ELSEVIER

European Journal of Pharmacology 405 (2000) 89–101

ejp

www.elsevier.nl/locate/ejphar

Endogenous opioids and reward

Jan M. Van Ree *, Raymond J.M. Niesink, Leo Van Wolfswinkel, Nick F. Ramsey, Marleen (L.) M.W. Kornet, Wouter R. Van Furth, Louk J.M.J. Vanderschuren, Mirjam A.F.M. Gerrits, Caroline L. Van den Berg

Department of Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, Netherlands

Accepted 28 June 2000

Abstract

The discovery of endogenous opioids has markedly influenced the research on the biology of addiction and reward brain processes. Evidence has been presented that these brain substances modulate brain stimulation reward, self-administration of different drugs of abuse, sexual behaviour and social behaviour. There appears to be two different domains in which endogenous opioids, present in separate and distinct brain regions, are involved. One is related to the modulation of incentive motivational processes and the other to the performance of certain behaviours. It is concluded that endogenous opioids may play a role in the vulnerability to certain diseases, such as addiction and autism, but also when the disease is present, such as alcoholism.

Keywords: Endogenous opoid; Addiction; Reward; Brain stimulation reward; Self-administration; Sexual behaviour; Social behaviour

1. Introduction

Opium, morphine, and related drugs were fascinating substances for the ancient Greeks but also for people of the 21st century. These substances can control pain quite well in many patients, but can also evoke addiction. The concepts of addiction have changed following the discovery of neuropeptides in the brain that mimic the action of morphine (endogenous opioids) and of the presence in the brain of the machinery for the process of dependence. Endogenous opioids have been implicated in pain relief and addiction, but also in brain reward processes. Behaviours in which reward plays an important role may be controlled or at least be modulated by endogenous opioid systems. In this article, some of the findings on this topic of the last decades are summarised and discussed, with special emphasis on the procedures used in our laboratory, i.e. drug self-administration, intracranial electrical self-stimulation (ICSS), sexual behaviour and social behaviour (Van Ree and De Wied, 1988).

2. Reinforcement

Alterations in the organism's environment trigger sensory mechanisms and, thus, generate information, which is then conveyed to the central nervous system (CNS). This information and other inputs into the brain are integrated at several levels and can activate or inhibit the brain output systems, including motor systems, thus eliciting behavioural changes. The purpose of these behavioural changes is the adaptation of an organism to changes in environmental conditions, with the ultimate result that the survival of the organism or its species is ensured. The extreme of an environmental continuum is that the organism approaches a desirable (pleasant) and avoids a noxious (aversive) environment.

The setpoint of behavioural reactions is determined by genetic factors, but its value is continuously modulated by new experiences and, as a consequence, by acquired behavioural patterns. Behavioural reactions can be acquired through the association of stimuli that are originally neutral to innate reactions. The processes involved are types of associative learning. Two major classes of associative learning are distinguished: classical and instrumental conditioning. During classical conditioning, a concept which

* Corresponding author. Tel.: +31-30-2538807; fax: +31-30-2539032.
E-mail address: j.m.vanree@med.uu.nl (J.M. Van Ree).

0014-2999/00/$ - see front matter
PII: S0014-2999(00)00544-6

was introduced by Pavlov (1927), the organism learns about the relationship between one stimulus in its environment and another stimulus (the unconditioned and the 'neutral' conditioned stimulus). The unconditioned stimulus activates an established reflex and, thus, elicits an unconditioned reaction (e.g. the presence of food in the mouth results in salivation). Before conditioning, the conditioned stimulus does not elicit the unconditioned reaction. After association of the conditioned stimulus and the unconditioned stimulus, the conditioned stimulus evokes a conditioned reaction that resembles the unconditioned one. Classical conditioning allows the organism to predict the coherence between events in its environment. The conditioned stimulus, thus, becomes an anticipating signal for the occurrence of the unconditioned stimulus. The conditioned response can prepare the organism to deal with the result of the unconditioned stimulus more efficiently.

Instrumental conditioning, introduced by Thorndike (1913), refers to the process of learning about the relationship between a stimulus and the behaviour of the organism. When a certain behavioural act is followed by a favourable change in its environment, the organism tends to repeat this behaviour (law of effect). This change in environment can be the occurrence of a pleasant stimulus or the removal of an aversive or noxious stimulus. In instrumental conditioning, in contrast to classical conditioning, the (behavioural) response changes the probability that the unconditioned stimulus will appear, allowing the organism to have more-or-less control over its environment. Four types of instrumental conditioning can be distinguished: positive reinforcement (presentation of a pleasant stimulus), punishment (presentation of an aversive stimulus), negative punishment (removal of a pleasant stimulus) and negative reinforcement (removal of an aversive stimulus). The frequency of behavioural responses usually increases when positive or negative reinforcement is operative and decreases in the case of punishment, including negative punishment.

Many studies on positive reinforcement in experimental animals use lever manipulation as the behavioural response, and conditioning in such experiments is also termed operant conditioning. This type of conditioning is often investigated in the so-called "Skinner box" (Skinner, 1938). A typical experiment involves placing a hungry animal in a box in which a horizontal lever protrudes from a wall. Pressing the lever is followed by food presentation. The animal learns that this behavioural act is reinforced by food. Thus, when the animal is hungry and is placed in the same box, it is likely to press the lever to obtain food. The behavioural act in operant conditioning is termed 'operant' and the pleasant stimulus that tends to increase the frequency of the operant is called 'positive reinforcer'.

Operant conditioning has had a major influence on addiction research. Using the drug self-administration paradigm, it was shown that most, if not all, drugs of abuse could serve as positive reinforcer. In the literature, the concepts of reward and of (positive) reinforcement are often used to describe the effects of drugs of abuse. These terms have different meanings, however, in the sense that reward implies a positive subjective effect of a stimulus, whereas positive reinforcement is strictly a measure of the beneficial effect of a stimulus on acquisition or frequency of a required behavioural response. Thus, whereas reinforcement can be assessed experimentally, reward is a matter of interpretation of experimental findings. In translation to drugs of abuse, reward implies the positive subjective effect of the drug and positive reinforcement the facilitating effects of the drug on the learning of a required behavioural response.

3. Endogenous opioids and opioid receptors

The structural similarities between all substances with an opiate-like action and the discovery of opioid receptor agonists, mixed agonist–antagonists and antagonists, generated the concept of opioid receptors. Goldstein et al. (1971) used radiolabelled levorphanol to detect opioid binding sites in subcellular fractions of mouse brain. When radioligands with high specific activity became available, stereospecific opioid binding sites in the CNS were demonstrated. The finding of opioid binding sites and the finding that opioid antagonists exerted some intrinsic activity in opiate-naive subjects and could diminish nondrug-induced analgesia stimulated thoughts about endogenous compounds with opiate-like actions.

In this review, the term opioid will be used for all substances with an opiate agonistic action. Endogenous and exogenous opioids can be distinguished, depending on whether the substances are normally present in the body or not. The first indication for endogenous opioids came from studies showing that brain extracts contain opioid-like activity (Terenius and Wahlström, 1974). Further investigations led to the isolation and characterisation of the enkephalins (from the Greek 'in the head'), the first endogenous opioids to be discovered (Hughes et al., 1975). There appeared to be two pentapeptides, [Met^5]- and [Leu^5]-enkephalin. The structure of [Met^5]-enkephalin was also present in the N-terminal part of the earlier isolated C-fragment, part of the fat-mobilising pituitary hormone β-lipotropin (Bradbury et al., 1976). The C-fragment, later termed β-endorphin (from endogenous morphine), and the enkephalins were shown to induce similar actions as morphine in a number of in vitro and in vivo test procedures. Repeated administration of β-endorphin led to tolerance to its analgesic action and to morphine-like withdrawal symptoms upon challenge with naloxone (Van Ree et al., 1976; Wei and Loh, 1976). Furthermore, β-endorphin and the enkephalins were self-administered by laboratory animals, indicating the rewarding properties and addictive potential of these substances (Belluzzi and Stein, 1977; Van Ree et al., 1979). Thus, endogenous opioids may share all their

typical opioid-like actions with morphine, both after acute and chronic administration.

