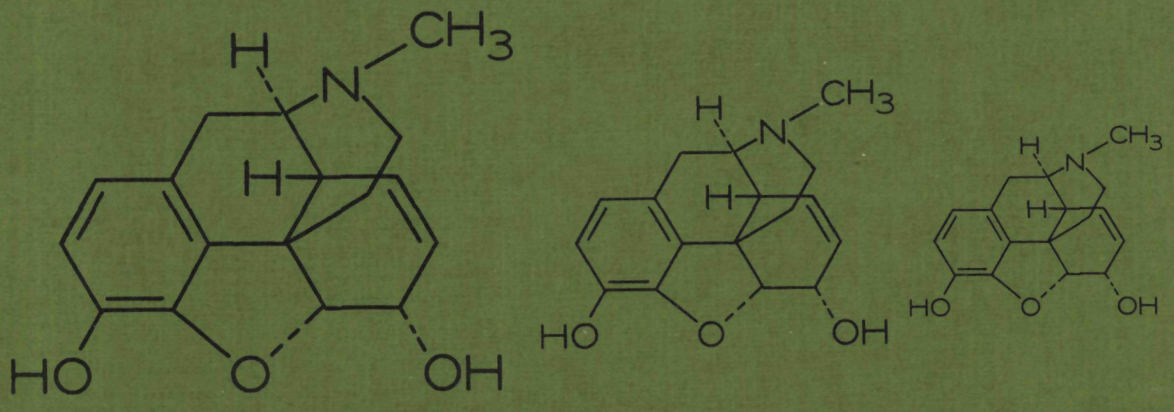
MORPHINE-INDUCED BEHAVIOUR OF CATS AND NEUROTRANSMISSION PROCESSES WITHIN RAPHE, STRIATAL AND SEPTAL NUCLEI

Introduction of a new method in psychopharmacology.



Anton A.H.P. Megens

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PROMOTOR : PROF.DR. J.M. VAN ROSSUM

CO-REFERENT: DR. A.R. COOLS

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PROEFSCHRIFT

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Science is but an exchange of ignorance for that which is another kind of ignorance

Lord Byron

The present dissertation is based upon the following papers:

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Cools, A.R., L.C.M. Gieles, H.J. Janssen and A.A.H.P. Megens, Morphine and its biphasic influence upon pharmacologically distinct dopaminergic systems within the feline caudate nucleus: a behavioural study, Eur. J. Pharmacol. 48, 67-85 (1978).

Megens, A.A.H.P. and A.R. Cools, Effects of intraseptal administration of cholinergic agents on morphine-induced behavior of cats, Psychopharmacology 66, 183-188 (1979).

Megens, A.A.H.P. and A.R. Cools, Presence of a particular subpopulation of dopamine receptors within the septal nuclei: a behavioural study on cats, Eur. J. Pharmacol. 71, 247-258 (1981).

Megens, A.A.H.P. and A.R. Cools, Intraseptally injected opiate agents: effects on morphine-induced behaviour of cats, submitted for publication.

Megens, A.A.H.P. and A.R. Cools, The septal nuclei and involvement in central dopamine-acetylcholine balance: a behavioural study on cats, submitted for publication.

CHAPTER 2

MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL FOR THE STUDY OF CENTRAL NEUROTRANSMISSION PROCESSES

CHAPTER 3

MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL TO STUDY THE ACTION OF MORPHINE ON NEUROTRANSMISSION PROCESSES WITHIN RAPHE AND STRIATAL NUCLEI

CHAPTER 4

MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL TO STUDY NEUROTRANSMISSION PROCESSES WITHIN THE SEPTAL REGION

CHAPTER 5

MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL TO STUDY FUNCTIONAL INTERACTIONS BETWEEN NEUROTRANSMISSION PROCESSES WITHIN SEPTAL AND STRIATAL NUCLEI

Behaviour - considered as the dynamic process by which a living organism attempts to control its input signals by output actions (Cools, 1981; Powers, 1973) - is mediated via a vast number of neuronal processes within the brain. Most of these processes are neurochemically regulated, which accounts for the ability of drugs to affect behaviour. Such drugs are used clinically for therapeutic treatment of patients suffering from nervous and mental disorders and for producing such valuable effects as analgesia, sedation or anaesthesia. In order to improve the effective use of drugs for these purposes, it is necessary to get more insight into the functional and pharmacological properties of central processes that are involved in the regulation of behaviour. Many disciplines are involved in this field, including biochemistry, neurochemistry, neuropharmacology, neurophysiology, neuroanatomy, physiological psychology, and psychiatry. Each of these uses its own characteristic methods with their own specific advantages and disadvantages.

This thesis deals with the effects of intracerebrally injected drugs on morphine-induced behaviour of cats. The aim of this work is to investigate whether the morphine-induced behaviour of cats, in combination with intracerebral injection of drugs, may be a new and suitable method for the study of the pharmacological and functional properties of neurotransmission[1] (NT-)processes in the brain. This method may have substantial advantages compared to other methods:

[1] Within the context of this thesis, the term neurotransmission is used in the broadest sense of the word, i.e. neuronal communication involving neurochemicals.

1. The intracerebral injection technique enables us to change pharmacologically the activity of specific NT-processes in anatomically circumscribed regions of the brain.
2. The highly structured organized behaviour of morphine-treated cats, together with their characteristic behaviour patterns, enables us to detect subtle changes in their behaviour following the intracerebral injection of drugs (Cools et al., 1974; Chapter 2).
3. The use of behaviour as an experimental variable enables us to perform experiments *in vivo* in unanaesthetized animals.
4. The drug-induced behaviour provides a test-situation in which the animals move freely, not constrained - as in avoidance or operant behaviour - by a learned task.
5. Results are obtained in acute experiments; training is not required.
6. An advantage of cats as experimental animals is that the larger dimensions of their brains compared with those of such other laboratory animals, so as mice or rats, allow more definite conclusions about any specific location of the intracerebrally evoked effects.
7. The morphine pretreatment enables us to study the action of morphine on central NT-processes. The cat is a suitable experimental animal for this purpose as most of the morphine-induced effects are similar in both man and cats (see sections 1.3 and 1.4)

Several aspects of NT-processes are selected in order to investigate whether the present method is, indeed, suitable for the study of central NT-processes:

1. <u>The functional activity of NT-processes at the behaviour level</u>
   The existence of NT-processes within a particular brain region can be established on the basis of histochemical and biochemical studies. Their functional activity, however, can be only established by assessing their ability to produce biological responses. These biological responses can be measured at different levels, e.g. at the electrophysiological, biochemical, physiological, or behaviour level. Especially, their functional activity at the behaviour level is of interest as it indicates which NT-processes may play a critical role in the ability of drugs to prevent or cure disturbed behaviour.

   Little is known about the functional activity, at the behaviour level, of NT-processes within the septal nuclei although this brain

structure has been implicated in a wide variety of behaviours, e.g. avoidance behaviour, operant responding, and food and water intake. In the present study, it is investigated a) whether the morphine-induced behaviour of cats may serve as a suitable tool for the disclosure of septal evoked effects, and b) whether the present method may enable us to establish the functional activity of septal NT-processes at the behaviour level. Septal, cholinergic processes are selected for the first purpose as these processes have already previously been implicated in behaviour (section 4.2). Septal, dopaminergic and endorphinergic processes are selected for the second purpose as these processes were previously unknown to be involved in behaviour.

2. The pharmacological character of receptor sites

It is known that multiple receptor sites exist for one and the same neurotransmitter. These receptor subtypes can be differentiated in several ways, e.g. biochemically, anatomically, histochemically and pharmacologically. Especially, the pharmacological character of receptor sites are of interest, e.g. for researchers, neurologists and psychiatrists, as it indicates how the activity of specific neuronal systems can be changed pharmacologically, e.g. in order to prevent or cure disturbed behaviour. It is investigated as to whether the present method may enable us to establish the presence and pharmacological character of particular subtypes of receptors within the septal nuclei. Dopamine receptors (section 4.3) and opiate receptors (section 4.4) are selected for this purpose as it is known that subtypes of receptors exist for both dopamine and opiates.

3. The flexibility of NT-processes

The flexibility of NT-processes, i.e. their ability to adjust their properties to changes in NT-activity, is a mechanism which enables NT-processes to maintain homeostasis. It is quite possible that this flexibility of NT-processes may play a critical role in the development of tolerance to and dependence on the effects of drug treatments. Flexibility has been reported especially for noradrenergic processes. Noradrenergic processes in the raphe nuclei have been implicated in the behaviour effects of morphine. Hence, it is investigated whether the present method may enable us to get more insight into the flexibility of noradrenergic processes within the raphe nuclei and to

investigate whether this flexibility may play a role in the development of tolerance to morphine (section 3.1).

4. Functional interactions between NT-processes

Brain functioning is dependent on interactions between NT-processes. Disturbance of normally occurring interactions between NT-processes is a major cause of nervous and mental disorders in man, e.g. an imbalance between central dopaminergic and cholinergic interactions, particularly within the caudate nucleus, is generally thought to be one of the etiological factors in Parkinson's disease. The functional relationship between NT-processes is of interest, e.g. for neurologists, as it enables them to treat the consequences of dysfunctioning NT-processes not only by manipulation of the disturbed processes, but also by manipulation of functionally related processes, e.g. both dopamino-mimetics and anticholinergics are beneficial in the treatment of Parkinson's disease. As there is some evidence that not only NT-processes in the caudate nucleus but also NT-processes in the septal nuclei may play an important role in the central dopamine-acetylcholine interactions and, consequently, in the etiology of Parkinson's disease, it is investigated whether the present method may enable us to achieve more insight into the functional relationship between dopaminergic activity in the caudate nucleus and a dopamine-acetylcholine balance in the septal nuclei (Chapter 5).

5. The action of morphine on NT-processes

Morphine, a drug belonging to the group of natural opium alkaloids, is known already over the centuries because of its therapeutic properties, which are used clinically for allaying severe pain and alleviating anxiety, and its euphoric properties, which account for its compulsive abuse by addicts (for the history of morphine see section 1.2). More knowledge of the mechanism of action of morphine is a prerequisite to achieve an improved therapeutic use and a decreased abuse of morphine. Much controversy exists about morphine's action on central NT-processes. This may be partly due to the fact that most studies investigate morphine's overall effect on complex neuronal systems, which may mask morphine's action on single neuronal systems, and partly due to the fact that most studies pay little attention to the time-pattern of the morphine-induced ef-

fects. It is investigated as to whether the present method may enable us to distinguish morphine's action on two pharmacologically distinct, dopaminergic processes in the caudate nucleus, and to establish a distinct role of these dopaminergic processes during different periods after morphine administration (section 3.2).

6. The action of morphine on opiate receptors
   Morphine affects NT-activity within a particular brain structure either directly via opiate receptors within that structure or indirectly via fibres afferent to that structure. It is investigated as to whether the present method may enable us to establish whether morphine's action on septal neuronal activity is mediated via an direct action on the septal opiate receptors or via an indirect action on septal afferent fibres (section 4.4).

The present investigations show that morphine-induced behaviour of cats is, indeed, a suitable tool for the study of pharmacological and functional properties of central NT-processes. In other words, it provides a new method for extending our knowledge of NT-processes in the brain.

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CHAPTER 1

MORPHINE-INDUCED EFFECTS: A GENERAL INTRODUCTION

1.1 INTRODUCTION

Morphine - belonging to the group of the natural opium alkaloids - is a well-known drug already over the centuries because of its therapeutic properties, which are used clinically for allaying severe pain and alleviating anxiety, and its euphoric properties, which account for its abuse by addicts. In this thesis, advantage is taken of another property of the opiate agonist, viz. its ability to evoke a particular, species-specific behaviour syndrome in cats. This morphine-induced behaviour in cats is, in combination with intracerebral injection of drugs, introduced as a new method for the study of the pharmacological and functional properties of neurotransmission processes in the brain.

As a general introduction to this subject, a survey is given of the overt effects of morphine in man and the cat, with the main emphasis on pure behaviour effects (section 1.3). Behaviour is closely related to various events taking place inside the organism. Accordingly, effects of morphine on such events may, at least partly, underly the behaviour effects of the opiate agonist. Therefore, a summary of the more specific effects of morphine in man and the cat is also given (section 1.4). It will be indicated that most of the morphine-induced effects in the cat are similar to those in man. This close similarity between man and the cat makes it feasible to use cats as experimental animals for the study of morphine's mechanism of action. More insight into this mechanism of action is a prerequisite for achieving an improved therapeutic use and a decreased abuse of morphine. The action of morphine on central neurotransmission processes is, therefore, one of the subjects investigated within the scope of this thesis. As an introduction to this subject, data from literature over the central effects of morphine in cats is reviewed (section 1.5). But, first of all, the fascinating history of morphine will be shortly summarized

(section 1.2). The experimental method, i.e. the analysis of the experimentally evoked effects on the morphine-induced behaviour of cats, is discussed in Chapter 2.

1.2 HISTORY OF MORPHINE

The therapeutic and euphoric properties of opiates like morphine have been known already for a very long time. Gradually, but in an ever increasing tempo, some aspects of the working-mechanism of these drugs have been disclosed. The history about the discoveries made in this field until now are fascinating, and, for those who are not acquainted with it, it is shortly summarized below (for references to the older literature, the reader is referred to Krueger et al. (1943)).

The ancient Sumerians (4000 B.C.) depicted in their hieroglyphics a plant of joy; it might be that they were acquainted with the euphoric properties of the poppy plant, Papaver Somniferum, which is indigenous to Asia Minor. Also Theophrastus (third century B.C.) mentioned the poppy juice in his writings. The air-dried juice of the un-ripe fruit of the poppy plant was called opium (after the Greek word for juice). Arabic physicians were acquainted with the analgesic and stopping action and, probably, with the narcotic and euphoric action of opium. Arabic traders introduced the drug into the Orient and China, where it was mainly used in the treatment of dysenteries. In the 16th century, the most important pharmacological properties of the drug were known in Europe, and it was already regularly used in medical sciences at that time. The opium preparation laudanum, which was compounded by Paracelsus (1493-1541), is still in use today. In 1803 the most important component of opium was isolated by the German pharmacist Sertüner; it was called morphine after Morpheus, the Greek god of dreams. Later on, other opiate alkaloids were discovered, e.g. codeine (1832) and papaverine (1848). By the middle of the 19th century the medical world began to use pure alkaloids rather than crude opium preparations. Pharmacists and chemists tried to synthesize substitutes for morphine. The synthesis of meperidine (pethidine), the first synthetic narcotic analgesic, may be considered as a great triumph. Nowadays, several synthetic opiates are commercially available; because of the difficulties involved in the synthesis, also many semi-synthetic deri-

vatives are prepared from morphine. The development of synthetic surrogates of morphine had important consequences for the clinical application of opiates and also for the progress in opiate research. Clinically, some synthetic agents have distinct advantages over morphine, e.g. a higher potency, a higher oral effectiveness, a lower addiction liability, or a longer duration of action. Methadone was not only useful as a morphine substitute, developed for pain relief, but it found also application for gradual discontinuation of morphine-abuse by the narcotic addict. Furthermore, methadone, being a racemate, could be resolved into an active levorotatory isomer and an inactive d-isomer and these two substances were used to establish stereospecificity of narcotic action. For similar purposes another enantiomorph pair was used more extensively, viz. levorphanol and dextrophan, which were synthesized shortly after methadone. In 1915, Pohl reported antagonism of morphine- and heroin-induced respiratory depression by N-allylnorcordeine. The true impact of this discovery was not realized at that time. In the period between 1941 and 1943, N-allylnormorphine was synthesized in an attempt to synthesize a morphine surrogate with fewer respiratory depressant properties and lower addiction liability than morphine (Hart and McCawley, 1944). It appeared that this compound could antagonize the action of morphine. In 1952, Eckenhoff reported a successful clinical usage of nalorphine as an antidote for narcotic overdosage. In 1953, Wikler et al. reported precipitation of withdrawal symptoms by nalorphine in subjects who had received morphine, methadone, or heroin. Since then, search for better narcotic antagonists led to the introduction of several specific antagonists (e.g. naloxone and naltrexone).

One of the best known properties of morphine and its congeners (e.g. heroin) is the addicting action characterized by the development of tolerance to and dependence on the drug's action. The Greek physicians were already acquainted with it, but opium smoking first became popular in the 18th century. The discovery of pure opium alkaloids, the invention of the hypodermic needle, the advent of opium smoking Chinese labourers and the extensive use in the U.S.A. of morphine among wounded Civil War soldiers led to an increased compulsive drug abuse. The recognition of this serious drawback of morphine stimulated a research for potent analgesics that would not lead to compulsive drug abuse.

Almost all the potent narcotic analgesics have similar characteristics with respect to their chemical structures. These minimal structural features usually associated with narcotic analgesic activity are: 1) a quaternary carbon atom, 2) an phenylgroup linked to this carbon, 3) a tertiary aminogroup linked to the quaternary carbon atom through a chain of 2 saturated carbon atoms, and 4) a relatively small group linked to the aminogroup.

The observations that the various opiate drugs had common structural properties, that enantiomerics of the narcotic drugs showed great differences in potency, and that structurally related specific opiate antagonists existed, indicates the existence in the brain of specific opiate receptors. Using low concentrations of radioactively labelled opiate agonists or antagonists of high specific activity and with the help of repeated washprocedures, stereospecific binding of these agents to brain homogenates was demonstrated in 1973. This was realized independently by three different groups of workers: Simon (1973), Snyder et al. (1974) and Terenius (1973). It is only reasonable that evolution did not produce specific receptor sites in animals for the sole purpose of receiving an external stimulus, such as narcotic drugs. Accordingly, the existence was postulated of endogenous substances within the brain that act on the opiate receptors. The existence of such endogenous opiate ligands was further strenghtened by the observations that electrical stimulation of the periaqueductal grey of rats produced analgesia (Reynolds, 1969) and that such stimulation-produced analgesia was antagonized by the opiate antagonist naloxone (Akil et al., 1976). Hughes (1974) isolated an endogenous substance with opiate activity; it was called "enkephalin" (after the Greek word for head) and appeared to be a mixture of two peptides, H-Tyr-Gly-Gly-Phe-Met-OH (Met-enkephalin) and H-Tyr-Gly-Gly-Phe-Leu-OH (Leu-enkephalin). Further research resulted in the discovery of other opiate-like peptides called "endorphins" (after endogenous morphine). According to the sequence of their discovery, the endorphins received the prescripts α, β and γ. It appeared that with exception of Leu-enkephalin all opiate-like peptides (opioid peptides) were fragments of the polypeptide β-lipotropin (β-LPH), viz. Met-enkephalin (β-LPH$_{61-65}$), α-endorphin (β-LPH$_{61-76}$), β-endorphin (β-LPH$_{61-91}$), and γ-endorphin (β-LPH$_{61-77}$). Various reviews appeared dealing with the discovery of the opiate receptors, the endogenous opiate

ligands, and their distribution over the brain (e.g. Adler, 1980; Chau-Pham, 1978; Frederickson, 1977; Goldstein, 1974; Miller and Cuatrecasas, 1978; Simon, 1981; Snyder, 1975, 1977; Snyder and Simantov, 1977). The Met- and Leu-enkephalin containing neuronal system seems to be comprised of many cellgroups with short fibre systems distributed over the brain. The β-endorphin containing system shows one cellgroup in the hypothalamus with major fibre paths coursing to several brain regions. The endogenous opioid peptides and their synthetic analogues have, in general, pharmacological properties similar to those of the exogenous opiates. It was found that the ranking order of potencies with which unlabelled ligands displaced binding of labelled ligands is dependent on the type of labelled ligand used. This indicated the existence of either a single class of opiate receptors that can exist in several conformational states or, alternatively, of multiple types of opiate receptors with distinct pharmacological properties. Indeed, the existence of more than one type of opiate receptor has been suggested (Lord et al., 1977; Martin et al., 1976; Wüster et al., 1979): the μ-receptor (for opiate alkaloids like morphine), the δ-receptor (for the enkephalins), the ε-receptor (for the endorphins), the σ-receptor (for SKF-10.047), and the κ-receptor (for ketocyclazocine).

At present, an impressive amount of work has been done in opiate research. Only a very small part of it is summarized above. But, as always in science, all this work has raised more questions than it has answered. In the present study, the attention is focussed on two main features of morphine: a) its ability to affect behaviour via changing the activity of central neurotransmission processes within particular brain regions (Chapter 3) and b) its ability to change the activity of central neurotransmission processes via stimulating one or more subtypes of opiate receptors (section 4.4). The experimental results obtained from this work may contribute to our knowledge of the central action of opiates. Eventually, this knowledge may lead to an improved therapeutic use and a decreased abuse of morphine by man.

1.3 OVERT EFFECTS OF MORPHINE IN MAN AND CATS

This section gives a survey of the overt effects of morphine in man and cats with most attention to the purely behaviour effects; more specific effects of morphine are discussed in section 1.4.

1.3.1 Overt effects of morphine in man

Respiratory depression, pupillary constriction and emesis - belonging to the most pronounced overt effects of morphine in man - are discussed in detail in section 1.4. Other effects produced by the opiate agonist at therapeutic analgesic doses (about 10 mg) are the following: drowsiness, loss of anxiety, lethargy, apathy, mental confusion, muscular relaxation, sleepiness and decreased sensitivity to internal and external stimuli. In the presence of pain, sedation and hypnosis may result from relief of pain and from its accompanying mental and physical exhaustion. Morphine produces euphoria and some feelings of improved mentation in post-addicts, but dysphoria and feelings of impaired mentation in non-addicted subjects. Morphine-induced sedation, though generally accompanied with diminished physical activity, does not produce any slurring of speech or marked motor inco-ordination. As the dose is increased (about 30 mg), the depression deepens to complete unconsciousness from which the subject can be aroused only with difficulty. Some individuals, particular women, are excited by morphine; however, convulsions are rarely seen in the human subject. Doses in excess of 100 mg produce serious symptoms, and doses over 250 mg are usually fatal. Tolerance may built up to a remarkable degree: doses as much as 4 g are without adverse effect in subjects chronically treated with morphine. Withdrawal from morphine causes yawning, lacrimation, sneezing, stuffy nose, mydriasis, chills, piloerection, perspiration, muscle twitching, diarrhea, nausea, restlessness, and a feeling of weakness; sleep, caloric intake, and weight are decreased following withdrawal (for references: Goldstein et al., 1974; Martin and Sloan, 1977; Pradhan and Dutta, 1977; Reynolds and Randall, 1957).

1.3.2 Overt effects of morphine in cats

Acute effects

Respiratory depression, pupillary dilatation, and emesis belong to the overt effects of morphine in cats and are discussed in detail in section 1.4. Other overt effects accompanying the pure behaviour effects are salivation, defaecation and micturation (Cools et al., 1974; Dhasmana et al., 1972; French et al., 1979b; Kayan and Mitchell, 1968; Labrecque and Domino, 1974).

Table 1.1 gives references to studies dealing with the behaviour effects of morphine in cats. These effects are clearly dose- and time-related: small doses produce a long period of sedation followed by some excitation, high doses produce mainly excitation preceded by a short period of sedation. Doses of 0.05 mg/kg have a mild sedative effect (Phillis et al., 1973). Daily treatment with doses of 0.2 mg/kg for five days produces an increase in operant responding (Djahanguire et al., 1966). A dose of 0.3 mg/kg totally eliminates sleep for approximately 6 hr; the cats adopt a sphinx-like posture during this period (Echols and Jewett, 1972). A dose of 0.5 mg/kg produces some excitation but only after a lapse of 40-60 min following intraperitoneal injection or 10-30 min following intravenous injection (Phillis et al., 1973): the cats appear inattentive moving the head in apparently random fashion, some cats show mild muscle spasms and they are more responsive to such external stimuli as noise. Following a dose of 1 mg/kg, the period of sedation is reduced to 30-45 min (intraperitoneal injections) or 10-20 min (intravenous injections) (Dhasmana et al., 1972; French et al., 1979b). During this period the animals lie quietly in the cage, they remain in the same position if undisturbed, and they do not react to mild external stimuli as opening of the cage door. Later on, the animals become more active: sitting becomes highly dominant and head movements increase. Additional effects observed are: licking, staring, scratching, and loud mewing. Remarkably, grooming does not occur at all (French et al., 1979b). With increasing doses the periods of sedation become shorter and the signs of excitation more pronounced as seen by increased locomotion. Cools et al. (1974) distinguish three behaviour phases, viz. depression, re-organization, and ritualization, which appear in succession following a dose of 5 mg/kg. Depression is a period of hypoactivity, re-organization a period of increasing activity, and

TABLE 1.1

References to studies dealing with the behaviour effects of morphine in cats

dose (mg/kg)	references (route of administration)
0.05	Phillis et al., 1973 (i.p.; i.v.)
0.2	Djahanguire et al., 1966 (s.c.)
0.3	Echols and Jewett, 1972 (s.c.)
0.5	Phillis et al., 1973 (i.p.; i.v.)
1.0	Dhasmana et al., 1972 (i.p.); French et al., 1979b (i.v.); Navarro and Elliott, 1971 (i.p.)
2.0	Chernov and Woods, 1965 (s.c.); French et al., 1979b (i.v.)
3.0	Labrecque and Domino, 1974 (s.c.)
4.0	French et al., 1979b (i.v.)
5.0	Cools et al., 1974 (i.p.); Dhasmana et al., 1972 (i.p.); Gunne, 1963 (s.c.); Van Dongen, 1980a (i.p.); Wikler, 1944 (i.v.; s.c)
10.0	Borrell and Borrell, 1975 (i.p.); Dhasmana et al., 1972 (s.c.); Guaza et al., 1979 (i.p.); Loewe, 1956 (s.c.); Moore et al., 1965 (i.v.); Navarro and Elliott, 1971 (i.p.); Wikler, 1944 (i.v.; s.c.)
15.0	Fertiziger et al., 1974 (i.p.); Mehta, 1975 (i.v.); Wikler, 1944 (i.v.; s.c.)
20.0	Dhasmana et al., 1972 (i.p.); Loewe, 1956 (s.c.); Norton and De Beer, 1956 (p.o.); Sturtevant and Drill, 1957 (s.c.)
30.0	Chernov and Woods, 1965 (s.c.); Gunne, 1963 (s.c.); Moore et al., 1965 (i.p.)

i.p. = intraperitoneal
i.v. = intravenous
s.c. = subcutaneous
p.o. = per os (oral)

ritualization a period of hyperactivity. Moreover, a restricted number of behaviour patterns is continuously repeated during ritualization. Other animals in the cage are ignored, and the cat's motor performances are poorly co-ordinated following this dose of morphine (Wikler, 1944).

A small number (20%) of the cats tested with a dose of 5 mg/kg exhibit a characteristic manic response, showing vigorous motor excitement with periods of rage-like activity and producing harsh, loud and prolonged cries (Dhasmana et al., 1972). This manic response occurs in 40% of the animals tested with a dose of 10 mg/kg and in all cats tested with a dose of 20 mg/kg (Dhasmana et al., 1972). The excitatory effects following a dose of 15 mg/kg have a duration of 6-8 hr (Guaza et al., 1979; Wikler, 1944). Muscle twitches are sometimes seen after a lapse of several hours following a dose of 15 mg/kg, but frank convulsions do not occur after this dose (Wikler, 1944). Following doses of 30 mg/kg, convulsions occur and 50% of the animals die within 4-8 hr (Chernov and Woods, 1965; Moore et al., 1965). Some degree of unrest is generally noted for more than 12 hr and is occasionally present even 24 hr after a dose of 30 mg/kg (Gunne, 1963).

As indicated above, acute injections of morphine produce both depressant and excitatory behaviour effects which appear in succession. When doses are low, the depression phase predominates and has a long duration; when doses are high, the sedation phase becomes shorter and the excitatory phase more intense. The behaviour effects of morphine following a moderate dose of 5 mg/kg are discussed in more detail in Chapter 2; it will be indicated that this dose of morphine produces a highly characteristic behaviour syndrome which enables us to detect subtle, experimentally-evoked changes in behaviour.

Intracerebroventricular injections of morphine produce behaviour effects similar to those of systemic administration of morphine (see below: intracerebral injections), which indicates that these effects are centrally mediated. Since morphine produces different effects when injected into different regions of the brain (see below: intracerebral injections), it can be stated that the behaviour effects of systemic administration of morphine are the overall effect of morphine's action at various regions of the brain. In the present study, we pay attention to morphine's central action (Chapter 3; section 4.4).

Catlin et al. (1978) report the behaviour effects of intravenous injections of β-endorphin (50-300 μg/kg). The cats assume a sitting or crouching position and, in general, they remain stationary for at least 7-10 min. The head is usually lowered, the eyes are open, and the cats stare. The animals remain alert, respond rapidly to a tap on the cage,

and follow attentively an object moving in front of it 5 min after injection. No abnormal movements are observed and no motor impairment occurs. Additional effects are: licking, tremors, defaecation and head shakes. In contrast to morphine, β-endorphin has no effect on respiratory rate or pupillary diameter, and it does not produce micturation. Moreover, grooming - which is absent following morphine - occurs following β-endorphin. Hence, it can be stated that the behaviour effects of systemic injections of β-endorphin are different from those of morphine. In contrast, intraventricularly administered β-endorphin produces effects very similar to those of morphine (see below: intracerebral injections). This discrepancy indicates that the opioid peptide does not readily pass the blood-brain barrier. Moreover, the observation that intraventricular injections of β-endorphin but not of Met-enkephalin produce effects similar to those of morphine (see below: intracerebral injections), suggests that the behaviour effects of the exogenous opiate morphine are mediated by the β-endorphin system rather than by the enkephalin system.

Chronic effects

In general, chronic administration of morphine results in the development of tolerance to and dependence on its effects. Much controversy, however, exists about the development of tolerance to and dependence on the behaviour effects of morphine in cats. This may be partly due to the absence of quantitative measures or detailed qualitative descriptions in most studies, and partly due to differences between studies in dose, duration and schedule of morphine treatment.

Djahanguire et al. (1966) report tolerance to morphine's effect on operant responding after daily injections of doses of 0.2 mg/kg for more than 12 days. Kayan and Mitchell (1968) fail to observe tolerance to the behaviour effects of morphine following 4 weeks of weekly injections and, for the next 2 weeks, daily injections of morphine in a dose of 1 mg/kg; but, in contrast to Djahanguire et al. (1966), these authors use no quantitative measures and they observe only gross behaviour. French et al. (1979b) - using quantitative measures - indicate that cats treated with daily injections of morphine (1-4 mg/kg) for 7 days develop partial tolerance to morphine's behaviour effects: the total time spent engaging in lying down increases whereas scores for sitting and moving decrease. Moreover, the same study shows that naloxone administered to cats daily

treated with this dose of morphine produces a withdrawal syndrome consisting of wet-dog shakes, urination, pupillary constriction, loud mewing, hyperpnea and a catatonic-like posture. Eddy and Himmelsbach (1936) report tolerance to morphine's overt behaviour effects after daily doses of 2 mg/kg administered for a long period of 2 months. In the absence of quantitative measures or detailed qualitative descriptions, Gunne (1963) fails to find tolerance, following 5 daily doses of 5-30 mg/kg. A considerable degree of tolerance is found, however, when cats are treated for a longer period of 30 days with daily doses of 10 mg/kg (Borrell and Borrell, 1975; Guaza et al., 1979). As described in these studies, nalorphine-induced withdrawal in the chronically treated animals produces excitement comparable to that observed following acute injections of morphine. Tolerance to the overt behaviour effects of morphine develops already after 10-12 days when progressively increasing doses of morphine (2-20 mg/kg/day or 9-30 mg/kg/day) are administered (Labrecque and Domino, 1974; Mullin and Phillis, 1974). These same studies report naloxone-induced withdrawal phenomena consisting of agitation, frequent mewing, miosis and rage reactions. About half of the cats die within 1 hr after naloxone administration preceded by clonic-tonic convulsions. Using the technique of implantation of morphine pellets (resulting in the daily delivery of 16-58 mg/kg), Huidobro and Lewin (1969) find profound tolerance around the 15th day. These authors describe nalorphine-induced withdrawal phenomena consisting of micturation, sneak walking, nasal and lacrimal secretion, mewing, polypnea, miosis, salivation, emesis and agitation; the cats neither obey nor recognize during the withdrawal period, they become irritable and aggressive. Hyperreflexia, tremors, and convulsions sometimes occur during the withdrawal period. Following daily doses of 40 mg/kg administered for 5 months, Tatum et al. (1929) find no tolerance but rather sensitization to morphine-induced excitation, i.e. an increased rather than a decreased response of the cats to morphine administration. The doses used, however, are quite high, and accumulation of morphine in the body may have influenced the development of tolerance. Finally, it is of interest to note that cats do self-administer morphine, suggesting the development of psychological dependence to morphine in cats (Kilbey and Ellinwood, 1980).

Summarizing, it can be stated that chronic administration of morphine results in the development of tolerance to and dependence on the behav-

iour effects of morphine. The rate and degree of tolerance development are dependent on the dose, duration and schedule of morphine treatment. When doses are small and intervals between doses long, the degree of tolerance may be low: quantitative measures or detailed descriptions are required for the disclosure of such a low degree of tolerance. When doses are higher and intervals shorter, the degree of tolerance may be more pronounced and visible by inspection of gross behaviour. Very high doses may cause accumulation of the drug in the body and, consequently, produce an increased rather than a decreased response to morphine. In general, the rate of tolerance development may be expected to be maximal in the case of a high and constant concentration of the drug at the receptor level. In the present study, we investigate whether morphine-induced changes in central neurotransmission processes within particular brain regions anyhow contribute to the development of tolerance to morphine's behaviour effects (section 3.1).

Intracerebral injections

Intraventricular injections of morphine (25-1000 µg) produce emesis, mydriasis, salivation, vocalizations and varying degrees of excitation (Clark and Cumby, 1978; Feldberg and Shaligram, 1972; Meglio et al., 1977; Moore et al., 1965). Low doses (25-100 µg) cause sudden quick head movements, fine head tremors, fixation of the eyes into space, and a diminished response to external stimuli; these effects are antagonized by naloxone (Meglio et al., 1977). Some animals become restless and pace throughout the cage (Clark and Cumby, 1978). Apparently, the effects are identical to those elicited by systemically administered morphine (Feldberg and Shaligram, 1972): initially, the cats are sitting on their hindquarters moving their head and forepaws. The cats stare vacantly and do not react to objects moving in front of their eyes. After some minutes a gradually increasing amount of compulsive locomotion is observed. The cats resist handling but without trying to bite or attack. The animals are not cataleptic.

Morphine (2 x 5 µg), bilaterally injected into or near the nucleus centralis oralis or the nucleus laterodorsalis tegmenti, produces behaviour inactivation (Van Dongen, 1980a). Morphine (10 µg) unilaterally injected into or near the substantia nigra causes dyskinetic movements and contralateral turning of the head (Broekkamp, 1976). A 1 hr localized

perfusion of the sensorimotor cortex with a morphine containing solution (1-5%) does not affect the behaviour of cats (Mullin and Phillis, 1974).

Intraventricular administration of β-endorphin (12.5 µg) produces within 15-20 min sudden quick head movements, head tremors, mild excitation, fixation of the eyes into space, and pupillary dilatation (Meglio et al., 1977). The cats barely respond to external stimuli and appear to have "visual hallucinations". The effects have a duration of about 1 hr and are antagonized by naloxone. Higher doses (25-50 µg) produce more pronounced effects which have a longer duration. Infusion into the third ventricle (10-20 µg) produces mydriasis, vocalizations, staring, and after a lapse of 30 min following the injection hyperexcitability and restlessness (Feldberg and Smyth, 1977). During the state of hyperexcitability, external stimuli cause the cat to make rapid, brisk movements usually leading to circling in the cage. Later on, circling occurs spontaneously. In some animals, signs of catalepsy are observed 1-1.5 hr after infusion. The effects are antagonized by naloxone.

Intraventricular injection of Met-enkephalin (100-400 µg) does not affect the behaviour of cats (Meglio et al., 1977). Infusion into the third ventricle of N-methyl-Met-enkephalinamide (150-180 µg) produces some mydriasis and, within 15 min, deep stupor and catalepsy; these effects have a duration of 1-2 hr, are antagonized by naloxone, but not mimicked by those of Met-enkephalin (30-400 µg) (Feldberg and Smyth, 1977).

As the intracerebral injection of morphine and related opioid peptides appears to be an effective tool to elicit behaviour changes, we used this technique to elucidate the nature of opiate receptors within a particular brain region (section 4.4).

Effects of drugs

High doses of the catecholamine-depleting drugs reserpine (2.5 mg/kg, given 24 hr before morphine) and tetrabenazine (75 mg/kg, given 18 hr before morphine) block the excitatory responses of cats to morphine administration (20 mg/kg) (Dhasmana et al., 1972). Lower doses of reserpine (0.1-0.5 mg/kg) administered several hours after morphine (20 mg/kg) also antagonize the behaviour excitation; in contrast, when administered several hours before morphine, these lower doses enhance the behaviour effects of morphine (Sturtevant and Drill, 1957). When a

single dose of reserpine (25-100 µg/kg) is given 90-180 min before morphine or when daily doses of reserpine are administered for 3 days (total dose 150-200 µg/kg), the last injection being given 150 min before morphine, the excitatory effects of morphine are enhanced; progressively lower doses tend to cause a greater increase in morphine's effects (Loewe, 1956). Cats pretreated with 2 intraventricular injections of 0.5 mg reserpine, one given 18 hr and the other 4 hr before an intraventricular morphine injection (0.75 mg), show enhanced excitation and some signs of catalepsy (Feldberg and Shaligram, 1972).

The dopamine antagonist chlorpromazine (2-20 mg/kg) partially antagonizes the behaviour excitation caused by morphine (20 mg/kg) (Loewe, 1956; Sturtevant and Drill, 1957). A similar result is obtained when the dopamine antagonist haloperidol is administered in a dose of 2.0 mg/kg to cats pretreated with morphine in a dose of 5 mg/kg (Cools et al., 1974) or in a dose of 0.5 mg/kg to cats treated with morphine in a dose of 20 mg/kg (Dhasmana et al., 1972). Haloperidol (50 µg) microinjected into the rostromedial part of the caudate nucleus blocks the morphine-induced behaviour when administered 20 min after morphine, but it potentiates the behaviour effects when administered 30-40 min after morphine (Cools et al., 1974).

The adrenergic blocker phenoxybenzamine (10-20 mg/kg) enhances the behaviour effects of morphine (10-20 mg/kg) given 145-210 min later (Loewe, 1956). When phenoxybenzamine (10 mg/kg) or propranolol (2.5 mg/kg) is administered 60 min before morphine (20 mg/kg), such an enhancement is not observed (Dhasmana et al., 1972). When noradrenaline (250 µg) or adrenaline (250 µg) is injected into the lateral ventricles 15 min before and 30 min after intraventricular morphine (0.75 mg), the morphine-induced excitation is greatly reduced (Feldberg and Shaligram, 1972). Noradrenaline (2 x 10 µg) bilaterally administered into the nucleus raphe linearis of cats pretreated with morphine (5 mg/kg) 20 or 30-40 min earlier completely suppresses morphine's behaviour effects (Cools et al., 1974).

The cholinergic blocker atropine (5 mg/kg) given 1 hr before morphine (20 mg/kg) does not affect the behaviour excitation (Dhasmana et al., 1972). The cholinergic agonist carbachol (2 x 2 µg) bilaterally injected into the nucleus raphe linearis enhances the morphine-induced excitation (Cools et al., 1974).

Pretreatment with the opiate antagonist naloxone (0.25-1.0 mg/kg) antagonizes the behaviour effects of morphine (1-4 mg/kg) (French et al., 1979b). Naloxone (2-10 µg) bilaterally injected into the region of the substantia nigra causes cats pretreated with morphine (5 mg/kg) to become hypoactive (Van Dongen, 1980b). Naloxone (2-10 µg) injected into the dorsolateral pontine tegmentum partially antagonizes the behaviour effects of morphine (5 mg/kg): the cats remain hyperactive but they show more "normal" behaviour activities (Van Dongen, 1980b).

