16%-solution always was preferred to the 32%-solution. After naltrexone, preference for 16%-solution was decreased. Drinking from the 32% solution was less influenced in both low and high dose trials.

Opioids in alcohol drinking

The study showed that naltrexone reduced ethanol intake in both experiments indicating that endogenous opioids were involved in chronic alcohol drinking (Exp I) as well in drinking after an imposed alcohol abstinence (Exp II). The doses used were low compared to rodent (Hubbel et al., 1986; Samson and Doyle, 1985; Volpicelli et al., 1986) and other monkey studies (Altshuler et al., 1980) and can be considered to be opioid specific in effect (Frenk and Rogers, 1979; Prunell et al., 1987).

The results might contribute to the discussion whether naltrexone exerts specific effects on animal alcohol consumption compared to water consumption (De Witte, 1984; Hubbell et al., 1986) or mainly produces a general suppression in ingestive behaviour (Samson and Doyle, 1985; Koob and Weiss, 1990).

In Experiment I the temporary reduction in water drinking was compensated for later on; by contrast ethanol intake remained suppressed. This is reminiscent of the rodent studies in which daily naltrexone treatment at first led to a suppression of both water and alcohol drinking (daily restricted supply of both), but after a few days the effect on water waned and on alcohol remained (Hubbell et al., 1986; Sandi et al., 1988). This suggests that naltrexone can interact with behaviours (including water drinking or eating) that specifically lead to positive reinforcement, provided that physiological demands (e.g. dehydration or starvation) are not critically involved.

In Experiment II the effect of naltrexone was specific for ethanol intake. Furthermore, it appeared that in both experiments the preference for 16%-solution was much higher than for the 32%-solution and the effect of naltrexone mainly concerned the consumption of this highest preferred ethanol solution of 16%. Therefore, we postulate that the opioid modulation by naltrexone specifically interacted with positively reinforced behaviour, rather than causing a general behavioural suppression.

Although in both experiments, the maximal reduction by naltrexone in net ethanol intake was quite comparable, results were not fully identical. A significant reduction after abstinence (Exp. II) was reached for a lower dose (0.17 $mg.kg^{-1}$) of naltrexone, which could indicate that imposed alcohol abstinence made the monkeys more sensitive for opioid modulation. Furthermore, the absolute ethanol intake levels in Experiment II remained higher than in Experiment I, and the effect of naltrexone after abstinence was more restricted in time than during continuous supply, being restricted to the period when an abstinence-induced increase in consumption occurred.

The difference in results of Experiments I and II suggest that opioid modulation had different effects after abstinence. With respect to the interaction of endogenous opioids and drinking after abstinence, several hypotheses are considered here.

A hypothesis has been formulated that the basis of recurrent ethanol-seeking behaviour is a reduced level of central β-endorphin, due to a feedback inhibition of central β-endorphin by chronic alcohol consumption (Genazzani et al., 1982; Gianoulakis et al., 1989; Volpicelli et al., 1986; Volpicelli et al., 1990). So it might be that recurrent relapse into alcoholic drinking is due to an acquired deficiency of central β-endorphin. Additional experimental support for an 'endorphin compensation' hypothesis comes from Sandi et al. (1989) who found that administration of β-endorphin in rats before alcohol drinking reduced alcohol consumption, and from Sinclair et al (1973) who found in rats that administration of an opiate agonist (morphine) during alcohol abstinence prevented an alcohol abstinence effect later on. Moreover, decreased β-endorphin levels also have been associated with cocaine- and heroin-seeking behaviour in rats (Sweep et al., 1988; Sweep et al., 1989). However, if decreased endorphin levels are mainly responsible for the relapse after abstinence, it is not clear why by antagonizing opiate receptors, i.e. inducing even less opioid activity, the abstinence-induced increase in the monkeys was suppressed (Hubbell et al., 1986; Czirr et al., 1987; Koob and Weiss, 1990).

In human alcoholism, high risk of relapse is frequently found to be related to the presence of environmental stimuli previously associated with drinking (Marlatt and George, 1984; Burish et al., 1981). It could be that the relapse in the monkeys was (partly) elicited by the renewed presence of alcohol ('priming'), or of drug-associated stimuli, independent of some state of internal deficiency (Stewart et al., 1984; Stewart and Vezina, 1988; Barnes 1988; Cornell et al., 1989). A recent hypothesis is that opioid activity can stimulate alcohol drinking in rats (Hubbel et al., 1987; Reid et al., 1987). If incentive-elicited motivation leads to enhanced opioid activity, this could explain why the antagonist naltrexone reduced the relapse-like drinking in the monkeys. Possibly, the smell

and taste of the solutions had functioned as conditioned incentive stimuli (Cornell et al., 1989) and had elicited alcohol drinking again in our monkeys.

Since the increase in ethanol intake after abstinence could not be completely abolished by the highest dose of 1.5 $mg.kg^{-1}$, it is conceivable that relapse drinking after abstinence was mediated by other, e.g. non-opoid reinforcement, systems (Stewart et al., 1984; Stewart and Vezina, 1988; Koob and Weiss, 1990; Samson et al., 1990). Immediate resumption of alcohol drinking after abstinence could be based on resistance to extinction of previously acquired ethanol-reinforced behaviour (Kalant et al., 1978, Barnes, 1988; Hand et al., 1989) and on incentive-stimulated behaviour (Stewart et al., 1984; Stewart and Vezina, 1988); both components might be mediated by different mechanisms (Cloninger, 1987; Hand et al., 1989; White, 1989). An interesting anatomical and neurochemical distinction between learned and motivated behaviour is made by White (1989), who distinguished stimulus-response memory (persistence of reinforced behaviour) and reward (approach behaviour is elicited by environmental stimuli).

The study of relapse-like alcohol drinking after imposed abstinence in monkeys, that show striking similarities with recurrent relapses in human alcoholism (Sinclair, 1971; Burish, 1981; Barnes, 1988; Winger, 1988), can be useful to further investigate new hypotheses and treatments for chronic alcohol drinking and relapse, a phenomenon of which the underlying mechanisms still are poorly understood (Mendelson and Mello, 1979; Horwitz et al., 1989; Barnes, 1988).

Although evidence is growing that the reinforcing effects of ethanol interact with endogenous opioid systems (Volpicelli et al., 19990), the precise mechanism(s) through which agonists and antagonists exert their actions, is not yet fully elucidated (Myers and Privette, 1989; Koob and Weiss, 1990; Lewis and June, 1990; Volpicelli et al., 1990). As for the possible clinical use of naltrexone, it seems important that, using low doses, apparently no compensatory reaction existed for the initial reduction in ethanol intake during continuous supply and that its effects were specific for ethanol intake during relapse. Furthermore, if alcoholism is to be regarded as an acquired ethanol-reinforced behaviour that is strongly resistant to extinction, this would explain why complete abstinence as currently employed in clinical treatment is only moderately effective: it does not produce extinction of the behaviour. Achievement of extinction by neuropharmacological means, might be a promising addition to the treatment of alcoholism.

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7. LOW DOSES OF MORPHINE REDUCE VOLUNTARY ALCOHOL CONSUMPTION IN RHESUS MONKEYS

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Abstract

Experimental opioid modulation has been found to influence the consumption of alcohol in animals. Whereas it has generally been agreed upon that opiate antagonists reduce alcohol consumption, the results with opiate agonists are less consistent. The present study reports on the effect of low doses of morphine in 8 adult male rhesus monkeys that had a free-choice in drinking water, a 16% and a 32% ethanol/water solution, a: during continuous ad libitum access (Experiment I) and b: after 2 days of alcohol abstinence (Experiment II). In both experiments each monkey received a single morphine injection (i.m.) in 5 different doses (0.03, 0.06, 0.17, 0.50, 1.50 $mg.kg^{-1}$); each morphine injection (i.m.) was placebo-controlled in a cross-over design. Consumption was measured from 16.00 hr in the afternoon (30 min after injection) to 08.30 hr the next morning. In Experiment I after 0.50 and 1.50 $mg.kg^{-1}$ of morphine ethanol intake and water consumption were both reduced during the first hours after injection; only ethanol intake remained reduced during the subsequent night. Effects lasted not longer than 24 hours. In Experiment II, morphine administered 30 min. before reintroduction of ethanol solutions selectively reduced ethanol intake at doses of 0.17, 0.50 and 1.50 $mg.kg^{-1}$; water consumption was unaffected. The reduction lasted for the subsequent night after the 2 highest doses. Obtained records of various spontaneous behavioural activities made it unlikely that the used dose range had induced some aspecific sedation; monkeys remained alert and active. The results are contradictory with studies, in which low doses of morphine stimulated alcohol drinking in rats. The present results seem to support the hypothesis that at least in monkeys morphine can compensate for the effects of alcohol.