After the discovery of another class of endogenous opioids, the dynorphins, (dyn… from Greek dynamis = power) (Goldstein et al., 1981), it appeared that most endogenous opioids are generated by enzymatic processing from three precursor molecules, pro-opiomelanocortin (POMC), pro-enkephalin and pro-dynorphin. Each of these precursors has a unique anatomical distribution throughout the CNS and in peripheral organs (Akil et al., 1984). The anterior and neurointermediate lobes of the pituitary gland are major sites of POMC biosynthesis. In the brain, there are two distinct nuclei that contain POMC neurons: the arcuate nucleus of the hypothalamus and the nucleus tractus solitarius. Projections from these neurons are distributed throughout the brain. The opioid β-endorphin is generated from POMC, as are α- and γ-endorphin and several nonopioid peptides, e.g. adrenocorticotropin (ACTH) and β- and γ-melanocyte-stimulating hormones (β- and γ-MSH). ProEnk containing neurons are widely distributed throughout the brain and consist of both local circuits and long projection neurons. ProEnk is the source of [Leu^5]- and [Met^5]-enkephalin and several extended forms of these pentapeptides. ProDyn-containing cell bodies have a characteristic widespread distribution throughout the CNS. ProDyn-containing neurons have both short and long projection pathways and can generate several opioid peptides, including α- and β-neoendorphin, dynorphin A and dynorphin B.

Martin et al. (1976) first postulated the existence of multiple types of opioid receptors. On the basis of their behavioural and neurophysiological findings in the chronic spinal dog, they distinguished between the μ-type (for morphine, which induces analgesia, hypothermia and miosis among others), the κ-type (for ketocyclazocine, which induces depression of flexor reflexes and sedation among others) and σ-type (for SKF10,047 or *N*-allylnormetazocine, which induces tachycardia, delirium and increased respiration among others). Later, a fourth type of opioid receptor, named δ (for vas deferens), was identified (Lord et al., 1977). Further research revealed that the σ-type receptor is nonopioid in nature, thus leaving three main type of opioid receptors, μ, δ and κ (Dhawan et al., 1996). These receptors, belonging to the family of seven transmembrane G-protein-coupled receptors, have been cloned using molecular biological techniques.

Interestingly, the different endogenous opioid ligands show some preference for different receptors: β-endorphin for μ, enkephalins for δ and dynorphins for κ. Subtypes of these receptors have been proposed (μ_1, μ_2; δ_1, δ_2; κ_1, κ_2, κ_3) (Dhawan et al., 1996) and some evidence is available for some other receptor types (e.g. the ε-receptor which is considered β-endorphin specific (Narita and Tseng, 1998)). The IUPHAR subcommittee on Opioid Receptors has proposed another terminology to distinguish the opioid receptors: OP1, OP2 and OP3 receptor for the δ-, κ- and μ-opioid receptors, respectively (Dhawan et al., 1996).

4. ICSS

ICSS is widely used to explore the involvement of particular brain circuits in reward. Typically, when an animal is equipped with an electrode placed in a 'positive' brain area and given the opportunity to perform a behavioural response, e.g. pressing a lever, that is followed by a short pulse train of electrical current via the electrode, it will initiate and maintain responding. Thus, the stimulation serves as an operant reinforcer (Skinner, 1938). The phenomenon of ICSS was described initially by Olds and Milner (1954), who observed this behavioural pattern in rats equipped with electrodes in the septal area of the brain. ICSS was suggested to be linked to brain circuits implicated in natural incentives, such as food and sexual contact. However, it appeared that a variety of brain structures, related and not related to natural incentives, could support ICSS (Wise, 1996). Although ICSS resembles other types of reward, it has some unique properties. In most stimulated sites the rewards are strong and immediately present during stimulation and last not much longer than the stimulus itself. The brain structures in which ICSS can be elicited have been termed as reward or pleasure centres. Whether these various brain structures belong to a single system or to multiple reward circuits operating in parallel is still a matter of debate.

In general, drugs of abuse facilitate ICSS in that the frequency current-response function is shifted leftwards in a parallel manner and/or the threshold for eliciting ICSS is decreased (see Van Ree et al., 1999). It seems that facilitation of ICSS is an effect that drugs of abuse have in common, despite the different pharmacological characteristics of these drugs. Thus, facilitation of ICSS may be relevant for the dependence-creating properties of drugs and worthwhile to analyse in detail, in order to understand the basic mechanisms of drug dependence.

Concerning the neurobiology of ICSS, catecholamines and especially dopamine have been implicated as important neurotransmitters in the reward circuit. Evidence that dopamine is involved in ICSS stems from anatomical studies, lesion experiments, pharmacological manipulations and neurochemical studies. It has been suggested that, in particular, the mesocorticolimbic dopamine system is important for ICSS (Wise, 1996).

The first report about the effect of morphine on ICSS was from Olds and Travis (1960). Self-stimulation behaviour was studied over a range of stimulus intensities in animals with electrodes implanted in the lateral hypothalamus, septal area or ventral tegmental area. Although it was found that morphine caused a significant decrease in response rate in most animals, an increase in the response

rate was also seen. Adams et al. (1972) reported that morphine decreased self-stimulation behaviour in rats with electrodes in the medial forebrain bundle during the first 2 h after drug administration. However, thereafter an increase in the response rate was observed. Morphine was administered for five consecutive days. By day 3, there appeared complete tolerance to the inhibitory effect on the response rate, but no tolerance to the stimulatory action of morphine. These findings of inhibitory and stimulatory effects of morphine after acute and repeated administration of the drug have been further analysed in a number of studies.

Morphine can stimulate and depress motor performance depending on several variables, such as the dose, the time between injecting and testing and the presence or absence of tolerance. Since in most ICSS studies a motor response is the measured variable, the effects of morphine on motor performance may interfere with the drug-induced changes in reinforcement, and may hamper the interpretation of the observed effects. A way to circumvent problems associated with performance changes in rewarded behaviour is to determine the threshold for that behaviour. Such a threshold method, usually associated with a low rate of motor performance, may measure reward-induced changes in behaviour more accurately and physiologically than methods that are highly dependent on motor performance.

Van Wolfswinkel and Van Ree (1985b) compared the effect of graded doses of morphine using three different procedures to measure the threshold of ICSS in rats with electrodes in the ventral tegmental area. The procedures were (1) determination of the response rate, i.e. the number of responses, to high and threshold currents, (2) measurement of the threshold current when the response rate was kept low and relatively constant, and (3) determination of the 'behavioural' threshold using a two-lever procedure in which a response on one lever resulted in the decreasing current being reset to a high current contingent on a response on the other lever. Morphine induced a slight decrease (low doses) and increase (high doses) of the threshold current in the response rate procedure, no effect in the constant response rate procedure and a dose-related decrease of the threshold current in the 'behavioural' threshold procedure. During this latter procedure, no change in response rate was observed after morphine treatment. The 'behavioural' threshold procedure, in which the rat can select its own threshold current, is theoretically the most insensitive to nonreward-related motor performance effects. The response rate is not used to calculate the threshold and is not affected by morphine treatment. In subsequent experiments, using the same 'behavioural' threshold procedure, no tolerance to the morphine-induced decrease in threshold was observed when morphine was administered for 15 days before ICSS testing (Van Wolfswinkel et al., 1985). In this experiment, a decrease in response rate was found, but only during the first two days of morphine treatment. Thus, enhanced brain reinforcement can be observed after acute and chronic treatment with morphine when a response rate-insensitive procedure is used to measure ICSS behaviour. This conclusion is consistent with the results of other experiments using threshold determinations of ICSS (Kornetsky and Bain, 1983).

A number of studies have addressed the site of action of morphine and other opioids in facilitating ICSS behaviour. Morphine injected bilaterally into the ventral tegmental/substantia nigra area, but not into the nucleus accumbens or the striatum, facilitated ICSS behaviour elicited via electrodes placed in the medical forebrain bundle (Broekkamp and Phillips, 1979). ICSS behaviour elicited via electrodes in the nucleus accumbens was facilitated by morphine injected in a low dose into the ventral tegmental area, using the 'behavioural' threshold method. Interestingly, morphine injected into the nucleus accumbens did not affect ICSS elicited via electrodes in the ventral tegmental area (Van Wolfswinkel and Van Ree, 1985a). The data collected so far provide evidence that the ventral tegmental area is a sensitive site for morphine and other opioids in facilitating ICSS reinforcement, although this may not be the only brain site.