Lysergic acid diethylamide (0.5 mg/kg) or the antihistaminic drug mepyramine (10 mg/kg) does not affect the excitement elicited by morphine (20 mg/kg) given 1 hr later (Dhasmana et al., 1972). Intraventricular 5-hydroxy-tryptamine (250 µg) does not affect the excitation elicited by intraventricular morphine (250 µg) (Feldberg and Shaligram, 1972). Systemic administration of 5-hydroxy-tryptophan (10 mg/kg) does not affect the behaviour effects of intraventricular β-endorphin (Hosobuchi et al., 1977). Pretreatment with the anticonvulsant drug diphenylhydantoin (30 mg/kg) given for a period of 4-6 days prior to morphine injection (15 mg/kg) antagonizes the behaviour effect of morphine (Fertiziger et al., 1974). Daily administration of cortisone (25 mg/kg) for 4 days enhances the excitatory effects of morphine (12 mg/kg) given on the 5th day (Winter and Flataker, 1951), whereas desoxycorticosterone daily given over 5-12 days in a total dose of 75-180 mg/kg diminishes the morphine-induced excitation (Loewe, 1956).

As indicated above, the morphine-induced behaviour response in cats is influenced by a wide variety of systemically or intracerebrally injected drugs. This indicates that the behaviour effects of morphine are mediated via a large number of neurotransmission processes in the brain. In other words, the behaviour effect of morphine is related to neurotransmission activity in various regions of the brain. Accordingly, changing neurotransmission activity should, in general, affect the behaviour response to morphine.

In the present study, we investigate whether pharmacological manipulation of particular neurotransmission processes in morphine-treated cats gives insight into morphine's ability to change behaviour via changing the activity of central neurotransmission processes (Chapter 3; section 4.4).

1.4 SPECIFIC EFFECTS OF MORPHINE IN MAN AND CATS

In the preceding section, overt effects of morphine have been discussed. More specific effects of the opiate agonist in man and cats are discussed in this section. For general information about the morphine-induced effects in other species the reader is referred to the following publications: Jaffe and Martin, 1975; Martin and Sloan, 1977; Pradhan and Dutta, 1977; Reynolds and Randall, 1957.

1.4.1 Analgesia

With regard to man

Analgesia is induced in man by administration of morphine in oral doses of 0.1-0.3 mg/kg (Jaffe and Martin, 1975). It is principally indicated in the treatment of constant dull or sharp pain, post-operative pain, as well as pain caused by coronary, pulmonary, or peripheral vascular trombosis, neoplasm, fractures, burns and renal or biliary colic (Pradhan and Dutta, 1977). The use of peridural or spinal opiates for analgesia without significant side-effects is promising (Behar et al., 1979; Dirksen and Nijhuis, 1980; Torda, 1979; Wang et al., 1979; Zenz, 1981).

With regard to cats

Analgesia is induced in cats by injections of morphine in doses of 0.25-4.0 mg/kg (Denavit-Saubié et al., 1978; Kayan and Mitchell, 1968; Malseed and Goldstein, 1979; Mitchell, 1964, 1966). Chronic treatment results in the development of tolerance to and dependence on this effect of morphine (Kayan and Mitchell, 1968; Mullin and Phillis, 1974); multiple injections of morphine have been reported to cause an increased rather than a decreased responsiveness to the analgesic test (Kayan and Mitchell, 1968). Pretreatment with tricyclic antidepressants enhances the antinociceptive action of morphine (Malseed and Goldstein, 1979). Analgesia is also obtained after intraventricular injections of morphine (150-200 µg) or β-endorphin (10-25 µg) or after intrathecal administration of morphine (40-80 µg) (Dey and Feldberg, 1976; Feldberg and Smyth, 1977; Meglio et al., 1977; Yaksh, 1978). Intraventricular injection of Met-enkephalin (30-400 µg) produces no or very weak analgesia; the more stable analogue N-methyl-Met-enkephalin, however, does produce analgesia after its intraventricular injection (Feldberg and Smyth, 1977; Meglio et al., 1977). Tolerance develops rapidly to the analgesia induced by intraventricular

injection of β-endorphin; the analgesia is restored if 5-hydroxy-tryptophan (10 mg/kg) is administered soon after β-endorphin (Hosobuchi et al., 1977). Analgesia is also obtained after intravenous injection of β-endorphin (Catlin et al., 1977). Injection of morphine (10 µg/kg) into the nucleus reticularis gigantocellularis causes suppression of the jaw opening reflex (Chan, 1979). Structures in the periaqueductal grey and the nucleus raphe magnus may also play a critical role in opiate-induced analgesia (Sessle et al., 1981b).

1.4.2 Respiratory depression

With regard to man

Respiratory depression is one of the most prominent actions of morphine, which, in man, reduces respiration rate, even in doses below the analgesic level. Doses only slightly larger than the therapeutic doses produce death from respiratory depression (Jaffe and Martin, 1975). Morphine depresses all components of respiratory activity, and may induce irregular breathing. Morphine depresses the response of the brain stem respiratory center to raised blood carbon dioxide tension. Voluntary control of respiration is also affected by morphine, and this is considered beneficial in the management of patients with pulmonary edema, in which the patients' effort to breath may aggrevate the underlying pathology (Pradhan and Dutta, 1977; Jaffe and Martin, 1975; Reynolds and Randall, 1957). In addicts stabilized on morphine, respiration is within normal limits; withdrawal from morphine causes an increased respiratory rate (Martin and Sloan, 1977).

With regard to cats

Morphine in doses of 1-10 mg/kg depresses respiration in cats (Flórez et al., 1968; Pentiah et al., 1966). Chronic treatment results in the development of tolerance to and dependence on this effect of morphine (Flórez et al., 1973). Morphine-induced respiratory depression is reversible by naloxone (Flórez and Mediavilla, 1977). Reserpine and para-chlorophenylalanine, but not 6-hydroxydopamine, antagonize the respiratory depression; pargyline and tranylcypramine enhance it (Flórez et al., 1973). Moreover, para-chlorophenylalanine facilitates, pargyline partially inhibits, and reserpine does not affect the development of tolerance (Flórez et al., 1973). Intravenous β-endorphin (10-500 µg/kg) does not

affect respiratory rate (Catlin et al., 1978). Morphine-induced respiratory depression is thought to be mediated via ventral surface areas in the brain stem involved in respiratory regulation (Flórez et al., 1968; Flórez and Mediavilla, 1977; Hassen et al., 1976; Pentiah et al., 1966; Pokorski et al., 1981). Morphine (10-50 µg) injected into the bulbar subarachnoid space induces respiratory depression qualitatively similar to that obtained after intravenous injection (Flórez et al., 1968). Met-enkephalin (1.6 µmol) directly applied to the ventral surface of the brain stem, consistently depresses respiration (Flórez and Mediavilla, 1977). Morphine (1-2 mg/kg, i.v.) selectively depresses the activity of pontile apneustic neurons and, to a lesser extent, of medullary inspiratory neurons in the midcollicular decerebrated cat (Hassen et al., 1976). In slightly sedated, freely moving cats or in bi-vagotomized paralysed cats, microelectrophoretically applied morphine and Met-enkephalin, and systemically administered morphine (0.1-0.5 mg/kg) depress the spontaneous and evoked firing of respiration related neurons in the region of the tractus solitarius, nucleus ambiguus and in the pons, nucleus parabrachialis medialis; these effects are antagonized by naloxone (Denavit-Saubié, 1978). Electrical stimulation of the periaqueductal grey and the nucleus raphe magnus suppresses neuronal functions associated with respiration and respiratory-related activities; as the effects were antagonized by naloxone, these neuronal systems may be involved in the opiate-induced effects on respiration (Sessle et al., 1981a).

1.4.3 Pupil reaction

With regard to man

In man, morphine produces constriction of the pupil (miosis). Tolerance to this miotic action does not develop readily, and, consequently, constricted pupils (pinpoint pupils) are observed in the morphine addict (Pradhan and Dutta, 1977; Reynolds and Randall, 1957). In cases of fatal poisoning, the pupils ultimately dilate due to asphyxia (Reynolds and Randall, 1957). Withdrawal from morphine causes mydriasis (Martin and Sloan, 1977). The precise mechanism for the miotic action is not established, but it is believed to be mediated through stimulation of the cranial nerve nucleus; miotic action is blocked by atropine and related cholinergic blocking agents (Pradhan and Dutta, 1977).

With regard to cats

In the cat, morphine in doses of 0.3 mg/kg and more, produces an opposite effect, viz. dose-dependent dilatation of the pupil (mydriasis) in a naloxone-reversible way (Denavit-Saubié et al., 1978; Echols and Jewett, 1972; Labrecque and Domino, 1974; Sturtevant and Drill, 1957; Wallenstein, 1979; Wallenstein and Wang, 1979; Wikler, 1944). Tolerance to this effect of morphine does not develop rapidly (Labrecque and Domino, 1974), but has been reported to occur (Huidobro and Lewin, 1969; Mullin and Phillis, 1974). Withdrawal from morphine causes miosis (French et al., 1979b; Huidobro and Lewin, 1969; Labrecque and Domino, 1974; Mullin and Phillis, 1974). Pupillary dilatation is also evoked by intraventricular injections of morphine (25-1250 µg), Met-enkephalin (1-2 mg), (D-Ala^2)-Met-enkephalinamide (3.1-400 µg), or β-endorphin (12.5 µg) (Clark, 1977; Clark and Cumby, 1978; Clark and Ponder, 1980; Meglio et al., 1977; Moore et al., 1965). Intravenous β-endorphin (10-500 µg/kg) does not produce mydriasis (Catlin, 1978). Morphine-induced mydriasis is accompanied by an increased firing rate of all light-sensitive, pupilloconstrictive neurons recorded from the pretectal region and the anterior oculomotor nucleus including the nucleus of the third nerve in sedated, immobilized cats (Wallenstein and Wang, 1979). Intravenously or topocally applied phenoxybenzamine antagonizes morphine-induced mydriasis indicating a peripheral source of sympathetic input as the basis for morphine-induced mydriasis (Wallenstein and Wang, 1979). In adrenalectomized animals, morphine (2 mg/kg) produces miosis instead of mydriasis but it still increases the firing rate of the light-sensitive neurons mentioned above (Wallenstein and Wang, 1979). These observations suggest that, in the cat, morphine activates the oculomotor nerve to produce miosis, but that this effect is masked by the morphine-induced release of catecholamines, mainly from the adrenal glands, which produce mydriasis (Wallenstein and Wang, 1979). Morphine-induced mydriasis is blocked by two intraventricular injections of reserpine (0.5 mg), one given 18 hr and the other given 4 hr before morphine (Feldberg and Shaligram, 1972). Morphine-induced mydriasis is not affected by systemic injections of chlorpromazine (20 mg/kg) (Sturtevant and Drill, 1957). It is worth mentioning that morphine-induced mydriasis is also commonly observed in other species (e.g. pigs, cows, sheep, goats, lions, tigers, bears and horses) in which morphine produces excitation of behaviour as

well (Jaffe and Martin, 1975; Pradhan and Dutta, 1977). Monkeys show also morphine-induced mydriasis but not the morphine-induced excitation of behaviour (Jaffe and Martin, 1975). Anyhow, behaviour excitation is not the cause of the morphine-induced mydriasis since anaesthetized cats also show pupillary dilatation after morphine administration (Wallenstein, 1979).

1.4.4 Emesis

With regard to man

Vomiting often occurs as undesirable side-effect of morphine action in man; the incidence of this side action is much greater in ambulatory patients (Reynolds and Randall, 1957).

With regard to cats

Morphine in doses higher than 0.3 mg/kg readily produces emesis in the cat (Reynolds and Randall, 1957). Vomiting is also observed after intravenous injections of β-endorphin (higher than 50 μg/kg) or after intraventricular injections of morphine (50-1000 μg), Met-enkephalin (1-2 mg) or (D-Ala^2-)-Met-enkephalinamide (50-400 μg) (Catlin et al., 1978; Clark, 1977; Clark and Cumby, 1978; Clark and Ponder, 1980; Costello and Borison, 1977; Moore, 1965). Tolerance develops rapidly to the emetic action of morphine (Costello and Borison, 1977; Labrecque and Domino, 1974). Lesions of the area postrema suppress morphine's emetic action (Moore, 1965). Apart from an emetic action morphine has also a late anti-emetic action: the emetic action is thought to be mediated via excitation of the medullary emetic chemoreceptor triggerzone and the anti-emetic action via the vomiting center within the medullary reticular formation (Costello and Borison, 1977).

1.4.5 Temperature response

With regard to man

In man, at therapeutic dosage morphine slightly reduces body temperature, but continued administration of higher doses may cause a hyperthermic response (Pradhan and Dutta, 1977). Withdrawal from morphine produces an increase in body temperature (Martin and Sloan, 1977).

With regard to cats

In the cat, morphine, administered in doses of 1-10 mg/kg, produces

dose-related hyperthermia; doses of 0.25-0.50 mg/kg do not affect body temperature (Clark and Cumby, 1978; French et al., 1978a, b). Chronic treatment results in the development of tolerance to and dependence on this effect of morphine (French et al., 1978b). The hyperthermic response is blocked by ergotamine (Banerjee et al., 1968), and enhanced by the dopamine antagonist pimozide or the serotonin uptake inhibitor fluoxetine (French et al., 1978a). Neither metiamide nor indomethacin do affect morphine-induced hyperthermia (Clark and Cumby, 1978). Intraventricular administration of morphine (2.5-1250 µg), β-endorphin (5-50 µg), Met-enkephalin (1-2 mg), or (D-Ala^2-)-Met-enkephalinamide (3.1-50 µg) also produces hyperthermia; higher doses of (D-Ala^2-)-Met-enkephalinamide (200-400 µg) result in a diminished hyperthermia and sometimes in hypothermia (Banerjee et al., 1968; Clark, 1977; Clark and Cumby, 1978; Clark and Ponder, 1980). Differences in the thermoregulatory effects of various opiate agents indicate the involvement of multiple types of opiate receptors (Clark and Ponder, 1980). Just as morphine-induced mydriasis, morphine-induced hyperthermia is commonly observed in other species in which morphine produces excitation of behaviour as well. Morphine-induced behaviour excitation is certainly not the cause of the hyperthermia, since the hyperthermic response to morphine is also observed in paralysed, sedated cats (Wallenstein, 1978).

1.4.6 EEG effects

With regard to man

In man, single therapeutic doses of opiates produce synchronization of the EEG, i.e. a shift towards increased voltage and lower frequencies, such as occurs in natural sleep or after a low dose of barbiturates (Jaffe and Martin, 1975). In post-addicts, morphine decreases REM (rapid-eye-movement) sleep, increases non-REM light sleep, and decreases non-REM deep sleep. It increases the waking state. The initial decrease in the REM state was followed by an increase in the third night in some subjects (Kay et al., 1969).

With regard to cats

In awake, unrestrained cats, morphine (1 mg/kg) produces cortical EEG activation, i.e. increased frequency and decreased amplitude; in awake, paralysed animals, morphine (10 mg/kg) causes convulsant EEG patterns in the hippocampus (Navarro and Elliott, 1971). Cats are awake for about

6 hr following administration of morphine (0.3 mg/kg, s.c.); the return to regular sleep patterns occurs after about 11 hr. A rebound increase in REM sleep is noted 11-17 hr after the morphine administration. The alerting action of morphine is blocked by naloxone, but not by α-methyltyrosine or 5-hydroxy-tryptophan (Echols and Jewett, 1972). In brain stem-transected cats, the effect of morphine (0.31-10.0 mg/kg) varies with the EEG pattern present prior to morphine administration: in EEG activated cats, no obvious EEG modification is observed while, in EEG synchronized animals, morphine causes an activation (Aiello-Malmberg, 1979; Labrecque and Domino, 1974). With larger doses of morphine, EEG activation is less consistent than with lower doses (0.31-1 mg/kg) (Labrecque and Domino, 1974). Chronic morphine treatment results in the development of tolerance to and dependence on the morphine-induced EEG activation (Labrecque and Domino, 1974). In cats trained to press for milk reward, morphine (0.1-0.4 mg/kg) causes a strong monophasic enhancement of post-reinforcement EEG synchronization (PRS); higher doses (0.6-1.0 mg/kg) have a biphasic action, viz. initial enhancement followed by strong suppression; doses of 1.0-1.2 mg/kg predominantly suppress PRS (Marczynski and Hackett, 1976).

1.4.7 Cardiovascular effects

With regard to man

In man, therapeutic doses of morphine produce little or no effect on cardiovascular function. Cardiac responses to morphine in patients with myocardial infarction are inconsistent and often produce pronounced hypotension and bradycardia. Hypovolemic patients are more susceptible to the vasodepressor action of morphine (Jaffe and Martin, 1975; Pradhan and Dutta, 1977). In addicts stabilized on morphine, heart rate and blood pressure are within normal limits; withdrawal from morphine causes an increase in blood pressure (Martin and Sloan, 1977).

With regard to cats

In unanesthetized cats, small doses of morphine (0.5-2.0 mg/kg) produce a fall in blood pressure whereas higher doses (2-10 mg/kg) mainly produce an increase in blood pressure (Feldberg and Wei, 1977; Grundy, 1971; Kayaalp and Kaymakcalan, 1966). The morphine-induced fall in blood pressure, which is accompanied with mild bradycardia, is also

observed after intracisternal administration (400 µg) but not after intraventricular administration (Feldberg and Wei, 1977; Grundy, 1971). The morphine-induced vasodepression is still present after cutting both vagi and is antagonized by naloxone (Feldberg and Wei, 1977). It is thought that the vasodepression and bradycardia result largely from removal of sympathetic tone to blood vessels and heart (Feldberg and Wei, 1977). The hypertension observed after higher doses is blocked by ganglion-blocking and sympathetic α-receptor blocking agents, and absent in spinal cats (Kayaalp and Kaymakcalan, 1966). In anaesthetized cats, morphine (2-10 mg/kg) causes a fall in blood pressure, which is antagonized by nalorphine (Kayaalp and Kaymakcalan, 1966). In brain stem-transected cats, morphine (0.31-10 mg/kg) causes a dose-related hypotension and a decreased heart rate; tolerance develops to these effects of morphine (Labrecque and Domino, 1974). In sedated, paralysed cats, the cardiovascular effects of morphine appear to be biphasic in that small doses (1-2 mg/kg) lower blood pressure and heart rate while larger doses (8-12 mg/kg) increase blood pressure and heart rate (Wallenstein, 1979). In adrenalectomized cats, small doses of morphine (1-2 mg/kg) decrease, but - in contrast to in unadrenalectomized cats - larger doses (8-12 mg/kg) do not longer increase blood pressure and heart rate (Wallenstein, 1979). This latter observation suggests a role for the adrenals in the excitatory phase. As a dose of 1 mg/kg of morphine affects blood pressure but not heart rate, these effects of morphine seem to be independent (Wallenstein, 1979). Naloxone antagonizes the cardiovascular effects of low doses of morphine, but enhances those of larger doses of morphine (Wallenstein, 1979). Structures at the dorsal surface of the medulla and in the nucleus gigantocellularis reticularis have been suggested as site of action for the cardiovascular effects of morphine (Feldberg and Wei, 1978; Hwa and Chan, 1981).

1.4.8 Effects on endocrine glands

With regard to man

Single or repeated administration of opioids has been shown to exert profound effects on hypothalamo-hypophysial target gland areas (George, 1971; Meites et al., 1979; Sloan, 1971). In man, acute single doses of morphine are either without effect or produce a liminal depression of corticosteroid excretion. Chronic morphine administration results in

suppression of corticosteroid excretion to which partial tolerance develops. The early abstinence syndrome in man is associated with signs of adrenal cortical stimulation, whereas during protracted abstinence the urinary levels of corticosteroids are normal. The morphine-induced effect on corticosteroid secretion is thought to be mediated via an altered release of adrenocorticotrophic hormone (Meites et al., 1979; Sloan, 1971). Acute administration of an opiate to post-addicts suppresses luteinizing hormone levels; chronic treatment is associated with decreased testosterone levels (Mendelson et al., 1980).

With regard to cats

In the cat, acute administration of morphine (1-10 mg/kg) increases adrenal corticosteroid release, while chronic morphine administration results in a reduced increase or even in a decrease of adrenal corticosteroid release (Borrell et al., 1975; French et al., 1979a; Guaza et al., 1979). Acute morphine (1-4 mg/kg) also causes elevated plasma levels of growth hormone (French et al., 1979a). The increase in growth hormone levels decreases as the dose of morphine is increased, which indicates that the effectiveness for growth hormone release is inversely related to morphine doses. Tolerance does not develop to the morphine-induced growth hormone release (French et al., 1979a). Opiate antagonist-induced withdrawal in tolerant cats results in an increase in corticosteroid secretion but it does not change growth hormone levels (Borrell et al., 1975; French et al., 1979a; Guaza et al., 1979). In the cat, single doses of morphine (10-60 mg/kg) decrease catecholamine levels in the adrenal medulla and increase catecholamine levels in blood and urine (Borrell et al., 1974; Elliott, 1912; Gunne, 1963; Maynert, 1967; Vogt, 1954; Way and Shen, 1971). In the anaesthetized cat, this effect of morphine remains absent (Borrell et al., 1974). When noradrenaline and adrenaline are evaluated separately, the effect of morphine on adrenomedullary function depends on the stage of morphine treatment (Guaza et al., 1979). Single doses of morphine (10 mg/kg) decrease the adrenal level of both catecholamines. No variations in adrenaline/noradrenaline ratio are observed. After 7 days of daily morphine (10 mg/kg) an increase in adrenal noradrenaline level is found together with a decrease in adrenal adrenaline level and a decrease in

the adrenaline/noradrenaline ratio. After 2 weeks of daily morphine treatment the adrenal contents of both catecholamines are again within the range of control animals. Nalorphine injection at this stage of morphine treatment results in increased levels of noradrenaline but decreased levels of adrenaline and a lower adrenaline/noradrenaline ratio. After 1 month of daily morphine, the adrenal levels of both catecholamines are elevated; no change in the adrenaline/noradrenaline ratio is observed. Borison et al. (1962) and Moore et al. (1965), attributed the release of catecholamines from the adrenal medulla after morphine treatment to the stimulation of chemoreceptors in the brain stem. These chemoreceptors are thought to be localized at the ventral surface of the brain stem most probably in the upper part of the medulla oblongata (Dey et al., 1975; Feldberg and Gupta, 1974).

1.4.9 Hyperglycaemia

With regard to man

Morphine produces transient hyperglycaemia (elevation of blood glucose) in humans, resulting from mobilization of liver glycogen through release of adrenaline from the adrenals (Pradhan and Dutta, 1977).

With regard to cats

In the cat, morphine (10 mg/kg) also produces hyperglycaemia (Borrell et al., 1975; Moore et al., 1965). Borison et al. (1962) and Moore et al. (1965) described a chemoreceptor in the brain stem which when activated by morphine causes the release of adrenaline and noradrenaline from the adrenal medulla, which is responsible for the increase in blood sugar. Hyperglycaemia is also produced by morphine (0.75 mg) or β-endorphin (60 µg) injected into the cerebral ventricles (Dey et al., 1975; Feldberg and Gupta, 1974; Feldberg and Smyth, 1977). The chemoreceptors are thought to be localized at the ventral surface of the brain stem, most probably in the upper part of the medulla oblongata (Dey et al., 1975; Feldberg and Gupta, 1974). Morphine-induced hyperglycaemia is reduced by intraventricular injection of 5-hydroxy-tryptamine, noradrenaline and adrenaline; reserpine pretreatment accentuates morphine-induced hyperglycaemia (Feldberg and Shaligram, 1972).

1.4.10 Comments

Various effects produced by morphine in man and the cat are discussed in detail above and summarized in Table 1.2. Most of the effects are similar in both man and cat. Moreover, the differences that exist between both species in morphine-induced effects seem to be not fundamental, but rather the consequence of differences between both species in the ratio of adrenal catecholamine levels as argued by the following. In such species as cats, which respond to morphine with excitation of behaviour, the ratio between the levels of adrenaline and noradrenaline within the adrenal gland is smaller than in such species as rats or humans, which respond to morphine with sedation of behaviour (Guaza et al., 1979). As morphine affects adrenal activity, and adrenaline and noradrenaline have differential effects on the various biological subsystems within the organism that are involved in the other morphine-induced effects, it is evident that differences between species in the ratio of adrenal catecholamine levels may underly differences in the various morphine-induced effects. This is on line with the observation

TABLE 1.2

Morphine-induced effects in man and cat

effects	man	cat
analgesia	+	+
behaviour activity	↓(occ. ↑)	↓→↑
blood pressure	occ. ↓	↓→↑
body temperature	↓→↑	↑
corticosteroid excretion	occ. ↓	↑
emesis	+	+
heart rate	occ. ↓	↓→↑
hyperglycaemia	+	+
physiological dependence	+	+
psychological dependence	+	+
pupillary diameter	↓	↑
respiratory depression	+	+
tolerance	+	+

+ = present; ↑ = increase; ↓ = decrease; →=higher doses; occ. = occasionally

that hypertension and mydriasis are not obtained by morphine administration to adrenalectomized cats (sections 1.4.3 and 1.4.7).

The similarity of the morphine-induced effects in man and the cat argues for the use of cats as an experimental animal for the study of morphine's mechanism of action. We have selected the cat as an experimental animal hoping that insight into morphine's actions in this species will lay a solid foundation for the understanding of morphine's actions in man.

1.5 CENTRAL EFFECTS OF MORPHINE IN CATS

The central effects of morphine in cats are discussed in this section. For information about the central effects of morphine in other species the reader is referred to the following publications: Brase, 1979; Clouet and Iwatsubo, 1975; Domino et al., 1976; Domino, 1979; Iwamoto and Way, 1979; Martin and Sloan, 1977; North, 1979).

1.5.1 Effects on neurotransmission processes

Acetylcholine

Morphine (0.5-5 mg/kg) enhances the release of acetylcholine from the cerebral cortex and into the cerebral ventricles of unanaesthetized cats; the effect is antagonized by naloxone, and chronic morphine treatment results in the development of tolerance and dependence (Mullin et al., 1973; Mullin and Phillis, 1974; Phillis et al., 1973). The effect on cortical acetylcholine release appears to be mediated subcortically as a one hour perfusion of the sensorimotor cortex with a 1-5% solution of morphine is ineffective (Mullin and Phillis, 1974). In the anaesthetized brain stem transected cat, morphine (0.1-5 mg/kg) depresses the release of acetylcholine from the cerebral cortex and into the cerebral ventricles; this effect is antagonized by naloxone (Beleslin and Polak, 1965; Jhamandas et al., 1971). In the unanaesthetized brain stem transected cat, the effect on cortical acetylcholine release appears to be dose-dependent, in that doses of 0.3-5.6 mg/kg or less yield depression, whereas higher doses (10-15 mg/kg) produce enhancement of acetylcholine release; chronic morphine treatment results in the development of tolerance to and dependence on both the increasing and

decreasing action of morphine (Labrecque and Domino, 1974; Mehta, 1975). Morphine (1-5 mg/kg) produces a dose-dependent and naloxone-reversible depression of both the resting and evoked release of acetylcholine from the caudate nucleus of the unanaesthetized brain stem transected cat (Yaksh and Yamamura, 1975). Morphine-induced behaviour excitation in the cat appears to be related to increased release of brain acetylcholine (Domino et al., 1976).

Noradrenaline

Vogt (1954) reports that morphine (30-60 mg/kg) decreases noradrenaline levels in the brain of cats. These findings are confirmed by Gunne (1963), Laverty and Sharman (1965), Maynert (1967), and Reis et al. (1969). Reis et al. (1969) show that morphine decreases levels of noradrenaline in the medial hypothalamus, the midbrain, cerebellum, and cervical cords but that it increases levels in the mammillary bodies and globus pallidus. Reduced levels of noradrenaline in the hypothalamus are obtained after intravenous (10 mg/kg), intraventricular (1 mg), but not intracisternal (1 mg) administration of morphine (Moore et al., 1965). Chronic morphine treatment results in the development of tolerance to the effect of morphine on brain noradrenaline (Gunne, 1963).

Dopamine

Intraventricular (1 mg) or systemic (10-30 mg/kg) administration of morphine to cats does not affect dopamine levels within the caudate nucleus; but, as it increases homovanillic acid levels, it may increase dopamine turnover (Laverty and Sharman, 1965; Moore et al., 1965). Indeed, locally administered opiate agents stimulate the *in vivo* release of dopamine from the caudate nucleus (Chesselet et al., 1981).

Serotonin

Morphine (30 mg/kg) slightly increases the content of serotonin and its metabolite 5-hydroxy-indoleacetic acid in the thalamus of the cat (Laverty and Sharman, 1965). Morphine (6 mg/kg) increases the release of 5-hydroxy-tryptamine from the cerebral cortex and the caudate nucleus of brain stem transected cats (Aiello-Malmberg, 1979).

1.5.2 Electrophysiological responses

Cerebral cortex

Administration of morphine (2 mg/kg) to immobilized cats has little effect on somatically evoked responses in the neocortex (McKenzie, 1964). Iontophoretic application of morphine or naloxone does not affect the spontaneous or acetylcholine-induced firing of neurons in the neocortex of anaesthetized or decerebrated cats (Duggan et al., 1976). Microiontophoretic administration of morphine excites cholino-excitable neurons in the cerebral cortex of decerebrated cats, but this effect is not antagonized by naloxone (Bioulac et al., 1975). Morphine (2 mg/kg) depresses somatically evoked responses, but it does not affect septally evoked responses in the hippocampal formation of immobilized cats (McKenzie, 1964). Nakamura and Mitchell (1972) demonstrate in a more detailed study that administration of morphine (1-4 mg/kg) to immobilized cats does not affect hippocampal or entorhinal excitability but that it reduces sensory input into the entorhinal area. Administration of morphine (0.5-2.0 mg/kg) to immobilized cats augments the spontaneous firing of hippocampal neurons; this effect is antagonized by naloxone (Chou and Wang, 1977). Microinjections of Leu-enkephalin produce epilepticformic changes in the hippocampal EEG of spinal cats which are antagonized by naloxone; tolerance develops rapidly to this opioid peptide-induced effect (Elazar et al., 1979).

Forebrain

Administration of morphine (0.5-2.0 mg/kg) to immobilized cats augments the spontaneous firing rate of neurons in the amygdala, lateral hypothalamus, caudate nucleus and septum (Chou and Wang, 1977; Robinson and Wang, 1979). Electrophoretic administration of morphine to decerebrated or anaesthetized cats excites neurons in the ventrobasal thalamus that have nicotinic receptors for acetylcholine; this effect is antagonized by naloxone (Duggan et al., 1976). In anaesthetized, immobilized cats, morphine (1-2 mg/kg) blocks the bradykinin-induced increase in thalamic unit activity (Lim et al., 1969). Electrophoretic administration of morphine to anaesthetized, immobilized cats depresses the glutamate-induced, the noxious stimuli-induced, and the spontaneous neuronal activity but not the acetylcholine-induced neuronal activity in the medial thalamus; naloxone does not antagonize this effect of

morphine but, in contrast, mimicks it (Duggan and Hall, 1977). Intravenous administration of morphine (0.5-1.5 mg/kg) mimicks electrophoretic administration of morphine in its effect on glutamate-induced neuronal activity in the medial thalamus, but it has inconsistent effects on the spontaneous and the noxious stimuli-induced firing of medial thalamic neurons. The effect of glutamate-induced firing is antagonized by naloxone (Duggan and Hall, 1977).

Midbrain

Administration of morphine (1-4 mg/kg) to immobilized cats depresses the somatically evoked responses in the ventral tegmental area, paralemniscal area, central grey, and mesencephalic reticular formation (McKenzie, 1964; Straw and Mitchell, 1964). Neurons in the periaqueductal grey are inhibited by iontophoretic administration of morphine, α-endorphin or Met-enkephalin; some of the neurons are excited, with or without a preceding inhibitory phase, particularly by morphine and α-endorphin (Gent and Wolstencroft, 1979b).

Hindbrain

Iontophoretic application of morphine, Met-enkephalin or α-endorphin inhibits neurons in the reticular formation of medulla and pons, and in the nucleus raphe magnus of unanaesthetized, decerebrated cats (Gent and Wolstencroft, 1976b). Iontophoretic administration of morphine, Met-enkephalin or Leu-enkephalin depresses also the firing rate of neurons in the nuclei reticularis gigantocellularis and pontis caudalis of unanaesthetized, decerebrated cats. None of these responses is blocked by naloxone (Gent and Wolstencroft, 1976a). Intravenous administration of morphine (1-4 mg/kg) excites medullary raphe neurons of decerebrated or anaesthetized cats. This effect is antagonized by naloxone (Anderson et al., 1977). Iontophoretic application of enkephalin reduces the spontaneous and noxious stimuli-induced firing of nociceptive neurons in the trigeminal nucleus caudalis of decerebrated cats. This effect is antagonized by naloxone (Anderson et al., 1978). Morphine (0.25-1.0 mg/kg) inhibits the spontaneous activity of cells in the locus coeruleus of anaesthetized, immobilized cats in a naloxone-reversible way; moreover, systemic administration of naloxone suppresses the inhibitory responses of locus coeruleus cells to stimulation of the hypothalamic arcuate nueleus, i.e. the origin of β-endorphin containing neurons

afferent to the locus coeruleus (Strahlendorf et al., 1980). Electrophoretic administration of morphine or Met-enkephalin depresses the firing of respiration related neurons in the region of the tractus solitarius, the nucleus ambiguus and in the pons, the nucleus parabrachialis medialis; these effects are mimicked by those of intravenous administration of morphine (0.1-0.5 mg/kg) and antagonized by naloxone (Denavit-Saubié, 1978).

1.5.3 Comments

Morphine produces a wide variety of effects, which are used and abused by the human being. Most of these effects are centrally mediated (sections 1.3 and 1.4). More knowledge about morphine's central action is a prerequisite to achieve an improved therapeutic use and a decreased abuse of morphine by man. We have chosen the cat as experimental animal in our studies on the central action of morphine. In section 1.4, we have already emphasized that morphine's actions in man and cats share many common features. The relatively large dimensions of the feline brain, together with the relatively high sensitivity of the cat to morphine, are considered as additional advantages of the use of cats in the study of morphine's central action. On the other hand, our knowledge about morphine's central action in cats is rather limited as discussed in the previous sections. Although there is already some evidence that at least cholinergic, noradrenergic, dopaminergic and serotonergic processes are involved in the central actions of morphine (see sections 1.5.1, 1.4), it is not yet possible to draw definite conclusions because of the following reasons: 1) The great variety of doses, routes of administration and experimental conditions preclude any comparison between the outcome of the available studies, 2) Morphine-induced changes within the brain are rarely analyzed in terms of being consequences of a direct or indirect action of morphine, 3) Morphine-induced changes are only partly analyzed in terms of being specific or not, 4) Almost no attention is paid to the time-course of the morphine-induced changes. In the present study, we pay particular attention to these factors. In all experiments, dose, route of administration and experimental conditions are fully standardized according to the outcome of pilot experiments. If necessary, experiments are included in order to separate direct and indirect actions of morphine and/or to establish the specificity of the

effects elicited. And, finally, attention is paid to effects appearing at different time-intervals after the administration of morphine.

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CHAPTER 2

MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL FOR THE STUDY OF CENTRAL NEUROTRANSMISSION PROCESSES

2.1 INTRODUCTION

Behaviour - considered as the dynamic process by which the living organism attempts to control its input signals by output actions (Cools, 1981; Powers, 1973) - is mediated via a large number of neuronal processes within the brain. Accordingly, changing the activity of these central processes will, in general, affect behaviour. This relationship between behaviour and central neuronal activity enables investigators to use behaviour as an experimental variable for the study of central processes. Most of these central processes are neurochemically regulated. Accordingly, the activity of these processes can be changed experimentally by intracerebral injection of drugs that have the ability to interfere specificly with these processes. The aim of the present thesis is to investigate, whether the morphine-induced behaviour of cats, in combination with intracerebral injections of drugs, may be a suitable method for the study of the functional and pharmacological properties of neurotransmission processes in the brain.

Intracerebral injection of drugs is chosen as a method to change the activity of central neurotransmission processes. In contrast to other techniques such as lesions or electrical stimulation, the intracerebral injection technique offers the possibility to affect single neuronal systems, rather than whole brain structures. These neuronal systems can be characterized both pharmacologically, taking advantage of the specificity of the intracerebrally injected drugs, and anatomically by histological verification of the injection sites.

Behaviour is chosen as an experimental variable for the study of central neurotransmission processes. Accordingly, the experiments are performed *in vivo* in unanaesthetized animals. As a direct consequence of the use of behaviour as experimental variable, only neurotransmission

processes can be studied that are somehow connected with behaviour. Knowledge of the functional and pharmacological properties, especially of these processes is of interest as it indicates, 1) which neuronal systems may play a critical role in disturbed behaviour, and 2) how the activity of these systems may be changed pharmacologically to prevent or cure disturbed behaviour.

Several test situations are available for the disclosure of experimentally-evoked changes in behaviour. Such gross behaviour effects as excitation, sedation, aggression, convulsions, tremors, defaecation, emesis, or urination can be disclosed in the open-field test situation. More subtle changes in behaviour, e.g. dealing with the organization of complex behaviour, are less evident in the open-field, and require a more specific test situation. In the present study, drug-induced behaviour is chosen. This drug-induced behaviour has the advantage compared to other test situations, such as avoidance behaviour or operant responding, that it can be studied in acute experiments without any preceding training trials.

On the basis of its highly structured organization and characteristic behaviour patterns, the morphine-induced behaviour of cats may serve as a suitable tool for the disclosure of experimentally-evoked changes in behaviour as will be argued below. An advantage of cats as experimental animals is that the larger dimensions of their brains compared with those of other laboratory animals, such as mice or rats, allow more definite conclusions about any specific location of the intracerebrally evoked effects. Apart from providing a suitable test situation, the morphine treatment offers also the possibility to study morphine's action on central processes.

Experimentally-evoked changes in the behaviour of morphine pretreated cats have been reported previously (Dhasmana et al., 1972; Feldberg and Shaligram, 1972; Fertiziger et al., 1974; French et al., 1979; Loewe, 1956; Sturtevant and Drill, 1957; Wikler, 1944; Winter and Flataker, 1951). In general, these authors used such terms as "frightened", "avoids handling", "rage", "mania", and "hyperactivity" to describe the morphine-induced behaviour. On the basis of the results of Cools et al. (1974), we have developed a more detailed description of the morphine-induced behaviour of cats. This detailed description, which uses both qualitative and quantitative measures, enables us to disclose

subtle changes in behaviour following the intracerebral injection of drugs.

Before considering both qualitative and quantitative aspects of the morphine-induced behaviour of cats in section 2.3, resp. 2.4, a general outline of the experimental procedure is given in section 2.2.

2.2 EXPERIMENTAL PROCEDURE

Only general aspects of the procedure are mentioned below; details of the procedure relevant to the individual experiments are described under the headings Materials and Methods in the various experimental sections.

Cats of both sexes are used. They are treated with an intraperitoneal injection of morphine (5 mg/kg) and observed for a period of 60 min immediately following the injection. Drugs are intracerebrally injected at variable time intervals after morphine is administered. The intracerebral injections are performed within a 2 min period during which the behaviour is not analyzed. The experiments are performed between 8.00 A.M. and 4.00 P.M.

The morphine-induced behaviour of cats greatly depends on the external stimulation in which it develops (Cools et al., 1974). Therefore, it may be useful to note that the experiments on morphine-pretreated cats described in this thesis are performed under strict experimental conditions:

1. Care is taken to prevent environmental changes during the course of the experiments. For instance, the experimental cage is placed in a separate room protected against disturbing influences such as changes in the environment, and observations are made via closed circuit television.
2. The cats are habituated to the experimental cage during 2 separate periods of 1 hr each and during a 15 min period immediately preceding each experiment.
3. The dimensions of the experimental cage are rather small (75 x 75 x 80 cm, or 88 x 66 x 61 cm).
4. The experiments are performed with a single animal in the cage in the absence of food, drinking water and a cat-box.

2.3 A DESCRIPTION OF THE BEHAVIOUR

In this section, a general description is given of the behaviour effects of morphine in cats under the experimental conditions mentioned above. This description partly replicates and partly extends the results of Cools et al. (1974).