Introduction

Numerous data have emerged supporting the existence of a relationship between alcohol and endogenous opioids (Davish and Walsh, 1970; Blum et al., 1977; Tabakoff and Hoffman, 1987; Olson et al., 1989). Experimental opioid modulation has been found to influence the consumption of alcohol in animals (Olson et al., 1989; Sandi et al., 1989; Messiha, 1989), although the underlying mechanisms are still under debate (Linseman, 1989; Volpicelli et al., 1990). More knowledge on this subject is of importance, because the endogenous opioid system could be a biological substrate for modifying excessive alcohol drinking and alcohol abuse in humans (Hubbell and Reid, 1990; Volpicelli et al., 1990). Although human alcoholism is a complex multifactorial syndrome (Van Dijk, 1979; Marlatt et al., 1988), the frequent desire to drink alcohol and the recurrent

relapses into the problematic drinking habit, despite the adverse consequences this may have for the alcoholic or the environment, are considered as its major problems (Jellinek, 1955; Dole, 1986; Horwitz et al., 1987).

We found that monkeys with experience in free-choice alcohol drinking after several days of imposed abstinence from alcohol supply, showed specific alcohol-directed behaviour, together with a temporarily increased alcohol consumption (Kornet et al., 1990a). This effect became stronger as the abstinence period lasted longer (up to 7 days), indicating that it could not be attributed to physical withdrawal reactions, which are known to subside within 48 hours in monkeys (Ellis and Pick, 1972; Winger, 1988). It was hypothesized (Sinclair and Li, 1989; Kornet et al., 1990a) that the observed behaviour might be mediated by the same mechanism(s) as the relapses in alcoholics after a (sometimes quite prolonged) period of abstinence (Marlatt and George, 1984; Horwitz et al., 1987).

In a previous study (Kornet et al., 1991 in press), alcohol drinking after abstinence in monkeys could be reduced by a single injection with naltrexone, an opiate antagonist, in doses from 0.17 to 1.50 $mg.kg^{-1}$. This suggests that endogenous opioid systems are involved in alcohol drinking after abstinence.

Some findings seem to indicate that opiate agonists can substitute for the effect of alcohol (Sinclair et al., 1973; Siegel, 1986; Volpicelli, 1986). This would imply that agonists can reduce alcohol drinking as well (Volpicelli et al., 1990). On the other hand, the opiate agonist morphine administered in non-sedative doses to rats enhanced alcohol drinking under a variety of conditions (Czirr et al., 1987; Linseman, 1989; Hubbell and Reid, 1990).

The present study was aimed at the influence of low doses of morphine on the alcohol consumption of rhesus monkeys. The effect of morphine was tested under two conditions: a: water and ethanol solutions remained continuously ad libitum available (Experiment I) and b: abstinence was imposed by interrupting the alcohol supply for 2 days; water remained continuously available (Experiment II).

Methods and Materials

Subjects

Subjects were 8 free-fed, male rhesus monkeys (*Macaca mulatta*), housed in single cages together in one room. Individual body weights were between 9.4 and 15.4 kg; ages between 9 and 14 years. The monkeys had about 4 years experience with alcohol drinking. They had spontaneously initiated and maintained alcohol drinking under conditions of unrestricted food and water access (for details see Kornet et al., 1990b); the last two years the alcohol supply consisted of a 16 and a 32% ethanol/water solution, concurrently available with drinking water. The

supply of 2 ethanol solutions permitted to make a differentiation between the amount of net ethanol ingested and individual preferences for different concentrated ethanol solutions. At the start of the present study, average individual net ethanol intakes varied between 1 and 7 $ml.kg^{-1}$ per day.

Alcohol Supply

Each cage was provided with 3 drinking nipples, concurrently providing for 24 hours per day: drinking water, a 16 and a 32% ethanol/water solution. The monkeys were fed in the morning with monkey chow (Hope Farms), fruit and vegetables at noon and a slice of bread at 14.30 hr.

Drug

Morphine hydrochloride (Pharmachemie BV, Haarlem, The Netherlands) was administered to the monkeys in their home cages by means of intramuscular injection in doses of 0.03, 0.06, 0.17, 0.50 and 1.50 $mg.kg^{-1}$. Each monkey received each dose once in Experiment I and once in Experiment II. Each injection with morphine was placebo (saline) controlled. Thus, five doses of morphine were tested in Experiment I in five separate trials, in which one morphine and one placebo injection was administered; five doses were tested in Experiment II in another five trials. In Experiment II one trial was carried out with one monkey less (Trial 6: 0.50 $mg.kg^{-1}$ of morphine; n=7), because this animal had to be treated for a hernia.

Experimental Procedure

The order of trials in which pairs of morphine and placebo injections were administered is given in Table 1 for Experiments I and II. Figure 1 illustrates the experimental procedure that was followed in Experiments I and II.

In Experiment I (a) the monkeys always had uninterrupted access to drinking water, a 16% and a 32% ethanol solution, 24 hours per day (shaded area). A dose of morphine was administered, at Tuesday 15.30 h, to half (n= 4) of the monkeys (a: black syringe), the other half (n=4) received placebo (a: white syringe); the next week, at Tuesday, morphine and placebo injections were reversed. In this way each dose of morphine was paired with a placebo injection in a cross-over design. Consumed volumes of ethanol solution and drinking water were recorded in units of milliliters fluid. Drinking bottles were immediately refilled after measurement. Consumption was measured at Monday (pre-treatment day), Tuesday (treatment day) and Wednesday (post-treatment day), 30 minutes after injection, for the time intervals (a: X) from 16.00 to 18.00 hr (i.e. the first two hours of measurement following injection) and from 18.00 to 08.30 hr the next morning (i.e. after an additional 14,5 hours period, including the night).

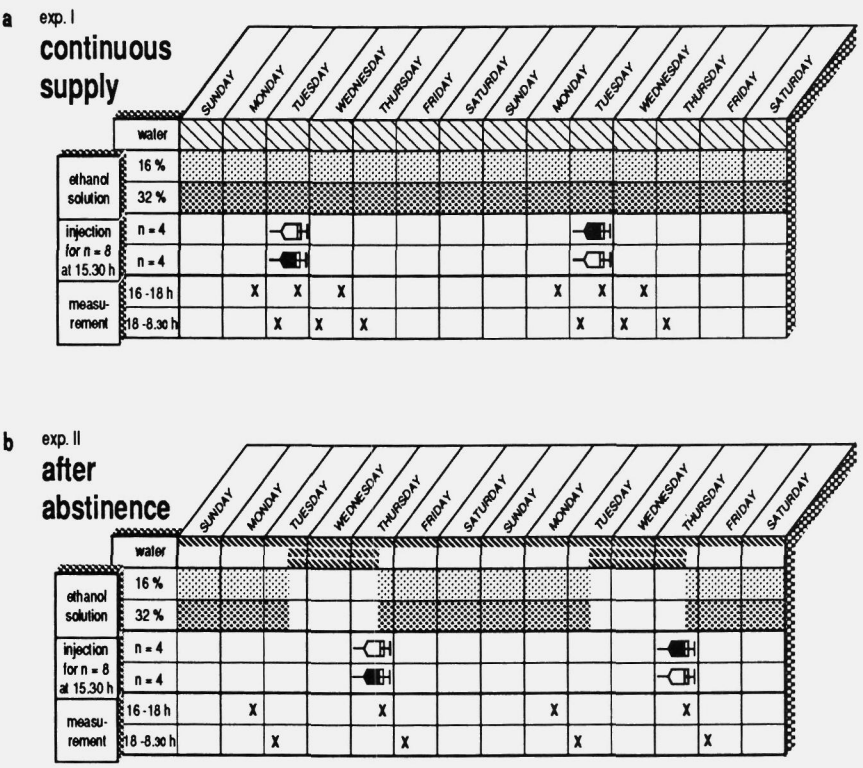

Figure 1. Experimental procedure used for each trial in Experiments I (a) and II (b). In Experiment I supply of alcohol and water was continuously available (shaded area). Consumed volumes of water and ethanol solutions were measured at Mondays (pre-treatment day), Tuesdays (treatment day) and Wednesdays (post-treatment day) from 16.00 to 18.00 hr (X) and from 18.00 to 08.30 hr in the next morning (X). At the first Tuesday at 15.30 hr 4 monkeys received morphine (black syringe), 4 received saline (white syringe); in the next week at Tuesday treatments were reversed. In Experiment II alcohol abstinence was enforced by substituting drinking water for ethanol solution from Tuesday 16.00 hr untill Thursday 16.00 hr (white area). At Thursday 15.30 hr 4 monkeys received morphine (black syringe), 4 received saline (white syringe); at 16.00 hr ethanol solutions were replaced again. The next week, this procedure was repeated, but treatments were reversed. Consumption was measured at Mondays (pre-abstinence day) and Thursdays (post-abstinence day) from 16.00 to 18.00 hr (X), and from 18.00 to 08.30 hr in the next morning (X).

In Experiment II (b) abstinence was imposed by interrupting the alcohol supply from Tuesday 16.00 h untill Thursday 16.00 hr (white area). In this period both ethanol solutions were substituted for water (shaded area). At Thursday 16.00 hr the ethanol solutions were made available again. A dose of morphine was administered (b: black syringe) to four monkeys at Thursday 15.30 hr (30 min before reintroduction of alcohol supply), 4 (for dose 0.50 mg.kg^{-1}: 3) monkeys received placebo (b: white syringe). In the next week this procedure was repeated, but at Thursday injections were reversed, so that each injection with morphine was paired with a placebo injection in a cross-over design. Consumption was measured at Monday (pre-abstinence) and Thursday (post-abstinence) in the same way as in Experiment I.