A useful approach to investigate the role of endogenous opioids in certain behaviours is to analyse the effects of opioid antagonists on that behaviour. A number of studies have been performed dealing with opioid antagonists and ICSS (Schaefer, 1988). It appears that the decreasing effect of opioid antagonists depends on where the stimulation electrode is placed and on the method used. Only a few studies have been performed using rate-independent procedures of ICSS. When the threshold for ICSS was measured in rats with electrodes in the ventral tegmental area, it was consistently found that a rather high dose of naloxone raised the threshold for ICSS (Van Wolfswinkel and Van Ree, 1985b). Using the 'behavioural' threshold procedure, and testing and treating the animals repeatedly with naloxone, it appeared that the naloxone-induced increase in the threshold became more pronounced during the 3 weeks of the experiment (Van Wolfswinkel et al., 1985). Interestingly, this effect persisted for at least three days after discontinuation of naloxone treatment. It was concluded that blockade of opioid receptors may induce long-term changes in the setpoint of ICSS. Accordingly, continuous subcutaneous administration of naloxone shifted ventral tegmental ICSS rate-frequency curves to the right, without suppressing behavioural performance (Hawkins and Stein, 1991).

In conclusion, there seems to be evidence that endogenous opioid systems are involved in ICSS. The data collected so far point to a modulatory role of endogenous opioids rather than that reward from ICSS is mediated by endogenous opioids. More studies are needed, in particular, after chronic blockade of endogenous opioids, to delineate more precisely the significance of endogenous opioids for ICSS.

5. Self-administration

Drug self-administration is the most widely used model for the experimental analysis of drug addiction and is based on the concepts of operant conditioning. The administration of a drug of abuse is made contingent upon a behavioural response of the animal. This response may consist of alleyway running, arm choice in Y-maze and drinking of flavoured solutions, yet most studies use lever-pressing as the behavioural act. An increase in the frequency of the response provides evidence that the drug is self-administered and, thus, serves as a positive reinforcer.

In 1940, Spragg first suggested that drugs could function as positive reinforcers (Spragg, 1940). His suggestion was based on experiments with chimpanzees, which were made physically dependent on morphine by daily treatment with morphine for several months. Then the animals learned to select one out of two boxes concealing a syringe filled with a morphine solution, which would subsequently be administered to the animal by the experimenter. The monkeys opened the box containing the morphine syringe more often than the other box that contained food.

Self-injection by animals was first reported by Headlee et al., (1955), who demonstrated that physically dependent rats would self-administer morphine via the intraperitoneal route. In the early 1960s, several investigators developed techniques for intravenous self-administration in rats and monkeys. Using this method, it was demonstrated that both opioid-dependent and opioid-naive animals would press a lever to receive injections of morphine (Weeks, 1962; Deneau et al., 1969). It became clear that besides morphine a wide variety of psychoactive drugs from different pharmacological classes could serve as positive reinforcers in animals. These drugs include psychomotor stimulants, such as amphetamine and cocaine, dissociative anaesthetics, such as barbiturates and benzodiazepines, ethanol, Δ^9-tetrahydrocannabinol, phencyclidine and nicotine. In general, drugs that are self-administered by animals are abused to some extent by humans. Conversely, drugs that fail to initiate or maintain self-administration behaviour in animals have no or little abuse potential in humans. It should, however, be noted that not all drugs are equally powerful as positive reinforcers in animals. For instance, nicotine is self-administered under a narrower unit dose range than opioids and cocaine. Nonetheless, the drug self-administration model can serve as a useful model for the prediction of the abuse potential of drugs in humans (Van Ree, 1979; Collins et al., 1984).

Although the positive reinforcing effects of a drug are the most important stimuli in self-administration behaviour, other factors may contribute significantly to operant behaviour and, thus, self-administration behaviour. These factors include, among others, conditioned or secondary reinforcement and negative reinforcement. Distinctive, neutral environmental stimuli that are repeatedly associated with the primary reinforcing effects of a drug can acquire (secondary) reinforcing properties through classical conditioning (Stewart et al., 1984). These stimuli are then called conditioned or secondary reinforcers. Although the primary reinforcing effects of the drug mainly determine the initiation of self-administration behaviour, the conditioned or secondary reinforcers maintain this behaviour over time, even in the absence of the primary reinforcer. The effects of conditioned reinforcers diminish over time when the drug injection is no longer available. In animals made physically dependent on drugs, an additional factor influencing self-administration behaviour is exerted by negative reinforcement, i.e. the animals will continue to self-administer a drug to alleviate or overcome the presumably aversive (negative) state of withdrawal (Koob et al., 1989).

Drug-taking behaviour, in general, and opioid self-administration, in particular, is controlled by a number of variables. The unit dose of drug delivered is one of the main factors which determines the ultimate level of drug intake during self-administration. It has been argued that the amount of drug taken can serve as a useful index of the reinforcing efficacy for drug injection (Van Ree et al., 1978). The development of physical dependence and tolerance has been regarded in the past as being critically involved in opioid addiction. However, experimental evidence does not support an important role of both tolerance development towards the reinforcing effects of opioids and of physical dependence for opioid self-administration behaviour (Van Ree et al., 1999). In addition, a number of predisposing variables can affect drug-taking behaviour. For example, it was found that prenatal exposure to opioids could facilitate the development of drug self-administration (Ramsey et al., 1993). Emotional stress appeared to enhance morphine and cocaine self-administration in drug-naive animals (Ramsey and Van Ree, 1993; Kuzmin et al., 1996). Also the genotype has been shown to be a genetic determinant of drug-taking behaviour. In fact, genetic and environmental factors may interact to determine the sensitivity for opioid reinforcement (Kuzmin et al., 1996). These kinds of studies are relevant for delineating risk factors in the aetiology of drug addiction.

Mediation of the reinforcing effects of opioids through activation of opioid receptors has been demonstrated in several studies by using opioid receptor antagonists (Mello and Negus, 1996). Intravenous morphine self-administration by rats and monkeys was attenuated by systemic administration of the opioid antagonists naloxone, naltrexone and nalorphine. Negus et al. (1993) showed that the μ-opioid receptor, in particular, plays an important role in the reinforcing effects of heroin in rats. They found that pretreatment with the μ-opioid receptor-antagonist β-funaltrexamine produced a significant increase in heroin intake, while some doses produced an extinction-like pattern of responding. These results were quantitatively similar to the effects of lowering the unit dose of heroin per

injection. Pretreatment with the δ-opioid receptor-antagonist naltrindole also produced a significant increase in heroin intake, but no extinction-like pattern, suggesting that the δ-opioid receptors might also be involved in opioid reinforcement, albeit less pronounced. Treatment with the κ-opioid receptor agonist U50,488H (*trans*-3, 4-dichloro-*N*-methyl-*N*-(2-1-pyrrolidinyl)-cyclohexyl-benzeace tamide) dose dependently decreased the intake of morphine when offered in unit doses that readily initiated self-administration behaviour. In addition, treatment with U50,488H induced proper self-administration behaviour with lower, subthreshold unit doses of morphine. It was found that activation of the κ-opioid receptor with U50,488H produced an almost parallel shift to the left of the inverted U-shaped dose–response curve for morphine self-injection rates, indicating an increased sensitivity of the animals to the reinforcing effects of morphine. Similar effects were observed with cocaine self-administration in rats (Kuzmin et al., 1997b). Thus, although μ-opioid receptors mediate the reinforcing effects of opioids, κ-opioid receptors may be involved in modulation of drug-taking behaviour.

The finding that animals self-administer morphine and heroin into the ventricle (Amit et al., 1976; Van Ree et al., 1979) strongly suggests that central loci subserve the reinforcing effects of opioids. Further, the lack of effect of opioid antagonists, which are not able to pass the blood–brain barrier (i.e. quaternary opioid receptor antagonists) on opioid self-administration supports the involvement of central opioid systems in opioid reinforcement (Koob et al., 1984). To localise the central site of the reinforcing action of opioids, two procedures are typically applied. One procedure is intracranial opioid self-administration and the other is injection of an opioid receptor antagonist into discrete brain regions. The outcome of these studies indicates that opioid systems in specific areas in the brain are involved in the mediation of opioid reinforcement. In particular, the ventral tegmental area seems to be a sensitive site for opioid reinforcement (Van Ree and De Wied, 1980).

The involvement of central opioid systems in opioid reinforcement has also been studied with i.c.v. and intracerebral self-administration of endogenous opioids. Belluzzi and Stein (1977) first reported that opioid-naive animals will work for enkephalin injections delivered directly in the brain ventricles. They found self-administration of [Leu^5]-enkephalin and [Met^5]-enkephalin at rates proximately two to four times higher than controls. However, other investigators reported that naive animals did not self-administer [Met^5]-enkephalin i.c.v., whereas the animals readily self-administered heroin and the endogenous opioid β-endorphin (Van Ree et al., 1979).