During the first minute following morphine administration, the cat is slightly excited due to the injection itself: the cat is alert, paces throughout the cage, and may sometimes stand against the cage door in attempt to escape. Within 2 min after the injection, however, the morphine-induced behaviour changes appear, ushered in by repetitive movements of the mouth. These repetitive mouth movements, which appear in almost all animals, are particularly pronounced 2-4 min after the injection, but they may be present during the whole observation period of 60 min. In a small percentage of the animals, effects such as emesis, defaecation, and/or urination take place within the first 5 min following morphine administration accompanied with the corresponding species-specific behaviour phenomena. Gradually, the excitation diminishes, the cat lies down, and it adopts either the crouching or bending down posture. The animal becomes relatively hypoactive as measured by low frequencies of head movements, limb movements, and postural changes. This period, which is called the depression phase (Cools et al., 1974), continues until 10-15 min after the morphine injection.

At 10-15 min after morphine administration, the second behaviour phase or re-organization phase begins (Cools et al., 1974). During this phase of the morphine-induced syndrome, the cat becomes more and more active, gradually extending its behaviour activities. Head movements, limb movements and postural changes appear in succession. The movements, which are initially performed in a regular, fluent way, rapidly lose their normal character and are replaced by a great number of staccato-like motor elements which increase in rate and frequency and are of a disorderly character: in other words, it starts to re-organize its ongoing behaviour. When the animal is lying, it stands up and adopts a sphinx-like posture moving its head abruptly from one direction to another. Meanwhile, mydriasis has developed and the cat stares vacantly with its pupils maximally dilated and its eyes wide open. The animal appears alert, and it seems to track objects visually which are not present. The cat frequently raises, stretches, or shakes

its forepaws. Next, sideward movements appear: the cat scarcely raising its hindquarters, places its forepaws first to one, then to the other side. Somewhat later, such sideward movements lead to circling around stationary hindlimbs. At first, the cat circles once, and then sits down again; later on, it circles several times without stopping. The circling goes on with increasing speed, whilst the periods of circling become longer and/or more frequent. Apart from or instead of circling, some cats develop an alternative locomotor pattern, viz. running from one side of the cage to the other side, sometimes jumping against the walls. Again, the frequency of running gradually increases. Between periods of locomotion, the cat nearly always resumes its original sphinx-like posture moving its head from one direction to another and frequently raising, shaking or stretching its forepaws.

At about 30 min after morphine administration, the behaviour is stabilized: when time progresses, a restricted number of behaviour patterns is regularly repeated. This stabilized behaviour phase is called ritualization phase (Cools et al., 1974). During this period, the cat neither recognizes, nor friendly responds to the observer when he is approaching, nor does follow an object moving in front of its eyes. The morphine-induced behaviour patterns and movements displayed during this period are characteristic in the sense that they are:

1. Ritualized, i.e. restricted to a certain number of senseless behaviour patterns which are repeated in random order at variable time intervals, and
2. Stereotyped, i.e. the way in which single units are integrated into spatio-temporal patterns is completely fixed.

This ritualization period outlasts the 60 min observation period.

The morphine-induced syndrome is sometimes accompanied with mewing or salivation; profuse salivation may occur after multiple morphine injections. Grooming, washing, scratching or sleeping, which is frequently observed in control cats, is never or almost never seen in morphine-treated cats. Fragments of such behaviour, which are performed in an abortive way, are occasionally observed. The cats neglect their own vomits and faeces.

Summarizing, the morphine-induced behaviour of cats consists of three distinct behaviour phases, i.e. depression, re-organization, and ritualization, which appear in succession. Depression - ushered in by the occurrence of repetitive mouth movements, vomiting, defaecation, and/or urination - is characterized by hypoactivity and a crouching or bending down posture. Re-organization is characterized by the appearance of staccato-like head movements, limb movements, and postural changes which rapidly increase in rate and frequency; the movements are initially of disorderly character but become integrated into more complex behaviour patterns. Ritualization is characterized by the performance of ritualized and stereotyped behaviour patterns at a stable level. The morphine-induced movements and behaviour patterns are individual-specific: the exact nature and the intensity of the behaviour differs between animals. Some animals develop vigorous motor activity, other animals show only movements of head and limbs maintaining their sphinx-like posture. The sequence of behaviour events following morphine administration is relatively constant over the animals in spite of the individualistic differences in the intensity of the morphine-induced behaviour phenomena.

2.4 THE ANALYSIS OF THE BEHAVIOUR

A general description of the behaviour analysis is given in this section; more details are given under the headings Materials and Methods in the various experimental sections.

We have developed a detailed description of the morphine-induced behaviour of cats, which enables us to disclose subtle, experimentally-evoked changes in their behaviour. As indicated in the previous section, the morphine-induced behaviour consists of three distinct behaviour phases which appear in succession: depression, re-organization and ritualization. The development of these phases is described both qualitatively and quantitatively.

1. Qualitative description. The behaviour is described according to a standard ethogram (see section 2.5). Individual-specific behaviour patterns are described in detail. Special attention is paid to the spatio-temporal pattern of the behaviour. The presence of the mor-

phine-induced phases is determined by the various qualitative characteristics of these phases, mentioned in section 2.3.

2. Quantitative description (Figs. 3.1, 3.12 and 4.3). Frequencies of various behaviour items, e.g. head movements, limb movements, postural changes, locomotor patterns, etc. are recorded per min and are plotted as function of time. The presence of depression is characterized by low frequencies, the presence of re-organization by increasing frequencies, and the presence of ritualization by high, stabilized frequencies of the behaviour items.

Thus, both qualitative and quantitative measures are used to determine the presence of depression, re-organization and ritualization.

Experimentally-evoked changes in the morphine-induced behaviour may appear from the following:

1. Changes in the onsets of the behaviour phases, which are measured by averaging the points of time at which the frequencies of the behaviour items start to increase (onset re-organization = end depression) or become stabilized (onset ritualization = end re-organization).
2. Changes in the intensity of the behaviour phases, which are measured by changes in the frequencies of the various behaviour items with or without the display of new stereotyped and ritualized patterns.
3. Changes in the nature of the behaviour patterns, which are measured by the replacement of the stereotyped and ritualized behaviour patterns, either by new stereotyped and ritualized behaviour patterns or by normal behaviour patterns.

As a consequence of the individual-specific characteristics of the morphine-induced behaviour patterns, the frequencies of the behaviour items are only helpful in determining the changes in the behaviour of each individual animal. Averaging of the experimentally-evoked changes in the frequencies over the test group does, in general, not offer reliable indices for the drug-induced effects. Only after injections of drugs into the septal nuclei (Chapters 4 and 5), which primarily affected the frequencies rather than the nature of the behaviour patterns, it appeared possible to describe the drug-induced effects purely in terms of changes in frequencies of behaviour items averaged over the test group. As behaviour is the consequence of changes occurr-

ing within the brain, it can be expected that each distinct morphine-induced phase will be accompanied by its own characteristic changes in central neurotransmission processes. Hence, we studied these phases in terms of relatively independent phases.

2.5 ETHOGRAM

The following ethogram is used for the description of the feline behaviour (see also: Cools and Van Rossum, 1970; Leyhausen, 1956; Norton and De Beer, 1956; Van Dongen, 1980).

1. lying	the body resting on the ground - forepaws extended forwards, hindpaws under the body - forepaws folded under the body, hindpaws under the body - forepaws beside the body, hindpaws under the body - forepaws and hindpaws on one side of the body
2. sitting	the body upright, forelegs extended, and the hindquarters resting on the ground - position of the body low - position of the body high
3. standing	
4. walking	any forward or backward movement of the animal along a minimum distance of 40 cm in the case of discontinuous locomotion or along a fixed distance of 80 cm in the case of continuous locomotion
5. turning	any turning movement of the animal around a minimum angle of 180^{o} in the case of discontinuous turning or around a fixed angle of 360^{o} in the case of continuous turning (i.e. circling)
6. sideward movements	any turning movement of the animal around an angle of 90^{o}
7. swinging	small sideward movements or small movements backward or forward
8. jumping	
9. rearing	standing upright against the walls with only the hindlegs on the ground

10. falling	coming down suddenly from an upright position
11. rolling	moving by turning around the head-tail axis
12. stretching	extending the body and forelegs to full length
13. sharpening of the nails	
14. digging	
15. sniffing	
16. eating	chewing and swallowing of f.i. vomit (food is not present)
17. emesis	
18. defaecation	
19. micturation	
20. grooming	licking the fur
21. washing	licking a forepaw and next rubbing it over its head
22. scratching	the fur with the hindlegs
23. rubbing	pressing head and neck against cage or against observer's hands
24. head movements	every movement of the head
25. staring	
26. mouth movements	
27. tongue extrusions	
28. salivation	
29. swallowing	
30. yawning	
31. panting	
32. eyes	open/closed
33. pupil	miosis/mydriasis/normal diameter

34. limb movements	any movement of the limbs not involved in locomotion, rearing, digging, sharpening of the nails, washing, or scratching
35. body movements	any body movement involving limb movements
36. postural movements	any body movement that does not involve limb movements
37. miaowing	
38. growling	
39. hissing	

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MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL TO STUDY THE ACTION OF MORPHINE ON NEUROTRANSMISSION PROCESSES WITHIN RAPHE AND STRIATAL NUCLEI

3.1 SITE OF ACTION OF DEVELOPMENT OF PARTIAL TOLERANCE TO MORPHINE IN CATS

3.1.1 Summary

a) A behavioural study on the ability of various agents, locally administered into the nucleus linearis intermedius raphe, to modify morphine-induced behaviour in freely moving cats has provided evidence for the concept that changes within this nucleus and in the noradrenergic fibre system terminating therein are necessary for the development of partial tolerance.

b) Compounds such as l-propranolol and desglycinamide lysine-8-vasopressin, which are known to influence the development of tolerance, accelerate its development after administration into the raphe nucleus mentioned.

c) Selective, pharmacological manipulation with the β-like noradrenergic receptors within this raphe structure influenced the development of partial tolerance to morphine.

d) The present article also summarizes evidence in favour of the following concepts: i) morphine suppresses neuronal activity within the locus coeruleus and, accordingly, decreases the pulse-flow in the arising, noradrenergic fibres resulting in a decreased release of noradrenaline within the mentioned raphe nucleus and ii) development of partial tolerance to morphine is at least partially due to the ability of the noradrenergic, coeruleo-raphe system to develop hyperactivity after an initial hypoactivity.

3.1.2 Introduction

In a previous study (Cools et al., 1974), attention was focused on the role of the interrelationship between the dopaminergic fibres - terminating within the rostromedial part of the head of the caudate nucleus - and the serotonergic fibres - arising from the nucleus linearis intermedius raphe (NRL) and terminating within the antero-ventral part of the head of the caudate nucleus - in the morphine-induced behaviour in cats. In the present article, the role of the noradrenergic fibres - terminating within the NRL - occupies a central position; some of the results have been published in condensed form (Cools et al., 1974; Cools, 1977d).

Morphine elicits a complex behavioural syndrome in cats (Dhasmana et al., 1972; Loewe, 1956; Norton and De Beer, 1956; Sturtevant and Drill, 1957; Wikler, 1944). Under certain conditions (for details, see Cools et al., 1974) morphine elicits a syndrome, which is built up in three steps: a) depression phase, b) re-organization phase, and c) ritualization phase. Characteristic of the second phase is 1) the break-down of normal head movements into an increasing number of isolated, staccato-like movements, and the subsequent appearance of new patterns in which these single units are re-integrated, and 2) the break-down of normal postural and body movements into single movements of the head, neck, trunk and limbs, and the subsequent appearance of novel patterns in which these single elements are re-integrated; the resulting patterns are both stereotyped, i.e. continuously repeated in a specific sequence, and ritualized, i.e. restricted to a certain number of senseless patterns. Characteristic of the last phase is the presence of purely stereotyped and ritualized head movements, postural changes and locomotor patterns. Since the nature of the patterns displayed during the last phase is to a large degree determined by the sensory information available during the re-organization phase, which is dependent on factors such as the individual's developmental history, acquaintance with the experimental environment, etc., descriptions of the morphine-induced behaviour in terms of mechanisms bringing about the induced behaviour (see above) are preferable to descriptions in terms of the consequences of the induced behaviour, i.e. frightened, avoids handling, aggressive, etc.

It is known that the serotonin-containing cell bodies within the

raphe nuclei are innervated by noradrenergic fibres (Chu and Bloom, 1974; Dahlström and Fuxe, 1965; Fuxe, 1965; Lindvall and Björklund, 1974; Loizou, 1969; Swanson and Hartman, 1975). Behavioural studies using the micro-injection technique have shown that the raphe nuclei, especially the NRL, contain β-like noradrenergic receptors (Cools and Janssen, 1974; Cools et al., 1975). Selective stimulation of these receptors by means of intracerebral injections of rather small doses of l-noradrenaline suppresses the display of the stereotyped and ritualized patterns elicited by acute morphine treatment (Cools et al., 1974); selective inhibition of these receptors by means of l-propranolol potentiates the display of these patterns (Cools, 1977d). Since higher doses of l-noradrenaline and l-propranolol were required to influence the morphine-induced behaviour in cats which had developed a partial tolerance to morphine, it has been concluded that the development of partial tolerance to morphine in cats is accompanied by the occurrence of clearcut changes in the noradrenergic activity within the NRL (Cools, 1977d). This is in agreement with the general theory that brain noradrenaline is involved in some manifestations of acute and chronic morphine treatment of mice, rats and cats (for recent reviews: Clouet and Iwatsubo, 1975; Herz and Bläsig, 1974; Takemori, 1974). Apart from the fact that these findings suggested an important role for the noradrenergic mechanisms within the NRL in morphine-induced effects, they opened the question of whether the changes within the NRL are necessary for the development of partial tolerance rather than epiphenomena of the presence of partial tolerance.

In the present article, evidence is presented to demonstrate that selective, pharmacological interference with the noradrenergic activity within the NRL does influence the development of partial tolerance to morphine. Furthermore, evidence is presented to show that an endocrinologically inert fragment of vasopressin, i.e. desglycinamide lysine-8-vasopressin, a neuropeptide that potentiates the development of tolerance to morphine-induced analgesia in mice (Krivoy et al., 1974) and restores the retarded development of tolerance to morphine-induced analgesia in homozygous rats with hereditary diabetes insipidus (De Wied and Gispen, 1976), accelerates the development of partial tolerance to morphine when locally applied to the NRL of cats. Both sets of data underline the hypothesis that characteristic changes with-

in the NRL are necessary for the development of partial tolerance to morphine-induced behaviour in cats.

3.1.3 Materials and methods

Cats ranging in weight from 2.5 to 3.2 kg were used; the cats were not derived from an inbred strain.

In the first series of experiments, daily injections of morphine (5 mg/kg, i.p. given between 9.00 and 12.00 a.m.) were administered on 5 consecutive days in order to determine the changes characteristic of the development of tolerance (see also Cools, 1977d).

In the second series of experiments, animals pretreated with an i.p. injection of morphine (5 mg/kg) received an intracerebral injection of 0.9% NaCl, l-noradrenaline or l-propranolol into the NRL at the point of time that the ultimate morphine-induced syndrome was developed (see also Cools et al., 1974; Cools, 1977d). The purpose of these experiments was to determine the acute effects of these compounds.

In the third series of experiments, animals pretreated with an intracerebral injection of 0.9% NaCl, l-noradrenaline or l-propranolol into the NRL received an i.p. injection of morphine (5 mg/kg) 5-10 days after the intracerebral injection. The purpose of these experiments was to determine whether selective, pharmacological manipulation with the β-like noradrenergic receptors within the NRL produces long-term after-effects; these experiments were suggested by the finding that a single injection of l-propranolol into the NRL resulted in a decreased sensitivity to a second injection of l-propranolol (Gieles, Mortiaux, Megens and Cools, unpublished data).

In the final series of experiments, animals pretreated with an i.p. injection of morphine (5 mg/kg) and an intracerebral injection of l-propranolol or desglycinamide lysine-8-vasopressin (DG-LVP) given 40-50 min after the morphine injection received a second i.p. injection of morphine (5 mg/kg) on the day following this pretreatment; the purpose of these experiments was to determine whether compounds which accelerate the development of partial tolerance in mice and rats after systemic administration (Cowan and MacFarlane, 1975; De Wied and Gispen, 1976; Krivoy et al., 1974) also accelerate the development of partial tolerance to morphine in cats when locally applied to the NRL.

The spontaneously occurring and drug-induced behaviour was recorded

on videotapes by means of a closed TV circuit. The tapes provided objective and continuous records, which were analyzed with the help of the following parameters (Fig. 3.1): 1) determination of the frequency of head movements (Aa), body movements including the single movements of the limbs (Ab), postural movements (Ac) and distinct types of stereotyped and ritualized patterns (Ad); 2) determination of the onset of the re-organization phase (= end of the depression) by averag-

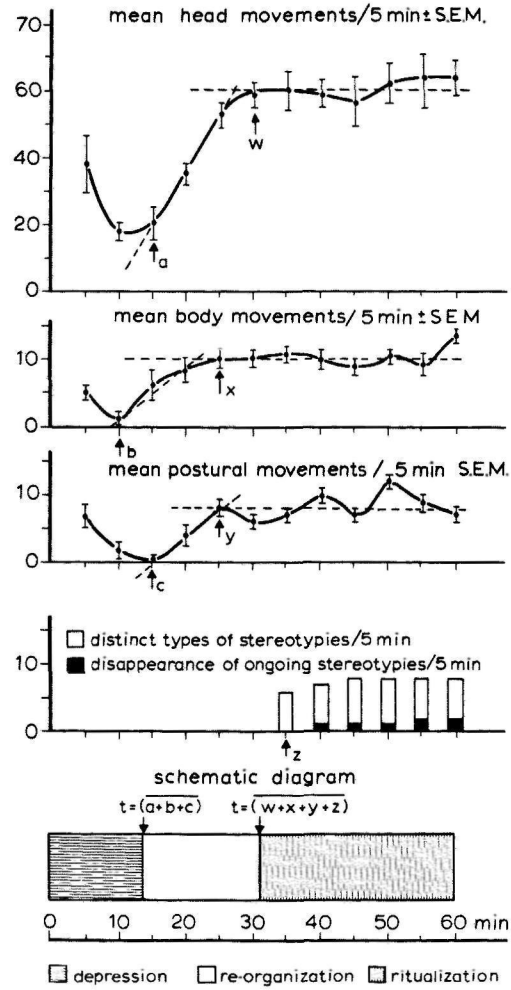

Fig. 3.1 Behavioural effects following a systemic injection of morphine (5 mg/kg, i.p.) in a male cat: illustration of the determinants of morphine-induced behaviour.

ing the points of time at which the frequencies of the head, body and postural movements started to increase; 3) determination of the onset of the ritualization phase by averaging the points of time at which the frequencies of the head, body and postural movements became stabilized and the stereotyped and ritualized patterns appeared.

As illustrated in Fig. 3.2, the drug action was determined as follows: a) immediate onset of the display of the stereotyped and ritualized patterns following intracerebral administration of the drug during the re-organization phase (= potentiation of the ritualization); b) increase in frequencies of head, body and postural movements with or without the display of new stereotyped and ritualized patterns follow-

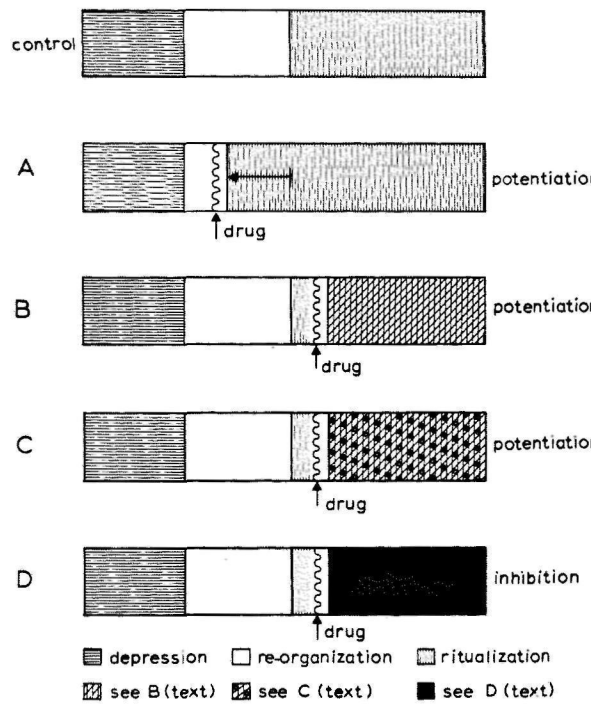

Fig. 3.2 A schematic diagram of the determinants of the effects of drugs (intracerebrally injected at time = ↑) upon morphine-induced ritualization.
A = immediate onset of ritualization after drug administration
B = change in "kinetic melody" of ongoing stereotypies
C = increased frequency of ongoing behaviour and/or new stereotypies
D = suppression of morphine-dependent behaviour

ing intracerebral administration of the drug during the ritualization (= potentiation of the ritualization). Since drugs which are able to produce effects a) and b) may produce slight changes in the "kinetic melody" of the ongoing stereotyped and ritualized patterns, which cannot be described as completely new stereotypies, changes in the "kinetic melody" of the ongoing stereotypies following the administration of *lower* concentrations of these agents are accordingly classified as *potentiation, score* 1 in contrast to the overt symptoms mentioned above, which are classified as *potentiation, score* 2 (see also Figs. 3.6 and 3.7); c) partial or complete suppression of the ongoing stereotyped and ritualized patterns and/or decreased frequencies of the head, body and postural movements following intracerebral administration of the drug during the ritualization (= inhibition of the ritualization; see also Fig. 3.5). In the experiments discussed below, determinants b) and c) were used.

Each observation period lasted 60 min; a period of 15 min preceding the first injection was used as control. In general, cats were placed in the observation cage (75 x 75 x 80 cm) 15 min before the control recordings. Precautions were taken in order to prevent the occurrence of changes in the external environment during the experimental session. All animals were habituated to the cage during two sessions of 1 hr each. Cats treated more than once on different days were always tested at the same time of day. The whole testing procedure, including the transport of the animal to and from the experimental cage, was kept as constant as possible. Since the sensitivity to morphine changes during the year (maximum sensitivity: March and July; minimum sensitivity: December and May (Mortiaux, Gieles, Megens, Jansen and Cools, unpublished data)), the experiments presented below were performed in periods marked by an intermediate sensitivity: January, April, June, September, October and November.

Cats receiving intracerebral injections were prepared as previously described (Cools and Van Rossum, 1970). Short, double-barreled, stainless steel cannulae were stereotaxically implanted in such a way that the injection needle could reach the NRL (using the co-ordinates of Snider and Niemer (1964): A = 1.5, L = 0.3, H = -4.0). Cannulae were placed at an angle of 10^{o} in reference to the mid-sagittal plane (Cools and Janssen, 1974; Cools et al., 1975). Upon recovery from the operation,

placing responses, righting and pupillary reflexes were checked. In addition, food-intake, wakefulness and reactivity to visual and acoustic stimuli were estimated; only cats which responded normally during the second post-operation week were used in the experiments.

Drug solutions (1 µl) were injected through injection needles, which extended into the brain tissue about 2 mm below the tip of the embedded cannulae. The following substances were used: l-noradrenaline HCl (Fluka A.G., Switzerland), l-propranolol (gift of I.C.I., United Kingdom), desglycinamide lysine-8-vasopressin (gift of Organon International B.V., The Netherlands), and morphine H_2SO_4 (Chemische Pharmaceutische Fabrieken, The Netherlands). Apart from morphine, which was always dissolved in 0.9% NaCl, all compounds were dissolved either in 0.9% NaCl or in aqua bidest. Injections of solvents only produced a slight arousal during the first 3 min following the injection regardless of the pretreatment.

After completion of the experiments, the brain was removed and prepared as previously described (Cools and Van Rossum, 1970); visual inspection of a series of brain slices (about 100 µm) caudally from a knife-cut along the track of the guide cannula was made to determine the sites of injections. Only experiments in which the injection sites were restricted to the target area shown in Fig. 3.3 are discussed below.

3.1.4 Results

Development of partial tolerance to morphine

The effects of daily administration of morphine (n = 14) are shown in Fig. 3.4; this figure shows that the onset of the re-organization and ritualization phase was significantly retarded from the third day ($p < 0.001$; Students t-test).

Acute effects of l-noradrenaline and l-propranolol upon morphine-induced behaviour

l-Noradrenaline (10 µg), locally administered into the NRL during the ritualization phase triggered by a single injection of morphine, immediately decreased the frequencies of the head, body and postural movements and, in addition, suppressed the ongoing stereotyped and ritualized patterns during a period of about 7 min. An example is given in Fig. 3.5. The results of all experiments (n = 9) are summarized on the left side of Fig. 3.8, and show that l-noradrenaline inhibited the morphine-induced

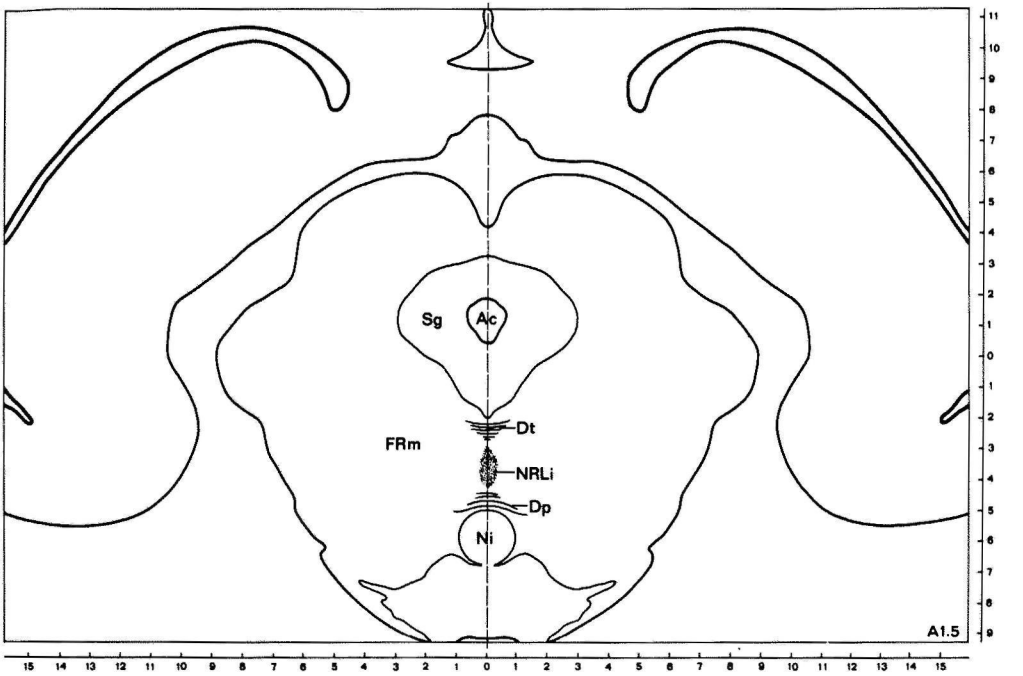

Fig. 3.3 Semi-diagrammatic outline of one anterior frontal plane of the cat brain showing the nucleus linearis intermedius raphe. Ac = aquaductus cerebri; Dt = decussatio tegmenti; NRli = nucleus linearis intermedius raphe; Dp = decussatio peduncularum; Ni = nucleus interpeduncularis; Sg = substantia grisea centralis.

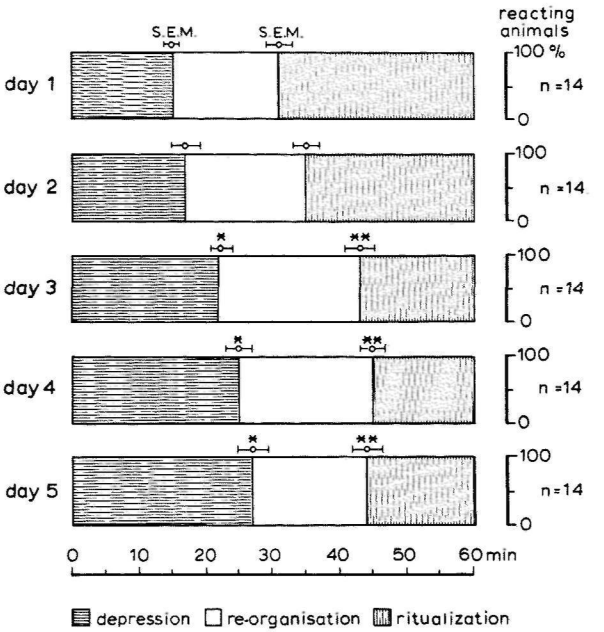

Fig. 3.4 Effects of subsequent, daily injections of morphine upon the duration of morphine-induced depression, re-organization and ritualization (morphine: 5 mg/kg, i.p. given at time = 0, each day).

** onset of re-organization on day 3-5 is significantly different from that on day 1 ($p < 0.01$; Students t-test)*

*** onset of ritualization on day 3-5 is significantly different from that on day 1 ($p < 0.001$; Students t-test)*

ritualization in all cases.

1-Propranolol (8 µg), locally administered into the NRL during the ritualization phase triggered by a single injection of morphine, produced a slight change in the nature of the ongoing stereotyped and ritualized patterns in *some* animals (an example is given in Fig. 3.6), whereas the same dose produced increased frequencies of head, body and postural movements and/or the display of completely new types of stereotyped and ritualized patterns in *other* animals (an example is given in Fig. 3.7). A dose of 10 µg 1-propranolol always produced the last mentioned effects. The results of all experiments, in which 10 µg was given

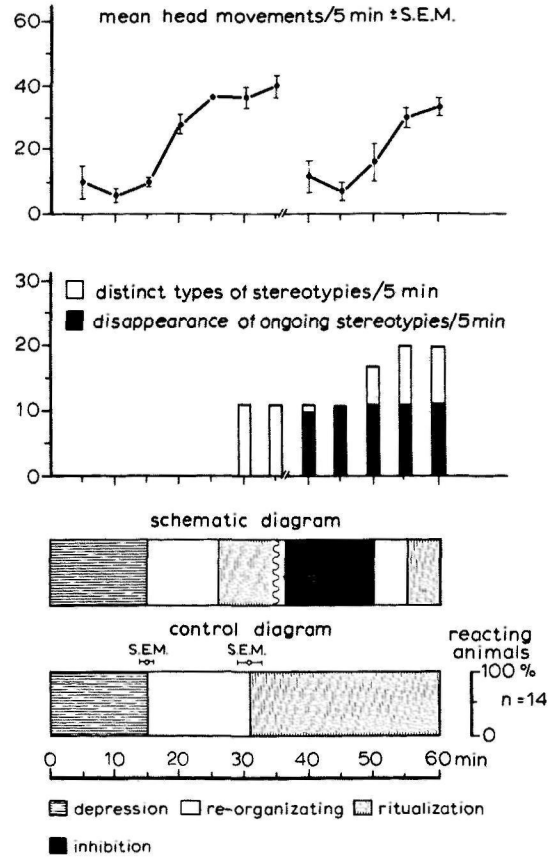

Fig. 3.5 Behavioural effects of injections of l-noradrenaline (10 µg/µl at time = 35) into the nucleus linearis intermedius raphe upon the morphine-induced ritualization in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): suppression of ongoing behaviour.

(n = 7), are summarized on the left side of Fig. 3.8, and show that l-propranolol potentiated the morphine-induced ritualization phase in all cases.

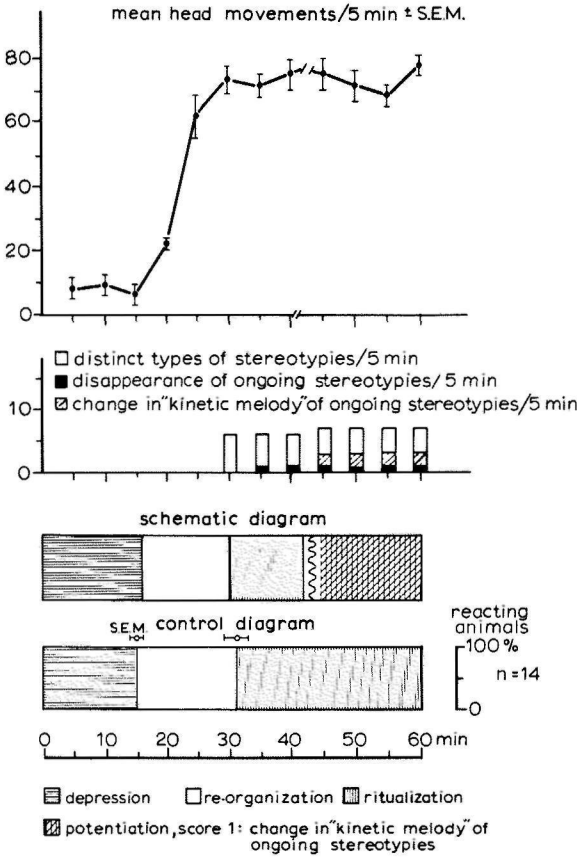

Fig. 3.6 Behavioural effects of injections of l-propranolol (8 µg/µl at time = 40) into the nucleus linearis intermedius raphe upon the morphine-induced ritualization in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): change in "kinetic melody" of ongoing stereotypies.

Effects of pretreatment with l-noradrenaline and l-propranolol upon morphine-induced behaviour

As can be seen on the right side of Fig. 3.8, intracerebral injections of l-noradrenaline (10 µg) and l-propranolol (10 µg) given 5-10 days prior to morphine, potentiated and suppressed respectively the display

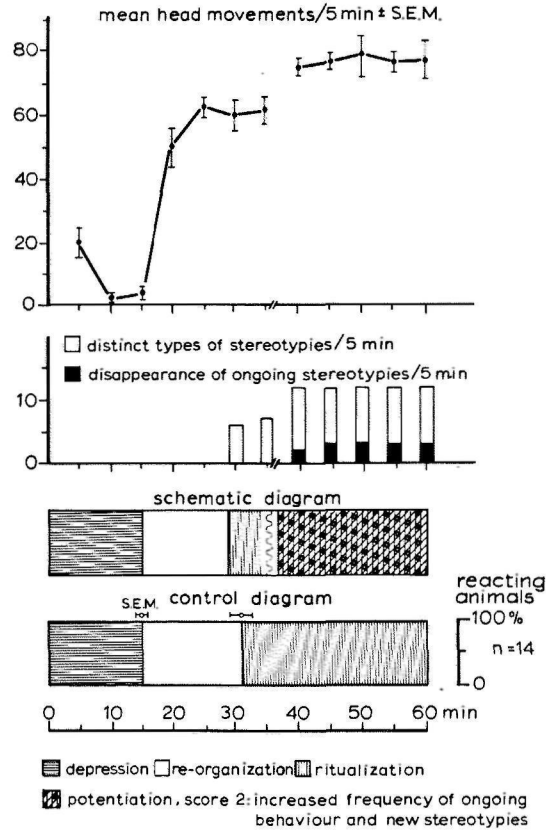

Fig. 3.7 Behavioural effects of injections of l-propranolol (8 µg/µl at time = 35) into the nucleus linearis intermedius raphe upon the morphine-induced ritualization in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): increased frequency of ongoing behaviour and display of new stereotypies.

of stereotyped and ritualized patterns: l-noradrenaline accelerated the onset of the ritualization phase ($p < 0.05$; Students t-test), whereas l-propranolol suppressed the ongoing behaviour during a period of about 12 min. In other words, a pretreatment with these noradrenergic agents produced effects diametrically opposite to those elicited by these agents in cats treated with morphine during the same session (Fig. 3.8,

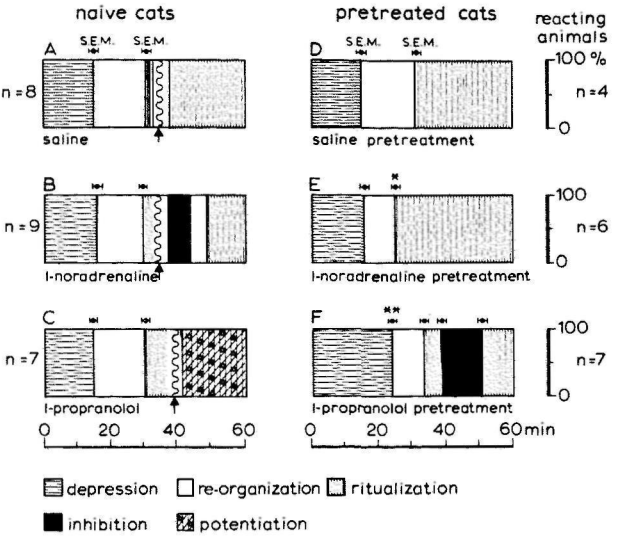

Fig. 3.8 Left side: behavioural effects of injections of saline (1 µl: A), l-noradrenaline (10 µg/µl: B) and l-propranolol (10 µg/µl: C) into the nucleus linearis intermedius raphe upon the behaviour elicited by morphine (5 mg/kg, i.p. given at time = 0): B = inhibition of ritualization; C = potentiation of ritualization.
Right side: effects of similar, intracerebral injections given 5-10 days prior to morphine (5 mg/kg, i.p. given at time = 0): E = potentiation of ritualization; F = inhibition of ritualization.
** onset of ritualization in E is significantly different from that in D ($p < 0.05$; Students t-test).*
*** onset of re-organization in F is significantly different from that in D ($p < 0.05$; Students t-test).*

cf. right and left side).

Effects of desglycinamide lysine-8-vasopressin and l-propranolol upon development of partial tolerance to morphine

Desglycinamide lysine-8-vasopressin (DG-LVP: 1 µg), locally administered into the NRL during the ritualization phase triggered by a single injection of morphine, produced a) an increase in frequencies of the head, body and postural movements, and b) the display of new stereotyped and

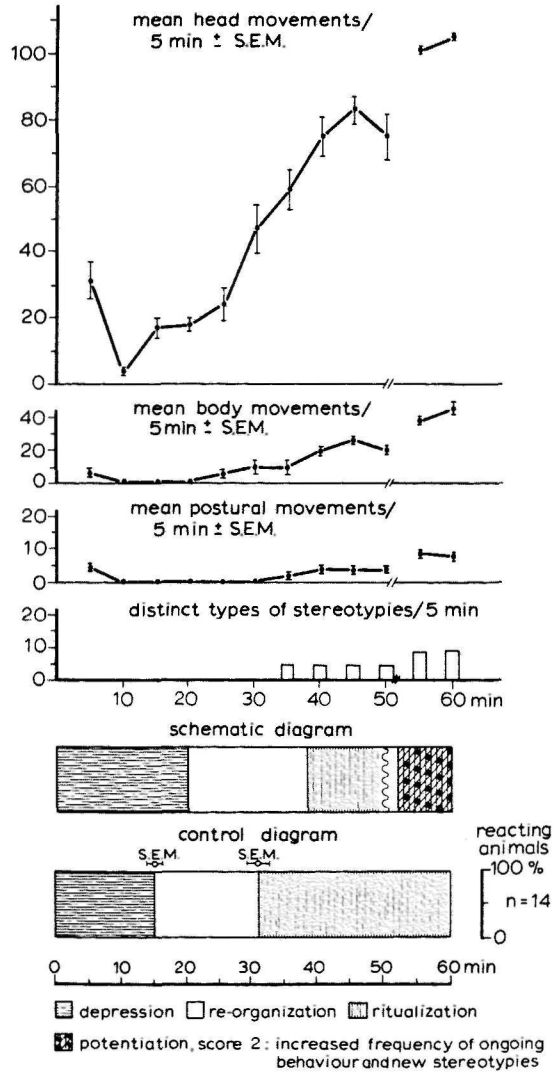

Fig. 3.9 Behavioural effects of injections of desglycinamide lysine-8-vasopressin (DG-LVP: 1 µg/µl at time = 50) into the nucleus linearis intermedius raphe upon the behaviour elicited by morphine (5 mg/kg, i.p.) given to a male cat at time = 0: potentiation of ritualization.

ritualized patterns; the number of new patterns varied from animal to animal. An example is given in Fig. 3.9. The results of all experiments (n = 6) are summarized on the left side of Fig. 3.11, and show that DG-LVP potentiates the morphine-induced ritualization phase in all cases. A second injection of morphine on the day following the above-mentioned experiments resulted in a significant retardation of the onset of the ritualization phase ($p < 0.001$; Students t-test). An example is given in Fig. 3.10. The results of all experiments (n = 6) are summarized on the right side of Fig. 3.11, and show that a pre-

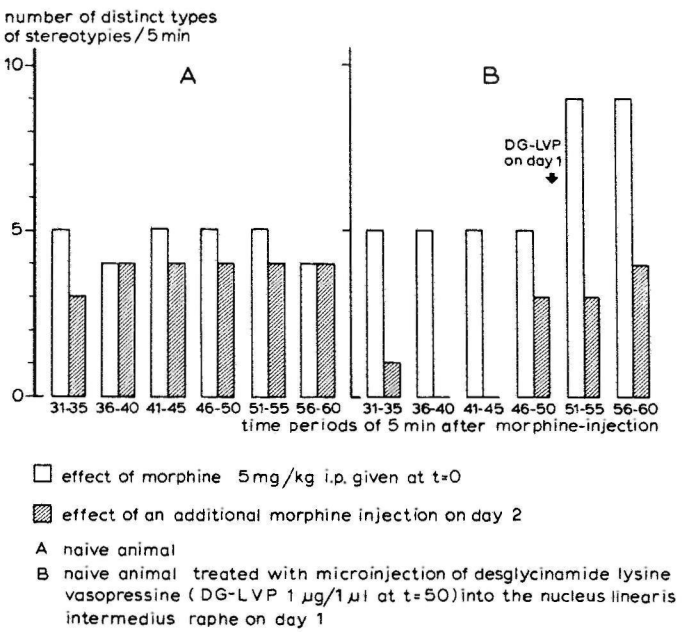

Fig. 3.10 Left side (A): behavioural effects of injections of morphine (5 mg/kg, i.p. given at time = 0) upon the number of distinct types of stereotypies in a male cat on day 1 (open column) and on day 2 following an additional injection of morphine (arced column; 5 mg/kg, i.p. given at time = 0). Right side (B): behavioural effects of injections of desglycinamide lysine-8-vasopressin (1 µg/µl, locally applied into the nucleus linearis intermedius raphe at time = 50) upon behaviour elicited by morphine (5 mg/kg, i.p. given at time = 0) in a male cat on day 1 (open column) and by an additional injection of morphine (5 mg/kg, i.p. given at time = 0) on day 2 (arced column). Note that the increase on day 1 (t_{51}-t_{60}) is followed by a decrease on day 2 (t_{31}-t_{45}).

treatment with DG-LVP produced effects characteristic of chronic treatment with morphine (cf. Fig. 3.4, day 3, 4 and 5, with Fig. 3.11, right side: C). The outcome of similar experiments with l-propranolol (10 µg) is presented in the same Fig. 3.11: pretreatment with l-propranolol also produced effects characteristic of chronic treatment with morphine (cf. Fig. 3.4, day 3, 4 and 5, with Fig. 3.11, right part: D).