During the days of imposed abstinence the monkeys were checked six times per day for physical withdrawal reactions that might occur in rhesus monkeys within 48 hours, like hyperactivity, tremor, sickness, irritability and convulsions (Ellis and Pick, 1972; Myers et al., 1972; Friedman, 1980).

Effects of morphine on behavioural activities

Since morphine could have sedated the monkeys, the behaviour of two animals (monkeys 2U and 1DW), that showed clear morphine-induced effects in net ethanol intake, was video taped at pre-abstinence day (b: Monday) and post-abstinence day (b: Thursday) between 16.00 and 17.00 hr. This was done once under placebo and once when treated with the highest dose of morphine (1.50 mg.kg^{-1}). Time spent in various spontaneous activities as described in Table 3 was determined from the recorded video samples.

Data analysis

Total net ethanol and drinking water

Daily consumed volumes of the two ethanol solutions were transformed into ml.kg^{-1} net ethanol and then summed to determine the total net ethanol intake. Consumed volume of drinking water was also transformed into ml.kg^{-1} water consumption. Across the measurement period from 16.00 to 08.30 hr (i.e. a period of 16,5 hours) intakes following morphine injection were compared with intakes following the paired placebo injection.

Effect of imposed abstinence (Exp. II only)

The effect of alcohol abstinence on subsequent total net ethanol intake and consumption of drinking water, was determined by comparing the intake following abstinence (post-abstinence) with the intake at the day before abstinence

(pre-abstinence). Previous studies (Kornet et al., 1990a; Kornet et al., in press) indicated that the effect of a two-day alcohol abstinence was particularly prominent within the first two hours of renewed supply. In the present study, the effect of imposed abstinence also was analysed separately for the first two hours of renewed supply and for the subsequent night period.

Time course

To study the time course of the effect of morphine, we separated the effect of morphine during the first two drinking hours after injection (from 16.00 to 18.00 hr) from the effect during the subsequent night period (from 18.00 to 08.30 h). The possibility that the effect lasted longer than 24 hours was evaluated by comparing intakes, between 16.00 and 08.30 hr, at post-treatment days (a: Wednesday) that followed placebo, with post-treatment days that followed morphine treatment days.

Concentration preference

Relative preference for the three available fluids (drinking water, 16% and 32% ethanol solution) following placebo and morphine injection was determined by comparing consumed amounts of the fluids. Statistical analysis made clear that with respect to total net ethanol intake (from 16.00 to 08.30 h) in Experiment I, morphine was mainly effective at the two highest doses (0.50 and 1.50 $mg.kg^{-1}$), and in Experiment II, at the three highest doses (0.17, 0.50 and 1.50 $mg.kg^{-1}$). For ease of survey, data of concentration preference are here presented for Experiment I as a: "ineffective doses" (0.03, 0.06, 0.17 $mg.kg^{-1}$) and b: "effective doses" (0.50 and 1.50 $mg.kg^{-1}$); for Experiment II as a: "ineffective doses" (0.03, 0.06 $mg.kg^{-1}$) and b: "effective doses" (0.17, 0.50 and 1.50 $mg.kg^{-1}$).

Statistics

Variance in intake between monkeys was considerable larger than within monkeys. Therefore we used statistics for related measures, comparing the effects of different experimental conditions within each subject. The data for total net ethanol intake and water consumption did not meet the assumptions for parametric analysis; pair-wise comparisons were carried out by use of a non-parametric test (Wilcoxon matched pair signed rank test, Siegel, 1956). Reliability analysis was performed to check within-subject consistency (Kirk, 1968; Friedman, 1988).

Results

EXPERIMENT I: CONTINUOUS ALCOHOL SUPPLY

Total Net Ethanol

Measurement period 16.00 - 08.30 h: Total net ethanol intakes at pre-treatment days (see Fig. 1a: Monday) in weeks for morphine injection were not significantly different from those at pre-treatment days in the matched weeks for placebo injection (Wilcoxon tests p's>0.05). Within-subject reliability of total net ethanol intake for matched pre-treatment days was R = 0.99.

Figure 2 (upper panel) shows the mean total net ethanol intake at treatment day (Fig. 1a: Tuesday) following a morphine and its paired placebo injection, as a function of the dose. Paired comparisons between morphine and placebo revealed a significant decrease in total net ethanol intake after 0.50 $mg.kg^{-1}$ and after 1.50 $mg.kg^{-1}$ of morphine. Within-subject reliability was R=0.96.

Time Course: To analyse the time course of the effect of morphine, effects were determined seperately for the first two hours shortly after injection (from 16.00 to 18.00 hr) and for the subsequent night (from 18.00 to 08.30 h3). Figure 3 (upper panel) displays the mean difference in net ethanol intake after morphine and placebo, shortly after injection and during the subsequent period. Shortly after injection, net ethanol intake was reduced significantly after 0.06 $mg.kg^{-1}$, 0.50 and 1.50 $mg.kg^{-1}$. In the subsequent period, net ethanol intake remained reduced after 0.50 and 1.50 $mg.kg^{-1}$ of morphine.

Comparison of total net ethanol intakes (measured between 16.00 and 08.30 hr) 24 hours after placebo and morphine injections (Fig. 1a: Wednesday), showed no significant differences. This indicates that after 24 hours there were no longer effects of morphine on ethanol intake.

In addition, the mean total net ethanol intake measured at placebo days for Experiment I and at pre-abstinence days for Experiment II (see Table 1b: Trials 1 to 10) pointed out that morphine injections had not changed net ethanol intake in the long run either.

Drinking Water

Measurement period 16.00 - 08.30 h: Consumptions of drinking water at pre-treatment days for morphine and paired placebo weeks were not significantly different (Wilcoxon tests p's>0.05). Within-subject reliability was R=0.85.

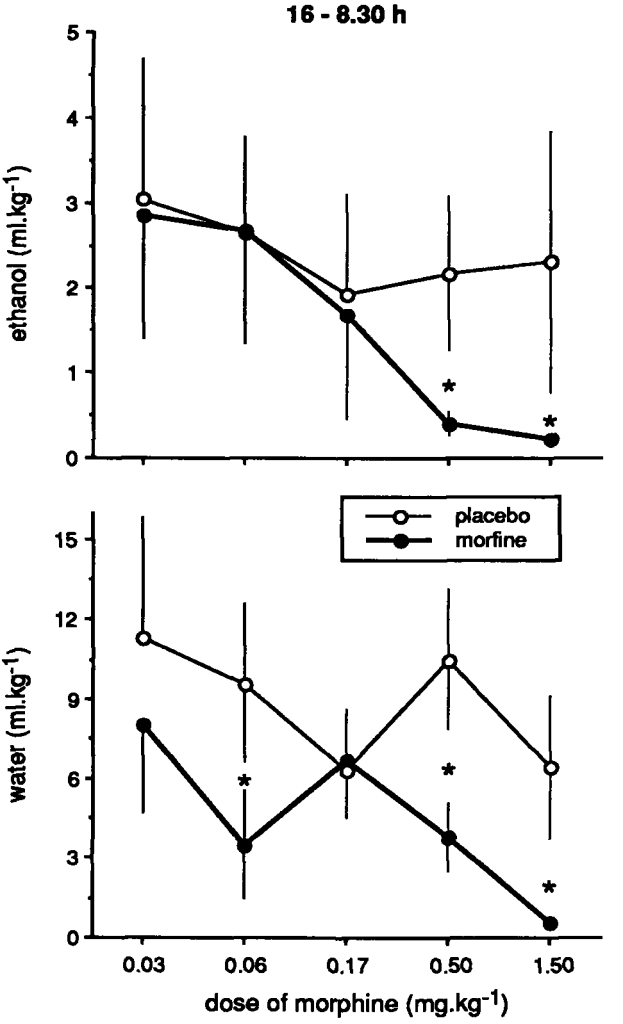

Figure 2. Mean (+SE) consumed total net ethanol (ml.kg^{-1}) (upper panel) and mean (+SE) consumed drinking water (ml.kg^{-1}) (lower panel) during continuous alcohol supply (measured from 16.00 to 08.30 hr) after paired morphine and placebo injections, for a dose range of 0.03 - 1.50 mg.kg^{-1} .
**significant difference between morphine and placebo, Wilcoxon matched pairs test p<0.05; ** p<0.01.*

Figure 2 (lower panel) illustrates that at treatment day (see Fig. 1a: Tuesday) water consumption after morphine injections at a dose of 0.06 mg.kg^{-1}, 0.50 mg.kg^{-1} and 1.50 mg.kg^{-1} appeared to be less than after placebo. Within-subject reliability at treatment days was R=0.80.

Time Course

Figure 3 (lower panel) illustrates the effect of morphine compared to placebo (shown in difference scores), shortly after injection (from 16.00 to 18.00 hr), and during the subsequent measurement period (from 18.00 to 08.30 hr). Shortly after injection, water consumption was significantly reduced after 1.50 mg.kg^{-1}. In the subsequent period, water consumption was lower at a dose of 0.06 mg.kg^{-1} of morphine. Comparison of water consumption 24 hours after placebo or morphine injections revealed that only once water consumption at post-morphine day was lower at the dose of 1.50 mg.kg^{-1} of morphine (Wilcoxon $p<0.05$). Within-subject reliability for post-treatment days was rather low (R=0.75).