Opioids have the ability to regulate the activity of the endogenous opioid systems, an action which, in turn, may be responsible, at least in part, for the reinforcing effects of opioids (Trujillo et al., 1993). Most studies in this respect were done by treating animals with opioids. Although the conclusion of these studies was that endorphin and dynorphin systems in different brain areas are affected by opioids, the relevance of most of the observed changes for opioid reinforcement is unclear. In addition, some studies were performed in animals self-administering opioids. Sweep et al. (1989) demonstrated that intravenous heroin self-administration for five daily 6-h sessions resulted in a decrease in levels of β-endorphin immunoreactivity in the septum when measured immediately following the fifth self-administration session. At the time of the scheduled session on day 6, 18 h later, the heroin self-administering animals showed marked decreases in β-endorphin immunoreactivity in several areas of the anterior limbic system, such as the nucleus accumbens, the septum, the hippocampus and the rostral striatum. Interestingly, similar findings were found in animals self-administering cocaine. The authors suggested that the change in levels of β-endorphin and related peptides in these areas might reflect an involvement of endogenous opioids in the processes underlying psychic dependence. Moreover, these findings are of particular interest because they address the functional interface between changes in endogenous opioid levels and drug dependence, in contrast to studies in which drugs are administered by the experimenter.

The discovery in the brain of endogenous opioids and their role in reward processes has stimulated ideas about the involvement of these substances in the reinforcing and dependence-creating properties of opioids, but also of nonopioid drugs of abuse. Most studies in this respect concern cocaine and ethanol. In a human study with cocaine abusers, it was found that chronic treatment with the opioid-antagonist naltrexone reduced euphoria and the 'crash' from an intravenous cocaine injection (Kosten et al., 1992). This finding suggests that the endogenous opioid system may be involved in certain aspects of cocaine addiction. Results from animal studies in which the effect of opioid blockade on cocaine self-administration was studied generally seem to confirm such an involvement (Mello and Negus, 1996). During the initiation phase of cocaine self-administration (i.e. in drug-naive animals), treatment with naltrexone decreased cocaine intake in rats, presumably by attenuation of the reinforcing effects of cocaine (De Vry et al., 1989). Naltrexone caused a rightward shift in the dose–response curve for cocaine, indicating that cocaine is less reinforcing after opioid blockade. A similar shift in the dose–response curve for cocaine has been observed in mice treated with naloxone (Kuzmin et al., 1997a). The proposed involvement of opioid systems in the reinforcing effects of cocaine is also supported by the observation that chronic treatment with naltrexone followed by a naltrexone-free interval facilitated the initiation of cocaine self-administration, probably by enhancing the reinforcing effects of cocaine (Ramsey and Van Ree,

1990). With regard to the local opioid systems in the brain, treatment with naltrexone in the ventral tegmental area, but not in the nucleus accumbens, the caudate putamen, the central amygdala, or the medial prefrontal cortex, attenuated cocaine self-administration behaviour (Ramsey et al., 1999). Thus, opioid systems in the ventral tegmental area may modulate the initiation of cocaine self-administration.

The above-mentioned effects of naloxone and naltrexone suggest a role of the μ-opioid receptor in cocaine self-administration. But the other opioid receptors also seem to play a role in this respect. Following a number of days of stable cocaine intake by rats, acute blockade of the δ-opioid receptor by naltrindole reduced the self-administration of cocaine (Reid et al., 1995). The reduction of cocaine intake seems to be dependent on the dose of cocaine offered (Negus et al., 1995). The involvement of κ-opioid receptors in cocaine reinforcement has also been demonstrated. Treatment with the selective κ-opioid agonists U50,488H and spiradoline dose dependently decreased cocaine self-administration in rats (Glick et al., 1995; Kuzmin et al., 1997b). Interestingly, Kuzmin et al. (1997b) showed that treatment with U50,488H induced proper self-administration behaviour with lower subthreshold unit doses of cocaine, doses that did not initiate self-administration under control conditions. In fact, the dose–response curve for cocaine reinforcement was shifted to the left. This finding suggests that activation of κ-opioid systems increases the sensitivity to the reinforcing effects of cocaine. Accordingly, the κ-opioid receptor antagonist nor-binaltorphimine shifted the dose–response curve for cocaine reinforcement to the right (Kuzmin et al., 1998). Thus, it seems that blockade of μ-, δ-, or κ-opioid receptors may make the animals less sensitive to cocaine reinforcement, while activation of κ-opioid receptors may result in the opposite.

A number of studies have investigated the effect of administration of cocaine to rats on the levels of endogenous opioids, the expression of opioid mRNA and opioid receptors in the brain (Trujillo et al., 1993). However, the relevance of these effects for cocaine reinforcement and dependence is not easy to understand. Studies on animals self-administering cocaine may be more relevant in this respect. Sweep et al. (1989) found marked decreases in the levels of β-endorphin immunoreactivity in the anterior part of the limbic system (i.e. nucleus accumbens, septum, hippocampus and rostral striatum) in animals self-administering cocaine. Further, using an in vivo autoradiographic technique, a decrease in opioid receptor occupancy was found in restricted subcortical brain regions of animals self-administering cocaine, including limbic areas (i.e. lateral septum, ventral pallidum, nucleus stria terminalis and amygdala) and some regions of the hypothalamus and thalamus (Gerrits et al., 1999). The decrease in opioid receptor occupancy is probably due to the release of endogenous opioids in these particular brain regions. Interestingly, both these changes (i.e. decreased β-endorphin immunoreactivity and increased endogenous opioid release) were present just before the next scheduled cocaine self-administration session would have taken place, thus when the desire or need for the drug is assumed to be high. This might suggest an involvement of endogenous opioids, and possibly β-endorphin, in the processes underlying the need for cocaine. When the same methodologies were used in rats that just had completed their daily cocaine self-administration session, it appeared that levels of β-endorphin immunoreactivity in the brain were hardly changed and that fewer opioid receptors were occupied in many brain areas, including the nucleus accumbens (Sweep et al., 1989; Gerrits et al., 1999). Daunais et al. (1993) investigating the effect of cocaine self-administration on the expression of dynorphin mRNA, found an increased expression in the patch-like areas of the dorsal, but not ventral, striatum. Because repeated high doses of cocaine for 6–7 days were necessary to induce this effect, the authors concluded that the increased dynorphin mRNA expression did not underlie the acute reinforcing effects of cocaine but was more associated with long-term adaptation and sensitization.

Evidence for an involvement of the endogenous opioid systems in ethanol reinforcement and addiction is provided by studies with opioid receptor antagonists and agonists (Herz, 1997; Spanagel and Zieglsgänsberger, 1997). Opioid receptor antagonists, such as naltrexone and naloxone, decrease ethanol self-administration in rodents and monkeys under a variety of different experimental conditions. The majority of studies on the involvement of endogenous opioids in ethanol reinforcement have used an oral self-administration paradigm. Altshuler et al. (1980), using rhesus monkeys experienced with alcohol intake, found that chronic treatment with naltrexone on a daily basis for 15 days dose dependently decreased intravenous ethanol administration by as much as 50%. A later study with alcohol-drinking rhesus monkeys supported this finding, in that the total oral ethanol intake was reduced by acute treatment with naltrexone in a graded dose-dependent manner (Kornet et al., 1991). The effect of opioid blockade was found in nondeprived animals and during conditions of continuous and concurrent supply, but it has also been investigated in alcohol abstinence studies. In rhesus monkeys that had drunk alcohol for about 1 year, short and longer periods of imposed interruptions of alcohol supply (up to 7 days) led to a temporary increase in ethanol intake ('catch-up' phenomenon) and a subsequent relapse in the preinterruption drinking habit (Kornet et al., 1990). Blockade of opioid receptors with naltrexone after 2 days of imposed abstinence dose dependently reduced the abstinence-induced increase in ethanol intake after renewed presentation of ethanol (Kornet et al., 1991). Interestingly, a lower dose of naltrexone was effective in reducing ethanol intake after imposed abstinence as compared to

during continuous supply of alcohol, suggesting a role for endogenous opioids in the 'catch-up' phenomenon (Kornet et al., 1991).