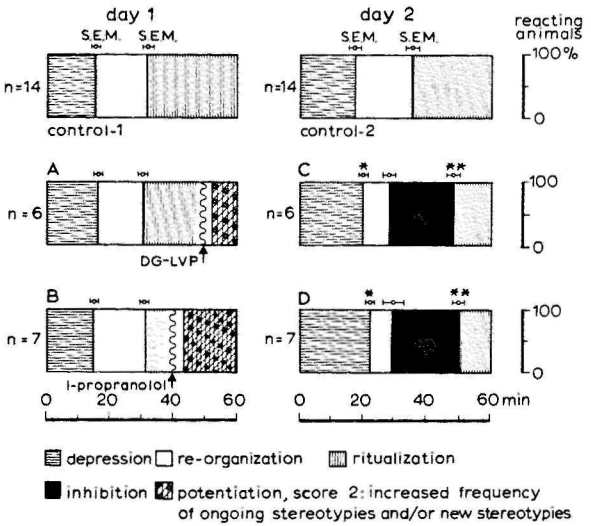

Fig. 3.11 Left side (day 1): behavioural effects of injection of desglycinamide lysine-8-vasopressin (DG-LVP: 1 µg/µl) and l-propranolol (10 µg/µl) into the nucleus linearis intermedius raphe upon the behaviour elicited by morphine given at time = 0 (5 mg/kg, i.p.): A and B = potentiation of ritualization.
Right side (day 2): behavioural effects of an additional injection of morphine on day 2 at time = 0 (5 mg/kg, i.p.): C and D = inhibition of ritualization.
** onset of the re-organization is significantly retarded of control 2 ($p < 0.01$; Students t-test).*
*** onset of the ritualization is significantly retarded of control 2 ($p < 0.001$; Students t-test).*

3.1.5 Discussion

The data collected in the first series of experiments show that repeated exposure of cats to morphine results in the development of partial tolerance. Our findings are in agreement with those reported by Djahanguire et al. (1966), Eddy and Himmelsbach (1936) and Seevers and Deneau (1963), who also used doses lower than 10 mg/kg; in this context, it is important to mention that tolerance to morphine does not develop when higher doses are used (cf. Huidobro and Lewin, 1969; Seevers and Deneau, 1963; Tatum et al., 1929).

On the basis of the data collected in the second series of experiments and those reported earlier (Cools et al., 1974; Cools, 1977d), it can be concluded that increase and decrease of the noradrenergic activity within the NRL suppresses and potentiates respectively the morphine-induced phase marked by the display of the stereotyped and ritualized patterns. This, together with the fact that morphine *per se* triggers the display of these patterns, suggests that an acute injection of morphine (5 mg/kg, i.p.) directly or indirectly decreases the noradrenergic activity within the NRL. The literature on decreased activity of brain noradrenaline in acute morphine intoxication is controversial (for recent reviews: see Clouet and Iwatsubo, 1975; Herz and Bläsig, 1974; Takemori, 1974), but findings such as a) potentiation of the morphine-induced analgesia following suppression of the noradrenergic activity (Watanebe et al., 1969), and b) potentiation of the morphine-induced effects upon the threshold for vocalization in rats following inhibition of the noradrenaline synthesis (Dahlström et al., 1975), are not in conflict with our suggestion. In this context, it is important to realize that the available literature only deals with the effects of systemically given agents.

Although various mechanisms of action such as a) decrease of the number of noradrenergic receptors, b) hyposensitivity of these receptors, c) inhibition of these receptors, d) increased re-uptake of noradrenaline, etc. might explain the occurrence of a decreased noradrenergic activity within the NRL of morphine-treated cats, changes triggered by morphine within the NRL can be excluded on the basis of the fact that morphine (1-10 µg) locally administered into this nucleus is behaviourally inert (Mortiaux and Cools, unpublished data). In this context, it is worthwhile to note that the ineffectiveness of locally applied morphine also excludes the possibility that putative interaction between propranolol and morphine or its receptors (De Feudis and Grosz, 1972; Goldstein,

1974) within the NRL underlies the ability of propranolol to influence morphine-induced behaviour. However, the ineffectiveness of locally administered morphine indicates that morphine may only indirectly influence noradrenergic activity within the NRL. Indeed, the following data suggest a different site of action. First, the noradrenergic terminals within the raphe nuclei belong to noradrenergic fibres arising from the locus coeruleus and/or subcoeruleus (Chu and Bloom, 1974; Lindvall and Björklund, 1974; Loizou, 1969). Second, morphine receptors are present within the locus coeruleus (Pert et al., 1975). Third, morphine suppresses the neuronal activity of cells within the locus coeruleus (Korf et al., 1974). On the basis of these data, it can be suggested that morphine may suppress the neuronal activity within the locus coeruleus by interference with morphine receptors and, accordingly, suppress the pulse-flow in the arising noradrenergic fibres, of which at least a part terminates within the NRL (cf. Swanson and Hartman, 1975); this mechanism of action may explain the finding that acute morphine appears to result in a decreased noradrenergic activity within the NRL. From this point of view, it becomes understandable that the ability of α-noradrenergic agents to influence morphine-induced effects in rats (Cicero et al., 1974) is not in conflict with the data in cats concerning the effectiveness of propranolol since the α-noradrenergic agents may, in particular, affect the receptive sites within the locus coeruleus. The presence of morphine receptors within the locus coeruleus on the one hand (Pert et al., 1975) and the ability of α-noradrenergic blockers to inhibit the binding of naloxone to rat brain homogenates on the other hand (Cicero et al., 1975) point in this direction.

The data collected in the third series of experiments show that noradrenaline and propranolol given 5-10 days before the morphine injection produce effects that are diametrically opposite to those elicited by these agents when given together with morphine. In other words, a β-noradrenergic blockade given 5-10 days earlier produces effects identical to those elicited by stimulation of the β-noradrenergic receptors in acute experiments, whereas stimulation of these receptors 5-10 days prior to morphine produces effects identical to those elicited by a β-noradrenergic blockade in acute experiments. These data clearly suggest that the noradrenergic mechanism within the NRL becomes hypoactive after a short-term increased exposure to noradrenaline and hyperactive after a short-term decreased exposure to noradrenaline;this fits in with the

observation that a single injection of a behaviourally ineffective dose of propranolol (5 µg) into the NRL changes the sensitivity of the β-noradrenergic mechanism in such a way that a behaviourally effective dose of propranolol (10 µg) given a few days later has lost its effectiveness (Gieles, Mortiaux, Megens and Cools, unpublished data). It is well-known that such phenomena follow a *long-term* stimulation, inhibition or denervation of noradrenergic postsynaptic receptors (Axelrod, 1974; Deguchi and Axelrod, 1973; Dismukes and Daly, 1974; Fleming and Trendelenburg, 1961; Fleming et al., 1973; Kakiuchi and Rall, 1968; Kalisker et al., 1973; Palmer, 1972; Palmer et al., 1976). The present data, however, suggest that such changes within the NRL appear after a *short-term* stimulation of the β-like noradrenergic receptors triggered by a physiologically occurring substance, i.e. noradrenaline. On the basis of comparable findings in biochemical studies on the pineal gland, i.e. the rapid development of subsensitivity of postsynaptic β-noradrenergic receptors after exposure to noradrenaline, Axelrod (1974) summarized the available evidence in favour of the concept that the mechanism responsible for such changes in pharmacological experiments may play a similar role in the regulation of physiologic processes.

Considering the above-mentioned properties of the noradrenergic raphe mechanism, and in view of the ability of a single injection of morphine to decrease the noradrenergic activity within the NRL, it becomes evident that animals treated with morphine must develop a long-lasting, hyperactivity within the NRL following the short-term morphine-induced hypoactivity. Furthermore, any subsequent injection of morphine should increase this after-effect more and more. It may then be expected that chronic treatment with morphine would be accompanied by an increased noradrenergic activity within the NRL; indeed, several studies have presented indirect evidence in favour of this (Ayhan and Randrup, 1972; Bläsig et al., 1975; Reis et al., 1970; Takagi and Kuriki, 1969; for reviews: Clouet and Iwatsubo, 1975; Cools, 1977d; Herz and Bläsig, 1974; Takemori, 1974).

With this in mind, the final series of experiments dealing with the role of propranolol and desglycinamide lysine-8-vasopressin (DG-LVP) can be discussed. NRL-injections of propranolol given together with a systemic injection of morphine one day before a second morphine injection significantly retards the onset of the ritualization phase on the

second test-day, i.e. it produces an effect characteristic of the development of partial tolerance to morphine (cf. Fig. 3.11, right part: D, with Fig. 3.4, day 3-5). Apart from the fact that these observations underline the notion that a pretreatment producing a short-term blockade of the β-like noradrenergic receptors within the NRL finally results in effects characteristic of a stimulation of these receptors in acute experiments, these data clearly indicate that selective blockade of these receptors accelerates the development of partial tolerance to morphine. This conclusion fits in very well with the recent study of Cowan and MacFarlane (1975) who found that repeated administration of propranolol given just prior to morphine also enhances the onset of tolerance to the morphine-induced analgesia in mice.

Summarizing the earlier mentioned considerations concerning the involvement of the β-like noradrenergic receptors within the NRL in some manifestations of morphine, it appears that: a) an acute morphine injection triggers a decreased noradrenergic activity within the NRL; b) chronic morphine injections result in the development of partial tolerance; c) development of partial tolerance to morphine is accompanied by an increased noradrenergic activity within the NRL; and d) a hyperactive noradrenergic activity within the NRL following a short-term blockade of this activity accelerates the development of partial tolerance to morphine.

On this basis, it can be postulated that the hyperactive, noradrenergic mechanism within the NRL - present in morphine-treated animals - significantly contributes to the development of partial tolerance to morphine. Considering the characteristic properties of this noradrenergic mechanism, it can also be postulated that the development of partial tolerance to morphine is due to the ability of the β-like noradrenergic mechanism within the NRL to develop a hyperactivity after a short-term suppression. Although it will be clear that this mechanism may be just one of the many factors contributing to the development of partial tolerance to morphine, it is highly important to realize that at least some aspects appear to be system-dependent rather than drug-dependent. This implies: a) compounds which influence the noradrenergic activity within the NRL should influence the development of partial tolerance to morphine; as discussed above, the effectiveness of l-propranolol in this respect points in this

direction. b) Manipulation with the system itself, i.e. the noradrenergic, coeruleo-raphe system, should influence the development of partial tolerance to morphine. Two very recent studies provide indirect evidence in favour of this. Laschka et al. (1976), using the intracerebral injection technique to precipitate morphine withdrawal in rats by means of morphine antagonists, report that the strongest withdrawal symptoms are evoked by injections into areas located in the anterior parts of the floor of the 4th ventricle and caudal parts of the periaqueductal grey matter, i.e. areas including the locus coeruleus and some raphe nuclei. Lewis et al. (1976), using the chemical lesion technique to modify the development of tolerance to morphine in rats, report that lesions in the mesencephalic noradrenergic bundle reduce various manifestations of chronic morphine administration. These data fit in with our above-mentioned hypothesis that morphine may suppress the neuronal activity in the locus coeruleus and, accordingly, decrease the pulse-flow in the arising noradrenergic fibres, resulting in a decreased release of noradrenaline within the NRL. c) Compounds which influence the development of partial tolerance to morphine should directly or indirectly influence the neurotransmitter systems within the NRL. The first piece of evidence in favour of this is provided by the present studies on the effectiveness of DG-LVP. As mentioned in the introduction, this neuropeptide is able to accelerate the development of partial tolerance to morphine in mice and to restore the retarded development of partial tolerance to morphine in rats with hereditary diabetes insipidus. As illustrated in Fig. 3.11, DG-LVP locally administered into the NRL produces a potentiation of the stereotyped and ritualized patterns elicited by morphine given 50 min prior to DG-LVP, whereas it retards the onset of such patterns elicited by a second morphine injection given on the next day. On the basis of these data, it can be concluded that DG-LVP not only mimics the effectiveness of l-propranolol, but also accelerates the development of partial tolerance to the morphine-induced effects (cf. Fig. 3.11, right side: C, with Fig. 3.4, day 3-5). It remains to be determined whether DG-LVP produces its effects in a way similar to that of l-propranolol, i.e. producing a short-term blockade of the β-like noradrenergic mechanism, which is followed by a long-term hyperactivity of this mechanism.

Summarizing, the present study has provided evidence in favour of the con-

cept that characteristic changes within the NRL - in particular, changes in the noradrenergic fibre system terminating within this structure - are essential prerequisites for the development of partial tolerance to morphine-induced behaviour in cats: a) compounds such as l-propranolol and DG-LVP, which are known to influence the development of tolerance, are highly effective in this respect after intracerebral administration of these agents into the NRL; and b) selective manipulation of the β-like noradrenergic receptors within the NRL does influence the development of tolerance to morphine.

Furthermore, we have summarized indirect evidence in favour of the following concepts: 1) morphine, *inter alia*, suppresses the neuronal activity within the locus coeruleus, and accordingly, decreases the pulse-flow in the arising noradrenergic fibres resulting in a decreased release of noradrenaline within structures such as the NRL; and 2) the development of partial tolerance to morphine is at least partially due to the characteristic feature of the noradrenergic coeruleo-raphe system to develop a hyperactivity after an initially produced hypoactivity.

3.2 MORPHINE AND ITS BIPHASIC INFLUENCE UPON PHARMACOLOGICALLY DISTINCT DOPAMINERGIC SYSTEMS WITHIN THE FELINE CAUDATE NUCLEUS: A BEHAVIOURAL STUDY

3.2.1 Summary

Behavioural changes subsequent to alterations in DA activity in the caudate nucleus were studied in freely moving cats pretreated with a systemic injection of morphine (5 mg/kg, i.p.). Intracerebral, bilateral injections of dopamine (DA) and haloperidol into the rostromedial part of the caudate nucleus, respectively increased and decreased DA activity within this area; similar injections of (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) and ergometrine into the anterodorsal part of this nucleus respectively increased and decreased DA activity within this region. The ability of intracerebrally injected drugs to influence the morphine-induced successively appearing "depression", "re-organization" and "ritualization" was a central aspect of this study. Administration of DA into the rostromedial part a) during re-organization, intensified

the symptoms characteristic of the re-organization and prolonged the duration of their display, b) before the re-organization, accelerated the onset of this re-organization and c) when made during ritualization suppressed this ritualization. Administration of haloperidol into this area a) during re-organization, suppressed the progressive development of the re-organization and b) when made during the ritualization, potentiated the ritualization. Administration of DPI into the anterodorsal part a) during re-organization immediately triggered the ritualization and b) when made during ritualization potentiated this ritualization. Finally, administration of ergometrine into the anterodorsal area a) during re-organization, immediately triggered a short-lasting period marked by symptoms characteristic of ritualization and prolonged the duration of the subsequent re-organization and b) during ritualization, intensified the ritualization for a short time, then suppressed the characteristic symptoms during a variable number of short-term periods. It is concluded that a) administration of DA into the rostromedial part and of ergometrine into the anterodorsal part mainly potentiated the "relatively early" morphine effects and inhibited the "late" morphine effects, and b) administration of haloperidol into the rostromedial part and of DPI into the anterodorsal part inhibited the "relatively early" morphine effects and potentiated the "late" morphine effects. The hypothesis is put forward that an acute injection of morphine (5 mg/kg, i.p.) affects different DA systems in a differential way: it is suggested that morphine *inter alia* produces a time-dependent, biphasic shift in the normally occurring "balance" between the DA activity within the rostromedial part of the caudate nucleus (DAe activity) and the DA activity within the anterodorsal part of the caudate nucleus (DAi activity) from an initial predominant DAe activity during the "relatively early" morphine effects towards a predominant DAi activity during the "late" morphine effects. The mechanisms underlying the behavioural effects elicited by modulation of DAi activity in morphine-treated cats are discussed. The present study is believed to offer data which might bridge the gap between the two diametrically opposite hypotheses concerning morphine's ability to affect central DA activity.

3.2.2 Introduction

While the interaction between morphine and central dopaminergic activity is the main point of many studies on morphine, the issue remains extremely controversial (for a recent review: Scheel-Krüger, 1976). According to some workers, morphine decreases postsynaptic dopamine (DA) activity (Kuschinsky and Hornykiewicz, 1972; Lal et al., 1975; Puri and Lal, 1973). Other workers favour the concept that this narcotic analgesic somehow increases postsynaptic DA activity (Cools et al., 1974; Iwamoto et al., 1976; Iwatsubo, 1976; Nakamura et al., 1973; Scheel-Krüger et al., 1977). A third group offers evidence that, depending on the dose used, morphine can both decrease and increase postsynaptic activity (for a review: Scheel-Krüger, 1976). As recent studies have shown that the brain contains more than one DA system, each one marked by its own functional and pharmacological properties (mice: Costentin et al., 1975; Di Chiara et al., 1976; rats: Cools, 1977a; guinea pigs: Costall and Naylor, 1975; cats: Cools et al., 1975; Cools and Van Rossum, 1976; Cools et al., 1976), it became attractive to investigate whether the apparent discrepancies in the literature might be ascribed to a differential action of morphine upon different DA systems within the brain. In the present study, this problem was attacked by studying the role of functionally, topographically and pharmacologically distinct DA sensitive striatal structures in the morphine-induced behavioural syndrome in cats.

Under certain conditions (Cools et al., 1974), morphine (5 mg/kg, given i.p. to cats) elicits a syndrome which is built up in three successive steps: a) depression phase, b) re-organization phase, and c) ritualization phase. The second phase, which starts about 15 min after the morphine injection, is characterized by the break-down of normal head, body and postural movements, into an increasing number of isolated, staccato movements, and the subsequent appearance of new head, body and postural movements in which the single units are re-integrated; the resulting patterns become both stereotyped, i.e. continuously repeated in a specific sequence, and ritualized, i.e. restricted to a certain number of senseless patterns. The last phase, which starts about 30 min after the morphine injection, is characterized by purely stereotyped and ritualized head movements, postural changes and locomotor patterns. An extensive description of this

syndrome is given elsewhere (Cools et al., 1974; Fig. 3.12).

Haloperidol, an antagonist of DA receptors, locally administered into the caput nuclei caudati rostromedialis (CRM area), modifies this syndrome in two opposite directions (Cools et al., 1974): a) when given at the onset of this phase, it suppresses the re-organization phase, and b) when given somewhat later, it potentiates the subsequent ritualization phase. Furthermore, it was shown that increased DA activity within the CRM area is an essential prerequisite for the development of the re-organization phase (Cools et al., 1974).

There is evidence that the caudate nucleus of cats contains two functionally and topographically distinct DA sensitive structures: a) the so-called excitation-mediating DA sensitive system (DAe system), which mainly terminates within the above-mentioned CRM area, and b) the so-called inhibition-mediating DA sensitive system (DAi system), which mainly terminates within the caput nuclei caudati anterodorsalis (r-CRM area) (Fig. 3.13; Cools et al., 1975, 1976; Cools and Van Rossum, 1976). Although it is not yet known whether there really exist two distinct types of DA receptors or, simply, two distinct states of one type of DA receptor (Creese et al., 1975), it has been found that each DA sensitive area can be selectively activated and inhibited by its own type of agonist and antagonist: the DAe system is activated by apomorphine and inhibited by low doses of haloperidol, and the DAi system is selectively activated by (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) and inhibited by ergometrine (Cools et al., 1976); a similar differentiation between different DA receptors and/or DA sensitive substrates appears to exist in the snail *Helix aspersa* (Struyker Boudier et al., 1974) and in the rat (Cools, 1977a).

As a decrease in the DAe activity within the CRM area produces symptoms which are to a large extent identical to those elicited by an increase in DAi activity within the r-CRM area (Cools et al., 1976; Cools, 1977c), the following questions emerged: Does morphine particularly affect DAe activity or does it also influence DAi activity in some way? If this is the case, does morphine also produce a biphasic change in this activity?

In our first study dealing with the role of the DA activity within the CRM area in morphine-treated cats (Cools et al., 1974), a purely descriptive method was used to analyze the effects produced. Later on,

it turned out that this syndrome could also be analyzed more quantitatively. In studies on the role of the noradrenergic coeruleo-raphe system in acute and chronic morphine-treated cats (Cools et al., 1977a), this semi-quantitative approach proved to be an considerable improvement. In view of this, the experiments dealing with DA activity within the CRM area were first repeated and analyzed by means of this new method before they were extended in so as to answer the questions mentioned above.

The data to be presented suggest that an acute injection of morphine directly or indirectly leads to a biphasic shift in the normally occurring "balance" between the DAe and DAi activity within the caudate nucleus during the development of the morphine syndrome.

3.2.3 Materials and methods

Both male and female cats ranging in weight from 2.5 to 3.2 kg were used; the cats were not derived from an inbred strain. In all experiments, the animals, which were chronically equipped with cannulae (see below), were pretreated with morphine (5 mg/kg, i.p. given between 9.00 and 12.00 a.m.); each animal was used only once. In general, the cats were kept under natural illumination conditions; the experimental cage was illuminated in addition by means of a bulb (60 W) hanging about 100 cm above its floor.

In the first series of experiments, the pretreated animals received intracerebral injections of 0.9% NaCl, DA or haloperidol into the left and right CRM area at various time intervals after the morphine injection. In this context, it is worthwhile to note that DA was chosen instead of apomorphine because apomorphine, being a DAe agonist, can also inhibit DAi activity (Cools, 1977c).

In the second series of experiments, the pretreated animals received intracerebral injections of 0.9% NaCl, DPI or ergometrine into the left and right r-CRM area at various time intervals after the morphine injection. Neither tests in which DPI or ergometrine was administered into the CRM area nor tests in which haloperidol was injected into the r-CRM area were included since such injections turned out to be ineffective (Cools et al., 1976).

The drug-induced behaviour was recorded on videotapes by means of a closed TV circuit. The tapes provided continuous records, which were

analyzed with the help of the following parameters (Fig. 3.12): 1) determination of the frequency/min of a) head movements, b) body movements, including the single movements of the limbs, and c) postural movements, in order to facilitate the detection of qualitative changes (see also below); 2) determination of the onset of the re-organization phase (= end of the depression phase) by averaging the time points at which the frequencies of a) head, b) body and c) postural movements started to increase and continued to do so for a 15 min period; 3) determination of the number of distinct types of stereotyped and ritualized locomotor patterns/5 min; 4) determination of the onset of the ritualization phase (= end of the re-organization phase) by averaging the time points at which the frequencies of a) head, b) body and c) postural movements became stabilized for a 15 min period, and the stereotyped and ritualized locomotor patterns appeared; in this context, it should be mentioned that the above definition of the onset of the ritualization phase implies that the cats already displayed stereotyped and ritualized head, body and postural movements before that time, but did not yet show the stereotyped and ritualized locomotor patterns (see 3.2.1).

Each observation period lasted 60 min, and started immediately after the morphine injection. In general, the cats were placed in the observation cage (75 x 75 x 80 cm) 15 min before the morphine injection. Precautions were taken to prevent changes in the external environment during the experimental session. All animals were habituated to the cage in two 1 hr sessions. The whole testing procedure including the transport of the animal to the experimental cage was kept as constant as possible. Since the sensitivity of cats to morphine changes during the year (maximum sensitivity: March and July; minimum sensitivity: December and May) (Mortiaux, Gieles, Megens, Janssen and Cools, unpublished data), the experiments presented below were performed during periods of intermediate sensitivity: January, April, June, September, October and November.

The influence of intracerebrally injected drugs upon the morphine-induced re-organization was determined as follows: a) immediate onset of re-organization following intracerebral administration of the drug during depression; b) retardation of the onset of ritualization following the intracerebral administration of the drug during re-organization;

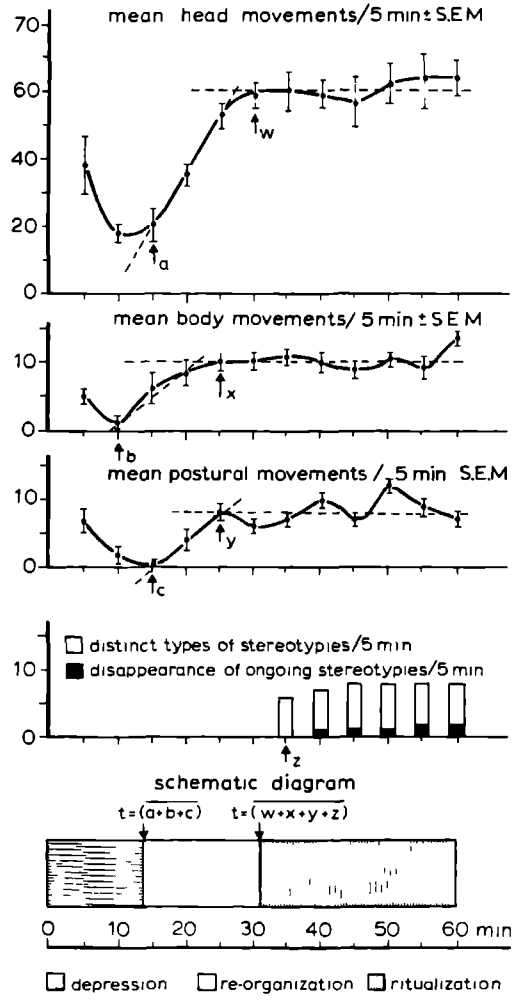

Fig. 3.12 Behavioural effects following a systemic injection of morphine (5 mg/kg, i.p.) in a male cat: illustration of the determinants of morphine-induced behaviour. The number of head, body and postural movements which were counted per min are represented as mean values/5 min ± S.E.M. The onset/termination of the various phases in terms of min following the morphine injection are represented as vertical axis inside the diagram outlining the different blocks depicted. For definition of the onset: see text.

and c) immediate termination of re-organization following the intracerebral administration of the drug during this phase. In this context, it should be noted that a) and b) could also be used as measures of the effectiveness of the drug-induced influence upon depression and ritualization respectively, i.e. a) = termination of depression, and b) = retardation of the onset of ritualization.

From the methodological point of view, it is useful to consider the following: as already mentioned, the morphine-induced syndrome is built up as three successively appearing phases. The characteristic features of each phase differ qualitatively from each other. Accordingly, the ability of an intracerebrally injected drug to accelerate the onset of a particular phase or to prolong its duration should simply be determined by analyzing the qualitative changes produced by that drug. As the recognition of such changes, however, required extensive and long-term training of the observers and, even thus errors remained possible, only a double-blind study with at least two independent observers per experiment could have offered reliable data. We therefore searched for quantitative parameters to facilitate the detection of qualitative changes. Thus, the onset of re-organization which is marked by the sudden appearance of isolated, staccato movements - which are recognizable by a well-trained observer - was in practice determined with the help of the number of movements which immediately increased because of the appearance of the isolated, staccato movements. In other words, the above-mentioned quantitative parameters were only used as tools to detect the occurrence of qualitative changes; no further use was made of the frequencies so obtained since neither calculation of group means of the absolute values nor calculation of group means of the relative changes offered a useful index for the changes occurring during or just before re-organization. Finally, all drugs which accelerated the onset of re-organization both increased the intensity of the symptoms characteristic of this phase and prolonged the duration of the phase, when these drugs were given shortly after the onset of re-organization. Since changes in the intensity were difficult to measure quantitatively, measurements of quantitative shifts in time were preferred to measurements of qualitative shifts in intensity for detecting the occurrence of drug-induced potentiation.

The influence of intracerebrally injected drugs upon the morphine-

induced ritualization was determined as follows: a) immediate onset of the display of the stereotyped and ritualized patterns following the intracerebral administration of the drug during re-organization; this effect could also be used as measure for the drug-induced influence upon re-organization, i.e. termination of re-organization; b) increase in the mean frequencies of head, body and postural movements for a period of at least 10 min and/or the display of new stereotyped and ritualized patterns following the intracerebral administration of the drug during ritualization; and c) partial or complete suppression of the ongoing stereotyped and ritualized patterns following the intracerebral administration of the drug during ritualization (= termination of ritualization). Since low doses of drugs - which given in higher doses are able to produce effects a) and b) - may produce slight changes in the nature of the head, body and postural movements without changing the overall nature of the stereotyped and ritualized patterns, such changes in the "kinetic melody" of the ongoing stereotypies were accordingly classified as *potentiation, score* 1 in contrast to the overt symptoms mentioned above, which were classified as *potentiation, score* 2 (Cools et al., 1977a).

In order to allow intracerebral injections into conscious, freely moving cats, the animals were prepared as previously described (Cools and Van Rossum, 1970). Short, double-barreled, stainless steel cannulae were stereotaxically implanted in such a way that the injection needle could reach the caput nuclei caudati rostromedialis (CRM area; co-ordinates according to Snider and Niemer (1974): A = 14-16, H = 14-16, L = 4-6) or the caput nuclei caudati anterodorsalis (r-CRM area; co-ordinates according to Snider and Niemer (1964): A = 18-19.5, H = 14-16, L = 3-5). Cannulae directed to the r-CRM area were placed at an angle of 10^{o} in reference to the mid-sagittal plane in order to bypass the lateral ventricle. Placing responses, righting and pupillary reflexes were checked upon recovery from the operation. In addition, food-intake, wakefulness and reactivity to visual and acoustic stimuli were estimated; only cats which responded normally during the second post-operation week were used in the experiments, which started at that time.

Drug solutions (5 µl) were injected through injection needles which extended into the brain tissue about 2 mm below the tip of the embedded cannulae. The following substances were used: dopamine hydrochloride (Koch-Light), haloperidol (Serenase^{R}, Janssen Pharmaceutica), (3,4-dihydroxyphenylamino)-2-imidazoline hydrochloride (DPI, Wander), ergo-

metrine maleate (Halewood Chemicals) and morphine sulphate (Chemische Farmaceutische Fabrieken). The doses shown in section 3.2.4 were based on the outcome of earlier studies (Cools et al., 1976) and refer to the salts. Apart from morphine which was always dissolved in 0.9% NaCl, all compounds were dissolved in either 0.9% NaCl or double-distilled water. Injections of solvents only produced slight arousal during the first 1-3 min following the injection regardless of the pretreatment.

After completion of the experiments, the brain was removed and prepared as previously described (Cools and Van Rossum, 1970); visual inspection of a series of brain slices (about 100 µm), caudally from a knife-cut along the track of the guide cannulae, was made to determine the sites of injections. Only experiments in which the injection sites were restricted to the target areas shown in Fig. 3.13 are discussed below.

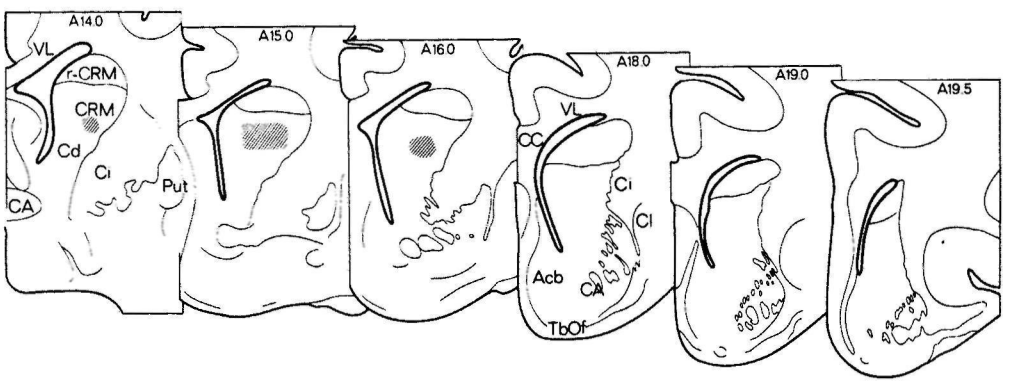

Fig. 3.13 Semi-diagrammatic outline of the functionally and pharmacologically distinct intracaudate dopamine-sensitive areas. All injections into the so-called CRM area were restricted to the shaded areas (CRM), whereas all injections into the so-called r-CRM area were restricted to the open area (r-CRM). Acb = nucleus accumbens; CA = commissura anterior; CC = corpus callosum; Cd = nucleus caudatus; Ci = capsula interna; Cl = claustrum; CRM = caput nuclei caudati, rostromedial part; Put = putamen; r-CRM = caput nuclei caudati, anterodorsal part; TbOf = tuberculum olfactorium; VL = ventriculus lateralis.

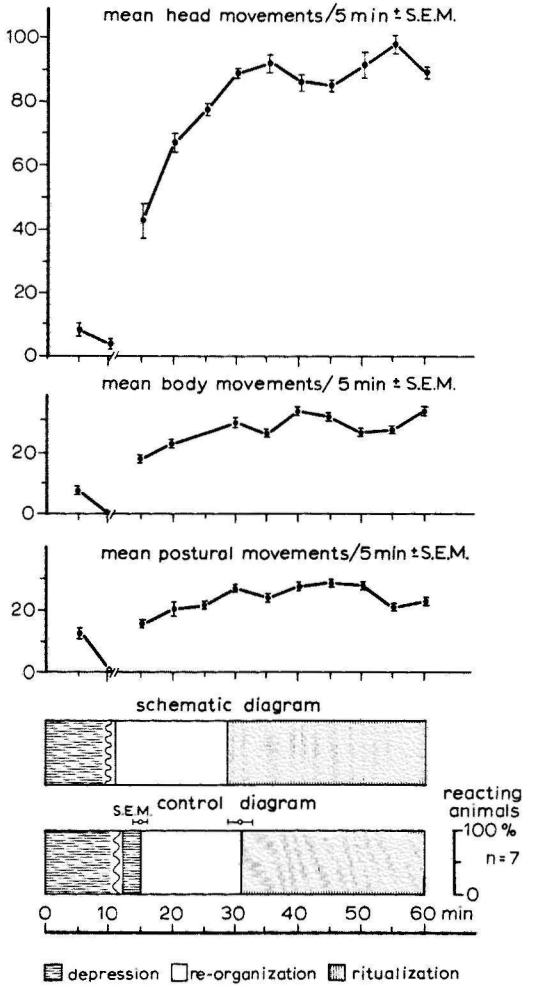

Fig. 3.14 Behavioural effect of bilateral injections of dopamine (10 µg/ 5 µl given at time = 10) into the CRM area upon the morphine-induced depression in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): immediate onset of the re-organization in the "dopamine-morphine"-treated cat. The control diagram at the bottom of the figure shows the effects of saline injections into the CRM area of morphine-treated cats (n=7). The number of head, body and postural movements counted per min are represented as mean values/5 min ± SEM. The onset/termination of the various phases in terms of min following the morphine injection (diagram of the "drug-morphine"-treated cat) or their mean values ± SEM (control diagram of the "saline-morphine"-treated cats; n = number of cats tested) are indicated by the vertical axes outlining the blocks. The percentage of animals showing a particular phase is given along the ordinate. The time of the intracerebral injections is indicated by the wave-like line.

3.2.4 Results

Morphine-induced "re-organization" and modulation of DA activity within the CRM area

DA (10 µg/5 µl per side), administered locally into the CRM area at the time when the morphine-induced depression had fully developed, immediately disrupted the ongoing depression, increased the frequencies of head, body and postural movements, and immediately triggered the display of the staccato movements. An example is given in Fig. 3.14; saline given at the same time remained ineffective in this respect. The results of all experiments (n = 9) are summarized in Fig. 3.16A, and show that the onset of re-organization was significantly accelerated by DA given under the conditions mentioned ($p < 0.05$; Students t-test). If a similar dose of DA was given at the time when the morphine-induced re-organization had fully developed (17-19 min following the morphine injection), it significantly retarded the onset of ritualization and, accordingly, prolonged the duration of re-organization from 16±2 min in "saline-morphine"-treated cats (n = 8) to 24±2 min in "dopamine-morphine"-treated cats (n = 7; $p < 0.05$; Students t-test). From the qualitative point of view, the intensity of the symptoms present during re-organization was strongly increased in comparison with that of symptoms in morphine-treated cats which received a placebo (saline) injection. The results, however, could not be analyzed quantitatively because: a) differences in frequencies of the head, body or postural movements did not simply reflect differences in intensities, b) no intra-individual comparison between placebo and drug injections could be made, and c) the inter-individual variability greatly varied.

Haloperidol (12.5 µg/5 µl per side), administered locally into the CRM area at the time when the morphine-induced re-organization had already developed, decreased the frequencies of the head, body and postural movements and suppressed the display of the staccato movements; those head movements which were already integrated in stereotyped and ritualized patterns remained unaffected (n = 9). An example is given in Fig. 3.15. If a similar dose of haloperidol was administered when the morphine-induced depression was still present, it completely suppressed the development of re-organization and ritualization. The results of the last-mentioned series of experiments (n = 11) are summarized in Fig. 3.16B.