Concentration Preference

In Table 2.1 the average volumes consumed from each of the three concurrently available solutions between 16.00 and 08.30 hr, are compared a: for "ineffective doses" (0.03, 0.06, 0.17 mg.kg^{-1}), and b: for "effective doses" (0.50 and 1.50 mg.kg^{-1}). With respect to placebo there was no concordance among the subjects in preference for water and the 16% solution: about 50% preferred the 16% solution to water, and 50% preferred water to the 16% solution. In most cases water and 16% solution was preferred over the 32% solution; only one animal always preferred ethanol solution over water. Between morphine and placebo there were no significant differences; neither for the "ineffective" nor for the "effective" doses. The "effective" doses of morphine significantly reduced the consumption from all three fluids.

Table 1. Experimental Trials

TRIALS	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*
1a. Doses										
EXP I	0.5	.17	1.5	.03	.06					
EXP II						0.5	.17	1.5	.03	.06
1b. ETHANOL	3.0	2.7	1.9	2.2	2.3	2.7	2.5	3.0	2.3	2.9

1a. Order of trials, in which a dose (mg.kg^{-1}) of morphine was administered, in Experiments I (trial 1 to 5) and II (trial 6-10).

1b. Corresponding mean total net ethanol intake (ml.kg^{-1}) between 16.00 and 08.30 hr, measured at placebo days (Exp. I, n=8) or pre-abstinence days (Exp. II, n=14) as a function of time.

Table 2. Concentration Preference

2.1 *Experiment I* solution	0%	16%	32%	Wilcoxon test 0-16	0-32	16-32
a. *"ineffective" doses*						
Placebo	106.7 (±21.2)	157.2 (±51.6)	11.6 (±3.5)	ns	***	**
Morphine	73.1 (±18.8)	153.8 (±51.1)	7.7 (±2.5)	ns	**	***
b. *"effective" doses*						
Placebo	100.7 (±21.6)	145.3 (±6.4)	6.4 (±1.8)	ns	**	***
Morphine	33.8 (±14.1)	13.7 (±4.3)	4.0 (±1.0)	ns	*	**
2.2 *Experiment II* solution	0%	16%	32%	0-16	0-32	16-32
a. *"ineffective" doses*						
Placebo	106.3 (±28.5)	185.5 (±44.6)	20.8 (±7.3)	ns	*	*
Morphine	87.7 (±31.7)	185.5 (±41.6)	17.7 (±5.9)	ns	0.06	**
b. *"effective" doses*						
Placebo	42.0 (±12.9)	174.1 (±45.2)	22.0 (±5.5)	*	ns	*
Morphine	29.2 (±13.1)	78.8 (±29.0)	10.4 (±3.0)	ns	ns	ns

Mean (±SE) consumed volumes (ml) of the separate, concurrently available fluids after placebo and morphine injections during the measurement period from 16.00 to 08.30 hr; Summarized in Experiment I (I) are data of: a. trials with "ineffective" doses of morphine (0.03, 0.06, 0.17 mg.kg^{-1}) and b. trials with "effective" doses (0.50 and 1.50 mg.kg^{-1}). Summarized in Experiment II (II) are data of: a. trials with "inefffective" doses of morphine (0.03 and 0.17 mg.kg^{-1}) and b. trials with "effective" doses of morphine (0.17, 0.50 and 1.50 mg.kg^{-1}).
*$p<0.05$; **$p<0.01$; ***$p<0.001$; Wilcoxon matched pairs test.*

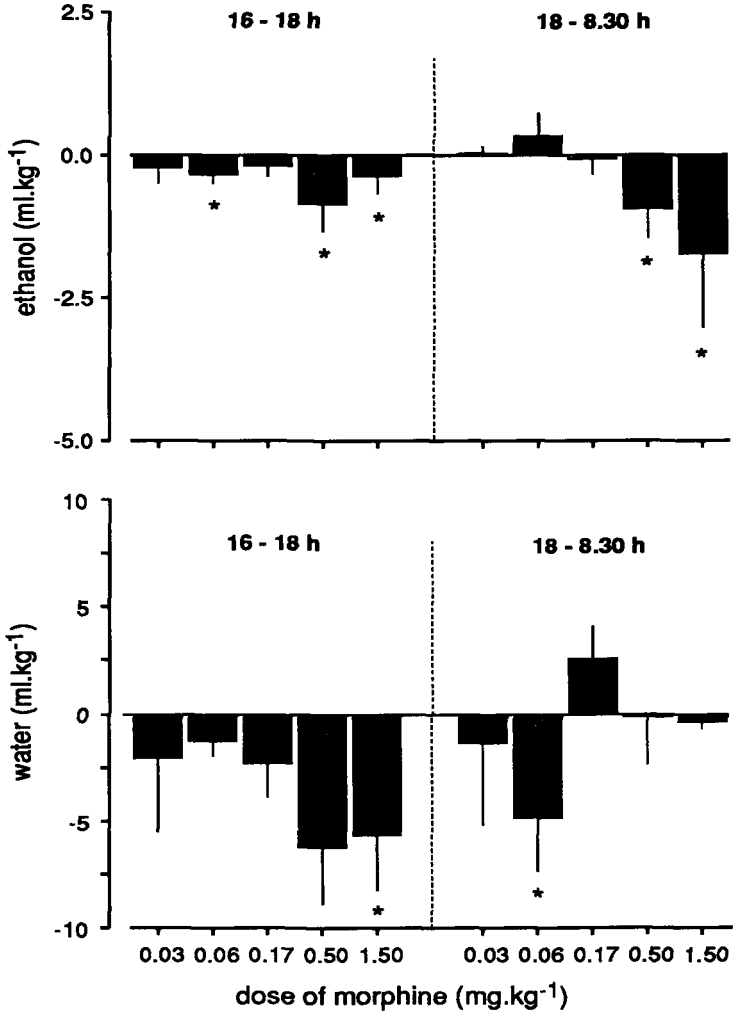

Figure 3.Time course of the effect of morphine on total net ethanol intake (upper panel) and on consumption of drinking water (lower panel) during continuous supply. Shown are the mean (+SE) differences between intakes after morphine and placebo during the measurement period shortly after injection (left: 16.00-18.00 hr) and during the subsequent measurement period (right: 18.00-08.30 hr).

**significant effect of morphine compared to placebo; Wilcoxon matched pairs test $p<0.05$.*

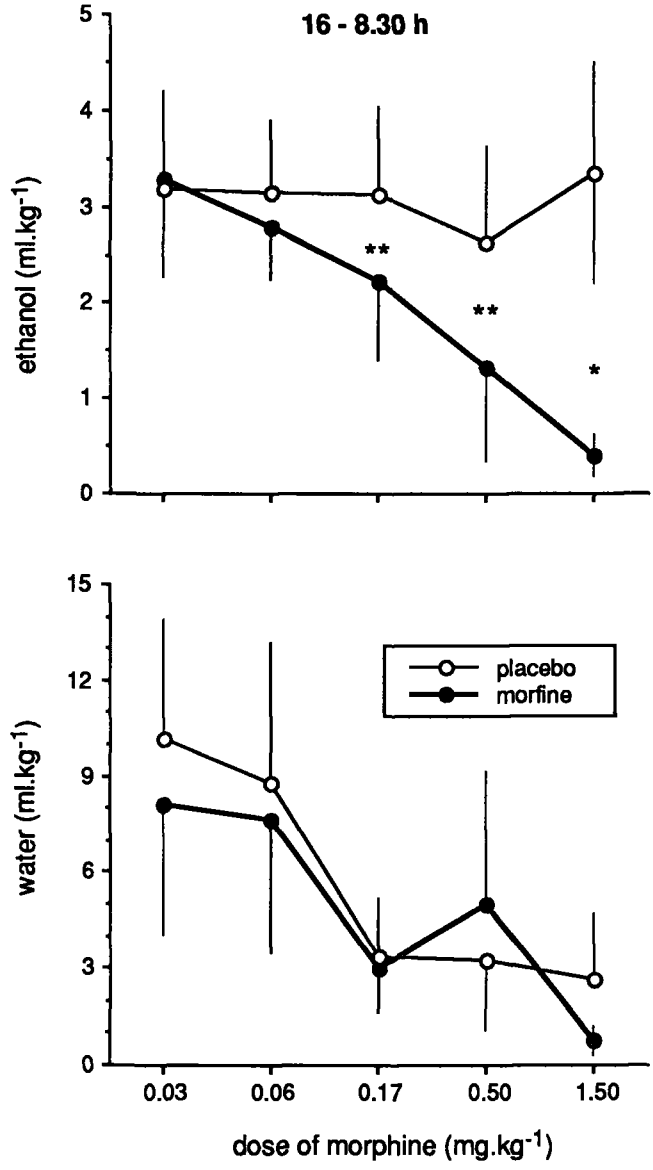

Figure 4. Mean (+SE) consumed total net ethanol (ml.kg^{-1}) (upper panel) and mean (+SE) consumed drinking water (ml.kg^{-1}) (lower panel) after imposed abstinence (measured from 16.00 to 08.30 hr) after paired morphine and placebo injections, for a dose range of 0.03 - 1.50 mg.kg^{-1}.
**significant difference between morphine and placebo, Wilcoxon matched pairs test $p<0.05$; **$p<0.01$.*