The results from the studies with opioid blockade suggest an involvement of endogenous opioids in ethanol reinforcement. Based on, among others, these findings, clinical studies have been undertaken to assess the effect of naltrexone treatment in alcoholics. During a 12-week, double blind, placebo-controlled trial, alcohol-dependent patients were treated with naltrexone hydrochloride (50 mg/day) in adjunct to psychosocial treatment following alcohol detoxification. Subjects taking naltrexone reported significantly less alcohol craving. The number of days in which alcohol was consumed was significantly decreased by naltrexone and relapse was reduced. Of the placebo-treated patients 95% relapsed after they drank alcohol again, while only 50% of the naltrexone-treated patients exposed to alcohol relapsed (Volpicelli et al., 1992). Additionally, a majority of the naltrexone-treated patients reported that the 'high' produced by alcohol was significantly less than usual. These findings were replicated and extended by O'Malley et al. (1996), who in addition found that the reducing effects of naltrexone on alcohol drinking, craving and relapse interacted with the type of supportive therapy the patients received. Naltrexone has recently received approval for the treatment of relapse in alcohol dependence, and thereby may offer a new treatment regimen in combination with psychosocial therapy to reduce relapse following alcohol detoxification. Another opioid receptor-antagonist nalmefene also has been reported to reduce alcohol consumption and to prevent relapse (Mason et al., 1994).

6. Sexual behaviour

The effects of opioids on male copulatory behaviour have been discussed for centuries. Already, in 1563, the physician García D'Orta debated that opium, which was generally considered to be an aphrodisiac, instead impaired sexual performance (Guerra, 1974). Since the use of narcotic psychotropic substances has become widespread, many reports have addressed the relationship between opioids and human sexual functions. Chronic use of high doses of opiates results in sexual dysfunctions with impaired libido and sexual performance. The impairment of copulatory behaviour appears to be dose-dependent, although some users have initially enhanced sexual appetite with moderate use of opiates. The combination of intact libido with decreased sexual performance, as reported by users after they have taken low doses of opiates, is reminiscent of idiopathic impotence. It was found that patients with idiopathic impotence showed improvement after treatment with the opioid receptor-antagonist naltrexone, suggesting an increased tone of endogenous opioids in the brain to be involved in the pathophysiology of this disorder (Fabbri et al., 1989).

Sexual behaviour is a type of natural incentive motivated behaviour. Together with hunger and thirst, it is commonly considered as a 'primary drive'. The homeostatic model of motivation can accommodate hunger and thirst, but for sexual behaviour the incentive motivational theory may be more appropriate (Mogenson and Philips, 1976). Motivation refers to the goal-seeking, purposive quality involving an expectation of a future state, e.g. reward, i.e. the strength of the tendency to engage in behaviour when taking into account not only internal factors but also appropriate external factors. An external factor that plays a role in stimulating motivation is often called an incentive. The incentive motivational theory stresses the importance of the interplay between internal factors, external incentives and cues predictive thereof.

Distinctions can de made between seeking sexual contact (the propensity to achieve copulation) and being able to complete the copulatory act. Although these processes may not be independent in the majority of normal situations, the distinction between sexual motivation and performance has proven to be useful (Sach and Meisel, 1988), and corresponds with the distinction between libido and potency. In classical ethological terminology, this corresponds with the distinction between the appetitive and the consummatory aspects of sexual behaviour. In animal studies, the evaluation of sexual motivation is complicated. Some authors have described effects on sexual motivation in terms of copulatory measures, but these reflect performance components as well. The central theme in the preferred behavioural paradigms to measure sexual motivation is the propensity to gain access to a receptive female, such as maze learning procedure or bar pressing. Sexual motivation can also be measured by partner preference with or without access to the female, conditioned place preference with a sexual reward or anticipatory level changing behaviour (in the bilevel box) (for reference, see Van Furth et al., 1995).

Systemically administered opioid agonists produce a reliable and specific naloxone-reversible inhibition of sexual performance (Pfaus and Gorzalka, 1987). Enhancement of sexual performance by systemically administered opioid receptor antagonists occurs, but under certain test conditions only. For example, systemic administration of a low dose of naloxone facilitated some aspects of sexual performance in the bilevel box when tested nocturnally, but not when tested during the light phase of the diurnal cycle, although performance was markedly impaired (Van Furth and Van Ree, 1994; Van Furth et al., 1994). The effects of morphine and naloxone on sexual motivation have been assessed using the bilevel box procedure, in which the number of level changes of a male rat in anticipation of a female is thought to reflect sexual motivation. Systemic administration of morphine increased the level-changing behaviour of male rats, indicating an enhancement of sexual motivation. Naloxone prevented the increase in anticipatory level changes over four weekly tests of sexu-

ally experienced males without prior experience in the bilevel box (Van Furth and Van Ree, 1994, 1996a,b; Van Furth et al., 1994). Rats with a high number of anticipatory level changes showed a gradual decrease over 4 weeks in anticipatory level changes when treated with naloxone (Van Furth et al., 1994). These and other studies suggest that endogenous opioids may have a stimulatory role in sexual motivation.

Evidence has been presented that the medial preoptic area of the brain is involved in the consummatory aspects of sexual behaviour, that is sexual performance, but not in sexual motivation (see Van Furth et al., 1995). A low dose of β-endorphin injected into the medial preoptic area inhibited copulatory activity in male rats. Sexual motivation was hardly affected by injection of β-endorphin into the medial preoptic area. When high doses of naloxone were injected into the medial preoptic area, sexual performance was facilitated, but this effect may be due to nonopioid mechanisms, since moderate doses were inactive in this respect. It has been reported that infusion of naloxone into the medial preoptic area facilitates copulatory performance in poor copulators, but impairs sexual behaviour in average copulators. These results suggest that administration of opioids, e.g. β-endorphin, into the medial preoptic area impairs sexual performance and that endogenous opioid systems in the medial preoptic area may be involved in sexual performance, although these systems are quiescent in normal, sexually active rats.

The mesoaccumbens dopamine system has been implicated in the neural processes of incentive motivation, in general, and also in sexual motivation (Robbins et al., 1989). The female directed behaviour of male rats that precedes and accompanies copulation was facilitated after infusion of morphine into the ventral tegmental area (Mitchell and Stewart, 1990). Local infusion of β-endorphin into the ventral tegmental area failed to affect the anticipatory level changing behaviour, but local infusion of naloxone prevented the increase in anticipatory level changes over four weekly tests (Van Furth and Van Ree, 1996c). This suggests that the endogenous opioid systems in the ventral tegmental area were already fully active. The percentage of sexually active rats decreased with repeated testing after naloxone injection into the ventral tegmental area, which may be due to the decreased sexual motivation and/or sexual reward of these rats. These results may suggest that opioidergic modulation in the ventral tegmental area of the mesoaccumbens dopamine system affects sexual motivation. Another brain area that has been implicated in the effects of opioids on sexual motivation is the corticomedial amygdala (McGregor and Herbert, 1992).

7. Social behaviour

For species that live in social groups, like humans and rats, survival requires that their actions are coordinated in both time and space with the behaviour of the other group members. Many psychopathological disorders, such as autism and schizophrenia, are accompanied by disturbances in social interactions. Such disturbances may contribute to the observed symptomatology and influence the disease process.

Studies of the development of social behaviour in the rat have shown that during this development specific periods can be distinguished, e.g. neonatal, juvenile and adult period, each period being characterised by separate categories of social interactions. During the neonatal period, in particular, mother–pup interactions are observed. Social contacts in juveniles are characterised by social play, the first extensive nonmaternal social contact. Social play begins at about 18 days after birth, peaks between 30 and 40 days, and then wanes at puberty, around 50–60 days. Social play is composed of behavioural patterns related to adult social, sexual and agonistic behaviour, although juvenile behaviour and adult social behaviour differ both in intensity, form and contextual settings. The third category, adult social interactions, is predominantly present during the subadult and adult period.

Social isolation has short- and long-term effects on a variety of social behaviours, including social, sexual and agonistic interrelations. An important critical factor in this respect is the age or developmental stage at which the isolation occurs. Maternal deprivation results in decreased social play and in aberrant sexual behaviour. Short-term isolation of juveniles results in an acute, transient increase in social play (Niesink and Van Ree, 1989). However, long-term isolation during the play period decreases adult social interactions, probably by decreasing social motivation, and increases aggressiveness (Van den Berg et al., 1999a). Especially, the acquisition of social play appears to be relevant for further social development and social coping. Social isolation of adult rats for 1 or 2 weeks enhances social interactions (Niesink and Van Ree, 1982).