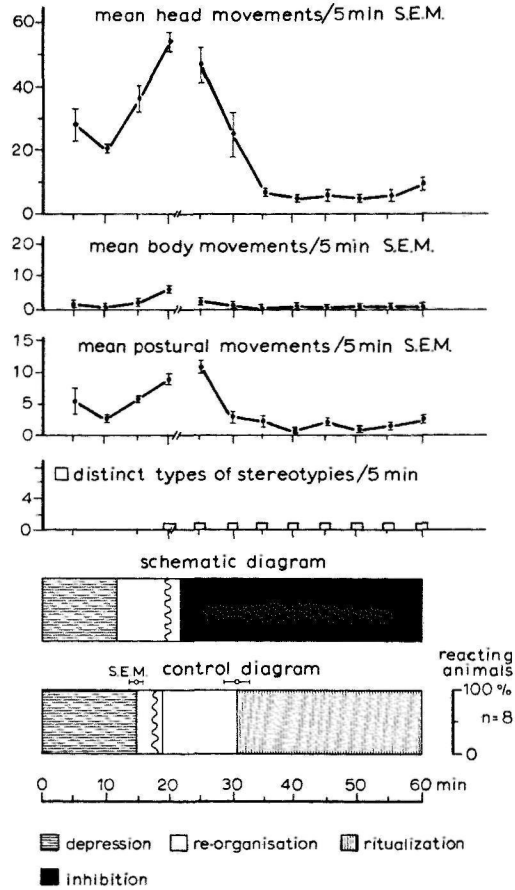

Fig. 3.15 Behavioural effect of bilateral injections of haloperidol (12.5 µg/5 µl given at time = 20) into the CRM area upon the morphine-induced re-organization in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): inhibition of re-organization and ritualization in the "haloperidol-morphine"-treated cat. The control diagram at the bottom of the figure shows the effects of saline injections into the CRM area of morphine-treated cats (n=8). For further explanations: see Fig. 3.14.

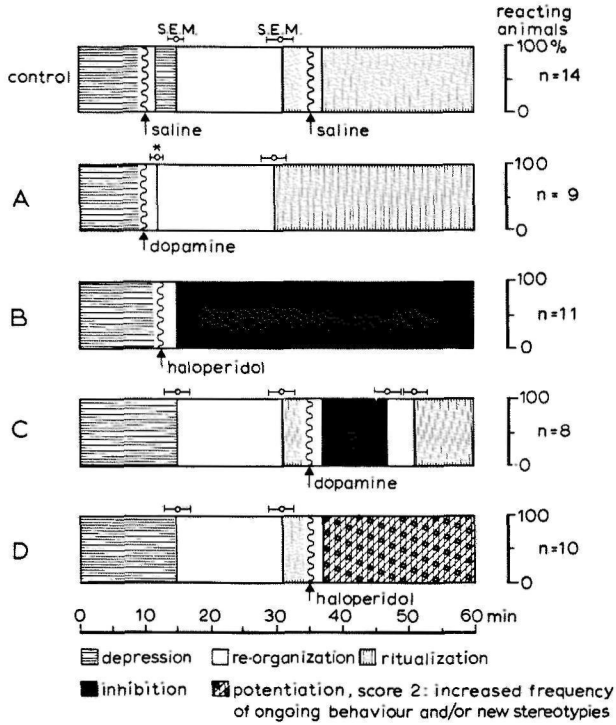

Fig. 3.16 Behavioural effect of bilateral injections of saline (5 µl given at time = ↑), dopamine (10 µg/5 µl given at time = ↑) and haloperidol (12.5 µg/5 µl given at time = ↑) into the CRM area upon the morphine-induced re-organization and ritualization (morphine: 5 mg/kg, i.p. given at time = 0): A = immediate onset of re-organization; B = immediate termination of re-organization, and inhibition of ritualization; C = immediate termination of ritualization followed by a short-term period marked by features of re-organization, and final return of ritualization; D = potentiation of ritualization. In the control diagram, morphine-treated cats having received intracerebral placebo injections at t = 10-12 min (n=7) and those having received placebo injections at t = 34-36 min (n=7) are taken together. For further explanations: see Fig. 3.14.

** onset of re-organization is significantly different from that in controls ($p < 0.05$; Students t-test).*

Morphine-induced "ritualization" and modulation of DA activity within the CRM area

DA (10 µg/5 µl per side), administered locally into the CRM area at the time when the morphine-induced ritualization had fully developed, immediately suppressed the display of the ongoing stereotyped and ritualized patterns for a period of about 7 min; the ritualization then returned after a short period marked by features characteristic of re-organization. Saline injections given at the same time remained ineffective in this respect. The results of these experiments (n = 8) are summarized in Fig. 3.16C.

Haloperidol (12.5 µg/5 µl per side), administered locally into the CRM area at the time when the morphine-induced ritualization was fully developed, immediately increased the mean frequencies of the head, body or postural movements during the next 10 min and, in addition, produced new types of stereotypies (Table 3.1). The results of these experiments (n = 10) are summarized in Fig. 3.16D.

TABLE 3.1

Effects of bilateral injections of haloperidol (12.5 µg/5 µl per side) applied locally into the CRM area at about 34-36 min after an i.p. injection of morphine (5 mg/kg) in cats (n = 10).

	Controls (n=7) $X_{post}-X_{pre}$	Haloperidol (n=10) $X_{post}-X_{pre}$	Mann-Whitney U-test
head movements	-1 ± 21	170 ± 35	$p < 0.002$
body movements	5 ± 11	140 ± 20	$p < 0.002$
postural movements	6 ± 4	77 ± 6	$p < 0.002$
% cats showing new stereotypies	0	100	

The difference between the total number of each type of movements displayed during the 10 min period following the intracerebral injection (post) and that displayed during the 10 min period preceding the intracerebral injection (pre) is calculated for each animal. Mean differences ± S.E.M. are given.

DPI (10 µg/5 µl per side), administered locally into the r-CRM area at the time when the morphine-induced re-organization had fully developed immediately introduced a shift from this phase towards the ritualization phase; saline injections remained ineffective in this respect. An example is given in Fig. 3.17. All cats (n = 7) immediately showed stereotyped and ritualized head, body and postural movements whose nature depended on the nature of the behavioural patterns displayed during the first 1-5 min following the injection. The following observation illustrates this point: cat no. 7745 defecated at a particular spot in the cage during the first post-injection minutes. The animal returned many times to that spot during the next 45 min, then displayed all the movements characteristic of defecation. If a similar dose of DPI was administered when the morphine-induced depression was still present (n = 3), the animals maintained their lying posture and repeated those head movements which had been displayed during the first post-injection minutes; most often, lowering of the nose towards the floor was seen. In other words, DPI immediately ritualized the ongoing behaviour. About 15-20 min after DPI injection, i.e. when the morphine-induced ritualization normally started in morphine-treated cats with either no intracerebral injection at all or a placebo (saline) injection, the number of distinct types of stereotypies increased. An example is given in Fig. 3.18. In 80% of the animals tested, an additional change in the form of increased intensity occurred at about 25-30 min after the DPI injection (not shown). The results of these experiments (n = 10) are summarized in Fig. 3.20C, and show that DPI given under the conditions described immediately triggered the display of the ritualization.

Ergometrine (10 µg/5 µl per side), administered locally into the r-CRM area at the time when the morphine-induced re-organization was fully developed, initially introduced a shift from this phase towards a ritualization-like phase, i.e. the isolated, staccato head movements and single movements of the head, neck, trunk and limbs were integrated into complete patterns which were repeated from time to time during a period of 3-7 min. These patterns subsequently disappeared and symptoms characteristic of re-organization appeared. Finally, the ritualization phase started; the onset of the last phase was significantly retarded ($p < 0.05$;

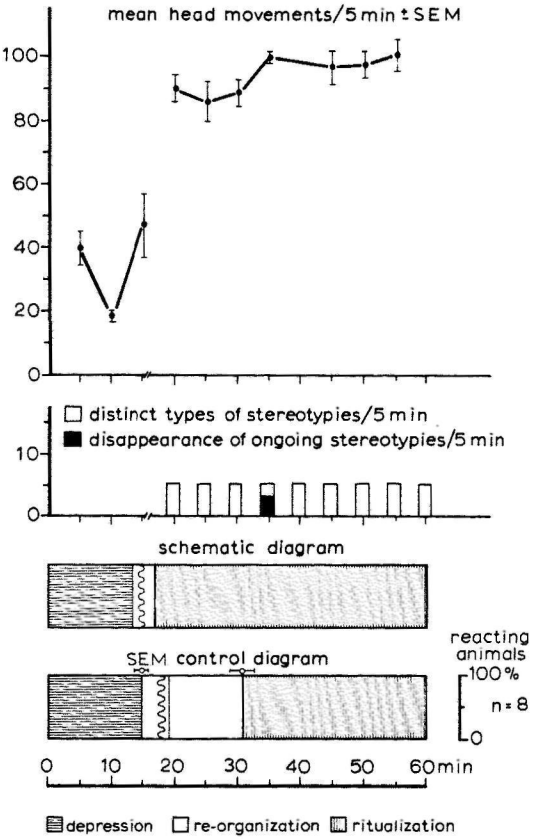

Fig. 3.17 Behavioural effect of bilateral injections of (3,4-dihydroxyphenylamino)-2-imidazoline (DPI, 10 µg/5 µl given at time = 15) into the r-CRM area upon the morphine-induced re-organization in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): immediate onset of the ritualization in the "DPI-morphine"-treated cat. The control diagram at the bottom of the figure shows the effects of saline injections into the r-CRM area of morphine-treated cats (n=8). For further explanations: see Fig. 3.14.

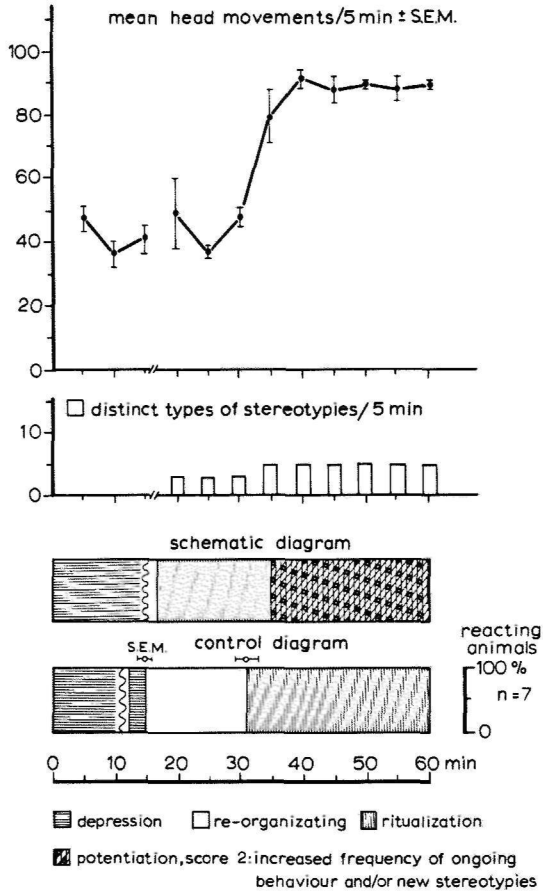

Fig. 3.18 Behavioural effect of bilateral injections of (3,4-dihydroxyphenylamino)-2-imidazoline (DPI, 10 µg/5 µl given at time = 15) into the r-CRM area upon the morphine-induced depression in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): immediate onset of the ritualization of the ongoing behaviour and additional potentiation at time = 35 in the "DPI-morphine" treated cat. The control diagram at the bottom of the figure shows the effects of saline injections into the r-CRM area of morphine-treated cats (n=7). For further explanations: see Fig. 3.14.

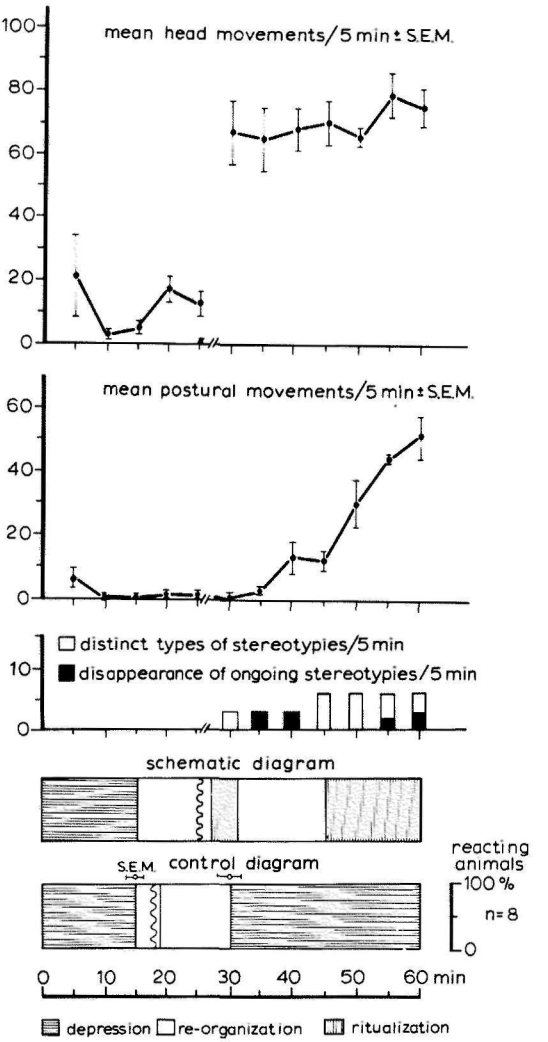

Fig. 3.19 Behavioural effect of bilateral injections of ergometrine (10 µg/5 µl given at time = 25) into the r-CRM area upon the morphine-induced behaviour in a male cat (morphine: 5 mg/kg, i.p. given at time = 0): immediate onset of a short-term ritualization, prolongation of the subsequently appearing re-organization and retardation of the onset of the subsequent ritualization in the "ergometrine-morphine"-treated cat. The control diagram at the bottom of the figure shows the effects of saline injections into the r-CRM area of morphine-treated cats (n=8). For further explanations: see Fig. 3.14.

Students t-test). An example is given in Fig. 3.19. From the qualitative point of view, the intensity of the symptoms present during re-organization and ritualization was strongly increased in comparison with that of symptoms in morphine-treated cats with either no intracerebral injection at all or a placebo (saline) injection. As already mentioned, the results could not be analyzed statistically. The results of this series of experiments (n = 10) are summarized in Fig. 3.20D and show that under the conditions described initially triggered symptoms characteristic of ritualization, intensified the successive re-organization and ritualization (not shown) and significantly retarded the onset of ritualization.

Morphine-induced "ritualization" and modulation of DA activity within the r-CRM area

DPI (10 µg/5 µl per side), administered locally into the r-CRM area at the time when the morphine-induced ritualization had fully developed, produced an increase in the mean frequencies of the head, body or postural movements (86% of the tested animals) and in the number of distinct types of stereotyped and ritualized patterns (100% of the tested animals); saline injections remained ineffective in this respect (Table 3.2). The results of these experiments (n = 7) are summarized in Fig. 3.20A, and show that DPI potentiated the morphine-induced ritualization.

Ergometrine (10 µg/5 µl per side), administered locally into the r-CRM area at the time when the morphine-induced ritualization was fully developed, produced a complex picture. In 80% of the animals (n = 10), there was an increase in the mean frequencies of the stereotyped and ritualized head, body or postural movements within the first 1-7 min (mean duration: 5 $\pm$ 1.8 min) following the injection; in 62.5% of these animals, the increase was accompanied by changes in the "kinetic melody" of the ongoing stereotypies. In the remaining 20% of the animals, there was no increase in frequency but there were slight changes in the nature of the ongoing stereotypies, i.e. changes in the "kinetic melody". Following this initial period, in which the morphine-induced ritualization was apparently potentiated, all animals showed a variable number (3-5) of short-lasting periods (1-2 min), in which the stereotyped and ritualized patterns completely disappeared and were replaced by normal behavioural patterns; as the animals remained very active during these periods, only changes in the number of distinct types of stereotypies and not changes in the frequencies of the head, body and postural move-

TABLE 3.2

Effects of bilateral injections of DPI (10 µg/5 µl per side) applied locally into the r-CRM area at about 40-42 min after an i.p. injection of morphine (5 mg/kg) in cats (n = 7).

	Controls (n=6) $X_{post}-X_{pre}$	DPI (n=7) $X_{post}-X_{pre}$	Mann-Whitney U-test
head movements	-1 ± 21	50 ± 60	n.s.[1]
body movements	5 ± 11	99 ± 59	n.s.[1]
postural movements	6 ± 4	24 ± 18	n.s.[1]
% cats showing new stereotypies	0	100	

The difference between the total number of each type of movements displayed during the 10 min period following the intracerebral injection (post) and that displayed during the 10 min period preceding the intracerebral injection (pre) is calculated for each animal. Mean differences ± S.E.M. are given.

1 = as mentioned in the text, 86% of the "DPI-morphine"-treated cats did show increased frequencies. Note that all "DPI"-treated cats showed new stereotypies during the 10 min period following the DPI injection.

ments could be used as objective variables. These periods were, in turn, followed by short-lasting time blocks (about 1 min), in which the characteristic features of re-organization appeared. In two animals, normal behaviour and re-organization succeeded each other during a period of 7-9 min; ritualization then returned and remained unaffected. In most animals (n = 8), however, the short-lasting periods marked by normal behaviour did not return until re-organization had been followed by a short-term period of ritualization; in these animals, the sequence was repeated 3-6 times. The mean time during which ritualization was completely absent was 11 ± 2 min. The results of this group of experiments (n = 10) are partly summarized in Fig. 20B, and show that ergometrine initially potentiated the ongoing ritualization and, subsequently, inhibited this phase.

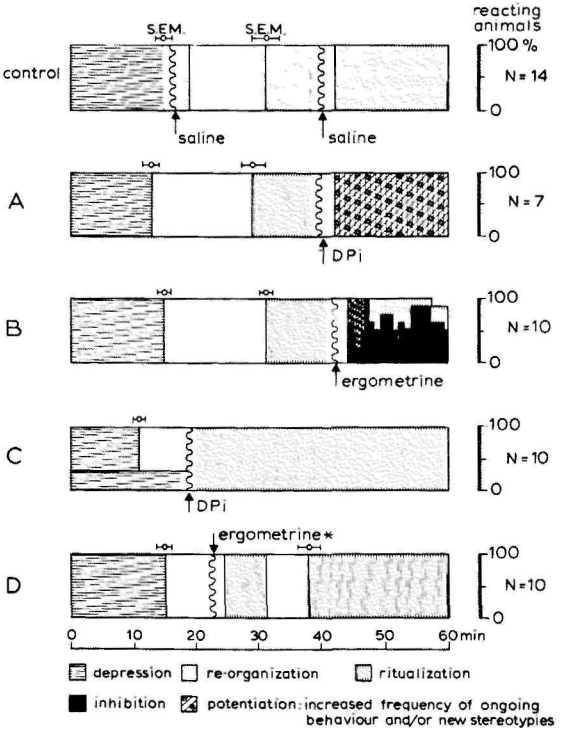

Fig. 3.20 Behavioural effects of bilateral injections of saline (5 µl given at time = ↑), (3,4-dihydroxyphenylamino)-2-imidazoline (DPI, 10 µg/5 µl given at time = ↑) and ergometrine (10 µg/ 5 µl given at time = ↑) into the r-CRM area upon the morphine-induced re-organization and ritualization (morphine: 5 mg/kg, i.p. given at time = 0): A = potentiation of the ritualization; B = short-term potentiation of the ritualization followed by inhibition of the ritualization; C = immediate ritualization of ongoing behaviour; D = short-term ritualization of ongoing behaviour, prolongation of the subsequent re-organization and retardation of the subsequent ritualization. In the control diagram, morphine-treated cats having received intracerebral placebo injections at t = 17-19 min (n=8) and those having received placebo injections at t = 40-42 min (n=6) are lumped together. For further explanations: see Fig. 3.14.

** onset of ritualization is significantly different from that in controls ($p < 0.05$; Students t-test).*

3.2.5 Discussion

Drug-induced changes in the DAe activity within the CRM area of morphine-treated cats

DA, locally administered into the caput nuclei caudati rostromedialis (CRM area) at different time intervals after an acute injection of i.p. given morphine, was found to: 1a) accelerate the onset of re-organization if given during the depression; 1b) prolong the duration of re-organization, retard the onset of ritualization and increase the intensity of the symptoms present during re-organization if given during re-organization; and 2) suppress the display of the stereotyped and ritualized patterns if given during ritualization. The results summarized in 1a-b suggest that re-organization, i.e. all effects appearing immediately after the depression and lumped together as "relatively early" morphine effects, required at least a relatively increased DA activity within the CRM area. In contrast, the results summarized in 2 suggest that ritualization, i.e. all effects appearing immediately after re-organization and lumped together as "late" morphine effects, required instead a relatively decreased DA activity within the CRM area. Haloperidol given under experimental conditions comparable to those used for DA also elicited two distinct types of effects: 1) if given during re-organization, haloperidol suppressed the progressive development of re-organization, and 2) if given during ritualization, haloperidol increased the number of distinct types of stereotypies. Thus, haloperidol produced effects diametrically opposite to those elicited by DA: a) inhibition of the "relatively early" morphine effects, and b) potentiation of the "late" morphine effects. These findings confirm the outcome of an earlier study (Cools et al., 1974). As haloperidol in the dose used inhibited DAe activity within the CRM area in a selective way, most likely via postsynaptic DA receptor blockade (Cools et al., 1976), it can be concluded that the effects observed were due to specific changes in DAe activity within the CRM area.

Drug-induced changes in the DAi activity within the r-CRM area of morphine-treated cats

DPI, a selective stimulant of DAi activity within the caput nuclei caudati anterodorsalis (r-CRM area) and administered into the r-CRM area at different time intervals after the morphine injection, was found to produce: 1) if given during re-organization, immediate re-

placement of the symptoms characteristic of re-organization by symptoms characteristic of ritualization, and 2) if given during ritualization, an increase in the number of distinct types of stereotypies. Thus, DPI also produced two distinct types of changes: a) suppression of the "relatively early" morphine effects, and b) potentiation of the "late" morphine effects. Ergometrine given under experimental conditions comparable to those used for DPI elicited the following effects at about 7 min after its administration: 1) if given during re-organization, an increased intensity of the symptoms displayed during re-organization in all cats and retardation of the onset of ritualization, and 2) if given during ritualization, suppression of the symptoms characteristic of ritualization. In other words, about 7 min after its injection ergometrine produced effects opposite to those elicited by DPI: a) potentiation of the "relatively early" morphine effects, and b) inhibition of the "late" morphine effects. Considering the specificity of the drugs in the doses used (Cools et al., 1976), it can be concluded that the effects observed were due to specific changes in DAi activity within the r-CRM area.

Ergometrine given at the onset of re-organization initially produced effects comparable to those triggered by injections of DPI (cf. Fig. 3.20D and C); the same holds true for the effects elicited by ergometrine given during ritualization (cf. Fig. 3.20B and A). Apart from its ability to produce a long-lasting decrease of DAi activity in rats (Cools et al., 1977b), ergometrine has recently been found to elicit a short-lasting increase in DAi activity before the decrease in DAi activity (Cools, 1977b). Although such a biphasic effect of ergometrine administered into the r-CRM area of naive cats was not detected, the ergometrine-induced inhibition of DAi activity only became visible after a period of 10-15 min (Cools et al., 1975). Considering the data from the present study in the light of the earlier results with rats and cats, it is tempting to postulate that the effects induced by ergometrine immediately after its administration were also due to a short-term increase of DAi activity; additional experiments are however required to support this postulate.

Morphine and its biphasic influence upon the DAe-DAi "balance" within the feline caudate nucleus

There may exist a DAe-DAi "balance" within the brain of rats and cats (Cools and Van Rossum, 1976; Cools, 1977a-c). The present data support

Drugs	Injection-area	System/receptors	morphine-induced re-organization	morphine-induced ritualization	DA_e-DA_i balance re-organization	DA_e-DA_i balance ritualization
dopamine	CRM	DA_e ↑	↑	↓		
haloperidol	CRM	DA_e ↓	↓	↑		
DPI	r-CRM	DA_i ↑	↓	↑		
ergometrine	r-CRM	DA_i ↓	↑	↓		

Conclusion: re-organization: $DA_e/DA_i > 1$
ritualization · $DA_e/DA_i < 1$

Fig. 3.21 Schematic representation of the data collected in the present study. Hatched area: drug-induced increased activity. Black area: drug-induced decreased activity.

this concept (Fig. 3.21): 1) both DA administered into the CRM area and increasing DAe activity and ergometrine administered into the r-CRM area and decreasing DAi activity could potentiate the "relatively early" morphine effects and suppress the "late" morphine effects; 2) both haloperidol administered into the CRM area and decreasing DAe activity and DPI administered into the r-CRM area and increasing DAi activity could suppress the "relatively early" morphine effects and potentiated the "late" morphine effects. From this point of view, it appears difficult to exclude the possibility that "relatively early" morphine effects requiring predominantly DAe activity (Fig. 3.21) were due to a) a morphine-induced increase in DAe activity, b) a morphine-induced decrease in DAi activity, or c) a combination of a) and b). The same holds true for the "late" morphine effects which required predominantly DAi activity (Fig. 3.21). Detailed inspection of the data, however, reveals that the development of the whole morphine syndrome could only be prevented by suppression of DAe activity with haloperidol (Fig. 3.16B); in contrast, stimulation of DAi activity with DPI given under the same conditions did not inhibit, but actually accelerated the development of the morphine syndrome (cf. Figs. 3.16B and 3.20C). In other words, restoration of the disrupted DAe-DAi "balance" during the "relatively

early" morphine effects could only be achieved by inhibition of DAe activity. Accordingly, it can be concluded that only an increased DAe activity within the CRM area might underly the "relatively early" morphine effects.

With respect to morphine's ability to influence DAe and/or DAi activity during the "late" morphine effects, the situation is somewhat more complicated. As summarized in Fig. 3.21, DAi activity predominates during ritualization, i.e. the period marked by the presence of the "late" morphine effects. As suppression of DAi activity by means of ergometrine administration into the r-CRM area during ritualization did not result in a continuous display of normal behaviour but in periods marked by symptoms characteristic of re-organization, it can be concluded that DAe activity was still increased at that time. Accordingly, the "late" morphine effects were certainly not due to the presence of decreased DAe activity. All the data taken together suggest that the "late" morphine effects required strongly increased DAi activity within the r-CRM area, i.e. that they overcame the increased DAe activity within the CRM area.

Summarizing, it appears that morphine initially increased DAe activity within the CRM area and, subsequently, also increased DAi activity within the r-CRM area in such a way that the increased DAi activity could overcome the increased DAe activity. This hypothesis is consistent with the outcome of a recent study on morphine in rats (Moleman, 1977). The biochemical data collected in this elegant study also suggest that morphine affects the DA activity differently in different brain regions.

Finally it is of interest that a) suppression of DAe activity prevented the occurrence of phenomena triggered by changes in DAi activity, and b) changes in DAe activity immediately affected the symptoms elicited by changes in the DAi system; there is, indeed, some indirect evidence that these systems might be connected via GABA-ergic neurons (Cools, 1977c).

Current concepts on morphine's ability to influence central DA activity

Two opposing hypotheses emerge from the literature on morphine's ability to modify DA activity (see 3.2.1): 1) morphine may somehow decrease postsynaptic DA activity; and 2) morphine may somehow increase postsynaptic DA activity.

In particular the observation that there was some resemblance between the behavioural effects, often labelled as catalepsia or catatonia, elicited by high doses of morphine in rats and those produced by antagonists of DA receptors had led to the idea that morphine might inhibit DA activity. Additional data which are more or less in line with this concept have recently been summarized (Lal et al., 1975). In contrast, there is an increasing number of studies which indicate that morphine increases rather than decreases DA activity within the brain of mice, rats and cats. In mice, both blockade of postsynaptic DA receptors and the postulated stimulation of presynaptic DA receptors by extremely low doses of apomorphine - which is assumed to decrease DA release - can suppress the "running fit" (Scheel-Krüger et al., 1977). In rats also both blockade of postsynaptic DA receptors and the postulated stimulation of presynaptic DA receptors suppress both the behavioural activation characteristic of low doses of morphine and the so-called "late" behavioural activation following higher doses of morphine (Scheel-Krüger, 1976; Scheel-Krüger et al., 1977). In rats, morphine also increased the firing rate of DA cells within the substantia nigra (Iwatsubo, 1976; Lichtensteiger and Lienhart, 1975), the striatal DA turnover rate, the striatal DA-dependent adenylate cyclase activity, and the striatal DA-specific cyclic AMP concentration (Bonnett, 1975; Clouet et al., 1975; Costa et al., 1973; Iwatsubo and Clouet, 1975; Puri et al., 1975, 1976) and potentiates the stereotyped behaviour induced by intermediate doses of apomorphine (McKenzie and Sadof, 1974; Scheel-Krüger, 1976; Scheel-Krüger et al., 1977; Vedernikow, 1970). In cats, blockade of DA receptors attenuates the morphine-induced behaviour (Cools et al., 1974; Dhasmana et al., 1972). These and related data suggest that morphine somehow increases DA activity (see also: Iwamoto et al., 1976; Nakamura et al., 1973).

The present data are consistent with this hypothesis. Are they thus in conflict with the concept that morphine somehow inhibits DA activity? It is questionable whether or not morphine produces true catalepsy (Janssen, 1964; Papeschi et al., 1976) and whether, accordingly, morphine-induced effects such as the increased DA turnover rate are due to a direct or indirect postsynaptic DA receptor blockade. The fact that the ability of morphine to suppress certain behavioural symptoms is a characteristic feature of dopaminergic inhibitors (Lal et al.,

1975) points to a more complex situation. The present study, however, offers data which might bridge the gap between the apparent discrepancies: haloperidol did not only suppress the effects at least partly due to increased DA activity within the rostromedial part (the so-called increased DAe activity present during the re-organization) by inhibiting the DAe receptors, but it also potentiated those effects which were at least partly due to an increased DA activity within the anterodorsal part (the so-called increased DAi activity present during the ritualization) by increasing the difference between DAe and DAi activity. The present data suggest the following explanation for the results which led to the hypothesis that morphine may somehow inhibit DA activity: the so-called "late" morphine effects produced by a predominance of the DAi activity are 1) potentiated by antagonists of DAe activity, i.e. classic neuroleptics, because of the ability of neuroleptics to increase the DAe-DAi imbalance marked by a predominant DAi activity, and 2) inhibited by agonists of DAe activity, i.e. compounds such as apomorphine, because of their ability to correct this DAe-DAi imbalance. This, and the fact that the so-called "relatively early" effects of predominant DAe activity are inhibited by classic neuroleptics and potentiated by compounds such as apomorphine, indicates that the outcome of studies on the ability of DA agonists and antagonists to influence the morphine symptoms depends on the cluster of morphine-induced symptoms selected. As different clusters e.g. those described in the present study, follow one another it is clear that the time schedule used plays a crucial role in studies on morphine-DA interaction. The fact that morphine given to guinea pigs hardly affects the L-DOPA-induced dyskinesias, but suppresses the apomorphine-induced biting (Costall and Naylor, 1976) also points in this direction.

Apart from the fact that the present study may help to solve some of the discrepancies in the literature, it stresses that selective DAe and DAi agonists or antagonists, might be considered as essential tools for the determination of the actual influence of morphine upon central DA activity. It is noteworthy that in studies on the behavioural effectiveness of morphine applied locally to the caudate nucleus of cats, doses varying from 1 to 10 µg morphine sulphate dissolved in saline remained completely ineffective, under the conditions used in the present study (unpublished data). In contrast, intracerebral administration of morphine into the

substantia nigra was effective, although the symptoms were not identical to those elicited by an i.p. injection of morphine (Broekkamp, 1975). The ability of morphine to trigger an increase in DA activity within the caudate nucleus might thus be due to direct or indirect interference with the DA cell bodies rather than to a direct interference with structures within the DA synapses. This suggestion is supported by several studies in which the rat was used as experimental animal (Broekkamp, 1976; Broekkamp et al., 1976; Carenzi et al., 1975; Lienhart et al., 1975; Wiegant et al., 1977). It is important to note that this statement does not exclude the possibility that morphine may affect non-dopaminergic substrates within the caudate nucleus; actually, both the presence of morphine receptors within the neostriatum (Pert and Snyder, 1973; Pert et al., 1976) and the ability of striatally applied morphine (1-10 µg) to modify the flinch and jump thresholds in rats (Jacquet and Lajtha, 1973) point in this direction.

Finally, the dissimilarity between the behavioural effects elicited by intranigral injections of morphine and those triggered by morphine applied systemically in cats emphasizes the nearly "classic" view that morphine needs to affect many different systems within the brain in order to produce its complex effects. The present study only points out that particular changes in central dopaminergic systems do play a role in eliciting the morphine-induced behavioural symptoms. Whether or not these changes in the DA systems are triggered by interaction of morphine with endorphin, enkephalin or other receptors remains an open question.

Functional aspects of the DAi system

In previous discussions of the role of the DA system (Cools, 1973, 1975; Cools and Van den Bercken, 1977) it has been suggested that increased DAe activity results in a) a break-down of previously established chains of motor responses enabling the organism to establish completely new behavioural patterns (Cools, 1973), and b) a break-down of previously established links between different concepts and conceptual frames enabling the organism to establish completely new concepts and conceptual frames (Cools, 1975). The mechanism underlying these phenomena has been described elsewhere (Cools and Van den Bercken, 1977). Although the present data are not sufficient to delineate the role of the DAi system

in this respect, they do show that stimulation of the DAi system prevents the display of completely new patterns and triggers a process resulting in the more or less permanent display of the behaviour present at the onset of the drug action. Considering the functional changes induced by modulation of the DAi system in morphine-treated cats, the characteristic features of the DAe-DAi "balance" and the function of the DAe system, it is tempting to postulate that the DAi system may actually inhibit the process activated by the DAe system, i.e. it may prevent the break-down of the previously established chains of motor responses and, as a result, hinder the organism in the introduction of new patterns. In this respect the DAi system appears to differ from the serotonergic, raphe-striatal system whose functioning is indirectly activated by the DAe system, since the 5-HT system appears to hinder the organism in the introduction of new patterns through its ability to condition ongoing behaviour (Cools et al., 1974). This postulate requires further investigation.

3.3 GENERAL COMMENTS

The studies presented in this chapter deal with morphine's action on pharmacologically defined neurotransmission processes within anatomically circumscribed regions of the brain, i.e. 1) noradrenergic processes within the nucleus linearis intermedius raphe (section 3.1), and 2) dopaminergic processes within the caudate nucleus (section 3.2). The results show that morphine's effect on behaviour is related to the activity of these neuronal systems. Accordingly, they may form crucial links between specific target sites of morphine in the brain and the behavioural consequences of morphine's central action.
The results also demonstrate that administration of morphine suppresses the activity of the noradrenergic processes in the raphe nuclei and increases the activity of the dopaminergic processes in the caudate nucleus. In other words, modulation of morphine-induced behaviour by intracerebrally injected drugs has proven to be a valuable method for the study of morphine's action on specific neurotransmission processes in the brain.

Much controversy exists in literature about the development of tolerance to morphine's behaviour effects in cats (section 1.3.2: chronic effects). In the present study, a semi-quantitative approach

is used for the analysis of the morphine-induced behaviour effects. This approach enabled us to demonstrate partial tolerance to morphine's behaviour effects after administration of morphine (5 mg/kg/day) for a short period of only 3 days (section 3.1). This result may attest to the sensitivity of the analysis-method used since in other studies dealing with this subject higher doses of morphine and/or longer periods of treatment were required to reveal the development of tolerance to the behaviour effects of morphine (section 1.3.2: chronic effects).

Considering the involvement of the noradrenergic, coeruleo-raphe system in the development of tolerance to morphine's behaviour effects (section 3.1), a few additional remarks have to be made. The depressant action of morphine and related opioid peptides on neuronal cells within the locus coeruleus seems established (North, 1979; Strahlendorf et al., 1980), and the development of tolerance to this action of morphine has been demonstrated (Aghajanian, 1978). Furthermore, naloxone injected into the locus coeruleus region partially antagonizes the behaviour responses of cats to morphine administration (Van Dongen et al., 1979). All this data is on line with a role for the noradrenergic, coeruleo-raphe system in the development of tolerance to the behaviour effects of morphine in cats.

Noradrenergic processes within the raphe nuclei become hypoactive after a short-term increased exposure to noradrenaline and hyperactive after a short-term decreased exposure to noradrenaline. It is now widely documented that target cells are able to respond to changes in ambient ligand concentration, e.g. by changing the number and/or affinity of their surface receptors, and various mechanisms have been proposed to explain these phenomena (Catt et al., 1979; Triggle, 1980). It is indicated in the present study that the flexibility of noradrenergic processes plays an important role in the development of tolerance to the effects of morphine. Presumably, it may play a similar role in the development of tolerance to the effects of other drugs (cf. Tabakoff et al., 1978, 1979).

Morphine exerts a differential, biphasic influence on the activity of two pharmacologically distinct dopaminergic systems in the caudate nucleus, i.e. the DAe- and the DAi-systems (section 3.2). It has been suggested that the DAe-system receives its neurons from the substantia

nigra and that the DAi-system receives its neurons from the ventral tegmental area (Cools and Van Rossum, 1980). Hence, it might be that morphine interacts differentially with the activity of the dopaminergic systems originating in these brain regions. Such a differential action of morphine on distinct dopaminergic systems has been previously reported (Alper et al., 1980; Moleman and Bruinvels, 1979; Nazarro et al., 1980). The present results indicate that it is important in studies on morphine's central action 1) to specify in detail which neuronal system is studied, and 2) to indicate the time-course of the morphine-induced effects.

The exact way in which morphine affects dopaminergic activity in the caudate nucleus is unclear. Systemic administration of morphine produces an increase in the activity of the dopaminergic, nigro-striatal neurons (Jurna, 1981; Iwatsubo and Clouet, 1977; Lee et al., 1977; Nowycky et al., 1978). Morphine may produce this effect via a direct or indirect action on the mesencephalic, dopaminergic cell bodies (cf. Broekkamp et al., 1979a, b; Joyce and Iversen, 1979; Phillips and Le Piane, 1980; Stinus et al., 1980; Van Dongen et al., 1979). On the other hand, the dopaminergic terminals in the striatum contain presynaptically localized opiate receptors (Gardner et al., 1980; Murrin et al., 1980; Pollard et al., 1978), and locally applied opiate agents affect the metabolism and release of striatal dopamine (Biggio et al., 1978; Chesselet et al., 1981). Therefore, morphine may affect dopaminergic activity in the striatum alternatively via an action on the dopaminergic terminals. Consequently, morphine's action on striatal dopaminergic activity has to be considered as an overall effect of the opiate agonist on both the cell bodies and the terminals of the dopaminergic neurons.

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CHAPTER 4

MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL TO STUDY NEUROTRANSMISSION PROCESSES WITHIN THE SEPTAL REGION

4.1 THE SEPTAL REGION: AN INTRODUCTION

4.1.1 Introduction

The septal region, or septum, is a midline brain structure located in the telencephalon ventral to the anterior portion of the corpus callosum and connected with many other brain structures, particularly the hippocampus and the hypothalamus (see section 4.1.2). In humans, little is known about the function of the septum in behaviour. A kind of coma has been reported with lesions of the septum (Smythies, 1970), while hyperreactivity has been found with tumors of the septum (Smythies, 1970; Zeman, 1958). Septal structures have been, furthermore, implicated in the etiology of memory deficits (Smythies, 1970) and schizophrenia (Domino, 1976; Farley et al., 1978). Numerous animal studies have been performed to extend our knowledge of septal functioning in behaviour. The results of these experiments showed involvement of the septum in a wide variety of behaviours and led to theories about a critical role of septal structures in e.g. emotionality, response inhibition, incentive motivation, or integration of information (for reviews: Fried, 1972; Grossman, 1976; Donovick et al., 1979b). An orderly pattern of function, however, has not yet emerged. Recent years have provided evidence that the septum is a heterogeneous structure with regard to its cytoarchitectonics, neurochemistry and anatomical connections. Only a few behaviour studies have taken into account this heterogeneity of the septum. Most studies used the lesion or electrical stimulation technique to change experimentally septal activity. Such techniques are rather aspecific as they affect whole brain structures rather than single neuronal systems. Moreover, brain lesions cause secondary changes in the brain, which may partly account for the effects following the lesions, and electrical stimulation affects fibres

passing through the stimulated brain region. In other words, interpretation of the effects observed in those studies is not easy. It is not surprising, therefore, that a uniform theory about septal function in behaviour is still lacking.