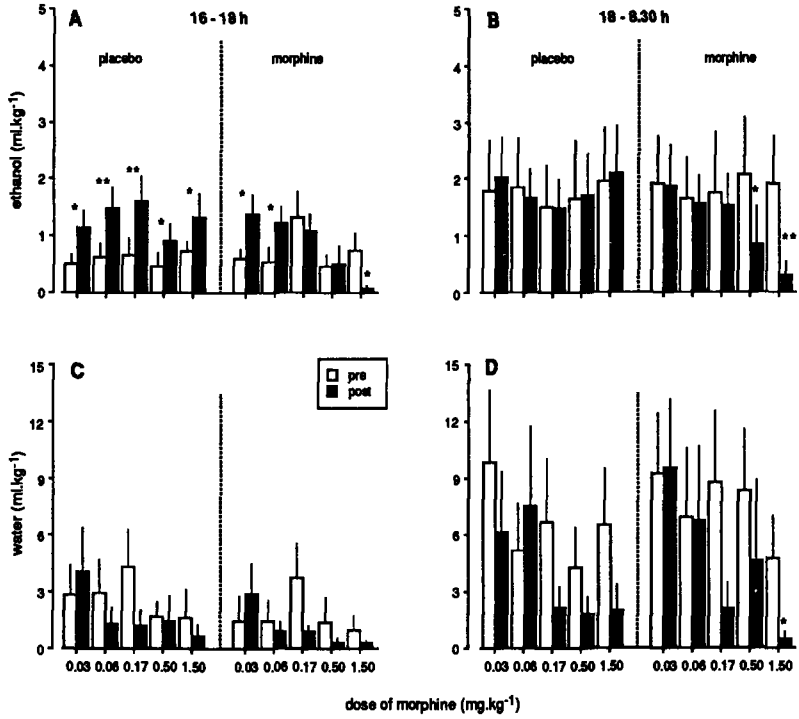

Figure 5. Mean (+SE) consumed net ethanol intake (A and B) and water (C and D) in ml.kg^{-1} at pre-(open bars) and post-(black bars) abstinence days for paired placebo (left) and morphine treatments (right) during the measurement period shortly after injection (left: 16.00-18.00 hr) and during the subsequent measurement period (right: 18.00-08.30 h) across a dose range of 0.03 - 1.50 mg.kg^{-1}.
**significant difference between pre-and post-abstinence day, Wilcoxon matched pairs test *p<0.05; **p<0.01.*

EXPERIMENT II: AFTER IMPOSED ABSTINENCE

Total Net Ethanol

Measurement period 16.00 - 08.30 h

Paired comparisons indicated that the total net ethanol intakes at the two pre-abstinence days of a trial (i.e. one for placebo and one for morphine treatment) were not significantly different from each other (Wilcoxon tests p's>0.05). Reliability within subjects of ethanol intake at pre-abstinence days was R=.99.

The results for morphine and placebo, administered after 2 days of alcohol abstinence, are shown in Figure 4 (upper panel) as a function of the dose.

Morphine reduced the total net ethanol intake significantly after 0.17 mg.kg^{-1}, 0.50 mg.kg^{-1} and 1.50 mg.kg^{-1}. Within-subject reliability at treatment days was R=0.98.

Effect Of Imposed Abstinence/ Time Course

The effect of alcohol abstinence on total net ethanol intake, shortly after renewed supply (from 16.00 to 18.00 h) and during the subsequent time interval (from 18.00 to 08.30 h) is shown in Figures 5A and 5B, for placebo and morphine treatments. Figures 5A (16.00 - 18.00 hr) and 5B (18.00 - 08.30 h)r show that with placebo the increase in total net ethanol intake after abstinence occurred specifically during the first two hours of renewed alcohol supply (16.00 - 18.00 hr). With respect to morphine, after the dose of 0.17 mg.kg^{-1} and of 0.50 mg.kg^{-1} post-abstinence net ethanol intake between 16.00 - 18.00 hr (Fig. 5A) was not significantly different from pre-abstinence intake; after the dose of 1.50 mg.kg^{-1} net ethanol intake was even significantly less than before abstinence. In the subsequent period (18.00 - 08.30 hr) net ethanol intake was below pre-abstinence level after 0.50 mg.kg^{-1} and 1.50 mg.kg^{-1} of morphine.

Comparison between morphine and placebo treatments revealed a significant difference in ethanol intake at the dose of 0.17, 0.50 and 1.50 mg.kg^{-1} for the period between 16.00 and 18.00 hr; for the period between 18.00 and 08.30 hr at the dose of 0.50 and 1.50 mg.kg^{-1}. The mean differences in ethanol intake between placebo and morhpine are presented as a function of the dose in Figure 6 (upper panel).

Drinking Water

Measurement period 16.00 - 08.30 hr: Paired comparisons indicated that the water consumption at the two pre-abstinence days of a trial (i.e. one for placebo and one for morphine treatment) were not significantly different from each other (Wilcoxon tests p's>0.05). Within-subject reliability as R=0.85.

Figure 4 (lower panel) shows the effect of morphine on the consumption of drinking water after alcohol abstinence. No difference in effect between placebo and morphine was observed. Independent of the kind of treatment, the water consumption appeared to be lower in three of the five trials. Within-subject reliability at treatment days was R=0.89,

Effect Of Imposed Abstinence/Time Course: After alcohol abstinence and placebo treatment no significant effect on water consumption during the first two hours (Fig. 5C: from 16.00 to 18.00 hr) of renewed alcohol supply, nor during the subsequent night period (Fig. 5D: from 18.00 to 08.30 hr), was found. Morphine reduced post-abstinence water consumption as compared to pre-abstinence

consumption only once, during the night period (Fig. 5D: from 18.00 to 08.30 hr) after the dose of 1.50 mg.kg^{-1}. Figure 6 (lower panel) shows the mean differences between morphine and placebo treatments as a function of the dose for the two separate measurement periods. No significant changes were found.

Concentration Preference

In Table 2.2 consumed volumes from each of the three concurrently available fluids are compared in trials a. with "ineffective doses" (0.02 and 0.06 mg.kg^{-1}) and b: with "effective doses" (0.17, 0.50 and 1.50 mg.kg^{-1}). After placebo, there was no concordance among the subjects with regard to preference for water or 16% solution in the "ineffective" dose trials: 50% preferred water to 16% solution, 50% did the reverse. Water and 16% solution both were preferred over 32% solution. In the "effective" dose trials 16% solution was significantly preferred over water and over 32% solution.

No concordance existed in preference for water versus 32% solution. After morphine, results were comparable to results found after placebo in the "ineffective" dose trials. In the "effective" dose trials, preference for the various fluids could no longer be well distinguished. Morphine appeared to have reduced the drinking from the 16% (Wilcoxon $p<0.001$) as well as from the 32% solution (Wilcoxon $p<0.05$).

Behavioural Activities

During abstinence days there were no overt signs of physical withdrawal reactions, that could be expected in monkeys to peak within 24 hours (Winger, 1988).

Table 3 gives the amount of time (per cent) spent in the various spontaneous behavioural activies between 16.00 and 17.00 hr, at 1: pre-abstinence day (see Fig. 1b: Monday, no injection) and 2: post-abstinence day (see Fig.1b: Thursday, injection at 15.30 hr), after a: a placebo injection and b: 1.50 mg.kg^{-1} of morphine.

After morphine, resting (i.e. sleeping, lying, sitting) was not changed as compared to pre-abstinence and placebo day. Movement (manipulate, standing, walking) was still present after morphine and not remarkably lower than at pre-abstinence day. Standing and drinking was highest after placebo. Self-manipulation and self-agression was decreased after morphine, while scratching was markedly increased. This scratching was not focussed on the spot of injection, but was performed over the whole body. Stereotyped behaviour clearly increased after morphine in 2U, but decreased in 1DW. Monkey 2U showed in general less reactive behaviour after morphine; this was most outspoken for eye press.