The neuronal systems and substances underlying social interactions are not well understood. Among the substances that have been implicated as such are the endogenous opioids. This has been concluded from studies showing effects of morphine agonists and antagonists on social interactions and on the consequences of social isolation. Some neurochemical observations further support the importance of endogenous opioid systems for social behaviours.

Morphine administration disrupts maternal behaviour, but the mechanism of its action is not well understood. Social separation of pups from their mother results in a marked increase in vocalisation. This vocalisation is diminished by administration of low doses of morphine and increased by treatment with opioid antagonists, suggesting that opioid stimulation can mimic feelings of social comfort normally given by the presence of the mother (Panksepp et al., 1994).

Although there seems no obvious direct benefit, social play appears to be a necessary form of social contact since

the opportunity to play can be used as a reward. As opioid systems are involved in reward processes (Van Ree et al., 1999), they are suggested to be involved in social play as well. Accordingly, treatment with morphine agonists enhances social play and treatment with opioid antagonists reduces social play (Niesink and Van Ree, 1989; Vanderschuren et al., 1997). Detailed analyses have revealed that especially the μ-opioid receptor system is involved in this respect (Vanderschuren at al., 1995a). Another line of evidence indicating the involvement of opioids in social play comes from autoradiographic studies in which brain opioid receptor occupancy is visualised and opioid release in vivo is measured indirectly. During social play, opioid peptides are released in distinct nuclei of the brain (Vanderschuren et al., 1995c). Also the observation that prenatal exposure to morphine elevates the levels of social play underscores the important role of opioid systems in this social behaviour of young rats (Hol et al., 1996).

Isolation of juveniles during weeks 4 and 5 of age increases body weight and sucrose consumption during the isolation period and decreases adult social interactions. These effects are reversed by treatment with morphine during the isolation period, suggesting that morphine might act as a substitute for the release of endogenous opioids during social play (Van den Berg et al., 1999c). In addition, juvenile isolation results in regiospecific increases in μ-opioid binding sites measured in adulthood (Van den Berg et al., 1999d). This increase is particularly present in the basolateral amygdala and in the bed nucleus of stria terminalis. Interestingly, morphine treatment of isolated rats reverses this upregulation in both brain areas. A general upregulation of κ-opioid binding sites has been observed after juvenile isolation, predominantly in the cortical regions, the hippocampus and the substantia nigra. This upregulation is, however, not affected by morphine treatment during the isolation period. The number of δ-opioid binding sites is changed neither by juvenile isolation nor by morphine treatment. Thus, juvenile isolation during the play period has long-term effects on the number of specific opioid receptors in distinct brain areas, further implicating the brain opioid systems in (disturbed) social interactions. Interestingly, the number of μ-receptors in the basolateral amygdala appears to be negatively correlated with the amount of social exploration in adult rats.

Adult social interactions are also stimulated by administration of β-endorphin and morphine-related compounds, and decreased by opioid antagonists, although the findings so far are not always consistent (Van Ree and Niesink, 1983). Endogenous opioid systems may be particularly involved in situations in which social interactions are modulated. Indeed, opioid release, measured indirectly, is — although slightly — different in rats isolated for 7 days as compared to socially housed animals (Vanderschuren et al., 1995b). As mentioned, isolation of juveniles during weeks 4 and 5 of age decreases social interactions of adult rats and this effect is counteracted by treatment with morphine during the isolation period. This effect of morphine is accompanied by enhanced opioid peptide release in some cortical brain regions and the ventral tegmental area (Van den Berg et al., 1999b). Both social activity and opioid release are unaffected by morphine treatment in nonisolated rats. Thus, there seems to be a relationship between social activity and opioid peptide release during social contact.

In conclusion, evidence is accumulating that endogenous opioid systems are involved in social interactions and in disturbances of social contact. This may have consequences for the underlying pathological processes and for the pharmacotherapy of psychiatric disorders in which impairments in social interactions are prominent, such as autism and conduct disorders.

8. Concluding remarks

The reviewed data suggest that endogenous opioids are involved in various reward processes in the brain and in rewarded behaviours. There appear to be two different domains in which endogenous opioids are involved. One is related to the modulation of incentive motivational processes, in which opioid systems in the ventral tegmental area and probably the mesolimbic dopamine system are involved. This involvement may explain the modulation by endogenous opioids of electrical self-stimulation behaviour and initiation of cocaine self-administration and may be pertinent to sexual motivation. The other domain is the performance of certain behaviours involving endogenous opioids present in separate and distinct brain regions. Examples are opiate self-administration (ventral tegmental area), sexual performance (medial preoptic area), play and social behaviour (amygdala?) and probably the desire or craving for drugs and alcohol (limbic regions). It can be hypothesised that endogenous opioids play a role in the vulnerability to certain diseases, such as addiction (ventral tegmental area) and autism (amygdala), but also when the disease is present, such as alcoholism (limbic regions). Research on exogenous and endogenous opioids remains a fascinating field.

References

Adams, W.J., Lorens, S.A., Mitchell, C.L., 1972. Morphine enhances lateral hypothalamic self-stimulation in the rat. Proc. Soc. Exp. Biol. Med. 140, 770–771.

Akil, H., Watson, S.J., Young, E., Lewis, M.E., Khachaturian, H., Walker, J.M., 1984. Endogenous opioids: biology and function. Annu. Rev. Neurosci. 7, 223–255.

Altshuler, H.L., Phillips, P.E., Feinhandler, D.A., 1980. Alteration of ethanol self-administration by naltrexone. Life Sci. 26, 679–688.

Amit, Z., Brown, Z.W., Sklar, L.S., 1976. Intraventricular self-administration of morphine in naive laboratory rats. Psychopharmacology 48, 291–294.

Belluzzi, J.D., Stein, L., 1977. Enkephalin may mediate euphoria and drive-reduction reward. Nature 266, 556–558.

Bradbury, A.F., Smyth, D.G., Snell, C.R., Birdsall, N.J.M., Hulme, E.C., 1976. C-fragment of lipotropin has a high affinity for brain opiate receptors. Nature 260, 793–795.

Broekkamp, C.L., Phillips, A.G., 1979. Facilitation of self-stimulation behavior following intracerebral microinjections of opioids into the ventral tegmental area. Pharmacol. Biochem. Behav. 11, 289–295.

Collins, R.J., Weeks, J.R., Cooper, M.M., Good, P.I., Russell, R.R., 1984. Prediction of abuse liability of drugs using IV self-administration by rats. Psychopharmacology 82, 6–13.

Daunais, J.B., Roberts, D.C.S., McGinty, J.F., 1993. Cocaine self-administration increases preprodynorphin, but not c-*fos*, mRNA in rat striatum. NeuroReport 4, 543–546.

De Vry, J., Donselaar, I., Van Ree, J.M., 1989. Food deprivation and acquisition of cocaine self-administration in rats: effect of naltrexone and haloperidol. J. Pharmacol. Exp. Ther. 251, 735–740.

Deneau, G., Yanagita, T., Seevers, M.H., 1969. Self-administration of psychoactive substances by the monkey. Psychopharmacologia 16, 30–48.

Dhawan, B.N., Cesselin, F., Raghubir, R., Reisine, T., Bradley, P.B., Portoghese, P.S., Hamon, M., 1996. International Union of Pharmacology: XII. Classification of opioid receptors. Pharmacol. Rev. 48, 567–592.

Fabbri, A., Jannini, E.A., Gnessi, L., Moretti, C., Ulisse, S., Franzese, A., Lazzart, R., Fraioli, F., Franjese, G., Isidori, A., 1989. Endorphins in male impotence: evidence for naltrexone stimulation of erectile activity in patient therapy. Psychoneuroendocrinology 14, 103–111.

Gerrits, M.A.F.M., Wiegant, V.M., Van Ree, J.M., 1999. Endogenous opioids implicated in the dynamics of experimental drug addiction: an in vivo autoradiographic analysis. Neuroscience 89, 1219–1227.

Glick, S.D., Maisonneuve, I.M., Raucci, J., Archer, S., 1995. Kappa-opioid inhibition of morphine and cocaine self-administration in rats. Brain Res. 681, 147–152.

Goldstein, A., Lowney, L.I., Pal, B.K., 1971. Stereospecific and nonspecific interactions of the morphine congener levorphanol in subcellular fractions of mouse brain. Proc. Natl. Acad. Sci. U.S.A. 68, 1742–1747.