More knowledge of the pharmacological and functional properties of single neuronal systems within the septum is a prerequisite to enable investigators to study septal function in behaviour in more detail. In the present study, therefore, we introduce the morphine-induced behaviour of cats in combination with intracerebral injection of drugs as a new method for the study of the pharmacological and functional properties of neurotransmission processes within the septum. This method, i.e. the analysis of the effects of intraseptally injected drugs on the morphine-induced behaviour of cats, may have substantial advantages compared to other methods as has been discussed in the Preface of this thesis. Several aspects of septal neurotransmission processes are selected in order to investigate whether the present method is indeed suitable for the purpose mentioned above: e.g. 1) the functional activity of specific neurotransmission processes within the septum at the behaviour level, 2) the pharmacological character of specific receptor sites within the septum, 3) the functional relationship between distinct neurotransmission processes within the septum, 4) the action of morphine on the activity of specific neurotransmission processes within the septum, and 5) the action of morphine on septal opiate receptors. Cholinergic (section 4.2), dopaminergic and noradrenergic (section 4.3), endorphinergic and enkephalinergic (section 4.4), and glutamatergic processes (section 4.5) are studied within this context since the existence of these processes within the septum has been extensively demonstrated (section 4.1.4). As a general introduction to the experimental studies, a survey is given of the morphology (section 4.1.2), anatomical connections (section 4.1.3), and neurochemistry (section 4.1.4) of the septum.

4.1.2 The morphology of the septum

The septal region comprises a collection of basal, medial telencephalic nuclei. The boundaries of this group of nuclei are the fornical commissure which is posteriorly situated, the anterior rudiment of the hippocampus anteriorly, the corpus callosum dorsally, the lateral ven-

tricle and the nucleus accumbens laterally, and the olfactory tubercle and the preoptic area ventrally. The septum is elongated anteriorly and tends to become wider posteriorly. In the human, the septum is divided into septum pellucidum and septum verum, the dorsal and ventral division respectively.

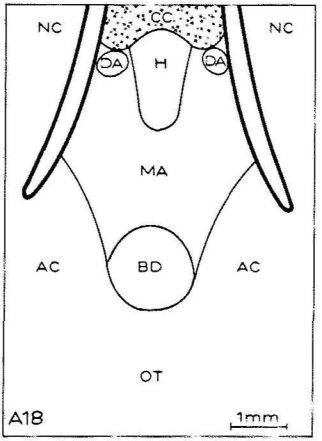
Most investigators have the conception that the septum consists of two principal divisions: a medial and a lateral division. Nevertheless, the comparative studies of Andy and Stephan (1959, 1961, 1964, 1966, 1968, and 1976) suggest a different organizational plan. The major septal divisions recognized in those studies are the medial, dorsal, lateral, and caudal groups. Andy and Stephan's subdivision of the septum in the cat is given in Table 4.1; the dimensions and the relative anatomical position of the various septal nuclei are visualized in Fig. 4.1. This subdivision of the septum is used in the experimental

TABLE 4.1

Subdivision of the septum in the cat as proposed by Andy and Stephan (1964)

Dorsal group	nucleus septalis dorsalis, - pars anterior	(DA)
	- pars externa	(DE)
	- pars intermedius	(DM)
	- pars interna	(DI)
Ventral group	nucleus septalis lateralis, - pars externa	(LE)
	- pars interna	(LI)
Medial group	nucleus septalis medialis, - pars anterior	(MA)
	- pars posterior	(MP)
	nucleus of the diagonal bond of Broca,	
	- pars dorsalis	(BD)
	- pars ventralis	(BV)
Caudal group	nucleus septalis fimbrialis	(F)
	nucleus septalis triangularis	(A)
	bed nucleus of the anterior commissure	(C)
	bed nucleus of the stria terminalis,	
	- pars anterior	(TA)
	- pars externa	(TE)
	- pars interna	(TI)

The abbreviations between brackets refer to Fig. 4.1.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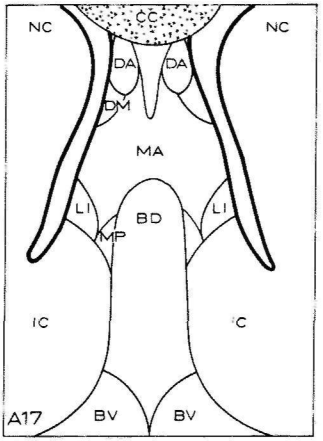
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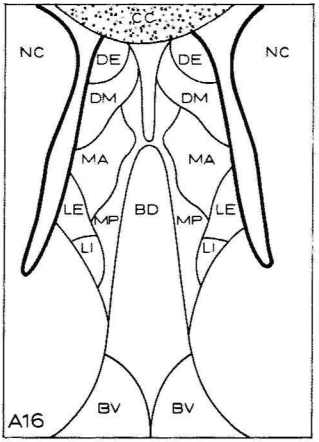
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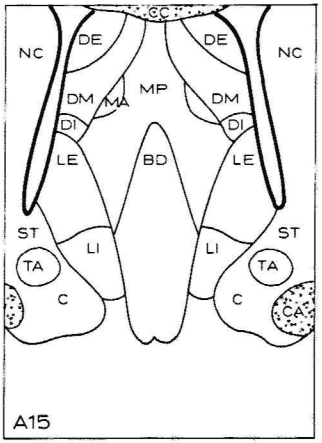
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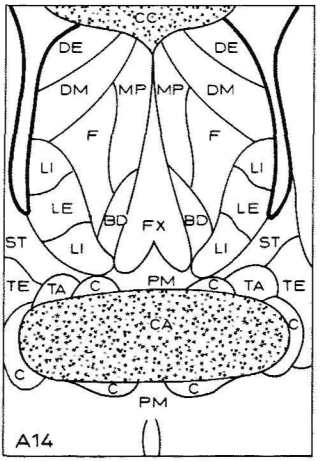
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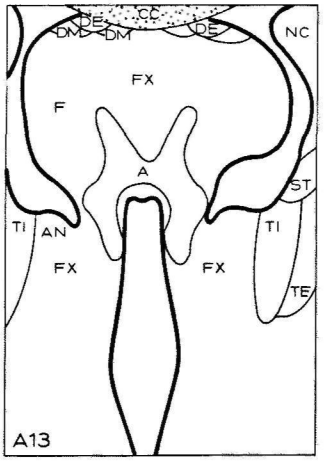
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Fig. 4.1 Subdivision of the septum in the cat as proposed by Andy and Stephan (1964). The figure shows 6 transversal sections (stereotaxic co-ordinates: A 13.0 - A 18.0).
Abbreviations:
A = nucleus septalis triangularis; Ac = nucleus accumbens; An = nucleus anterodorsalis thalami; BD = nucleus of the diagonal band of Broca pars dorsalis; BV = nucleus of the diagonal band of Broca pars ventralis; C = bed nucleus of the anterior commissure; CA = commissura anterior; DE = nucleus septalis dorsalis pars externa; DI = nucleus septalis dorsalis pars interna; DM = nucleus septalis dorsalis pars intermedius; F = nucleus septalis fimbrialis; Fx = fornix; H = anterior continuation of the hippocampus; IC = islands of Calleja; LE = nucleus septalis lateralis pars externa; LI = nucleus septalis lateralis pars interna; MA = nucleus septalis medialis pars anterior; MP = nucleus septalis medialis pars posterior; NC = nucleus caudatus; OT = olfactory tubercle; PM = nucleus preopticus medianus; ST = stria terminalis; TA = bed nucleus of the stria terminalis pars anterior; TE = bed nucleus of the stria terminalis pars externa; TI = bed nucleus of the stria terminalis pars interna.

sections of this chapter to describe the localization of the injection sites. Most anatomical studies on the septum, however, use a subdivision similar to that described by Swanson and Cowan (1979) for the rat. Hence, Swanson and Cowan's subdivision is used for the description of the anatomical connections of the septum in section 4.1.2. Table 4.2 indicates how the various septal divisions recognized by Swanson and Cowan correspond to the nuclear groups of Andy and Stephan.

TABLE 4.2

Subdivision of the septum in the rat as proposed by Swanson and Cowan (1979)

Lateral division	lateral septal nucleus, - dorsal part	(DA,DE,DM,DI)
	, - ventral part	(LE,LI)
	, - intermediate part	(MA,MP)
	septohippocampal nucleus	(MA,MP)
Medial division	medial septal nucleus	(BD)
	nucleus of the diagonal band	(BV)
Posterior division	septofimbrial nucleus	(F)
	triangular septal nucleus	(A)
Ventral division	bed nucleus of the stria terminals	(TA,TE,TI,C)

The abbreviations between brackets refer to the corresponding septal division of Andy and Stephan in Table 4.1.

The cytoarchitectonic structure of the septum is extremely complex (Andy and Stephan, 1964; Swanson and Cowan, 1979). More than one class of neurons is found within each of the septal nuclei. The dendrites of many of the cells in the medial septal and diagonal band nuclei extend for considerable distances beyond the cytoarchitectonic boundaries of these nuclei. Accordingly, neuronal activity in adjoining nuclei may influence directly the activity of neurons in the medial and diagonal band nuclei, and indirectly the neuronal centers to which these latter nuclei project. The dendrites of the cells in other nuclei tend to be more restricted but potentially they have the same property, especially when they adjoin the medial and diagonal band nuclei.

4.1.3 The anatomical connections of the septum

This section gives a survey of the anatomical connections of the septum. The evidence for the existence of these connections is derived from the anatomical studies referred to in Table 4.3. This table shows, furthermore, which anatomical techniques (degeneration, horseradish peroxidase, or autoradiography) and which experimental animals were used in those studies. The connections demonstrated in cats and rats are discussed below. References to the anatomical studies are numbered in brackets in the text and listed in Table 4.3. Electrophysiological, biochemical and/or histochemical data are mentioned to supplement the anatomical evidence. The neurochemistry of the septum is discussed in more detail in section 4.1.4.

Telencephalon

The cortex. The most important connections of the septum with cortical regions are those with hippocampal and adjacent subicular and entorhinal areas (see below: hippocampal formation). Septal connections with other cortical areas have been reported rarely.

Fibres afferent to the septum and arising in the pyriform cortex have been reported to terminate in the diagonal band nucleus (41).

Fibres efferent from the septum have been reported to course from the diagonal band nucleus to the anterior limbic cortex in the cat (27). In the rat, the diagonal band nucleus projects to the occipital cortex; this projection may be cholinergic (20).

The hippocampal formation. Massive reciprocal connections of the septum with the hippocampal formation (including the hippocampus proper, the dendate gyrus, and the adjacent subicular and entorhinal regions) belong to the best identified neuronal pathways in the brain.

Fibres afferent to the septum arising in the hippocampal formation have been reported in both the cat (51, 53, 54) and the rat (10, 34, 41, 53, 54, 59, 60). In the cat, it has been demonstrated (51, 53, 54) that degenerating fibres arising in ventral parts of the hippocampal formation pass through the fimbria and lateral aspect of the fornix to terminate in massive quantities throughout the lateral septal nucleus; degenerating fibres arising in dorsal parts of the hippocampal formation pass through the medial aspect of the fornix and terminate, to a lesser extent, in the medial septal nucleus. In the rat, this topographical

TABLE 4.3

Afferent connections of the septum

	anatomical techniques		
	degeneration	horseradish peroxidase	autoradiography
Telencephalon			
cortex (with exception of hippocampal formation)	41(r)	28(m)	
hippocampal formation	51,53,54(c);41,53,54(r); 53,54(rb);53,54(ge)	10(r);26(m)	34,59,60(r)
olfactory tubercle	41(r)		
amygdala	41(r)	48(r)	29(c);29(r)
Diencephalon			
habenula			22(r)
thalamus		2(r)	
hypothalamus	9(c);41(r)	2,48(r);28(m)	44,46,61(c);12,43,44,45, 58,60(r);44,46(m);1(gu)
Mesencephalon			
ventral tegmental area	30,31,35,41(r)	2,17,31,35(r);28(m)	4,17,35,56(r)
interpeduncular nucleus			
raphe nuclei	11(r)	2,48(r);28(m)	6(c);3,7,11(r)
Rombencephalon			
locus coeruleus	31,35(r)	2,32,35,48(r);28(m)	

Efferent connections of the septum

	anatomical techniques		
	degeneration	horseradish peroxidase	autoradiography
Telencephalon			
cortex (with exception of hippocampal formation)	23(l);24(m)	20(r)	27(c)
hippocampal formation	36,41(r);52(c)	27(c);33,42,47(r);16(m);25(p)	27(c);12,33,60(r);25(p)
olfactory tubercle	52(c);41(r)	15(c);50(r)	12(r)
amygdala			27(c);12,33,60(r)
Diencephalon			
habenula	39,52(c);39,41(r);39(m)	37(c);14,21,37(r);37(m)	27(c);12,33,60(r);25(p)
thalamus	52(c);40(m);23(l)	33(r)	27(c);12,33(r);25(p)
hypothalamus	52(c);41(r);55(m);23(l)	5,33(r);8(p)	27,57(c);12,18,33,60(r);25(p)
Mesencephalon			
ventral tegmental area		33,38(r)	27(c);12,33,60(r);25(p)
interpeduncular nucleus		13(r)	27(c);60(r)
raphe nuclei			60(r)
Rombencephalon			
locus coeruleus	19(c)		

The numbers refer to the appended list of references (page 134) and the letters in brackets to the experimental animals used: c = cat, ge = gerbil, gu = guinea pig, l = lizard, m = monkey, p = pigeon, r = rat, rb = rabbit

List of references (appendix Table 4.3)

1 = *Anderson and Shen, 1980*
2 = *Assaf and Miller, 1977*
3 = *Azmitia and Segal, 1978*
4 = *Beckstead et al., 1979*
5 = *Berk and Finkelstein, 1981*
6 = *Bobillier et al., 1976*
7 = *Bobillier et al., 1979*
8 = *Bouillé et al., 1977*
9 = *Chi and Flynn, 1971*
10 = *Chronister and De France, 1979*
11 = *Conrad et al., 1974*
12 = *Conrad and Pfaff, 1976*
13 = *Contestabile and Flumerfelt, 1981*
14 = *Crutcher and Davis, 1980*
15 = *Dennis and Kerr, 1976*
16 = *De Vito, 1980*
17 = *Fallon and Moore, 1978*
18 = *Garris, 1979*
19 = *Heath and Harper, 1976*
20 = *Henderson, 1981*
21 = *Herkenham and Nauta, 1977*
22 = *Herkenham and Nauta, 1979*
23 = *Hoogland et al., 1978*
24 = *Kemper et al., 1972*
25 = *Krayniak and Siegel, 1978*
26 = *Krayniak et al., 1979*
27 = *Krayniak et al., 1980*
28 = *Krayniak et al., 1981*
29 = *Krettek and Price, 1978*
30 = *Lindvall, 1975*
31 = *Lindvall and Stenevi, 1978*
32 = *Mason and Fibiger, 1979*
33 = *Meibach and Siegel, 1977a*
34 = *Meibach and Siegel, 1977b*
35 = *Moore, 1978*
36 = *Mosko et al., 1973*
37 = *Parent et al., 1981*
38 = *Phillipson, 1979*
39 = *Powell, 1968*
40 = *Powell, 1973*
41 = *Raisman, 1966*
42 = *Sakanaka et al., 1980*
43 = *Saper et al., 1976*
44 = *Saper et al., 1978*
45 = *Saper et al., 1979a*
46 = *Saper et al., 1979b*
47 = *Segal and Landis, 1974a*
48 = *Segal and Landis, 1974b*
49 = *Segal, 1976*
50 = *Shafa and Meisami, 1977*
51 = *Siegel and Tassoni, 1971a*
52 = *Siegel and Tassoni, 1971b*
53 = *Siegel et al., 1974*
54 = *Siegel and Edinger, 1976*
55 = *Simmons and Powell, 1972*
56 = *Simon et al., 1979*
57 = *Stoddard-Apter and MacDonnell, 1980*
58 = *Swanson, 1976*
59 = *Swanson and Cowan, 1977*
60 = *Swanson and Cowan, 1979*
61 = *Troiano and Siegel, 1975*

organization has been somewhat more specified in studies utilizing the horseradish peroxidase and the autoradiography technique (34, 59, 60): fibres arising in the rostro-dorsal part of the hippocampal formation project to the dorsal part of the lateral septal nucleus, while those from successively more caudo-ventral parts of the hippocampal formation project to progressively more ventral parts of the lateral septal nucleus. The majority of the hippocampo-septal fibres are excitatory (De France, 1976; De France et al., 1973a, b, 1976; Edinger et al., 1973; Gutnick and Feldman, 1977; McLennan and Miller, 1974a). Most of the hippocampo-septal neurons use glutamate as neurotransmitter (Fonnum and Walaas, 1978; Fonnum et al., 1979; Malthe-Sörenssen et al., 1980a, b; Nitsch et al., 1979; Storm-Mathisen and Opsahl, 1980; Taxt and Storm-Mathisen, 1979; Walaas and Fonnum, 1980b; Zaczek et al., 1979).

Fibres efferent from the septum and terminating in the hippocampal formation have been reported in both the cat (27, 52) and the rat (33, 36, 41, 42, 47, 60). In both species, the fibres arise in the medial septal nucleus/diagonal band complex, course by way of the dorsal fornix and the fimbria, and distribute widely to most parts of the hippocampal formation. The septo-hippocampal pathway is topographically organized in both the cat (27, 52) and the rat (33, 47): septal cells positioned near the midline project through medial parts of the dorsal fornix to terminate in the dorsal hippocampal formation, while fibres originating from cells situated more laterally project through progressively more lateral parts of the fimbria to terminate in progressively more posteroventral regions of the hippocampal formation. The septo-hippocampal neurons are excitatory (Andersen et al., 1961; Bird and Aghajanian, 1975; Biscoe and Straughan, 1966; Bland et al., 1974; Brücke et al., 1963) and use acetylcholine as neurotransmitter (Bajgar et al., 1977; Dudar, 1975, 1977; Lewis and Shute, 1967; Mellgren and Srebro, 1973; Moroni et al., 1978b; Mosko et al., 1973; Sethy et al., 1973; Smyth, 1974).

The olfactory tubercle. Evidence is scarce for afferents to the septum originating from the olfactory tubercle. In the rat, it has been reported that degenerating fibres arising in the olfactory tubercle course to the rostral aspect of the lateral septal nucleus (41). On the other hand, the existence of fibres efferent from the septum and terminating in the olfactory tubercle seems well established both in

the cat (15, 52) and the rat (12, 41, 50). This projection originates, particularly, in the diagonal band nucleus.

The amygdala. Afferents to the septum arising in the amygdala have been reported in both the cat (29) and the rat (29, 41). In both species, the amygdala projects via the stria terminalis to the bed nucleus of the stria terminalis in a topographically ordered way (29): the medial and posterior amygdaloid nuclei and the amygdalo-hippocampal area project to the medial part of the bed nucleus, the basolateral and the central amygdaloid nuclei project to the lateral part of the bed nucleus, and the basomedial amygdaloid nucleus projects to a region partly overlapping the medial and lateral divisions of the bed nucleus of the stria terminalis. A minor projection was also reported (29) from the amygdala to the ventral part of the lateral septal nucleus.

Also fibres efferent from the septum and terminating in the amygdala have been reported. In the cat, the vertical limb of the diagonal band nucleus projects partly via the medial forebrain bundle to the medial amygdaloid nucleus (27). In the rat, the septum projects in two distinct pathways to the amygdala: 1) the bed nucleus of the stria terminalis projects via the stria itself to the medial, central and anterior amygdaloid nuclei (12, 60), 2) the diagonal band nucleus projects partly via the medial forebrain bundle to the anterior and basal amygdaloid nuclei (12, 33). The latter projection may be cholinergic (Bajgar, 1977).

Diencephalon

The habenula. Evidence is scarce for afferents to the septum originating in the habenular complex. The existence of such connections has been reported in the rat (22, 48) but not in the cat: the medial habenula projects sparsely via the stria medullaris to the septofimbrial and triangular nuclei (22, 48), whereas the lateral habenula projects, more heavily, to the ventrolateral septum by way of the fasciculus retroflexus and the medial forebrain bundle (22).

The existence of fibres efferent from the septum and terminating in the habenula have been extensively reported in both the cat (27, 37, 39, 52) and the rat (14, 21, 33, 37, 39, 41, 60). In both species, the projection arises particularly in the septofimbrial nucleus. Projections from the septofimbrial nucleus, the vertical limb of the

diagonal band nucleus, and the ventral part of the lateral septal nucleus course via the stria medullaris to the medial habenular nucleus. In addition, fibres arising in the bed nucleus of the stria terminalis have been reported to terminate in the habenula (37, 39). In the cat, a projection has been reported from the bed nucleus of the anterior commissure to the lateral habenula. In the rat, it has been demonstrated (60) that the projection from the septofimbrial nucleus to the medial habenular nucleus is topographically organized such that axons of cells in the anterodorsal part of the nucleus end in a vertical band within the medial part of the medial habenular nucleus, and progressively more ventrolaterally placed neurons in the septofimbrial nucleus innervate progressively more lateral bands within the habenula. Moreover, the anterodorsal part of the septofimbrial nucleus projects to the rostral two-thirds of the medial habenular nucleus while the posteroventral part of the septofimbrial nucleus projects to the entire rostrocaudal extent of the medial habenular nucleus (60). Also the projection from the vertical limb of the diagonal band nucleus is topographically organized as demonstrated in the rat (21, 33): cells situated near the midline project to the medial habenula while progressively more laterally positioned cells project to progressively more lateral parts of the habenula. Electrophysiological support for the existence of septal projections to the lateral habenula is given by the studies of Mok and Mogenson (1972a, b). The septal projection to the habenula complex are at least partly cholinergic (Gottesfeld and Jacobowitz, 1979; Kataoka et al., 1977).

The thalamus. Afferents to the septum arising in the thalamus have not been reported in the cat. In the rat, a projection has been reported from the anterior thalamic nuclei to the lateral septal nucleus (2).

Efferents from the septum to the thalamus have been reported more extensively, both in the cat (27, 52) and in the rat (12, 33). In the cat, the lateral and medial septal nuclei project to the nucleus reuniens (27, 52) and the nucleus anterior medialis (52). In the rat, efferents from the diagonal band nucleus distribute to the anteromedial nucleus (33), the parataenial nucleus (33), and the mediodorsal thalamic nucleus (12, 33). This projection is topographically organized (33): a) fibres from the midline region of the vertical limb of the diagonal band nucleus and medial septal nucleus project to the anteromedial

nucleus, b) fibres of cells situated in slightly more lateral portions of the vertical limb and medial septum project to the parataenial nucleus, c) fibres of cells positioned still more laterally project to the mediodorsal nucleus. The dorsal septum appears to have a sparse projection to the midline thalamus (33). From the bed nucleus of the stria terminalis axons course to the parataenial and periventricular nuclei (12).

The hypothalamus. Projections afferent to the septal nuclei arising in the hypothalamus have been reported in both the cat (44, 46, 60) and the rat (2, 9, 12, 41, 43, 44, 45, 48, 58, 60). In the cat, the ventromedial nucleus projects through the medial forebrain bundle to the medial part of the bed nucleus of the stria terminalis, and the ventral part of the lateral septal nucleus (46). Furthermore, the anterior hypothalamic area projects to the zone between the medial and lateral septal nuclei and to the ventral part of the lateral septal nucleus (44, 46). Fibres have been reported that arise in the lateral preoptic-hypothalamic area and course via the medial forebrain bundle to terminate in the lateral septal nucleus and in the intermediate zone between the lateral and medial septal nuclei (61). In the rat, it is demonstrated (45, 58, 60) that the lateral preoptic and lateral hypothalamic areas, from the level of the premammillary nucleus caudally and the supraoptic nucleus rostrally, project to the medial septal-diagonal band complex. This projection is topographically organized in such a manner that the more caudal cells in the lateral hypothalamic area project diffusely to the nucleus of the diagonal band, while the more rostral cells in the lateral preoptic area project to the medial septal nucleus as well. Other studies suggest that the lateral hypothalamic area projects to the lateral septal nucleus (2), that the anterior hypothalamic area projects to the ventral part of the lateral septal nucleus (44), that the ventromedial nucleus projects to the bed nucleus of the stria terminalis and the adjacent part of the lateral septal nucleus (43), and that the medial preoptic area projects to the septum via the diagonal band (12). Septal afferents arising in the arcuate nucleus contain β-endorphin and adrenocorticotrophic hormone (Bloom et al., 1978; Rossier et al., 1977; Watson et al., 1977b, 1978a). Other hypothalamic-septal neurons contain vasopressin and oxytocin (Buijs, 1978; Buijs et al., 1978; Dogterom et al., 1978). The innervation from the lateral preoptic area may be cholinergic (Emson, 1978).

Septal efferents coursing to the hypothalamus have been reported in both the cat (27, 52, 57) and the rat (12, 18, 33, 41, 60). In the cat, the lateral septal nucleus projects to the preoptic area (27, 52), the lateral hypothalamus (27, 52) and supramammillary region (27). The diagonal band nucleus projects to the preoptic area and the lateral hypothalamus (27, 52). Fibres arising in the bed nucleus of the anterior commissure are distributed to the preoptic region, lateral hypothalamus, supramammillary region, and the posterioraspect of the medial mammillary nucleus (27). Septal efferents have also been reported to terminate in the medial hypothalamus (57). In the rat, the lateral septal nucleus sends fibres through the medial forebrain bundle to the medial preoptic and anterior hypothalamic areas (5, 18, 60), the dorsomedial nucleus (5, 33, 60), the lateral hypothalamic area (5, 18, 33, 60), the mammillary body (60), the posterior hypothalamic nucleus (5), the ventromedial hypothalamic nucleus (5), and the paraventricular hypothalamic nucleus (5). Furthermore, the medial septal nucleus/diagonal band complex projects through the medial forebrain bundle to the medial and lateral preoptic areas (18, 60), to the lateral hypothalamic area (18, 60), and the mammillary complex (18, 33, 60). The bed nucleus of the stria terminalis projects through the medial forebrain bundle to most parts of the hypothalamus, including the preoptic area (12, 60). The dorsal septal nucleus projects to the lateral preoptic area, lateral hypothalamus, and periventricular hypothalamus (33). Some of the septo-hypothalamic connections may be cholinergic (Bajgar et al., 1977).

Mesencephalon

The ventral tegmental area. Fibres efferent to the septal nuclei arising from the ventral tegmental area have been extensively reported in the rat (2, 4, 17, 30, 31, 35, 41, 56) but not in the cat. The fibres originate from the ventral part of the ventral tegmental area and course via the medial forebrain bundle to terminate mainly in the medial part of the lateral septal nucleus (31, 35). The projection is topographically organized (17): more rostrally located cells project more laterally in the septum, whereas more caudally situated cells project to a more medial septal region. The axons arising in the ventral tegmental area are, at least partly, excitatory (Assaf and Miller, 1977). Moreover, the majority of the neurons are dopaminergic (Lindvall and Stenevi, 1978; Moore, 1978) although also non-dopaminergic cells have been re-

ported to project from the ventral tegmental area to the septum (Deniau et al., 1980; Thierry et al., 1980).

Septal efferents to the ventral tegmental area have been reported in both the cat (27) and the rat (33, 38, 60). In the cat, the fibres originate in the horizontal limb of the diagonal band nucleus and course via the medial forebrain bundle (27). In the rat, the fibres arise in the ventral part of the lateral septal nucleus (60), the horizontal limb of the diagonal band nucleus (33, 38), the medial septal nucleus (60), and the bed nucleus of the stria terminalis (38, 60).

The interpeduncular nucleus. Septal afferents arising in the interpeduncular nucleus have not yet been demonstrated. On the other hand, there is some evidence for septal efferents to the interpeduncular nucleus in both the cat (27) and the rat (13, 60). In the cat, the fibres arise in the diagonal band nucleus (27). In the rat, the fibres arise in the diagonal band nucleus, septo-fimbrial and triangular nuclei, and course via the stria medullaris, the nucleus habenularis and the formatio retroflexus (13, 60). The neurons originating in the diagonal band are thought to be cholinergic (Gottesfeld and Jacobowitz, 1978; Kataoka et al., 1977; McGeer et al., 1979).

The raphe nuclei. Septal afferents arising in the raphe nuclei have been reported in the cat (6) and the rat (2, 3, 7, 11, 48). In the cat, the fibres arise in the dorsal and central superior nuclei (6). In the rat, the fibres arise in the central superior nucleus (2, 3, 7, 11, 48) and, to a lesser extent, in the dorsal raphe nucleus (3, 11). The central superior raphe cells project to the medial septal nucleus, whereas the dorsal raphe cells project to the lateral septal nucleus (3). The responses of septal cells to stimulation of the medial and dorsal raphe nuclei are complex (Segal, 1976), but the projection from the central superior nucleus to the medial septal nucleus is thought to be predominantly inhibitory (Assaf and Miller, 1978). The projections from the raphe nuclei to the septal nuclei are mainly serotonergic (Assaf and Miller, 1978; Geyer et al., 1976; Halaris et al., 1976; Kellar et al., 1971).

Septal efferents to the raphe nuclei have been reported in the rat (60). The fibres arise in the medial septal nucleus and the bed nucleus of the stria terminalis, and terminate in the dorsal and central superior raphe nuclei.

Rhombencephalon

The locus coeruleus. Septal afferents from the locus coeruleus have been reported in the rat (31, 32, 35, 48) but not in the cat. The projection arises from the dorsal part of the locus coeruleus, courses via the medial forebrain bundle, and terminates particularly in the medial septal and diagonal band nuclei, the bed nucleus of the stria terminalis and, to a lesser extent, in the lateral septal and the septofimbrial nuclei (31, 35). These neurons are, at least partly, noradrenergic (Lindvall and Stenevi, 1978; Moore, 1978; Segal, 1976).

Septal efferents to the locus coeruleus have been reported only in the cat (19). These fibres arise in the rostral part of the septum.

Summary

Summarizing, the septum is connected with several other brain regions (Fig. 4.2). These connections take three major routes: 1) the medial forebrain bundle (particularly the connections with the hypothalamus), 2) the dorsal fornix/fimbria (particularly the connections with the hippocampal formation), and 3) the stria medullaris (particularly the connections with the habenular complex). Most of the connections are reciprocal. The afferent fibres terminate mainly in the lateral septal nuclei while the efferent fibres arise chiefly in the medial septal nuclei. It has to be taken into account, however, that the dendrites of medial septal cells extend for considerable distances in lateral septal regions (section 4.1.2) and that intrinsic connections exist between the lateral and medial septal nuclei (Krayniak et al., 1980; Raisman, 1966; Swanson and Cowan, 1979). Hence, the afferent fibres terminating in the lateral septal nuclei may also influence both directly and indirectly the activity of neurons in the medial septal nuclei, and, consequently, the activity of the septal efferent fibres arising in the medial septal nuclei. This internal organization of the septum provides the basis for "feed-back" circuits involving the septum and brain regions with which it is reciprocally connected, e.g. the hippocampal formation, the hypothalamus and the ventral tegmental area.

The best documented input of the septum arises from the hippocampal formation, the hypothalamus, the ventral tegmental area, the raphe nuclei, and the locus coeruleus. Each of the various neuronal pathways terminating within the septum uses its own characteristic neurotrans-


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Fig. 4.2 Brain regions connected with the septum; modified diagram after Nieuwenhuys et al. (1979)
1 = nuclei septales; 2 = nucleus of the diagonal band of Broca; 3 = tuberculum olfactorium; 4 = nuclei amygdalae; 5 = bed nucleus of the stria terminalis; 6 = nuclei preoptici; 7 = nuclei hypothalami; 8 = nuclei mammillares; 9 = nuclei thalami; 10 = nuclei habenulae; 11 = nucleus interpeduncularis; 12 = area ventralis tegmenti; 13 = formatio hippocampi; 14 = nucleus raphe dorsalis; 15 = nucleus raphe centralis superior; 16 = locus coeruleus.

mitter, e.g. glutamate for fibres arising in the hippocampal formation, acetylcholine, β-endorphin, adrenocorticotrophic hormone, vasopressin and oxytocin for fibres arising in hypothalamic regions, dopamine for fibres arising in the ventral tegmental area, serotonin for fibres arising in the raphe nuclei, and noradrenaline for fibres arising in the locus coeruleus. (The neurochemistry of the septum is discussed in more detail in section 4.1.4) In the present study, the functional and pharmacological properties are investigated of cholinergic (section 4.2), dopaminergic and noradrenergic (section 4.3), endorphinergic and enkephalinergic (section 4.4), and glutamatergic (section 4.5) neurotransmission processes within the septum.

The output of the septum is directed to the hippocampal formation, the hypothalamus, the habenula, and, to a lesser extent, the amygdala, the thalamus, the ventral tegmental area, and the interpeduncular nucleus. Most of these efferent connections seem to be cholinergic. The data that 1) septal output is mainly cholinergic, 2) septal input arises from such neuronal centers as the noradrenergic locus coeruleus, the serotonergic raphe nuclei, and the dopaminergic ventral tegmental area, and 3) most of the connections are reciprocal, suggest a critical role for septal structures in mediating reciprocal interactions between cholinergic and other neurotransmission processes. In Chapter 5, we pay attention to a role of septal structures in the interactions between central cholinergic and dopaminergic neurotransmission processes. An imbalance of such interactions, particularly within the caudate nucleus, is generally thought to be one of the etiological factors in Parkinson's disease. As described in Chapter 5, it will be investigated whether also septal structures may be involved in such interactions.

4.1.4 The neurochemistry of the septum

A wide variety of chemical substances can act as neurotransmitter in the brain (Barchas et al., 1978). Some of these substances may act within the septum. Functional and pharmacological properties of several neurotransmission processes within the septum are investigated within this study: 1) cholinergic processes (section 4.2), 2) dopaminergic and noradrenergic processes (section 4.3), 3) endorphinergic and enkephalinergic processes (section 4.4), and 4) glutamatergic processes (section 4.5). This section is an introduction to this subject and gives a survey

of these septal neurotransmission processes with special attention to the aspects investigated within the present study. For the sake of completeness, septal neurotransmission processes that are not the subject of the present study are also discussed.

Acetylcholine

Acetylcholine and its synthetizing and degrading enzymes (cholineacetyltransferase and acetylcholine-esterase, respectively) are present within the septum (Chronister et al., 1976; Domino, 1976; Fonnum and Walaas, 1978; Harkmark et al., 1975; Hoover et al., 1978; Jacobowitz and Palkovits, 1974; Kimura et al., 1981; Palkovits et al., 1974; Srebro et al., 1976; Uchimura et al., 1978). The presence of these cholinergic markers is not surprising in view of the existence of massive cholinergic projections of the septum to e.g. the hippocampal formation, the hypothalamus, the habenula, the nucleus interpeduncularis, and the amygdala (section 4.1.2). On the other hand, acetylcholine is also active within the septum itself: 1) intraseptal injections of cholinergics affect several aspects of behaviour and physiological functioning, such as avoidance behaviour (Hamilton and Grossman, 1964; Hamilton et al., 1968; Kelsey and Grossman, 1975), emotional behaviour (Hernandez-Peon et al., 1963; MacPhail and Miller, 1968), anti-nociception (Metys et al., 1979), food- and water-intake (Grossman, 1964), and urinary excretion (Saad, 1975), 2) intraseptal administration of acetylcholine depresses septal neuronal activity (Segal, 1974), and 3) histochemical studies suggest that the septum is innervated by cholinergic fibres (Chronister et al., 1976; Emson, 1978; Harkmark et al., 1975; Srebro et al., 1976). The origin of the cholinergic fibres afferent to the septum is unknown at present: they may be either intrinsic, cholinergic neurons with the septum itself (Chronister et al., 1976; Harkmark et al., 1975; Srebro et al., 1976), or axon collaterals of the cholinergic, septo-hippocampal pathway (Harkmark et al., 1975; Srebro et al., 1976; Swanson and Cowan, 1979), or fibres from extraseptal origin, e.g. from the lateral preoptic area (Emson, 1978). The studies dealing with the effects of intraseptally injected cholinergic agents (see above) used drugs with a predominantly muscarinic character; the effectiveness of these drugs suggest that septal acetylcholine receptors are, at least partly, muscarinic ones.

One of the aims of the present study is to investigate whether morphine-induced behaviour of cats, in combination with intraseptal injection of drugs, may serve as a suitable method for the study of pharmacological and functional properties of septal neurotransmission processes (section 4.1.1). A prerequisite for the successful use of this method is that the morphine-induced behaviour of cats has to be related to septal neuronal activity. As intraseptal injection of cholinergic drugs has been demonstrated to affect septal neuronal activity, and to produce a variety of behaviour and physiological effects (see above), intraseptal injection of cholinergics is chosen as a tool to investigate whether morphine-induced behaviour of cats may indeed serve as a suitable variable for experimentally-evoked changes in septal neuronal activity (section 4.2).

Dopamine

Recent histochemical, pharmacological, and biochemical studies have indicated the existence of a substantial dopaminergic innervation of the septum (Brownstein et al., 1974b; Collu et al., 1980; Fallon and Moore, 1978; Garey, 1976; Garey and Heath, 1974; Kizer et al., 1976; Koslow et al., 1974; Lindvall, 1975; Lindvall and Stenevi, 1978; Moore, 1978; Robinson et al., 1979; Saavedra and Zivin, 1976; Versteeg et al., 1976). The dopamine containing fibres - belonging to the mesolimbic dopamine system - arise from the mesencephalic ventral tegmental area (A-10 cell group) and terminate heavily in the medial part of the lateral septal nucleus (Lindvall and Stenevi, 1978; Moore, 1978). The dopaminergic innervation of the septum is at least partly excitatory (Assaf and Miller, 1977). The dopaminergic afferents of the septum exert a tonic inhibitory action on the cholinergic, septo-hippocampal neurons (Robinson et al., 1979). This action on the septo-hippocampal pathway is presumably mediated via inhibitory, γ-aminobutyric acid containing interneurons (Wood et al., 1979). The dopamine containing neurons innervate the septum in two distinct histochemical patterns, viz. "smooth" and "fine-varicose" patterns (Lindvall and Stenevi, 1978; Moore, 1978), which may reflect the presence of distinct types of dopamine receptors (Cools and Van Rossum, 1976). Binding studies have also suggested the existence of a particular subpopulation of dopamine receptors within the septum (Howlett et al., 1979; Köhler et al., 1979).

Binding to this type of dopamine receptor is not "classical", i.e. the "classical" dopamine agonist apomorphine and antagonist haloperidol show low affinity for this binding site (Howlett et al., 1979). Nevertheless, both apomorphine and haloperidol do affect septal neuronal activity (Assaf and Miller, 1977; Robinson et al., 1979). Accordingly, more than one type of dopamine receptor may be present within the septum. In this context, it is useful to note that septal dopamine is not adenyl-cyclase linked (Collu et al., 1980).

Little is known about the functional activity of the septal dopaminergic processes at the behaviour level. Hence, the ability of intraseptally injected dopaminergic agents to affect behaviour is investigated (section 4.3). Attention is also paid to the possible existence of more than one type of dopamine receptor within the septum (section 4.3).

Noradrenaline

The septal nuclei are relatively rich in noradrenaline (Brownstein et al., 1974b; Garey, 1976; Koslow et al., 1974; Lindvall and Stenevi, 1978; Moore, 1978; Versteeg et al., 1976). The noradrenergic innervation of the septum arises almost equally from the locus coeruleus and from nuclei in the caudal brain stem (Lindvall and Stenevi, 1978; Moore, 1978). The axons originating in the locus coeruleus form a dense innervation of medial septal structures (anterior hippocampus, medial septal nucleus, and the diagonal band nucleus) and the bed nucleus of the stria terminalis, whereas the noradrenergic innervation arising from the brain stem is most pronounced in lateral septal structures (lateral septal nucleus and the bed nucleus of the stria terminalis) (Lindvall and Stenevi, 1978; Moore, 1978). Stimulation of locus coeruleus neurons or intraseptal application of noradrenaline decreases spontaneous neuronal activity within the septum (Segal, 1974, 1976; Winokur and Beckman, 1978). Hence, at least the innervation originating from the locus coeruleus is inhibitory. The noradrenergic afferents of the septum exert an excitatory influence on the cholinergic, septohippocampal neurons (Robinson et al., 1978). Binding studies have demonstrated the existence of both α- and β-noradrenergic receptors within the septum (U'Prichard et al., 1980). Intraseptal injections of noradrenergic agents affect avoidance behaviour (Grossman, 1964;

Kelsey, 1976), operant responding (Kelsey, 1976), and urinary excretion (Camargo et al., 1979).