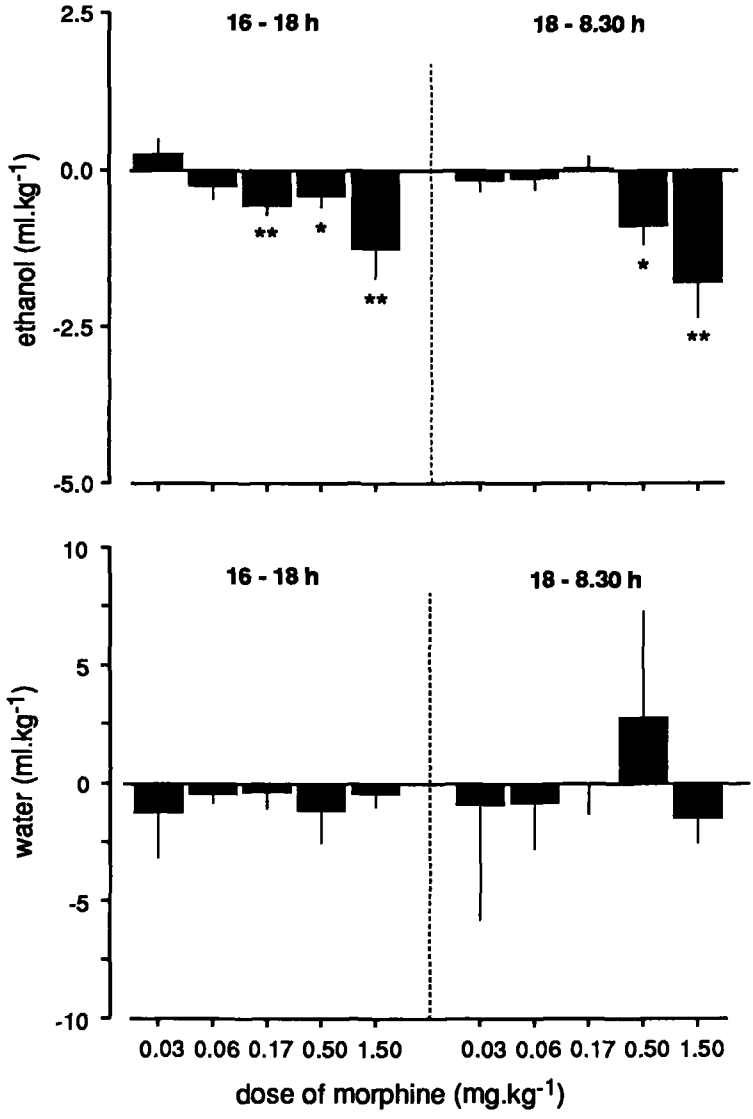

*Figure 6. Time course of the effect of morphine on total net ethanol intake (upper panel) and on consumption of drinking water (lower panel) after abstinence. Shown are the mean (+SE) differences between intakes after morphine and placebo during the measurement period shortly after injection (left: 16.00-18.00 hr) and during the subsequent measurement period (right: 18.00-08.30 hr). *significant effect of morphine compared to placebo; Wilcoxon matched pairs test $p<0.05$; **$p<0.01$.*

Table 3. Behavioural Activities

monkey		2U			1DW	
experimental day injection	pre no	post placebo	post morphine	pre no	post placebo	post morphine
General Activities						
sleep	0.4	0.8	0.0	0.0	0.0	0.0
lying	0.0	1.0	0.1	0.1	0.4	0.0
sitting	86.8	86.2	78.9	87.3	85.3	93.8
manipulate	0.3	7.0	0.1	1.5	0.8	0.1
standing	2.6	11.3	2.3	2.5	4.8	1.3
walking	0.2	1.2	1.0	0.2	0.3	0.3
Drinking						
0%	0.0	0.0	0.0	0.0	0.0	0.0
16%	0.0	1.7	0.0	0.8	4.1	0.0
32%	0.0	0.2	0.0	0.0	0.0	0.0
Self-directed Behaviours						
s. manip.	16.5	13.3	1.2	20.1	13.6	1.0
s. sex	0.0	0.0	0.0	0.0	0.0	0.0
s. aggres	0.1	0.5	0.0	0.4	0.4	0.0
scratch	0.7	1.0	15.9	0.3	0.6	29.8
Locomotor Stereotypies						
	10.3	1.0	17.1	9.6	9.1	4.5
Reactive Behaviours						
bounce	0.2	0.4	0.1	0.9	0.7	0.8
eye press	11.8	6.4	0.3	0.2	0.3	0.8
yawn	0.4	0.8	0.0	0.1	0.1	0.0
aggres	0.5	0.3	0.0	0.6	0.1	1.4

Time spent (per cent) of behavioural activities of monkeys 2U and 1DW, between 16.00 and 17.00 hr, at pre-abstinence day (pre) and post-abstinence day (post): a. after placebo and b. after 1.50 mg.kg^{-1} of morphine. Behavioural categories used are:

General Activities
sleep: no activity, eyes closed
sitting, lying: no displacement
standing: on 4 or 2 legs
manipulate: cage, environment
walking: active displacement
drinking: drink response at one of the drinking nipples.

Self-directed Behaviours
self-manipulate: grooming, caressing of own body,
self-sex: stimulation of own genitals,
self-aggression: biting and fighting own body, and/or
self-scratch: with nails of foot or hand.

Locomotor Stereotypies	*Reactive (i.e. environment-sensitive) Behaviours*
repetitive pattern of walking or summersaulting	*eye-press:* with hand pressing on eye or eye-brow after change in environment, *bounce*: jumping up and down, *aggression:* threatening neighbours, *yawn:* commonly reflecting some emotional uneasiness.

By contrast, monkey 1DW seemed to have somewhat increased his reactive behaviour after morphine; this was most outspoken for agression towards the environment. Although behaviour was not quantitatively analysed for the other monkeys, no clear signs of sedation could be discovered. The aspecific scratching appeared to occur in all monkeys as a result of morphine.

Discussion

The effect of morphine on ethanol intake was quite comparable in case of continuous and ad libitum access to ethanol solutions and water (Exp I) or after two days of abstinence (Exp II). The two highest doses of morphine reduced ethanol intake within a short-term period, i.e. within the first 2.5 hours after injection, as well as for a longer period. Effects were no longer observed 24 hours after injection. Across the whole study average net ethanol intakes did not really change over time. Consumption of drinking water was also reduced by morphine in Experiment I, but mainly for a short period. After abstinence (Exp II) morphine treatment had hardly any effect on water intake.

In a previous study with the same monkeys, we found under similar conditions as in the present study, that the opiate antagonist naltrexone in a dose range from 0.17 to 1.5 $mg.kg^{-1}$ (Kornet et al., 1991, in press) and naloxone (0.50 $mg.kg^{-1}$, unpublished data) also reduced alcohol drinking. The effect then was also specific for ethanol intake after abstinence, and like in the present study water consumption during continuous supply was reduced for a shortlasting period. The observed low specificity of the effect for ethanol intake during continuous supply seems to agree with the hypothesis that opioids interfere with the positive reinforcing effects of several ingesta, including water (Hubbell et al., 1987; Kirkham, 1990; Yeomans et al., 1990), but not with critical physiological needs, which activate negative reinforcement mechanisms (Hubbel et al., 1986; Reid et al., 1987; Koob and Weiss, 1990). This could explain why the effect on water was generally of a shorter duration than the effect on ethanol; specific negative reinforcement mechanisms with respect to fluid balance (need for water) could have prevailed

later on. With respect to ethanol intake, activation of negative reinforcement mechanisms presumably was not involved. The temporary increase in ethanol intake in Experiment II suggests that abstinence from alcohol caused a specific change with respect to the positive reinforcing effects of ethanol. This could explain why morphine treatment after abstinence exerted the most influence on ethanol intake.

The fact that at first sight an opiate agonist as well as an antagonist can produce similar results is puzzling However, for the used dose-range with a maximum of 1.5 $mg.kg^{-1}$, the effect of naltrexone was mainly restricted to the temporary period of abstinence-induced increase (Kornet et al., 1991, in press) while morphine was effective during the subsequent night period as well. Furthermore, the suppression by morphine on ethanol intake was larger (maximal 92%) compared to naltrexone (maximal 50%). These differences might indicate that the agonist had interacted with alcohol drinking in a different way than the antagonist (Linseman, 1989; Koob and Weiss, 1990).

The results in the present study are contradictory to a number of rodent studies, in which opiate antagonists decrease, and small doses (1 or 2 $mg.kg^{-1}$) of opiate agonists increase specifically the intake of alcohol solutions (Hubbell and Reid, 1990) under a variety of circumstances (Czirr et al., 1987; Hubbell et al., 1987; Reid et al., 1987). Variables that could explain (Hubbell and Reid, 1990) why an opiate agonist sometimes would not potentiate alcohol drinking (e.g. intake level, dose, and time course), do not seem responsible for the suppression of alcohol drinking in our monkeys. Monkeys ingested more net ethanol than the required amount for rats (more than 0.3 $g.kg.h^{-1}$); spontaneous ethanol ingestion by these monkeys had been shown earlier to induce positive blood alcohol levels (Kornet et al., 1990b). High doses of morphine can suppress drinking by inducing some state of satiety due to extensive agonism at opiate receptors (Reid et al., 1987), or by producing general debilitating effects (Hubbell and Reid, 1990). Compared to the studies in rats (1.0 to 2.5 $mg.kg^{-1}$) we actually used very low doses (0.02 to 1.5 $mg.kg^{-1}$). The lowest doses that did not suppress intake, did not enhance intake either. Also, there were no indications of general sedation or debilitating effects by these doses of morphine in our monkeys nor in other monkeys (Cuthbert et al., 1989). In fact, the observed behavioural effects might even be more in line with other studies in which low doses cause excitatory effects (Stewart et al 1984) as indicated by an increased aspecific scratching. Finally, a drinking period of 1.5 to 2 h to measure consumption after injection has been proposed as optimal to reveal the enhancing effects of morphine (Hubbell et al., 1986). As we measured intakes during the first 2 and after the subsequent 16,5 hours following injection, it is not likely that we overlooked a possible

enhancing effect of morphine due to measuring after a too short or a too long period.