Goldstein, A., Fischli, W., Lowney, L.I., Hunkapiller, M., Hood, L., 1981. Porcine pituitary dynorphin: complete amino acid sequence of the biologically active heptadecapeptide. Proc. Natl. Acad. Sci. U.S.A. 78, 7219–7223.

Guerra, F., 1974. Sex and drugs in the 16th century. Br. J. Addict. 69, 269–290.

Hawkins, M., Stein, E.A., 1991. Effects of continuous naloxone administration on ventral tegmental self-stimulation. Brain Res. 560, 315–320.

Headlee, C.P., Coppock, H.W., Nichols, J.R., 1955. Apparatus and technique involved in a laboratory method of detecting the addictiveness of drugs. J. Am. Pharm. Assoc. 44, 229–231.

Herz, A., 1997. Endogenous opioid systems and alcohol addiction. Psychopharmacology 129, 99–111.

Hol, T., Niesink, M., Van Ree, J.M., Spruijt, B.M., 1996. Prenatal exposure to morphine affects juvenile play behavior and adult social behavior in rats. Pharmacol. Biochem. Behav. 55, 615–618.

Hughes, J., Smith, T.W., Kosterlitz, H.W., Fothergill, L.A., Morgan, B.A., Morris, H.R., 1975. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258, 577–580.

Koob, G.F., Pettit, H.O., Ettenberg, A., Bloom, F.E., 1984. Effects of opiate antagonists and their quaternary derivatives on heroin self-administration in the rat. J. Pharmacol. Exp. Ther. 229, 481–486.

Koob, G.F., Stinus, L., Le Moal, M., Bloom, F.E., 1989. Opponent process theory of motivation: neurobiological evidence from studies of opiate dependence. Neurosci. Biobehav. Rev. 13, 135–140.

Kornet, M., Goosen, C., Van Ree, J.M., 1990. The effect of interrupted alcohol supply on spontaneous alcohol consumption by rhesus monkeys. Alcohol Alcohol. 4, 407–412.

Kornet, M., Goosen, C., Van Ree, J.M., 1991. Effect of naltrexone on alcohol consumption during chronic alcohol drinking and after a period of imposed abstinence in free-choice drinking rhesus monkeys. Psychopharmacology 104, 367–376.

Kornetsky, C., Bain, G., 1983. Effects of opiates on rewarding brain stimulation. In: Smith, J.E., Lane, J.D. (Eds.), The Neurobiology of Opiate Reward Processes. Elsevier Biomedical Press, Amsterdam, pp. 237–253.

Kosten, T., Silverman, D.G., Fleming, J., Kosten, T.A., Gawin, F.H., Compton, M., Jatlow, P., Byck, R., 1992. Intravenous cocaine challenges during naltrexone maintenance: a preliminary study. Biol. Psychiatry 32, 543–548.

Kuzmin, A., Semenova, S., Zvartau, E.E., Van Ree, J.M., 1996. Enhancement of morphine self-administration in drug naive, inbred strains of mice by acute emotional stress. Eur. Neuropsychopharmacol. 6, 63–68.

Kuzmin, A.V., Gerrits, M.A.F.M., Van Ree, J.M., Zvartau, E.E., 1997a. Naloxone inhibits the reinforcing and motivational aspects of cocaine addiction in mice. Life Sci. 60, PL257–PL264.

Kuzmin, A.V., Semenova, S., Gerrits, M.A.F.M., Zvartau, E.E., Van Ree, J.M., 1997b. κ-Opioid receptor agonist U50,488H modulates cocaine and morphine self-administration in drug-naive rats and mice. Eur. J. Pharmacol. 321, 265–271.

Kuzmin, A.V., Gerrits, M.A., Van Ree, J.M., 1998. Kappa-opioid receptor blockade with nor-binaltorphimine modulates cocaine self-administration in drug-naive rats. Eur. J. Pharmacol. 358, 197–202.

Lord, J.A., Waterfield, A.A., Hughes, J., Kosterlitz, H.W., 1977. Endogenous opioid peptides: multiple agonists and receptors. Nature 267, 495–499.

Martin, W.R., Eades, C.G., Thompson, J.A., Huppler, R.E., Gilbert, P.E., 1976. The effects of morphine- and nalorphine-like drugs in the nondependent and morphine-dependent chronic spinal dog. J. Pharmacol. Exp. Ther. 197, 517–532.

Mason, B.J., Ritvo, E.C., Morgan, R.O., Salvato, F.R., Goldberg, G., Welch, B., Mantero-Atienza, E., 1994. A double-blind, placebo-controlled pilot study to evaluate the efficacy and safety of oral nalmefene HCl for alcohol dependence. Alcohol Clin. Exp. Res. 18, 1162–1167.

McGregor, A., Herbert, J., 1992. The effects of β-endorphin infusions into the amygdala on visual and olfactory sensory processing during sexual behaviour in the male rat. Neuroscience 46, 173–179.

Mello, N.K., Negus, S.S., 1996. Preclinical evaluation of pharmacotherapies for treatment of cocaine and opioid abuse using drug self-administration procedures. Neuropsychopharmacology 14, 375–424.

Mitchell, J.B., Stewart, J., 1990. Facilitation of sexual behaviors in the male rat associated with intra-ventral tegmental area injections of opiates. Pharmacol. Biochem. Behav. 35, 643–650.

Mogenson, G.J., Philips, A.G., 1976. Motivation: a Psychological Construct in Search of a Physiological Substrate. In: Sprague, L.M., Epstein, A.N. (Eds.), Prog. Psychobiol. Physiol. Psychol. vol. 6 Academic Press, New York, pp. 189–234.

Narita, M., Tseng, L.F., 1998. Evidence for the existence of the beta-endorphin-sensitive "epsilon-opioid receptor" in the brain: the mechanisms of epsilon-mediated antinociception. Jpn. J. Pharmacol. 76, 233–253.

Negus, S.S., Henriksen, S.J., Mattox, A., Pasternak, G.W., Portoghese, P.S., Takemori, A.E., Weinger, M.B., Koob, G.F., 1993. Effect of antagonists selective for μ-, δ- and κ-opioid receptors on the reinforcing effects of heroin in rats. J. Pharmacol. Exp. Ther. 265, 1245–1252.

Negus, S.S., Mello, N.K., Portoghese, P.S., Lukas, S.E., Mendelson, J.H., 1995. Role of δ-opioid receptors in the reinforcing and discriminative stimulus effects of cocaine in rhesus monkeys. J. Pharmacol. Exp. Ther. 273, 1245–1256.

Niesink, R.J., Van Ree, J.M., 1982. Short-term isolation increases social interactions of male rats: a parametric analysis. Physiol. Behav. 29, 819–825.

Niesink, R.J., Van Ree, J.M., 1989. Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. Neuropharmacology 28, 411–418.

O'Malley, S.S., Jaffe, A.J., Rode, S., Rounsaville, B.J., 1996. Experience of a "slip" among alcoholics treated with naltrexone or placebo. Am. J. Psychiatry 153, 281–283.

Olds, M.E., Milner, P., 1954. Positive reinforcement produced by electrical stimulation of the septal area and other regions of rat brain. J. Comp. Physiol. Psychol. 47, 419–427.

Olds, M.E., Travis, R.P., 1960. Effects of chlorpromazine, meprobamate, pentobarbital and morphine on self-stimulation. J. Pharmacol. Exp. Ther. 128, 397–404.

Panksepp, J., Nelson, E., Siviy, S., 1994. Brain opioids and mother-infant social motivation. Acta Paediatr. Suppl. 397, 40–46.

Pavlov, I.P., 1927. Conditioned Reflexes. Oxford Univ. Press, Oxford.

Pfaus, J.G., Gorzalka, B.B., 1987. Opioids and sexual behavior. Neurosci. Biobehav. Rev. 11, 1–34.

Ramsey, N.F., Van Ree, J.M., 1990. Chronic pretreatment with naltrexone facilitates acquisition of intravenous cocaine self-administration in rats. Eur. Neuropsychopharmacol. 1, 55–61.

Ramsey, N., Van Ree, J.M., 1993. Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naive rats. Brain Res. 608, 216–222.

Ramsey, N.F., Niesink, R.J., Van Ree, J.M., 1993. Prenatal exposure to morphine enhances cocaine and heroin self-administration in drug-naive rats. Drug Alcohol Depend. 33, 41–51.