In the present study, the ability of intraseptally injected α-noradrenergic agents to affect behaviour is investigated (section 4.3).

Opioid peptides

The septal nuclei, particularly the lateral septal nucleus, the diagonal band nucleus, and the rostral part of the bed nucleus of the stria terminalis, receive afferents originating in the basal hypothalamus that secrete the opioid peptide β-endorphin (Bloom et al., 1978; Law et al., 1979; Rossier et al., 1977; Watson et al., 1977b). Enkephalins are also present in the septum (Hökfelt et al., 1977; Hong et al., 1977; Johansson et al., 1978; Rossier et al., 1977; Sar et al., 1978; Simantov et al., 1977; Wamsley et al., 1980; Watson et al., 1977a). Cell bodies of enkephalin containing neurons are present in the caudal ventromedial part of the lateral septal nucleus, and in the lateral part of the bed nucleus of the stria terminalis, whereas terminals are found in the medial part of the lateral septal nucleus, and in the bed nucleus of the stria terminalis. The medial septal nuclei do not contain enkephalins. The septal nuclei, with exception of the bed nucleus of the stria terminalis and the triangular nucleus, show a low density of opiate receptors as measured by autoradiographic localization of diprenorphine binding (Atweh and Kuhar, 1977; Pert et al., 1976). The existence of multiple types of opiate receptors within the septum has been reported. The medial, lateral, and triangular septal nuclei contain δ-receptors; the bed nucleus of the stria terminalis contains μ-receptors; the diagonal band nucleus contains both types of opiate receptors; the presence of κ-receptors within the septum seems less established (Duka et al., 1981; Wood and Stotland, 1980). Septal cells respond to administration of morphine (Dafny et al., 1979; Dafny and Rigor, 1980; Robinson and Wang, 1979). Moreover, intraseptally applied opiate agents, particularly β-endorphin, depress the activity of the cholinergic, septo-hippocampal neurons (Botticelli and Wurtman, 1981; Moroni et al., 1977, 1978a). This inhibitory effect is mediated via intrinsic, γ-aminobutyric acid containing interneurons in the septum (Wood et al., 1979). The inhibitory effect on the septo-hippocampal neurons is not mediated via an action of morphine on the dopaminergic

terminals in the septum (Wood et al., 1979) although it has been reported that the dopaminergic afferents of the septum contain presynaptically localized opiate receptors (Pollard et al., 1977).

As mentioned above, morphine affects septal neuronal activity. It is investigated in the present study (section 4.4) whether this effect of morphine is mediated via a direct action on septal opiate receptors or via an indirect action on afferents to the septum. Moreover, it is investigated whether septal opiate receptors are a site of action for morphine to produce its behaviour effects. In addition, septal opiate receptors are characterized pharmacologically.

Glutamate

High levels of glutamate are present in the lateral septal nucleus and the anterior diagonal band nucleus (Walaas and Fonnum, 1980). Microiontophoretically applied glutamate has an excitatory effect on cells in the dorsal part of the lateral septal nucleus (McLennan and Miller, 1974). The hippocampo-septal projection uses, at least partly, glutamate as neurotransmitter (Fonnum and Walaas, 1978; Fonnum et al., 1979; Malthe-Sörenssen et al., 1980a, b; Nitsch et al., 1979; Storm-Mathisen and Opsahl, 1980; Taxt and Storm-Mathisen, 1979; Walaas and Fonnum, 1980; Wood et al., 1979; Zaczek et al., 1979). The lateral septal cells which receive the glutamatergic afferents from the hippocampus are most probably intrinsic, γ-aminobutyric acid containing interneurons (Malthe-Sörenssen et al., 1980a).

In the present study (section 4.5), it is investigated whether the septal glutamatergic processes are functionally active at the behaviour level.

Serotonin

Serotonin is present within the septum (Koslow et al., 1974; Schofield and Everitt, 1981; Steinbusch, 1981). The serotonergic afferents of the septum arise from the central superior and dorsal raphe nuclei (Assaf and Miller, 1978; Geyer et al., 1976; Halaris et al., 1976; Kellar et al., 1971). The central superior nucleus projects to the medial septal nucleus whereas the dorsal raphe cells terminate in the lateral septal nucleus (Azmitia and Segal, 1978). The responses of septal cells to stimulation of the serotonergic afferents are quite complex (Segal, 1976); at least the afferents arising from the central

superior nucleus may be inhibitory (Assaf and Miller, 1978), which is further strenghtened by the data that septal neuronal activity is suppressed by intraseptally applied serotonin (Segal, 1974).

γ-Aminobutyric acid (GABA)

GABA and its synthetizing enzym glutamate decarboxylase are present within the septum (Earley and Leonard, 1978; Fonnum et al., 1979; Palkovits et al., 1974; Tappaz et al., 1976), and particularly concentrated in the diagonal band and lateral septal nuclei (Walaas and Fonnum, 1980). High affinity uptake of GABA is lower in the medial than in the lateral septal nucleus (Taxt and Storm-Mathisen, 1979). GABA-ergic drugs are functionally active in the septum (De France et al., 1975; Wood et al., 1979), and microiontophoretically applied GABA depresses the activity of septal cells (Herz and Gogolak, 1965; McLennan and Miller, 1974b; Segal, 1974). Septal interneurons may use GABA as neurotransmitter (De France et al., 1975; Fonnum et al., 1979; McLennan and Miller, 1974b). Septal GABA containing neurons exert an inhibitory action on the cholinergic, septo-hippocampal neurons (Revuelta et al., 1979).

Adrenocorticotrophic hormone (ACTH)

The ventral part of the lateral septal nucleus and the bed nucleus of the stria terminalis are innervated by ACTH-containing neurons arising in the basal hypothalamus (Hostetter et al., 1979; Krieger et al., 1977; Pelletier and Leclerc, 1979; Watson et al., 1978a). Intraseptally applied ACTH increases the activity of the cholinergic, septo-hippocampal neurons (Botticelli and Wurtman, 1981). Specific uptake of a behaviourally potent $ACTH_{4-9}$ analog in the septum has been reported (Rees et al., 1980; Verhoef et al., 1977a, b).

Substance P (SP)

SP-containing neurons are present in the septum (Cuello and Kanazawa, 1978; Kerdelhue et al., 1981). SP-containing cell bodies are concentrated in the medial and ventromedial part of the lateral septal nucleus and in the bed nucleus of the stria terminalis (Ljungdahl et al., 1978a, b). The lateral septal nucleus is innervated by SP-containing neurons arising from a region ventral to it (Paxinos et al., 1978). Intraseptally applied SP decreases the activity of the cholinergic, septo-

hippocampal neurons; this effect is not mediated via septal γ-aminobutyric acid containing interneurons (Malthe-Sörenssen et al., 1978; Wood et al., 1979). Intraseptally applied SP also affects avoidance behaviour (Stäubli and Huston, 1980).

Vasopressin (VP)

VP-containing neurons originating from the hypothalamus terminate within the lateral and dorsal septal nuclei (Brownfield et al., 1978; Buijs, 1978; Buijs and Swaab, 1979; Buijs et al., 1978; Dogterom et al., 1978; Glick and Brownstein, 1980). VP, locally applied in the dorsal septal nucleus affects avoidance behaviour (Kovacs et al., 1979).

Luteinizing hormone-releasing factor (LRF)

LRF-containing cells are found throughout the septal nuclei; some of these cells project to the habenula and interpeduncular nuclei, while others form small clusters within the septum itself (Barry, 1978; Kerdelhue et al., 1981; Silverman and Krey, 1978). Septal cells respond to microiontophoretically applied LRF (Poulain and Carette, 1977).

Angiontensin II (A-II)

Specific A-II binding and A-II containing nerve terminals are present in the lateral septal nucleus (Fuxe et al., 1976; Sirett et al., 1977). Septal cells respond to intravenously or microiontophoretically applied A-II (Huwyler and Felix, 1980; Simonnet et al., 1980).

Thyrotropin-releasing hormone (TRH)

Particularly the lateral and dorsal septal nuclei contain TRH (Brownstein et al., 1974a; Kerdelhue et al., 1981). Septal cells respond to microiontophoretically applied TRH (Winokur and Beckman, 1978).

Histamine

Histamine receptors of the H1 type are found in the bed nucleus of the stria terminalis and in the diagonal band nucleus; lower levels occur in the lateral septal nucleus (Palacios et al., 1981). Cells in the medial septal and diagonal band nuclei respond to iontophoretically applied histamine (Carette, 1978).

Oxytocin

Septal afferents originating from the hypothalamus contain oxytocin (Buijs, 1978; Buijs et al., 1978; Dogterom et al., 1978). Oxytocin is not found in the lateral septal nucleus (Buijs and Swaab, 1979). Oxytocin, locally applied in the dorsal septal nucleus affects avoidance behaviour (Kovacz et al., 1979).

Miscellaneous

Neurons containing gonadotropin releasing hormone are present throughout the septal nuclei (Marshall and Goldsmith, 1980). α-Melanocyte stimulating hormone is found particularly in the bed nucleus of the stria terminalis (Jacobowitz and O'Donohue, 1978; O'Donohue et al., 1979). Neurotensin occurs in the lateral septal and diagonal band nuclei and in the bed nucleus of the stria terminalis (Young and Kuhar, 1981). These latter nuclei contain also somatostatin (Bennett-Clarke et al., 1980). Androgen receptors are present in the septum (Liederberg et al., 1980). Septal cells respond to iontophoretically applied testosterone (Yamada, 1979). Estrogen binding sites are found in the lateral septal nucleus, the triangular nucleus, and the bed nucleus of the stria terminalis (Keefer and Stumpf, 1975; Martinez-Vargas et al., 1976).

4.2 EFFECTS OF INTRASEPTAL ADMINISTRATION OF CHOLINERGIC AGENTS ON MORPHINE-INDUCED BEHAVIOUR OF CATS

4.2.1 Summary

Behavioural effects of intraseptal administration of cholinergic agents were analyzed in cats pretreated with morphine 15-20 min or 40 min earlier. Drug-induced changes were measured both quantitatively (onset, duration, and frequency of behavioural items) and qualitatively (presence or absence of stereotyped and ritualized behaviour patterns). Carbachol and scopolamine given 15-20 min after morphine accelerated and retarded, respectively, the normal development of the morphine syndrome: carbachol resulted in the display of stereotyped and ritualized patterns, i.e. symptoms characteristic of cats pretreated 30-40 min earlier with morphine, whereas scopolamine reduced the frequency of the behavioural items in comparison with controls, and retarded the onset of stereotyped and ritualized patterns. Carbachol given 40 min after morphine also affected the fully developed morphine syndrome: it increased the frequency of stereotyped and ritualized patterns displayed at that time. This dose-dependent effect, mimicked by arecoline and the combination of acetylcholine and physostigmine, could be antagonized by atropine and scopolamine, which *per se* had no influence on the stereotyped and ritualized patterns. It is concluded that the degree of cholinergic muscarinic activity within the septal nuclei at least partly determines the genesis of the morphine syndrome in cats. The data are discussed in view of the hypothesis that systemically administered morphine and intraseptally applied cholinergic agents affect the cholinergic septo-hippocampal system in a common way via distinct sites of action at the level of the septal nuclei.

4.2.2 Introduction

Septal nuclei play an important role in the effects of morphine on hippocampal acetylcholine (ACh) release (Moroni et al., 1978a). Furthermore, relatively high concentrations of morphine receptors and endogenous ligands are present in this brain area (Pert et al., 1976; Rossier et al., 1977). Still, direct evidence for a role of the septal nuclei in the behavioural effects of morphine is not yet available.

Cholinergic terminals are present within the septal nuclei (Chronister

et al., 1976; Fonnum and Walaas, 1978b; Harkmark et al., 1975; Hoover et al., 1978; Kobayashi et al., 1978); moreover, ACh is functionally active in this brain area (Grossman, 1976; Segal, 1974). Alteration of intraseptal cholinergic activity via the intracerebral injection technique was, therefore, chosen as a tool to affect septal activity in morphine-treated cats and to study involvement of the septal nuclei in morphine-induced behaviour of cats.

The behavioural effects of morphine as shown in our laboratory during the last few years, are described elsewhere in detail (Cools et al., 1974, 1977, 1978). In summary, morphine (5 mg/kg, i.p.) elicits a syndrome which is built up in three successive steps: depression phase, re-organization phase, and ritualization phase. The second phase (starting 10-20 min after morphine injection) is characterized by the breakdown of normal head, limb and postural movements into an increasing number of isolated, staccato movements and the subsequent appearance of new head, limb and postural movements in which the single units are re-integrated. The resulting abnormal patterns are stereotyped, i.e. the way in which the single units are integrated into particular spatio-temporal patterns is completely fixed, and they become ritualized in the sense that they are repeated in random order at variable time intervals. The last phase (starting 25-35 min after morphine injection) is characterized by the display of stereotyped and ritualized behaviour patterns which have reached a more or less stabilized level of frequency. This syndrome can be analyzed in terms of 1) changes in onset, duration, and frequency of operationally defined behavioural items, and 2) presence or absence of stereotyped and ritualized patterns (Fig. 4.3) (Cools et al., 1977, 1978).

There is considerable evidence that the intracerebral administration of the same compound can influence the nature of morphine-induced re-organization and ritualization in a different manner (Cools et al., 1978). Therefore, the current study investigated the effects of cholinergic agents administered intraseptally during the re-organization and ritualization phases.

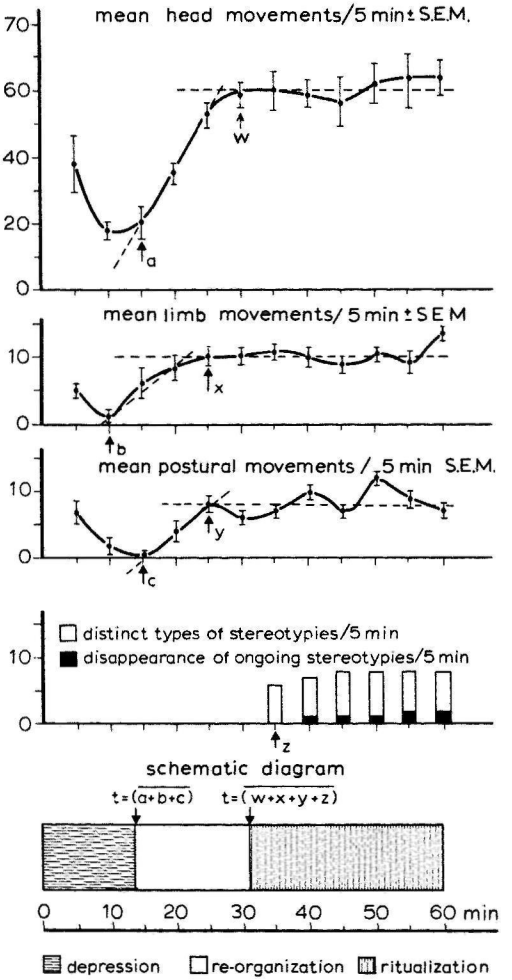

Fig. 4.3 Behavioural effects following morphine (5 mg/kg, i.p.) in a male cat: illustration of the determinants of morphine-induced behaviour. The number of head, limb, and postural movements counted per min are represented as mean values/5 min ± S.E.M. The onset and termination (min) of the phases following morphine injection are represented as vertical axis inside the diagram outlining the different blocks.
For definition of the onset, see text.
Reproduced from Cools et al. (1978).

4.2.3 Materials and methods

Experimental procedure

Adult cats of both sexes (2.0 - 3.5 kg) were used. Cannulae aimed at the septal area were bilaterally implanted under pentobarbital anesthesia (Nembutal, 40 mg/kg, i.p.). The diameters of the inner and guide cannulae were 0.5 and 0.8 mm, respectively (Cools and Van Rossum, 1970). After 14 days the animals were inspected and cats showing behavioural signs of brain damage were discarded. The intracerebrally administered drugs were injected bilaterally with a Hamilton injection syringe. Each injection took about 3 s; a volume of 0.5 µl was used in all experiments, except for the experiment in which a combined ACh/physostigmine injection was given (1.0 µl). The solutions were freshly prepared before each experiment.

The following agents were used: acetylcholine jodide (Fluka); arecoline bromide (Serva); atropine sulphate (De Onderlinge Pharmaceutische Groothandel); carbamylcholine chloride (carbachol; Sigma); physostigmine sulphate (De Onderlinge Pharmaceutische Groothandel); scopolamine bromide (De Onderlinge Pharmaceutische Groothandel); and morphine chloride (De Onderlinge Pharmaceutische Groothandel). All agents were dissolved in distilled water, except for morphine which was dissolved in saline (0.9% NaCl). The doses (referring to salts) were selected on the basis of pilot studies and earlier data (Hamilton and Grossman, 1969; Kelsey and Grossman, 1969; Macphail and Miller, 1968). All animals were pretreated with morphine (5 mg/kg, i.p.), except for cats used in the final series of experiments.

In the first series of experiments, cholinergic agonists and antagonists were intraseptally administered to cats pretreated 15-20 min earlier with morphine. In the second series of experiments, these agents were intraseptally administered to cats pretreated 40 min earlier with morphine; particular attention was paid to the muscarinic nature and dose-dependency of the effects elicited. In the final series of experiments, cholinergic agents were intraseptally administered to non-pretreated cats to detect their effectiveness *per se*.

After completion of the experiments, the animals were killed with an overdose of pentobarbital and transcardially perfused with saline (0.9% NaCl) and formaldehyde solution (4%). Brain slices (about 100 µm) were made along the tracks of the guide cannulae to determine the sites of injection. The injection sites were mapped with the help of the atlas

of Snider and Niemer (1964). Only experiments in which both left and right injection sites were restricted to the septal nuclei are discussed.

Observation procedure and behavioural analysis

First, each animal was familiarized with the observation cage during two 1 hr periods and adapted to the intracerebral injection procedure by inserting an empty needle into the target area. The observation cage (88 x 66 x 61 cm) was soundproof and equipped with a ventilator producing background noise. The animals were placed into the observation cage 15 min before the experiments. The observation started immediately after the morphine injection and lasted 60 min. Morphine was administered not more than four times to the same animal with a minimum intertrial interval of 10 days. This dose and schedule resulted in no signs of abstinence and tolerance, except for increased salivation.

The morphine-induced behaviour was recorded on videotapes by means of a closed-circuit television. The tapes provided continuous records which were analyzed according to the following parameters (Fig. 4.3; Cools et al., 1978): number/min of 1) head movements, 2) limb movements, 3) postural movements, and 4) individual-specific stereotyped and ritualized patterns; onset of the re-organization phase (i.e. the end of the depression phase) by averaging the points of time at which the frequencies of 1) head, 2) limb, and 3) postural movements started to increase and continued to do so for a period of 15 min; onset of the ritualization phase (i.e. the end of the re-organization phase) by averaging the points of time at which the frequencies of 1) head, 2) limb, and 3) postural movements became stabilized for a period of 15 min, and the stereotyped and ritualized limb movements or turning and walking patterns appeared. In this context, it should be mentioned that some animals showed only stereotyped and ritualized limb movements, whereas other animals initially showed these movements and, later on, integrated them into walking and/or turning patterns. As an increase of such locomotor patterns always implied an increase in limb movements, changes in the number of limb movements were only measured in case no changes in the number of ritualized locomotor patterns were elicited.

The effects of intracerebrally injected agents upon morphine-induced behaviour were analyzed in terms of changes in the same parameters. In cats receiving intraseptal injections 40 min after morphine, the following procedure was chosen to detect significant changes in ongoing behaviour: first, the number of stereotyped and ritualized limb movements or

turning and walking patterns was counted for 10 min before and after the intracerebral injection; second, the differences between both periods were calculated per animal; and, finally, these differences in drug-treated cats were compared with those in controls (Mann-Whitney U-test). As the stereotyped and ritualized turning and walking patterns were the most characteristic features of the fully developed syndrome, the analysis of changes in animals which showed stereotyped and ritualized locomotor patterns was restricted to these turning and walking patterns.

4.2.4 Results

Alteration of cholinergic activity in the septal nuclei during the morphine-induced re-organization phase

The overall effects were analyzed with the parameters summarized in Table 4.4. A qualitative description of these effects is given below.

Carbachol (1.0 µg/0.5 µl per side, n = 8) given 17 ± 1 min after morphine interrupted the normal development of morphine-induced behaviour. In contrast to distilled water-treated control animals, which continued to show increased head, limb and postural movements until 24 min after morphine (8 min after intraseptal injection) (Table 4.4), all carbachol-treated animals almost immediately maintained the frequencies of these items at a more or less fixed level and displayed individual-specific stereotyped patterns which became ritualized. Within 2 ± 1 min after intraseptal injection, during which two animals exhibited short-lasting (2-3 min) hyperactivity, the carbachol-treated cats in fact showed all symptoms characteristic of the ritualization phase. As shown in Table 4.4, the ritualization period I started at a significantly earlier point of time and lasted 8-17 min (mean ± S.E.M. 11 ± 1 min). Next, the frequencies of behavioural items started to increase for about 6 min, whereas the ritualized stereotyped patterns disappeared at the same time; i.e. some patterns disappeared and new patterns were introduced. In other words, a second re-organization phase appeared (Table 4.4). Finally, the animals started again to maintain the frequencies of behavioural items at a more or less fixed level and to display stereotyped patterns, which became ritualized for the remaining part of the observation period (Table 4.4, ritualization II).

The cholinergic antagonist scopolamine (10 µg/0.5 µl per side, n = 10) given 16 min after morphine also affected the normal development of

TABLE 4.4

Behavioural effects caused by intraseptal administration of distilled water (n=7), carbachol (n=8), and scopolamine (n=10) during the morphine-induced re-organization phase

	distilled water	carbachol	scopol-amine
morphine injection	0	0	0
re-organization I, onset	10 ± 1	12 ± 1	11 ± 1
intraseptal injection	16	17 ± 1	16
re-organization I, end	24 ± 2 (8 ± 2)[c]	17 ± 1	-
ritualization I, onset	24 ± 2 (8 ± 2)[c]	(19 ± 1)[a] (2 ± 1)[c]	(41 ± 3)[b] (25 ± 2)[c]
end	60 (> 36)[d]	30 ± 1 (11 ± 1)[d]	60 (> 19)[d]
re-organization II, onset	-	30 ± 1	-
end	-	36 ± 3 (6 ± 2)[d]	-
ritualization II, onset	-	36 ± 3	-
end	-	60 (> 24)[d]	-

Characteristic points of time are represented as number of minutes after morphine injection (mean ± S.E.M.)

a = carbachol vs. control: $p < 0.014$, two-tailed Mann-Whitney U-test
b = scopolamine vs. control: $p < 0.002$, two-tailed Mann-Whitney U-test
c = interval after intraseptal injection (min)
d = duration (min)

the morphine syndrome. In this case, however, the animals started to show fluctuations in frequencies of head, limb and postural movements; the ongoing increase in frequency was suppressed for a period of about

25 min. About 41 min after morphine, the cats started to maintain the frequencies at a more or less stabilized level and to display individual-specific stereotyped patterns which then became ritualized. In other words, scopolamine retarded the progress of the re-organization phase and delayed the ritualization phase in a significant way (Table 4.4, ritualization I, onset).

Alteration of cholinergic activity in the septal nuclei during the morphine-induced ritualization phase

Carbachol (1.0 µg/0.5 µl per side, n = 20) given 40 min after morphine resulted in increased frequencies of ritualized turning and walking patterns within 1-2 min. As shown in Fig. 4.4, this effect was significant in comparison to controls. The movements became even more compulsive and ritualized; in fact, all behaviour patterns were persistently repeated in exactly the same way. The duration of this effect was longer than the observation period of 60 min, although intermittent periods of

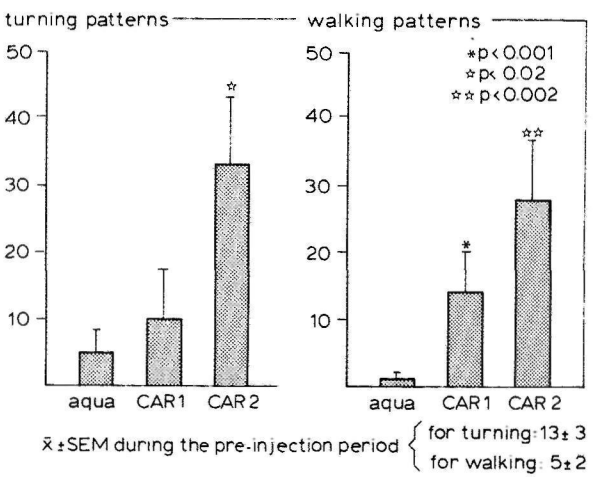

*Fig. 4.4 Behavioural effects caused by intraseptal administration of distilled water (AQUA, n=8), 0.5 µg carbachol (CAR 1, n=8), and 1.0 µg carbachol (CAR 2, n=20) at 40 min after morphine during the ritualization phase: mean differences in numbers of two ritualized locomotor patterns 10 min before and after intracerebral injection. For statistical analysis the individual differences measured in drug-treated cats were compared with those of controls (Mann-Whitney U-test, * one-tailed, ☆two-tailed).*

of lower activity were sometimes seen. As shown in Fig. 4.4, the effects were dose-dependent: 0.5 µg carbachol (n = 8) was less effective than 1.0 µg.

The muscarinic agonist arecoline (5 µg/0.5 µl per side, n = 5) given 40 min after morphine produced a significant increase in the number of ritualized limb movements when compared with controls (Fig. 4.5). Figure 4.5 shows that the same holds true for the injection of a combined solution of ACh and the acetylcholinesterase (AChE) inhibitor physostigmine (respectively, 8.0 and 6.0 µg/1.0 µl per side, n = 3).

Local pretreatment with the cholinergic blockers scopolamine (10 µg/0.5 µl per side, n = 9) or atropine (10 µg/0.5 µl per side, n = 2) given 5 min earlier antagonized the effect of 1.0 µg carbachol (Fig. 4.6). Intraseptal scopolamine (10 µg/0.5 µl per side, n = 4) or atropine (10 µg/0.5 µl per side, n = 4) alone had no effect on the behavioural items displayed at that time.

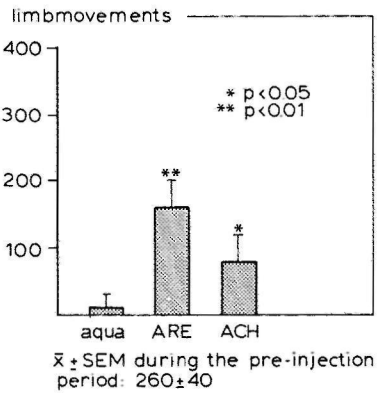

Fig. 4.5 Behavioural effects caused by intraseptal administration of distilled water (AQUA, n=8), arecoline (ARE, n=5) and acetylcholine/eserine (ACH, n=3) 40 min after morphine during the ritualization phase: mean differences in number of ritualized limb movements 10 min before and after intracerebral injection. For statistical analysis the individual differences measured in drug-treated cats were compared with those in controls (one-tailed Mann-Whitney U-test).

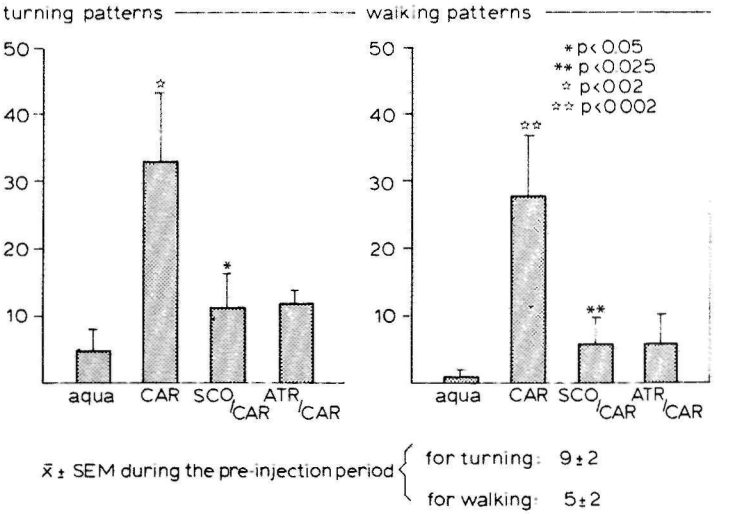

Fig. 4.6 Behavioural effects caused by intraseptal administration of distilled water (AQUA, n=8), carbachol (CAR, n=20), scopolamine/carbachol (SCO/CAR, n=9), and atropine/carbachol (ATR/CAR, n=2) 40 min after morphine during the ritualization phase: mean differences in numbers of two ritualized locomotor patterns 10 min before and after the intracerebral injection. For statistical analysis the individual differences measured in carbachol-treated cats were compared with those of controls (two-tailed Mann-Whitney U-test), whereas the individual differences in the scopolamine/carbachol and in the atropine/carbachol treated animals were compared with those in the carbachol treated animals (one-tailed Mann-Whitney U-test).

Alteration of cholinergic activity in the septal nuclei of naive cats

Intraseptal arecoline (5 µg/0.5 µl per side, n = 6), scopolamine (10 µg/0.5 µl per side, n = 2), or atropine (10 µg/0.5 µl per side, n = 2) did not affect the scores of the behavioural items used in the analysis of the morphine-induced behaviour in an unequivocal way. Carbachol (1.0 µg/0.5 µl per side, n = 9), however, resulted in a slight but not significant increase in locomotor activity during the 10 min observation period (n = 7, Mann-Whitney U-test). Two cats showing mydriasis started

to growl after 5-6 min, and one cat showed swallowing movements after 5 min. Furthermore, all cats displayed a number of threat, fear, and flee expressions and/or postures as soon as the experimenter approached the cage at the end of the observation period (cf. "affective defensive reaction", Macphail and Miller (1968)). Finally, two cats which were observed for a longer period exhibited hyperventilation and tongue protrusions about 20 min after injection. These effects were less pronounced in cats receiving a smaller dose of carbachol (0.5 µg/0.5 µl per side, n = 3).

4.2.5 Discussion

The present study indicates that cholinergics applied into the septal nuclei of morphine-treated cats affect the behaviour of these animals. Although leakage of the intraseptally injected drugs to the lateral ventricles might have occurred, the small injection volume used (0.5 µl) together with the short latency (within 1-2 min also for medial sites) justifies the assumption that the drugs injected have mainly affected the cholinergic mechanisms within the septal nuclei. In this context, it is of interest to note that the display of a symptom like hyperventilation required a much longer latency, suggesting that this effect was not mediated via septal cholinergic processes (cf. Macphail and Miller, 1968).

In general, both quantitative (onset, duration, and frequency of behavioural items) and qualitative (presence or absence of stereotyped and ritualized behaviour patterns) measures were used to describe the changes in the morphine-induced behaviour following the intracerebral injections.

The drug-induced changes elicited by injections given 15-20 min after systemic morphine were, in fact, qualitative in nature: 1) display of stereotyped and ritualized patterns during a period in which the controls had just shown an ongoing increase in the frequencies of head, limb and postural movements (this apparent shift from the re-organization phase towards the ritualization phase was seen in carbachol-treated cats); and 2) interruption of the ongoing increase in the frequencies of the various items together with postponement of the onset of stereotyped and ritualized patterns (this apparent retardation of the development of the morphine-induced behaviour was seen in scopolamine-treated cats).

The drug-induced changes elicited by agents given 40 min after systemic morphine were, in fact, quantitative in nature, i.e. increase in the number of stereotyped and ritualized limb movements during the period in which controls had maintained the frequencies of these movements at a stabilized level. This effect was seen in arecoline-treated cats and, to a different degree, in ACh/physostigmine-treated animals. A comparable effect, i.e. an increase in the number of stereotyped and ritualized walking and turning patterns (which implies an increase in limb movements) was seen in carbachol-treated cats.

Considering the specificity of the observed effects, the following arguments are offered to emphasize the involvement of cholinergic, especially muscarinic, processes. First, the carbachol-induced effect was dose-dependent (Fig. 4.4). In this context, it is useful to mention that 0.5 µg carbachol appeared to be a threshold dose (Fig. 4.4), whereas 1.0 µg carbachol had already affected the behaviour of naive cats slightly (thus, testing lower or higher doses was not considered). Second, the muscarinic agent arecoline and the combination of ACh and physostigmine (agents lacking the aspecific effects of carbachol) mimicked the effects of carbachol given 40 min after morphine administration. As it might be expected, due to different potencies of carbachol, arecoline, and ACh/physostigmine, these drugs produced quantitatively different, but qualitatively similar effects (Figs. 4.4 and 4.5). And, finally, the muscarinic cholinergic antagonists scopolamine and atropine inhibited the carbachol-induced effect in a qualitatively similar way (Fig. 4.6). All these findings support the conclusion that the carbachol-induced changes were mediated through muscarinic, cholinergic processes within the septal nuclei. Indeed, it is known that muscarinic receptors are present within this brain structure (Grossman, 1976; Kobayashi et al., 1978).

The final question deals with the possible role of these cholinergic processes in the genesis of morphine-induced behaviour. As mentioned, the development of the syndrome could be accelerated by carbachol and retarded by scopolamine. Especially the scopolamine-induced inhibition of the normal development of the morphine syndrome suggests that septal cholinergic processes are essential links in the chain of responses triggered by morphine. This, together with the observation that admin-

istration of cholinergics into the septum of naive cats resulted in no significant effect on the scores of the behavioural elements investigated, makes it rather unlikely that the observed effects were simply the sum of morphine- and cholinergic-induced behavioural changes. The present data, however, are insufficient to exclude this possibility. On the other hand, there is hard evidence that both systemic morphine and intraseptally applied cholinergic agents can affect the cholinergic septo-hippocampal system.

1. Intraseptal or systemic morphine decreases the turnover rate of ACh within the hippocampus of the rat (Moroni et al., 1978a; Schmidt and Buxbaum, 1978). Such a decreased hippocampal ACh turnover rate appears to be due to a morphine-induced decrease in activity of cholinergic septo-hippocampal neurons originating from the medial septal nuclei (Dudar, 1975; Lewis and Shute, 1967; Mellgren and Srebro, 1973; Moroni et al., 1978b; Rommelspacher and Kuhar, 1974; Sethy et al., 1973).

2. Local application of ACh inhibits the neural activity of cells in the medial septal nuclei, in which cholinergic terminals are concentrated (Fonnum and Walaas, 1978b; Harkmark et al., 1975; Segal, 1974).

Thus, both systemic morphine and intraseptally applied cholinergic drugs can affect the same cholinergic septo-hippocampal system. Nevertheless, the fact that morphine is unable to change the turnover rate of ACh within the septal nuclei (Zsilla et al., 1977) excludes the possibility that morphine might have affected the cholinergic septo-hippocampal neurons by modifying the cholinergic input of the medial septal cells. Accordingly, it is attractive to postulate that systemic morphine and intraseptally applied cholinergic agents did affect the cholinergic septo-hippocampal system in a common way via two distinct sites of action at the level of the septal nuclei: cholinergics via interference with a direct or indirect cholinergic input on medial septal cells, and morphine via interference with a direct or indirect noncholinergic input on these cells. From this point of view, it becomes possible to understand why the cholinolytics given 40 min after morphine injection did not affect the display of stereotyped and ritualized patterns although the cholinomimetics did. At that time, the influence of septal cholinergic mechanisms on the septo-hippocampal system might have been so small, in comparison to that of morphine, that a further decrease of the cholinergic influence on the septal cells remained ineffective.

As a final remark, the morphine-induced behaviour proved to be a reliable tool for detecting functional deficits of the septal nuclei. Although the present paradigm is insufficient to examine the nature of the deficit itself (cf. Donovick et al., 1979a), it appeared to be a valid and reliable tool for examining the nature and specificity of the neurotransmission processes underlying septal deficiencies. In more sophisticated paradigms, this knowledge can be used in studies analyzing the nature of the deficit itself.

The present study shows that the degree of activity in septal muscarinic processes at least partly determines the genesis of the morphine-induced behaviour in cats. On the basis of the present and earlier reported behavioural, biochemical, and electrophysiological data, it is suggested that systemic morphine and intraseptally applied cholinergic agents affect the cholinergic septo-hippocampal system in a common way via two distinct sites of action at the level of the septal nuclei.

4.3 PRESENCE OF A PARTICULAR SUBPOPULATION OF DOPAMINE RECEPTORS WITHIN THE SEPTAL NUCLEI: A BEHAVIOURAL STUDY ON CATS

4.3.1 Summary

The behavioural effects of intraseptal administration of dopaminergic drugs (apomorphine, haloperidol, (3,4-dihydroxy-phenylamino)-2-imidazoline (DPI), ergometrine and dopamine) and α-noradrenergic drugs (oxymetazoline, noradrenaline and phentolamine) were analyzed in cats pretreated with morphine (5 mg/kg, i.p.). Changes in frequencies of stereotyped locomotor patterns were used for statistical evaluation of drug-induced effects. Taking advantage of the specificity of the drugs mentioned, a distinction could be made between effects mediated via excitation-mediating dopamine (DAe), inhibition-mediating dopamine (DAi) and α-noradrenaline (α-NA) receptors. Intraseptal injection of the DAi agonist DPI resulted in a decrease in the frequency of stereotyped locomotor patterns. This effect was dose-dependent and mimicked by that of intraseptally applied dopamine but not of any of the other drugs. Moreover, intraseptal injection of the DAi antagonist ergometrine inhibited

the effect of DPI. The DAe agonist apomorphine as well as the DAe antagonist haloperidol remained ineffective when applied in low doses. The α-NA antagonist phentolamine and a rather high dose of haloperidol produced a slight but significant increase in the frequency of locomotor patterns; intraseptally applied oxymetazoline counteracted the phentolamine-induced effect. It is concluded that the septal nuclei of cats contain functionally active, α-NA receptors as well as functionally active, dopamine (DA) receptors having pharmacological properties identical to those of DA receptors present within mesolimbic structures such as the nucleus accumbens: the so-called inhibition-mediating DAi receptors.

4.3.2 Introduction

In the past few years, evidence has been accumulated for the existence of more than one type of dopamine (DA) receptor in the brain (Cools and Van Rossum, 1976, 1980; Creese and Sibley, 1979; Garau et al., 1978; Kebabian and Calne, 1979; Norcross and Spehlmann, 1978; Roth, 1979; Titeler et al., 1978). The septal nuclei are one of the brain areas with a relatively high dopamine content (Brownstein et al., 1974b; Collu et al., 1980; Garey, 1976; Klemm et al., 1979; Saavedra and Zivin, 1976; Versteeg et al., 1976). Dopamine-containing neurons innervating the septal nuclei and arising from the mesencephalic ventral tegmental area (A10 cell group; Assaf and Miller, 1977; Lindvall and Stenevi, 1978; Moore, 1978) can be classified into two distinct groups, viz. "smooth" and "fine-varicose" dopamine neurons (Lindvall and Stenevi, 1978; Moore, 1978) which might reflect the presence of distinct types of DA receptors (Cools and Van Rossum, 1976, 1980). Binding studies also suggest the existence of more than one type of DA receptor in septal and other limbic brain areas (Blackburn et al., 1978; Howlett et al., 1979). In the present study, an attempt was made to elucidate the pharmacological nature of the DA receptors within the septal nuclei by analyzing the behavioural effectiveness of intraseptally applied agents that selectively interact with the so-called excitation-mediating dopamine (DAe) and inhibition-mediating dopamine (DAi) receptors (Cools and Van Rossum, 1976, 1980). DAe receptors are rather selectively activated by apomorphine and inhibited by haloperidol; in contrast, DAi receptors are selectively activated by (3,4-dihydroxy-phenylamino)-2-imidazoline (DPI)

and inhibited by ergometrine. Accordingly, attention was focussed on the effectiveness of these agents.

Besides dopaminergic afferents the septal nuclei also receive noradrenergic afferents from the locus coeruleus and other structures in the lower brain stem (Lindvall and Steveni, 1978; Moore, 1978). In addition, noradrenaline and noradrenaline (NA) receptors are present and functionally active within the septal nuclei (Brownstein et al., 1974b; Camargo et al., 1977, 1979; Kelsey, 1976; Robinson et al., 1978; Segal, 1974; Swanson and Hartman, 1975; U'Prichard et al., 1980; Young and Kuhar, 1980). As the DAe antagonist haloperidol and the DAi agonist DPI are not devoid of α-noradrenergic (α-NA) properties (Bevan et al., 1979; Cools, 1978; Peroutka et al., 1977; Struyker Boudier et al., 1975), some α-NA agents were also tested to determine the specific dopaminergic character of the effects elicited by the DA drugs.