Opiate antagonists as well as agonists in rats usually interact both with the duration, but not with the initiation of a drinking bout (Hubbell et al., 1987; Hubbel and Reid 1990). From Table 3 it can be noted that (at least in 2 monkeys with large morphine-induced effects on alcohol drinking), drinking after morphine was not initiated at all during the first hour after abstinence, although usually consumption rate then is highest (Kornet et al., 1990a). A possible difference in effect of morphine on drinking pattern of rodents and monkeys might be an important cue (Marglin and Reid, 1990) to explain the discrepancy of the present results and needs further investigation. Interestingly, a dopamine agonist and antagonist have been found both to decrease ethanol intake in rats (Koob and Weiss, 1990; Samson et al., 1990). Dopamine agonists sorted their effect by disrupting the initial high rate of responding at the beginning of a session, dopamine antagonists by abbreviating the duration of a drinking period. So morphine in our study seemed to have inhibited the initiation of drinking and may resemble in that respect the effect of a dopamine agonist in rats.

The difference of our results with the rodent studies might reflect a difference in species. Enhancing effects of agonists have been found as far as we know only in rats (Hubbell and Reid, 1990). Differences in the opioid system between rats and rhesus monkeys could perhaps (partly) account for the observed discrepancy since for example the distribution of opiate receptor types in the brain (Billington et al., 1990), the role of opioids in neuroendocrine regulation (in e.g. the pituitary, Mansour et al., 1986) and physiological effects (Cuthbert et al., 1989) appear to differ for various species. Rats can develop a similar abstinence-induced increase in alcohol intake as we reported for our monkeys (Sinclair and Senter, 1968). An interesting question is whether rats that show the alcohol deprivation effect, as described by Sinclair and Li (1989) will respond differently than monkeys to low doses of morphine.

It must be taken into account as well that the monkeys studied already were chronic drinkers. Opioids probably play a different role during acquisition and initial maintenance of alcohol drinking (Sandi et al., 1989) than when it has become a chronic habit (Hand et al., 1989; Kirkham, 1990). Acute doses of ethanol are known to stimulate endorphin release, but chronic ingestion as found in alcoholics (Gianoulakis et al., 1989) and in animals (Barret et al., 1987) during and after detoxification, produces a state of decreased endogenous endorphin activity. Even after a long period of abstinence (6 months e.g.), alcoholics still were found to have lowered endorphin levels (Gianoulakis et al., 1989). These findings led to the endorphin compensation hypothesis on alcohol seeking behaviour, in which an (acquired) deficiency of endorphins is held responsible for

a frequent desire for alcohol and relapses in drinking (Genazzani et al., 1982; Gianoulakis et al., 1989; Volpicelli et al., 1990). According to this hypothesis, drinking of alcohol will decrease by opioid agonism due to compensation for the opioid deficiency, as well as by opioid antagonism, due to blocking the reinforcing properties of alcohol (Volpicelli et al., 1990). A comparable relationship between lowered endorphin activity and heroin- and cocaine-seeking behaviour has been postulated by Sweep et al. (1988). The existence of a common neuronal circuitry for reinforcing actions of different addictive drugs has been proposed by a number of authors (Koob and Weiss, 1990; Wise and Bozarth, 1987; Van Ree, 1987), and it might as well be that the same (acquired) biochemical deficit provides a biological basis for the various types of drug addiction (Sweep et al., 1989). Following this line of reasoning, our monkeys could have acquired after chronic daily alcohol consumption an endorphin deficiency, that induced relapse drinking after the imposed alcohol abstinence; morphine could have compensated in our monkeys for an endorphin deficiency, hence leaving no reason to ingest alcohol for a while. Further research will be needed to give this hypothesis experimental support.

Alcoholics can abstain for quite long periods, before they relapse (Barnes, 1988; Marlatt and George, 1984; Stewart et al., 1984; Hand et al., 1989). It can be questioned if endorphin deficiency in abstinent alcoholics must be expected to produce a continuous state of alcohol seeking behaviour, representing some drive-reduction behaviour (Stewart and Vezina, 1988; Milano et al., 1989; Cornell et al., 1989). Since alcohol-induced disturbances in the opioid and other neurobiological systems are longlasting, it is possible that alcoholics rather develop an altered susceptibility for opioidergic modulations (Von Wartburg, 1990). Perhaps this could make them more vulnerable for exogenous (e.g. opiates, alcohol) or endogenous (e.g. central processes) influences, which then provoke relapse.

Conclusions on the interaction between opioid agonists and alcohol drinking also have impact on clinical practice. The results from the rodent studies suggest that methadone used in the treatment of heroin addicts could bear the risk to stimulate alcohol consumption (Hubbel and Reid, 1990; Olson et al., 1989). On the other hand, the present study and human data (Genazzani et al., 1982; Gianoulakis et al., 1989; Volpicelli et al., 1990) make it plausible that low doses of opiate agonists could compensate for alcohol-induced endorphin deficiency and thus help to stop craving (Siegel, 1986; Volpicelli et al., 1990). To further investigate these rather opposite predictions, non-human primate studies could provide an important contribution.

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SAMENVATTING

De inhoud van dit proefschrift is onderverdeeld in Deel I en Deel II. Deel I behandelt recente hypothesen omtrent alcoholverslaving (Hoofdstuk 1) en geeft een samenhangend overzicht van de vraagstellingen (Hoofdstuk 2) en de gevonden resultaten (Hoofdstuk 3) behorende bij de verschillende experimentele studies (Studies 1-7) die voor dit proefschrift zijn verricht. In Hoofdstuk 4 wordt ingegaan op de mogelijke toepassing van de bevindingen, beschreven in dit proefschrift, op het gebied van alcoholverslaving. Deel II bevat de wetenschappelijke publikaties over de verschillende experimentele studies.

Het aantal alcoholverslaafden in Nederland, als ook in andere westerse landen, wordt geschat op 10% tot 15% van de bevolking van 15 jaar en ouder. Zij vertegenwoordigen een heterogene groep van mensen met uiteenlopende sociaal-economische en culturele achtergronden. Hoe deze afwijking ontstaat en hoe deze afdoende te behandelen, is niet duidelijk. Waarschijnlijk ligt er een samenspel van psychologische, biologische als mede sociale factoren aan de basis van het ontstaan en het in stand houden van alcoholverslaving. Het meest kenmerkende aspect van alcoholverslaving is dat de gebruiker niet meer zelfstandig in staat blijkt voor langere tijd het alcoholgebruik te verminderen of te beëindigen ondanks de vele nadelige gevolgen die hij/zij hierdoor ondervindt. Behandeling van alcoholverslaving is gewoonlijk gebaseerd op het opleggen van totale onthouding van alcohol (abstinentie), veelal in combinatie met medische en psychologische/sociaalmaatschappelijke hulp. Helaas blijkt een groot aantal alcoholverslaafden terug te vallen in hun oude drinkpatroon na beëindiging van dergelijke behandelwijzen.

Het is aangetoond dat proefdieren zichzelf met juist die stoffen inspuiten, die bij de mens tot verslaving kunnen leiden, terwijl zij dit niet doen met voor de mens niet-verslavende stoffen. Het zichzelf toedienen van verslavende stoffen is dus ook bij proefdieren te bestuderen. Het gegeven dat verschillende psychoactieve stoffen (bv. heroïne, cocaïne, alcohol) uit aparte farmacologische klassen (narcotica, stimulantia, depressiva) verslaving kunnen induceren, heeft geleid tot de hypothese dat er een gemeenschappelijk werkingsmechanisme betrokken is bij de verschillende typen van verslaving. Hierbij wordt met name gedacht aan de rol van hersensystemen, die betrokken zijn bij positieve bekrachtiging en motivatie van gedrag. Dit zou kunnen betekenen dat beïnvloeding van dergelijke hersensystemen, bijvoorbeeld met behulp van specifieke (neurofarmacologische) geneesmiddelen, een nieuwe mogelijkheid voor behandeling van (alcohol)verslaving zou kunnen bieden.

Doel van het onderzoek beschreven in dit proefschrift was a) te onderzoeken of (bepaalde aspecten van) alcoholverslaving te bestuderen zou(den) zijn bij vrijwillig alcoholdrinkende rhesusapen *(Macaca mulatta)* en b) recente hypothesen omtrent de neurofarmacologische aspecten van alcoholverslaving te toetsen.

Studie 1 onderzocht het drinkgedrag van rhesusapen in een eerste kennismakingsperiode met ethanol/water oplossingen in verschillende ethanolconcentraties ("acquisitie fase"). Studie 2 beschrijft het drinkgedrag na een periode van ervaring met het drinken van alcohol ("voortzettingsfase"). In Studie 3 werd het effect onderzocht van tijdelijke onderbreking van het alcoholaanbod op het daaropvolgende drinkgedrag ("terugval na abstinentie").