Ramsey, N.F., Gerrits, M.A.F.M., Van Ree, J.M., 1999. Naltrexone affects cocaine self-administration in naive rats through the ventral tegmental area rather than dopaminergic target regions. Eur. Neuropsychopharmacol. 9, 93–99.

Reid, L.D., Glick, S.D., Menkens, K.A., French, E.D., Bilsky, E.J., Porreca, F., 1995. Cocaine self-administration and naltrindole, a δ-selective opioid antagonist. NeuroReport 6, 1409–1412.

Robbins, T.W., Cador, M., Taylor, J.R., Everitt, B.J., 1989. Limbic-striatal interactions in reward-related processes. Neurosci. Biobehav. Rev. 13, 155–162.

Sach, B.D., Meisel, R.L., 1988. The physiology of male sexual behavior. In: Knobil, E., Neill, J.D. (Eds.), The Physiology of Reproduction vol. 2 Raven Press, New York, pp. 1393–1485.

Schaefer, G.J., 1988. Opiate antagonists and rewarding brain stimulation. Neurosci. Biobehav. Rev. 12, 1–17.

Skinner, B.F., 1938. The Behavior of Organisms. Appleton-Century Crofts, New York.

Spanagel, R., Zieglgänsberger, W., 1997. Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. Trends Pharmacol. Sci. 18, 54–59.

Spragg, S.D.S., 1940. Morphine addiction in chimpanzees. Comp. Psychol. Monogr. 15, 1–132.

Stewart, J., De Wit, H., Eikelboom, R., 1984. Role of unconditioned and conditioned drug effects in the self-administration of opiates and stimulants. Psychol. Rev. 91, 251–268.

Sweep, C.G., Wiegant, V.M., De Vry, J., Van Ree, J.M., 1989. Beta-endorphin in brain limbic structures as neurochemical correlate of psychic dependence on drugs. Life Sci. 44, 1133–1140.

Terenius, L., Wahlström, A., 1974. Inhibitor(s) of narcotic receptor binding in brain extracts and cerebrospinal fluid. Acta Pharmacol. 35 (Suppl.1), 15.

Thorndike, E.L., 1913. The Psychology of Learning. Teachers College, New York.

Trujillo, K.A., Bronstein, D.M., Akil, H., 1993. Regulation of opioid peptides by self-administered drugs. In: Hammer, R.P. (Ed.), The Neurobiology of Opiates. CRC Press, Boca Raton, FL, pp. 223–256.

Van den Berg, C.L., Hol, T., Van Ree, J.M., Spruijt, B.M., Everts, H., Koolhaas, J.M., 1999a. Play is indispensable for an adequate development of coping with social challenges in the rat. Dev. Psychobiol. 34, 129–138.

Van den Berg, C.L., Kitchen, I., Gerrits, M.A., Spruijt, B.M., Van Ree, J.M., 1999b. Morphine treatment during juvenile isolation increases social activity and opioid peptides release in the adult rat. Brain Res. 830, 16–23.

Van den Berg, C.L., Pijlman, F.T., Koning, H.A., Diergaarde, L., Van Ree, J.M., Spruijt, B.M., 1999c. Isolation changes the incentive value of sucrose and social behaviour in juvenile and adult rats. Behav. Brain Res. 106, 133–142.

Van den Berg, C.L., Van Ree, J.M., Spruijt, B.M., Kitchen, I., 1999d. Effects of juvenile isolation and morphine treatment on social interactions and opioid receptors in adult rats: behavioural and autoradiographic studies. Eur. J. Neurosci. 11, 3023–3032.

Vanderschuren, L.J., Niesink, R.J., Spruijt, B.M., Van Ree, J.M., 1995a. Mu- and kappa-opioid receptor-mediated opioid effects on social play in juvenile rats. Eur. J. Pharmacol. 276, 257–266.

Vanderschuren, L.J., Stein, E.A., Wiegant, V.M., Van Ree, J.M., 1995b. Social isolation and social interaction alter regional brain opioid receptor binding in rats. Eur. Neuropsychopharmacol. 5, 119–127.

Vanderschuren, L.J., Stein, E.A., Wiegant, V.M., Van Ree, J.M., 1995c. Social play alters regional brain opioid receptor binding in juvenile rats. Brain Res. 680, 148–156.

Vanderschuren, L.J., Niesink, R.J., Van Ree, J.M., 1997. The neurobiology of social play behavior in rats. Neurosci. Biobehav. Rev. 21, 309–326.

Van Furth, W.R., Van Ree, J.M., 1994. Endogenous opioids and sexual motivation and performance during the light phase of the diurnal cycle. Brain Res. 636, 175–179.

Van Furth, W.R., Van Ree, J.M., 1996a. Appetitive sexual behavior in male rats: 1. The role of olfaction in level-changing behavior. Physiol. Behav. 60, 999–1005.

Van Furth, W.R., Van Ree, J.M., 1996b. Appetitive sexual behavior in male rats: 2. Sexual reward and level-changing behavior. Physiol. Behav. 60, 1007–1012.

Van Furth, W.R., Van Ree, J.M., 1996c. Sexual motivation: involvement of endogenous opioids in the ventral tegmental area. Brain Res. 729, 20–28.

Van Furth, W.R., Wolterink-Donselaar, I.G., Van Ree, J.M., 1994. Endogenous opioids are differentially involved in appetitive and consummatory aspects of sexual behavior of male rats. Am. J. Physiol. 266, R606–R613.

Van Furth, W.R., Wolterink, G., Van Ree, J.M., 1995. Regulation of masculine sexual behavior: involvement of brain opioids and dopamine. Brain Res. Rev. 21, 162–184.

Van Ree, J.M., 1979. Reinforcing stimulus properties of drugs. Neuropharmacology 18, 963–969.

Van Ree, J.M., De Wied, D., 1980. Involvement of neurohypophysial peptides in drug-mediated adaptive responses. Pharmacol. Biochem. Behav. 13 (Suppl.1), 257–263.

Van Ree, J.M., De Wied, D., 1988. Behavioral approach to the study of the rat brain. Discuss. Neurosci. 5, 1–60.

Van Ree, J.M., De Wied, D., Bradbury, A.F., Hulme, E.C., Smyth, D.C., Snell, C.R., 1976. Induction of tolerance to the analgesic action of lipotropin C-fragment. Nature 264, 792–794.

Van Ree, J.M., Niesink, R.J., 1983. Low doses of beta-endorphin increase social contacts of rats tested in dyadic encounters. Life Sci. 33 (Suppl.1), 611–614.

Van Ree, J.M., Slangen, J.L., De Wied, D., 1978. Intravenous self-administration of drugs in rats. J. Pharmacol. Exp. Ther. 204, 547–557.

Van Ree, J.M., Smyth, D.G., Colpaert, F.C., 1979. Dependence creating properties of lipotropin C-fragment (β-endorphin): evidence for its internal control of behavior. Life Sci. 24, 495–502.

Van Ree, J.M., Gerrits, M.A., Vanderschuren, L.J., 1999. Opioids, reward and addiction: an encounter of biology, psychology, and medicine. Pharmacol. Rev. 51, 341–396.

Van Wolfswinkel, L., Van Ree, J.M., 1985a. Site of rewarding action of morphine in the mesolimbic system determined by intracranial electrical self-stimulation. Brain Res. 358, 349–353.

Van Wolfswinkel, L., Van Ree, J.M., 1985b. Effects of morphine and

naloxone on thresholds of ventral tegmental electrical self-stimulation. Naunyn-Schmiedeberg's Arch. Pharmacol. 330, 84–92.

Van Wolfswinkel, L., Seifert, W.F., Van Ree, J.M., 1985. Long-term changes in self-stimulation threshold by repeated morphine and naloxone treatment. Life Sci. 37, 169–176.

Volpicelli, J.R., Alterman, A.I., Hayashida, M., O'Brien, C.P., 1992. Naltrexone in the treatment of alcohol dependence. Arch. Gen. Psychiatry 49, 876–880.

Weeks, J.R., 1962. Experimental morphine addiction: method for automatic intravenous injection in unrestrained rats. Science 138, 143–144.

Wei, E., Loh, H., 1976. Physical dependence of opiate-like peptides. Science 193, 1262–1263.

Wise, R.A., 1996. Addictive drugs and brain stimulation reward. Annu. Rev. Neurosci. 19, 319–340.