In Studie 4 en 5 werd ingegaan op aanwijzingen dat (fragmenten van) hormonen, naast hun klassieke endocriene werking, ook specifieke interacties aangaan met hersenfuncties en als zodanig helpen gedrag te reguleren. Studie 4 onderzocht de hypothese dat een vasopressine fragment, het neuropeptide desglycinamide-(Arg^8)-vasopressine (DGAVP), het aanleren van alcoholdrinken zou kunnen verminderen. Verstoringen van het hormonale evenwicht in de hersenen zijn mogelijk te relateren aan bepaalde vormen van psychopathologie, zoals verslaving. Studie 5 onderzocht de relatie tussen alcohol drinken en het neuro-endocriene profiel bij apen die hetzij met placebo (een nepbehandeling), hetzij met DGAVP waren behandeld.

Ons lichaam blijkt zelf opiaatachtige stoffen te produceren, zoals endorfinen, en beschikt over specifieke bindingsplaatsen (receptoren) voor dergelijke stoffen. Endorfinen hebben waarschijnlijk een functie in het ervaren van prettige gevoelens en euforie. Op grond hiervan is het idee geformuleerd dat endorfinen ook betrokken zijn bij het ervaren van de prettige (verslavende) effecten van andere opiaten, zoals morfine en heroïne, als ook bij het ervaren van de effecten van andere verslavende stoffen en wellicht zelfs van verslavende handelingen (bv. gokken). De relatie tussen endogene opiaatachtige functies en alcoholdrinken werd onderzocht in de laatste twee studies, waarin de effecten van een opiaatantagonist (naltrexon, Studie 6) en van een opiaatagonist (morfine, Studie 7) werden onderzocht bij apen, ervaren in het drinken van alcohol.

In totaal namen 28 mannelijke rhesusapen, geboren in het Primaten Centrum TNO, deel aan de hier gepresenteerde studies. Zij genoten een normaal dieet van brokken, fruit, groenten en brood. Elk dier kreeg een vrije keuze aangeboden tussen 2 ethanol/wateroplossingen en drinkwater gedurende 24 uur per dag. De gebruikte ethanolconcentraties varieerden tussen 2% en 32% (v/v). In 8 dieren is, na een acquisitiestudie (Studie 1), het gebruik van alcohol op langere termijn onderzocht, zodat het mogelijk was de effecten van ervaring met alcohol (Studie 2), van abstinentie (Studie 3) en van experimentele farmacologische behandeling (Studie 6, 7) te bestuderen. 20 Dieren namen deel aan een acquisitiestudie (4 weken alcoholaanbod), waarvan dagelijks de helft met placebo, de helft met DGAVP werd behandeld (Studie 4, 5). In Studies 4, 5, 6 en 7 werden experimenteel farmacologische middelen toegediend volgens een dubbel-blind en placebo-gecontroleerd protocol. Een van de redenen om apen te kiezen voor deze studies was dat ratten minder geschikt geacht werden voor een experimenteel drinkmodel waarin dieren uit zichzelf gaan drinken als ze dag en nacht de vrije keuze hebben tussen water en alcohol.

Bovendien, omdat de rhesusaap, een non-humane primaat, nauw verwant is aan de mens, zou het een geschikte soort kunnen zijn om een gecompliceerde stoornis als alcoholverslaving te kunnen bestuderen.

De bevindingen in Studies 1 en 4 lieten zien dat de rhesusapen spontaan alcohol leerden drinken met behulp van geen andere methode dan het vrijelijk beschikbaar maken van alcoholoplossingen. Er kon onderscheid gemaakt worden tussen de invloed van de smaak van alcohol en de gemiddelde dagelijkse netto ethanolinname (Studie 1). Alhoewel bij keuze uit twee ethanoloplossingen sterke concentraties minder werden geprefereerd, bleef de gemiddelde individuele netto ethanolinname per dag vrij constant, onafhankelijk van de concentraties van de aanwezige oplossingen. Na een periode van ervaring met alcohol werd echter vastgesteld dat bij toenemende ethanolconcentraties de gemiddelde dagelijkse netto ethanolinname toenam (Studie 2). Tevens bleek de smaakpreferentie verschoven te zijn in de richting van sterkere ethanoloplossingen. Gepostuleerd werd dat ervaring in alcohol drinken leidde tot minder invloed van aversieve factoren, waardoor alcohol drinken meer onder controle kwam van positieve bekrachtiging (Studie 2).

Na opgelegde interruptieperioden van het alcoholaanbod (1, 2 of 7 dagen), bleken de apen onmiddellijk weer alcohol te gaan drinken zodra alcohol beschikbaar kwam. Daarbij was de alcoholinname tijdelijk verhoogd (Studie 3). Tijdens onthouding waren geen onthoudingsverschijnselen waarneembaar. Bij apen manifesteren dergelijke verschijnselen zich gewoonlijk binnen 2 dagen. Het interruptie-effect op alcoholdrinken lijkt derhalve niet gericht op het tegengaan van onthoudingsverschijnselen (een vorm van "negatieve gedragsbekrachtiging"), maar lijkt eerder gebaseerd op een toestand van verhoogde motivatie, door het opnieuw kunnen toepassen van eerder aangeleerd gedrag (alcoholdrinken) dat gepaard werd met positieve bekrachtiging (effect van alcohol). Dit effect lijkt te relateren aan de terugval ("relapse") in verslaving bij mensen na een langere periode van abstinentie en/of behandeling.

De resultaten van Studie 4 ondersteunen de hypothese dat onder bepaalde omstandigheden het neuropeptide DGAVP de positiefbekrachtigende werking van verslavende stoffen, inclusief alcohol, kan verminderen. Tijdens een vrijwillige acquisitie periode van 4 weken lieten dieren die de eerste 2 weken dagelijks waren behandeld met placebo, een geleidelijke toename in alcoholconsumptie zien. Een dergelijke toename in alcoholconsumptie bleef echter uit bij de meeste dieren die de eerste 2 weken dagelijks met DGAVP waren behandeld. In twee DGAVP-behandelde individuen die bij aanvang van de acquisitie al een hoge alcoholinname vertoonden, leek DGAVP niet effectief. Een mogelijke verklaring is dat DGAVP vooral een modulerende werking op de positieve bekrachtiging van gedrag kan uitoefenen, indien dit gedrag zich nog aan het ontwikkelen is of aan verandering onderhevig is.

Studie 5 liet zien dat veranderingen zich voordeden gedurende de eerste weken van matig alcoholdrinken in de plasmaspiegels van hypofysevoorkwabhormonen, zoals β-endorfine, ACTH, prolactine en cortisol. Met name viel de

blijvende verhoging in plasma β-endorfinespiegel op. DGAVP-behandelde dieren verschilden niet duidelijk van de placebo-behandelde dieren. Opmerkelijk was dat 2 placebo-behandelde dieren die de grootste toename in alcoholinname lieten zien, een afwijkend neuro-endocrien profiel vertoonden. Dit zou kunnen wijzen op een relatie tussen neuro-endocriene stoornissen en verhoogde gevoeligheid voor alcoholverslaving.

De resultaten van Studie 6 en 7 komen overeen met de opvatting dat alcohol drinken kan worden gemoduleerd via opiaatachtige systemen. Toediening van lage doses opiaatantagonist (naltrexon) en lage doses opiaatagonist (morfine) bleek de alcoholconsumptie te verlagen. De effecten waren met name selectief na een periode van alcoholinterruptie (terugvaldrinken). Deze resultaten waren strijdig met bevindingen in studies bij ratten, waarin naltrexon een verlagend, maar morfine een verhogend effect had. De bevindingen in dit proefschrift lijken meer aan te sluiten bij de "endorfine compensatie" hypothese, die ervan uit gaat dat de verlaagde endorfine-activiteit aangetoond bij alcoholisten, gecompenseerd kan worden door alcohol (of opiaatachtige stimulatie anderszins, bijv. met morfine). De verlaging door naltrexon zou kunnen berusten op het blokkeren van opiaatachtige activiteit, waardoor extinctie van de drinkgedrag zou kunnen worden bewerkstelligd. Het verschil tussen effecten van opiaatachtige modulatie in alcohol drinkende ratten en apen is nog niet goed verklaarbaar, en verdient verder onderzoek.

Tot slot wordt ingegaan op de bijdrage van het beschreven onderzoek aan de bestrijding van alcoholverslaving. De gebruikte proefopzet bleek vooral geschikt om gedragsaspecten van alcoholgebruik te bestuderen, zoals positieve bekrachtiging door alcohol en terugval na abstinentie. Openlijke onthoudingsverschijnselen en/of medische complicaties kwamen niet voor. Terugval is de kern van (alcohol)verslaving. Het terugvalgedrag bij de rhesusapen biedt een waardevol experimenteel model om dit gedrag beter te leren begrijpen en te behandelen. Ook lijkt de rhesusaap geschikt om de mogelijke relatie tussen neuroendocriene eigenschappen en de ontvankelijkheid voor alcoholverslaving te onderzoeken. Voorts ondersteunen de beschreven resultaten de hypothese dat neurofarmacologische middelen, die van invloed zijn op de positieve bekrachtiging van gedrag, een nieuw perspectief kunnen bieden in de behandeling van alcoholverslaving. De klinische belangstelling voor dergelijke middelen is sterk in ontwikkeling. Het experimenteel verslavingsmodel in de rhesusaap zou belangrijk kunnen bijdragen tot de mogelijkheden van preklinisch onderzoek gericht op nieuwe behandelwijzen van alcoholverslaving.

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