The morphine-induced behaviour of cats has been demonstrated to be a reliable model to disclose changes in the functional activity of the septal nuclei (Megens and Cools, 1979). Consequently, this behavioural model was chosen as a tool in the present study to investigate the effects elicited by intraseptal microinjection of DA and NA agents. The results provide evidence for the existence of the DAi type of receptor within the septal nuclei and for their involvement in the regulation of morphine-induced behaviour of cats.

4.3.3 Materials and methods

General

Adult cats (2.0-3.5 kg) of both sexes were used. The animals were obtained from the breeding colony of the Central Animal Laboratory University of Nijmegen, and housed and fed under standard laboratory conditions. Cannulae aimed at the septal area were bilaterally implanted by means of a stereotaxic procedure under pentobarbital anaesthesia (Nembutal^{R}, 50 mg/kg, i.p.). The double-barrelled, stainless steel cannulae were fixed on the skull with acrylic dental cement; the diameter of the guide and inner cannulae were 0.8 mm and 0.5 mm respectively. After a 2 week recovery period, the animals showing no overt behavioural signs of brain destruction were habituated to the experimental cage and the injection procedure for two separate periods of 1 hr each. A quarter of an hour prior to each experiment, the animal was placed in this obser-

vation cage. The observation cage (88 x 66 x 61 cm) was soundproof and equipped with a ventilator producing background noise. With exception of the perspex front window the walls were opaque. During the experiments care was taken to prevent the occurrence of changes in the immediate surroundings of the cage. Behaviour was recorded on videotapes by means of closed-circuit television.

Drugs administered intracerebrally were injected manually by means of Hamilton injection syringes (diameter of the needle: 0.4 mm). Each injection took about 3 sec. In general, the volume injected was 0.5 µl. The drugs were dissolved in distilled water apart from the higher dose of haloperidol (10 µg), which was dissolved in 1% ascorbic acid solution to achieve better solubility. The solvents were sterile and apyrogenic. The solutions were prepared freshly before each experiment. The doses refer to the salts. The following agents were used: apomorphine hydrochloride (De Onderlinge Pharmaceutische Groothandel), ascorbic acid (Merck AG), (3,4-dihydroxy-phenylamino)-2-imidazoline hydrochloride (DPI; Wander), dopamine hydrochloride (De Onderlinge Pharmaceutische Groothandel), ergometrine maleate (Halewood Chemicals), haloperidol hydrochloride (Janssen Pharmaceutica), l-noradrenaline hydrochloride (Fluka AG), oxymetazoline hydrochloride (Ciba-Geigy), phentolamine methane-sulphonate (Ciba-Geigy), and yohimbine hydrochloride (Nogepha).

All intracerebrally applied drugs were tested in cats pretreated with a systemic injection of morphine dissolved in saline (morphine hydrochloride, De Onderlinge Pharmaceutische Groothandel, 5 mg/kg, i.p.). Agents proven to be effective in morphine-pretreated cats were also tested in non-pretreated cats to determine their effectiveness *per se*. The experiments were performed between 8.00 a.m. and 4.00 p.m. When animals were tested more than once, an intertrial interval of at least two weeks was used. Using this time-interval, no signs of tolerance or abstinence were seen apart from increasing salivation.

After the experiments the animals were sacrificed with an overdose of pentobarbital (Nembutal^{R}) and perfused intracardially with a formaldehyde solution (4-10%) containing the anticoagulant heparin (50 mg/l). Brain sections (ca. 100 µm) were made along the tracks of the guide cannulae. The injection sites were localized with the help of the atlas of Snider and Niemer (1964). Effects caused by bilateral injections into sites only one of which was localized within the septal nuclei were discarded.

Behavioural analysis in morphine-pretreated cats

Animals receiving an intraperitoneal (i.p.) injection of morphine were observed for a period of 60 min immediately following morphine administration. The morphine-induced behaviour of cats has been described extensively elsewhere (Cools et al., 1974, 1977, 1978; Megens and Cools, 1979). All intraseptally injected drugs were given 40-41 min after morphine: at that point of time, the animals normally show stereotyped behaviour. Changes in frequencies of stereotyped locomotor patterns were used for statistical evaluation of the drug-induced effects. In this study, single locomotor patterns were defined as: 1) any forward or backward movement of the animal along a minimum distance of 40 cm in the case of discontinuous locomotion, 2) any forward or backward movement of the animal along a fixed distance of 80 cm in the case of continuous locomotion, 3) any turning movement of the animal around a minimum angle of 180° in the case of discontinuous turning, 4) any turning movement of the animal around a fixed angle of 360° in the case of continuous turning. Both the total score of locomotor patterns over 10 min immediately preceding the intraseptal injection (X_{pre}) and the total score of locomotor patterns over 10 min following the intraseptal injection (X_{post}) were determined per animal. Behaviour was not analyzed during the 2 min injection period.

Drug-induced *increases* in locomotor activity were assessed by measuring the absolute difference per animal in number of locomotor patterns scored per animal during the pre- and post-injection period:

$$\Delta_{abs} = X_{post} - X_{pre}$$

This variable was calculated for all animals. The values obtained in that manner per test group were compared with the corresponding values for the appropriate control group.

As decreases in locomotor activity only occur in animals showing at least some locomotion during the pre-injection period, only animals with a minimal number of 10 locomotor patterns during the pre-injection period were used in the assessment of drug-induced *decreases*. Furthermore, since a decrease in the number of locomotor patterns measured by the variable Δ_{abs} is limited by the pre-injection value X_{pre}, the differences between pre- and post-injection values were expressed as percentage of the summated pre- and post-injection values:

$$\Delta_{rel} = \frac{X_{post} - X_{pre}}{X_{post} + X_{pre}} \times 100\% \qquad (X_{pre} > 10)$$

The values thus obtained per test group were compared with the corresponding values for the appropriate control group. Note the difference between this definition of relative changes and the more conventional definition of relative changes

$$\frac{X_{post} - X_{pre}}{X_{pre}} \times 100\%.$$

All statistical tests were two-tailed. When test and control group were composed of the same animals, the Wilcoxon matched pairs signed rank test was used; the Mann-Whitney U-test was applied in the remaining experiments. Note that the data in the tables are given as mean values $\pm$ S.E.M. notwithstanding the non-parametric statistics used. Although values of both Δ_{abs} and Δ_{rel} were calculated for all drug treatments, only values of Δ_{abs} are decisive for the presence of a drug-induced increase in contrast to the values of Δ_{rel}, which are decisive for the presence of a drug-induced decrease in number of locomotor patterns.

Behavioural analysis in non-morphinized cats

After a habituation period of a quarter of an hour the inner cannulae were unscrewed and a 10 min observation period started. Immediately after this reference period the injections were given; next, the animals were observed for another 20 min period. Since these experiments served as controls for drug effects in morphine-pretreated cats particular attention was paid to the items used in the analysis of morphine-induced behaviour.

TABLE 4.5

Behavioural effects elicited by drugs applied intraseptally 40-41 min after systemic morphine. Frequencies of stereotyped locomotor patterns are used as dependent variables

drug treatment[a,b]		n	Δ_{abs} (mean±SEM)	n[c]	Δ_{rel} (%) (mean±SEM)
distilled water	2 x 0.5 µl	15	6 ± 3^{f}	9	13 ± 8^{f}
apomorphine	2 x 5.0 µg	16	14 ± 10^{ns}	11	10 ± 10^{ns}
haloperidol	2 x 5.0 µg	11	13 ± 4^{ns}	4	23 ± 1^{ns}
haloperidol	2 x 10.0 µg	10	19 ± 5^{d}	4	28 ± 11^{ns}
DPI	2 x 10.0 µg	15	-11 ± 6^{d}	9	-36 ± 15^{d}
DPI	2 x 4.3 µg	7	-19 ± 10^{e}	6	-28 ± 13^{d}
DPI	1 x 5.0 µg	12	-36 ± 16^{e}	5	-32 ± 8^{e}
ergometrine	2 x 10.0 µg	10	14 ± 6^{ns}	4	6 ± 9^{ns}
oxymetazoline	2 x 10.0 µg	8	7 ± 6^{ns}	4	11 ± 10^{ns}
noradrenaline	2 x 10.0 µg	9	13 ± 8^{ns}	7	6 ± 11^{ns}
phentolamine	2 x 10.0 µg	19	18 ± 4^{e}	10	$16 + 3^{ns}$
phentolamine oxymetazoline	2 x 10.0 µg 2 x 10.0 µg	8	$1 \pm 2^{ns,g}$	4	-16 ± 15^{ns}

For each drug treatment mean values ± S.E.M. averaged over the whole test group are given for both Δ_{abs} *(defined as* $X_{post} - X_{pre}$*) and* Δ_{rel} *(defined as* $(X_{post}-X_{pre})/(X_{post}+X_{pre})$ *x 100%); for details see section Behavioural analysis in morphine-pretreated cats.*

a = all drugs were administered in a volume of 0.5 µl apart from haloperidol, which was given in a volume of 1.0 µl.

b = the agents were given bilaterally (2x) apart from 5 µg DPI, which was injected unilaterally (1x).

c = only animals with X_{pre} *(number of locomotor patterns displayed during the pre-injection period) > 10.*

d = p < 0.05, e = p < 0.02, ns = not significant,

f = not tested (drugs vs. distilled water)

g = p < 0.02 (phentolamine vs. phentolamine/oxymetazoline)

4.3.4 Results

Alteration of DAe activity within the septal nuclei of morphine-pretreated cats

After intraseptal injection of the DAe agonist apomorphine (bilaterally, 5 µg per side, n = 16) there was no change in the performance of stereotyped behaviour. The drug-induced change in number of locomotor patterns measured by Δ_{abs} and Δ_{rel} did not differ significantly from the corresponding values measured in distilled water-treated control animals (bilaterally, 0.5 µl per side, n = 15; Table 4.5). The same applied to the intraseptal injection of the DAe antagonist haloperidol (bilaterally, 5 µg per side, n = 11; due to solubility problems this agent was administered in a volume of 1 µl; Table 4.5). When administered in a higher dose (bilaterally, 10 µg/µl per side, n = 10), however, haloperidol resulted in a slight, but significant, increase in the number of locomotor patterns as measured by Δ_{abs} (Table 4.5).

Alteration of DAi activity within the septal nuclei of morphine-pretreated cats

Intraseptal injection of the DAi agonist DPI (bilaterally, 10.0 µg per side, n = 15; bilaterally, 4.3 µg per side, n = 7; unilaterally, 5 µg, n = 12) greatly affected the performance of stereotyped behaviour. As indicated by the values of Δ_{rel}, the various doses of DPI produced significant decreases in the number of locomotor patterns (Table 4.5). Locomotion was totally, or almost totally, absent within a few min after an effective injection of DPI; the drug-induced attenuation was most pronounced in animals displaying higher pre-injection levels of locomotor activity (Fig. 4.8). Although the method chosen does not offer hard information about changes in the duration of the drug-induced phenomena, DPI also appeared to shorten the total time spent in locomotor activity. The mean latencies $\pm$ S.E.M. of the effects elicited by the various doses of DPI were: 0.2 $\pm$ 0.2 min (2 x 10 µg), 0 $\pm$ 0 min (2 x 4.3 µg) and 1.5 $\pm$ 1.0 min (1 x 5 µg); additional characteristics are illustrated in Fig. 4.7. Even a unilateral injection of 5 µg DPI was effective (Table 4.5: Δ_{rel}; Fig. 4.7b). With respect to the dose-dependency of the DPI effect it must be stated that there was no difference in the magnitude of the effect elicited by bilaterally given doses of 10 µg and of 4.3 µg during the first 10 min post-injection period (Table 4.5: Δ_{rel}); however, the effect caused by 2 x 4.3 µg DPI

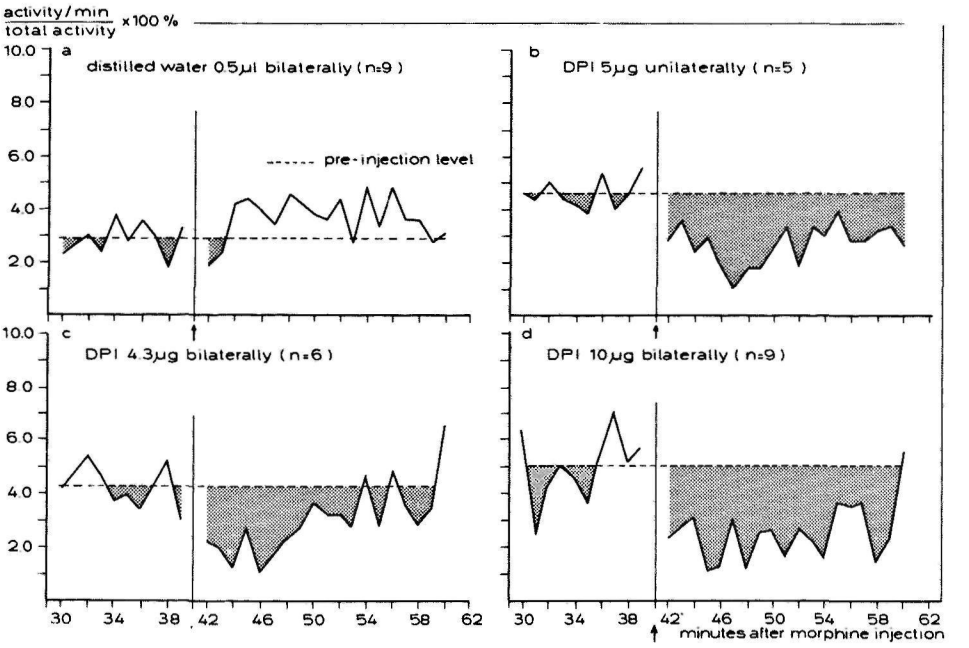

Fig. 4.7 Time-dependent development of drug-induced changes in frequencies of morphine-induced locomotor patterns elicited by intraseptal administration of: a) distilled water (0.5 µl, bilaterally, n=9); b) DPI (5 µg, unilaterally, n=5); c) DPI (4.3 µg, bilaterally, n=6); d) DPI (10 µg, bilaterally, n=9) 40-41 min after morphine injection. The number of locomotor patterns scored per animal per min is expressed as percentage of the total number of locomotor patterns scored for each individual animal during 10 min immediately preceding and 19 min immediately following the local injection. Presented are mean values of these percentages averaged over the whole test group (with exception of animals showing less than 10 locomotor patterns during the pre-injection period). This method takes into account the inter-individual differences in locomotor activity.

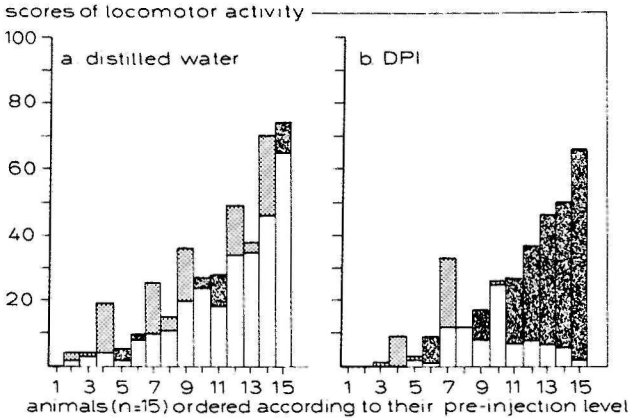

Fig. 4.8 Behavioural effects elicited by intraseptal administration of: a) distilled water (0.5 µl, bilaterally, n=15); b) DPI (10 µg, bilaterally, n=15) on morphine-induced locomotor activity. Animals were ranked in order of increasing scores of locomotor activity displayed during the pre-injection period of 10 min. These pre-injection scores per animal are represented in the figure as successive columns of increasing height. For each individual animal the scores for locomotor activity displayed during the post-injection period of 10 min are also given in the appropriate columns. Drug-induced differences between post- and pre-injection levels are illustrated as follows: ▨ increase, ■ decrease. Note the dependence of the DPI-induced effect on the pre-injection level.

had a shorter duration than the effect caused by 2 x 10 µg DPI (cf. Figs. 4.7c and 4.7d).

From a qualitative point of view, the behaviour following the DPI injection was not different from that before the injection: the movements themselves remained staccato and poorly co-ordinated, and the behavioural patterns were still stereotyped. In some animals, however, DPI produced a remarkable effect, i.e. the cats adopted a posture quite similar to the species-specific genital grooming posture for shorter or longer periods during which they did not start to groom. Occasionally,

this posture changed into lying, or almost lying, with extended forelegs. These particular postures were observed for a mean period of about 80 sec in 40% of the animals receiving the lowest dose of DPI (unilaterally 5 µg) and for a mean period of about 200 sec in 90% of the animals receiving the highest dose of DPI (bilaterally, 10 µg per side).

A number of animals in which DPI turned out to be effective were also given intraseptal dopamine (bilaterally, 10 µg per side, n = 8) or the α-NA agonist oxymetazoline (bilaterally, 10 µg per side, n = 7): dopamine, but not oxymetazoline, mimicked the DPI-induced effect (Table 4.6: Δ_{rel}).

TABLE 4.6

Behavioural effects elicited by drugs applied intraseptally 40-41 min after systemic morphine in DPI-sensitive animals. Frequencies of stereotyped locomotor patterns are used as dependent variables

drug treatment[a,b]		n	Δ_{abs} (mean±SEM)	n[c]	Δ_{rel} (%) (mean±SEM)
distilled water	2 x 0.5 µl	15	6 ± 3[f]	9	13 ± 8[f]
dopamine	2 x 10.0 µg	8	-21 ± 10[e]	8	-9 ± 4[e]
oxymetazoline	2 x 10.0 µg	7	13 ± 5[ns]	7	9 ± 4[ns]
DPI	1 x 10.0 µg	10	-39 ± 10[f]	10	-36 ± 10[f]
DPI ergometrine[d]	1 x 10.0 µg 1 x 10.0 µg	10	-18 ± 7[f,g]	10	-19 ± 6[f,g]

For each drug treatment mean values ± S.E.M. averaged over the whole test group are given for both Δ_{abs} (defined as $X_{post} - X_{pre}$) and Δ_{rel} (defined as $(X_{post}-X_{pre})/(X_{post}+X_{pre}) \times 100\%$); for more details see section Behavioural analysis in morphine-pretreated animals.

a = all drugs were administered in a volume of 0.5 µl.
b = the agents were given bilaterally (2x) apart from DPI and ergometrine, which were injected unilaterally (1x).
c = only animals with X_{pre} (number of locomotor patterns displayed during the pre-injection period) ≥ 10.
d = given 10 min prior to DPI
e = $p < 0.05$, ns = not significant, f = not tested (drugs vs. distilled water), g = $p < 0.05$ (DPI vs. DPI/ergometrine).

The DAi antagonist ergometrine (bilaterally, 10 µg per side, n = 10) alone did not modify the behavioural effects of morphine when administered intraseptally. However, intraseptal injection of ergometrine (unilaterally, 10 µg, n = 10) did antagonize the effect of DPI (unilaterally, 10 µg, n = 10): animals selected on basis of their apparent DPI effectiveness and treated unilaterally with both 10 µg DPI and 10 µg ergometrine, given 10 min earlier, showed a significantly reduced DPI effect (Table 4.6: Δ_{rel}).

Alteration of α-NA activity within the septal nuclei of morphine-pretreated cats

The intraseptal injection of the α-NA agonists oxymetazoline (bilaterally, 10 µg per side, n = 8) and noradrenaline (bilaterally, 10 µg per side, n = 9) did not affect the stereotyped locomotor patterns (Table 4.6). After bilateral injection of the α-NA antagonist phentolamine (bilaterally, 10 µg per side, n = 19) there was a slight, but significant, enhancement of locomotor activity (Table 4.6: Δ_{abs}). This enhancement was not present in animals receiving a combined solution of phentolamine and oxymetazoline (n = 8; Table 4.6: Δ_{abs}).

Alteration of DAe, DAi and α-NA activity within the septal nuclei of non-morphinized cats

The drugs affecting morphine-induced behaviour were also tested in non-pretreated animals. Neither haloperidol (bilaterally, 10 µg per side, n = 5) nor phentolamine (bilaterally, 10 µg per side, n = 5) was effective. Intraseptal injection of DPI (bilaterally, 10 µg per side, n = 13; unilaterally, 10 µg, n = 10; bilaterally, 4.3 µg per side, n = 8; unilaterally, 5 µg, n = 12), however, elicited one or more of the following elimination effects, viz. vomiting, urination and defecation (Table 4.7). Defecation was always followed by vomiting.

Similar effects were also observed after intraseptal injection of the α-NA agonist oxymetazoline (bilaterally, 10 µg per side, n = 8) in a small number of cats: vomiting after a latency of 13-16 min (n = 2) and defecation after a latency of 20-60 min (n = 2). Animals displaying DPI-induced vomiting (n = 3), defecation (n = 1) and urination (n = 3) did not show these effects when pretreated with the α-NA antagonist yohimbine (0.5 mg/kg, i.p.) given 30 min prior to DPI (bilaterally, 10 µg per side).

TABLE 4.7

Characterization of the DPI-induced elimination effects elicited in non-morphinized cats

	DPI 10 µg bil. n=13	DPI 10 µg unil. n=10	DPI 4.3 µg bil. n=8	DPI 5 µg unil. n=12
Vomiting				
number of effective animals	5 (38%)	5 (50%)	0 (0%)	1 (8%)
latency (mean ± SEM, min)	7.4±0.5	9.0±1.3	-	7
latency (range, min)	6-9	6-12	-	7
Defecation				
number of effective animals	2 (15%)	0 (0%)	0 (0%)	0 (0%)
latency (mean ± SEM, min)	4	-	-	-
latency (range, min)	4	-	-	-
Urination				
number of effective animals	2 (15%)	1 (10%)	0 (0%)	1 (8%)
latency (mean ± SEM, min)	7.5±3.5	12	-	7
latency (range, min)	4-11	12	-	7

Finally, none of the 44 morphine-treated cats receiving DPI displayed elimination effects with the exception of 2 animals showing defecation after injection of the highest dose of DPI (bilaterally, 10 µg per side) with latencies of 4 and 20 min.

Relation between the effect of DPI upon elimination and that upon the morphine-induced behaviour

No correlation was found between the occurrence of both DPI-induced effects as indicated by 2 sets of data: 1) data derived from animals receiving a single dose of DPI with and without morphine pretreatment (n = 34). During the 10 min period immediately after DPI injection, 17 out of the 34 animals when pretreated with morphine had frequencies of locomotor patterns significantly lower than those present during the

10 min pre-injection period ($p < 0.05$, two-tailed Mann-Whitney U-test). Only 5 out of these 17 animals showed elimination when only DPI treatment was given. Such a single DPI treatment given to cats in which DPI was unable to affect morphine-induced behaviour (n = 17) produced elimination in 4 animals. In other words, the occurrence of elimination in animals that showed the DPI effect on morphine-induced behaviour (viz. in 29% of the animals) was not different from that in cats not showing the DPI effect on morphine-induced behaviour (viz. in 24% of the animals).
2) data derived from the animals showing a significant DPI effect upon morphine-induced behaviour (n = 17). As mentioned above, only 5 animals showed elimination. These 5 animals showed a DPI-induced decrease in the morphine-induced locomotor activity ($\Delta_{rel} = -43 \pm 9\%$) that was not significantly different from that elicited by DPI in the remaining 12 animals ($\Delta_{rel} = -51 \pm 8\%$).

Localization of the injection sites

All injection sites were localized within the septal nuclei; the majority of these sites were concentrated in the anterior part of the septal nuclei (i.e. stereotaxic co-ordinates A15-A19; cf. Snider and Niemer, 1964). It might be useful to note that all DPI sites effective in attenuating locomotor activity in morphine-treated animals were, in addition, restricted to the nucleus septalis medialis pars anterior $\pm$ 1 mm (cf. Andy and Stephan, 1964).

4.3.5 Discussion

The intraseptal injection of DPI suppressed locomotor activity in morphine-pretreated cats. This effect of DPI was mimicked by that of intraseptal injection of dopamine and antagonized by intraseptal injection of ergometrine; neither apomorphine nor haloperidol in a low dosage was effective in this respect. These observations suggest that the septal nuclei contain DA receptors with pharmacological properties identical to those of the so-called inhibition-mediating dopamine (DAi) receptor (Cools and Van Rossum, 1976, 1980). To substantiate this statement the specificity of the effects elicited by the various agents has to be discussed.

The small injection volume (0.5 µl) together with the rather short latency for the DPI effect (ca. 1 min), even after unilateral injections into medially localized sites within the septal nuclei, argues for in-

volvement of septal receptors in the mediation of the DPI effect on stereotyped behaviour. This argument is strenghtened by the fact that the effective sites are found within a restricted area of the septal nuclei, viz. the nucleus septalis medialis pars anterior. It is when injected in this part of the septal nuclei that cholinergic and opiate agents likewise affected the behaviour of morphine-pretreated cats (Megens and Cools, 1979 and unpublished data). As there were no animals with both the left and right cannula implanted into purely medial or lateral structures, no definite conclusion could be drawn in this respect. To emphasize the locus specificity of the DPI-induced effect on morphine-induced behaviour it is worth mentioning that injections of DPI into regions adjacent to the septum, i.e. neostriatal structures potentiate but do not inhibit the morphine-induced behaviour (Cools et al., 1978).

The present results attest to the specific dopaminergic character of the DPI-induced suppression. The effect elicited by the DAi agonist was mimicked by dopamine, but not by α-NA agents, and antagonized by the DAi antagonist ergometrine. Although the dopamine-induced decrease was relatively small (Δ_{rel} = -8±4% (DA) vs. Δ_{rel} = -36±10% (DPI)), the effectiveness of higher doses was not assessed as increasing doses would have resulted in a higher spread of the injected drug (cf. Bondareff et al., 1970) and, accordingly, would have affected septal structures as well as regions adjacent to them. Anyhow, taken together, all the data clearly indicate that the DPI effect has a dopaminergic character. This dopaminergic character of the DPI effect is consistent with the high affinity of DPI for limbic dopamine receptors (cf. Blackburn et al., 1978). In this context, it is worth mentioning that the DPI effect mimicked to a certain degree the effect elicited by manipulation of the mesencephalic A9-A10 area (the origin of dopaminergic afferents to the septal nuclei) of morphine-treated cats. On the other hand, the DPI effect differed greatly from that elicited by manipulation of the locus coeruleus region (the origin of noradrenergic afferents to the septal nuclei) of morphine-treated cats (Van Dongen et al., 1979). Again, both sets of data emphasize the dopaminergic rather than the noradrenergic nature of the DPI-induced effect.

The DPI-induced elimination effects seem to have been mediated via a different neuronal system. First, the latency for the DPI effect on

morphine-induced behaviour ranged from 0 to 4 min in contrast to that for the elimination effects, which varied from 4 to 12 min. Secondly, the DPI effect on morphine-induced behaviour had a dopaminergic character (see above), whereas the elimination effects were most probably mediated via NA receptors (see below). And, finally, no correlation was found between the occurrence of both effects.

As mentioned, intraseptal injection of the DAi antagonist ergometrine antagonized the DPI effect on morphine-induced behaviour. This effect was not due to a simple summation of DPI-induced and ergometrine-induced behavioural changes since ergometrine injection *per se* did not affect the performance of stereotyped behaviour. Accordingly, the conclusion seems justified that the DPI antagonizing effect of ergometrine was specific. Whether or not the absence of an effect of ergometrine alone on morphine-induced behaviour implies a minimal firing rate of the system affected at the time of ergometrine injection (ca. 40 min after morphine administration) remains open for discussion.

The ability of a relatively high dose of haloperidol to potentiate the morphine-induced locomotor activity can be ascribed to its potency to antagonize α-NA receptors (Cools, 1978a; Peroutka et al., 1977). First, the septal nuclei contain α-NA receptors (Brownstein et al., 1974b; Lindvall and Stenevi, 1978; Moore, 1978; Segal, 1974; U'Prichard et al., 1980). Secondly, the α-NA antagonist phentolamine produced an effect that could be counteracted by the α-NA agonist oxymetazoline. And, las but not least, haloperidol mimicked phentolamine when applied intraseptally. This non-dopaminergic character of the effects elicited by a relatively high dose of haloperidol together with the ineffectiveness of the DAe agonist apomorphine excludes the involvement of the so-called DAe receptors in the dopaminergic DPI effect described above. Apart from this, the latter data show that not only dopamine receptors, but also α-NA receptors, are functionally active within the septal nuclei.

In the present study the postulated existence of the DAi type of receptor within the septal nuclei is based purely on pharmacological evidence. Considering the electrophysiological properties of DAi receptors found in the snail *Helix aspersa* (Struyker Boudier, 1975), one might expect that the above-mentioned DA receptors within the septal nuclei are also inhibitory at the electrophysiological level. Still, it has been reported that dopaminergic, septal afferents arising from the mes-

encephalic A10 region are excitatory in nature (Assaf and Miller, 1977). As the excitatory responses could be inhibited by haloperidol, these electrophysiological responses are apparently mediated by a type of DA receptors different from the receptors found in the present study.

As mentioned in the section 4.3.4, intraseptally applied DPI also produced vomiting, urination and defecation. In this case, the involvement of DAi receptors can be excluded on the basis of the following arguments. First, the DPI-induced vomiting, defecation and urination did not occur following pretreatment with the systemically administered α-NA antagonist yohimbine. This together with the fact that the intraseptally applied α-NA agonist oxymetazoline also elicited vomiting and defecation indicates that α-NA rather than DAi receptors appear to be involved in the DPI-induced autonomic responses; indeed, DPI has some α-NA agonistic activity (Bevan et al., 1979; Struyker Boudier et al., 1975). Secondly, the DPI-induced suppression of locomotor activity in morphine-treated cats appeared nearly immediately following the intraseptal injection, whereas the DPI-induced vomiting, urination and defecation required a latency of several minutes. And, finally, there was no relationship between the DPI-induced effect upon locomotor activity on the one hand and that upon vomiting, urination and defecation on the other.

In conclusion, the septal nuclei contain a particular subclass of DA receptors marked by pharmacological properties identical to those of the so-called DAi receptors, which are known to be present in structures such as the nucleus accumbens that are innervated by the dopaminergic, mesolimbic tract (Cools and Van Rossum, 1976, 1980).

As a final remark, the present results reinforce the earlier reported evidence for an important role of the septal nuclei in mediating the behavioural responses of cats to morphine (Megens and Cools, 1979); as demonstrated, stimulation of septal DA receptors attenuated the performance of stereotyped, morphine-induced behaviour. As it has been reported that stimulation of septal, cholinergic receptors potentiated stereotyped behaviour (Megens and Cools, 1979), it is evident that the septal nuclei contain dopaminergic and cholinergic systems that mediate mutually opposite effects.

4.4 INTRASEPTALLY INJECTED OPIATE AGENTS: EFFECTS ON MORPHINE-INDUCED BEHAVIOUR OF CATS

4.4.1 Summary

Behavioural effects of intraseptally administered (i.s.) opiate agents were analyzed in cats pretreated with intraperitoneally given (i.p.) morphine to characterize septal opiate receptors, and to investigate their involvement in the behavioural effects of morphine. The intraseptal injections affected only the frequencies of behaviour patterns; the nature of the behaviour patterns remained unchanged. The following results were obtained by injections (i.s.) 15-16 min after morphine (i.p.): 1) naloxone decreased frequencies of head and limb movements, 2) morphine was ineffective. The following results were obtained by injections (i.s.) 40-41 min after morphine (i.p.): 1) β-endorphin and, to a lesser extent, fentanyl increased frequencies of locomotor patterns, 2) morphine and Met-enkephalin were ineffective, 3) naloxone and naltrexone decreased frequencies of locomotor patterns in a dose-dependent way, 4) naloxone and naltrexone antagonized the effects of β-endorphin and fentanyl, 5) morphine did not attenuate the effect of naloxone. Injections (i.s.) of morphine and naloxone in non-morphinized animals were ineffective. It is concluded that 1) morphine (i.p.) does not affect behaviour via a direct action on septal opiate receptors, and 2) the receptors mediating the effects mentioned are most probably ε-type opiate receptors. The hypothesis is put forward that morphine (i.p.) increases the release of β-endorphin from hypothalamic-septal neurons.

4.4.2 Introduction

Since opiate receptors and neurons containing opioid peptides are distributed over various regions of the central nervous system (for reviews see: Adler, 1980; Miller and Cuatrecasas, 1978), the broad range of distinct effects, elicited by morphine and related opiate agonists might result from discrete actions of the opiates at specific regions of the central nervous system. The septal nuclei receive afferents, originating from the hypothalamus, that secrete the opioid peptide β-endorphin (Bloom et al., 1978; Rossier et al., 1977; Watson et al., 1977b). In addition, high densities of opiate receptors and

opioid peptides are present within the septal nuclei themselves (Bloom et al., 1978; Hong et al., 1977; Law et al., 1979; Rossier et al., 1977; Simantov et al., 1977), and opiate agents are functionally active in this brain region (Botticelli and Wurtman, 1981; Moroni et al., 1977, 1978a). Consequently, some of the opiate-induced effects may be mediated via septal opiate receptors. Septal opiate receptors are not involved in the analgesic effect of opiates: although septal lesions have been reported to antagonize morphine-induced analgesia (Castellano et al., 1975; Oliverio, 1974), intraseptal injections of morphine or β-endorphin do not evoke analgesia (Herz et al., 1970; Herz and Teschemacher, 1971; Moroni et al., 1977, 1978a). On the other hand, there is indirect evidence that septal opiate receptors are involved in the behavioural effects of opiates: 1) the septal nuclei have been implicated in a wide variety of behaviours (for reviews: Fried, 1972; Grossman, 1976), and 2) septal neuronal systems play an important role in the behavioural response of cats to morphine administration (Megens and Cools, 1979, 1981; Robinson and Wang, 1979). In the present study, an investigation was carried out into whether septal opiate receptors are indeed a site of action for opiates to produce their behavioural effects. Therefore, an analysis was carried out on the effects of intraseptally injected opiate agents on the behavioural phenomena elicited by systemic administration of morphine in cats.

The existence of multiple opiate receptors has recently been reported (Chang et al., 1980; Kachur et al., 1980; Lord et al., 1977; Martin et al., 1976; Wüster et al., 1979). In order to characterize septal opiate receptors in the present study, we determined the relative effectiveness of intraseptally injected opiate agonists (morphine, fentanyl, Met-enkephalin and β-endorphin) that have differential potencies on 3 distinct types of opiate receptors (Chang et al., 1979a, b; Law et al., 1979; Wüster et al., 1979): 1) the opiate alkaloid receptor or μ-receptor (fentanyl > β-endorphin > morphine > Met-enkephalin), 2) the enkephalin receptor or δ-receptor (Met-enkephalin > fentanyl > β-endorphin > morphine) and 3) the endorphin receptor or ε-receptor (β-endorphin > fentanyl >> Met-enkephalin, morphine). The opiate antagonists used, naloxone and naltrexone, have affinity for all 3 types of opiate receptors, although somewhat more for the μ-receptor (Chang et al., 1979a, b; Lemaire et al., 1978; Schulz et al., 1979).

The behavioural effects elicited by systemically administered morphine in cats have been extensively described elsewhere (Cools et al., 1974, 1977, 1978). Administration of a moderate dose of morphine (5 mg/kg, i.p.) causes the development of 3 behavioural phases which appear in succession: depression, re-organization and ritualization. Depression is characterized by hypoactivity, re-organization by increasing activity, and ritualization by stabilized hyperactivity. Furthermore, during re-organization the movements are staccato-like and uncoordinated; during ritualization stereotyped behaviour patterns, which develop during the re-organization, are regularly repeated. In the present study, the effects of intraseptally injected opiate agents were investigated both during the re-organization and ritualization phases in cats pretreated with morphine (5 mg/kg, i.p.).

4.4.3 Materials and methods

For a detailed description of the experimental conditions and procedures the reader is referred to a previous report (Megens and Cools, 1981); a general outline of the procedure is given below.

Stereotaxic operations were carried out in adult cats (2.0-4.0 kg) under pentobarbital anaesthesia (Nembutal^{R} or Narcovet^{R}, 50 mg/kg, i.p.): cannulas aimed at the septal nuclei were implanted bilaterally (Cools and Van Rossum, 1970; Megens and Cools, 1981). Intracerebral injections, either bilateral (2x) or unilateral (1x), were carried out manually with Hamilton syringes. The following agents were used: morphine hydrochloride (De Onderlinge Pharmaceutische Groothandel), fentanyl citrate (gift from Janssen Pharmaceutica), human β-endorphin and Met-enkephalin (gifts from Organon), naloxone hydrochloride and naltrexone hydrochloride (gifts from Endo Laboratories). The intracerebrally administered drugs were dissolved in distilled water (with the exception of the opioid peptides, which were dissolved in 0.01 n HCl); the intraperitoneally injected morphine was dissolved in saline (0.9% NaCl). All doses refer to the salts.

All the intraseptally applied drugs were tested in cats pretreated with intraperitoneal morphine (5 mg/kg) either during the re-organization phase (15-16 min after i.p. morphine) or during the ritualization phase (40-41 min after i.p. morphine). Morphine and naloxone were also tested in non-pretreated cats. When animals were tested more than once,

an intertrial interval of at least 2 weeks was used. Using this time-interval, no signs of tolerance or abstinence were seen, apart from increasing salivation.

After the experiments, the animals were sacrificed with an overdose of pentobarbital (Nembutal^{R} or Narcovet^{R}) and perfused intracardially with a formaldehyde solution (4-10%) containing the anti-coagulant heparin (50 mg/l). The brains were removed and sectioned along the tracks of the guide cannulae. The injection sites were localized with reference to Snider and Niemer's atlas (1964). The various parts of the septal nuclei in which the injection sites were localized were subdivided and named according to Andy and Stephan (1964).

Behavioural analysis 15-16 min after intraperitoneal morphine

The animals received an intraperitoneal injection of morphine (5 mg/kg) and were observed for a period of 60 min immediately following the morphine injection. Drugs were injected intraseptally 15-16 min after i.p. morphine. Drug-induced changes were measured both qualitatively (changes in the nature of stereotyped behaviour patterns) and quantitatively (changes in frequencies of head and limb movements). For analysis of the quantitative effects, the period of 44 min immediately following the intraseptal injections was subdivided into 11 periods of 4 min each. For each of these 4 min-periods, the scores of head and limb movements per animal were determined and expressed as percentages of the total number of head and limb movements displayed during the whole post-injection period of 44 min. In this way, the ongoing development of the morphine syndrome following the intraseptal injections was represented independently of inter-individual differences in activity. For statistical analysis the percentages determined for all animals in each test group were compared per 4 min-period with the corresponding percentages measured in control animals treated with distilled water (2-tailed Mann-Whitney U-test).

Behavioural analysis 40-41 min after intraperitoneal morphine

The animals received an intraperitoneal injection of morphine (5 mg/kg) and were observed for a period of 60 min immediately following the morphine injection. Drugs were injected intraseptally 40-41 min after i.p. morphine. Drug-induced changes were measured both qualitatively (changes in the nature of stereotyped behaviour patterns) and quantitatively

(changes in frequencies of locomotor patterns). The definition of locomotor patterns is given in a previous report (Megens and Cools, 1981). Both the total score of locomotor patterns over the 10 min immediately preceding the intraseptal injections (X_{pre}) and the total score of locomotor patterns over the 10 min immediately following the intraseptal injections (X_{post}) were determined per animal. Behaviour was not analyzed during the 2 min injection period. Further analysis of the effects was performed in a different way for the opiate agonists and antagonists, for reasons to be mentioned below.

Opiate agonists. The absolute difference in the number of locomotor patterns scored during the pre- and post-injection period per animal was determined by:

$$\Delta_{abs} = X_{post} - X_{pre}$$

This variable was calculated for all the animals. The values obtained in this way per test group were compared with the corresponding values determined for the appropriate control group (Mann-Whitney U-test).

Opiate antagonists. As might be expected, injection of opiate antagonists resulted in decreased frequencies of the morphine-induced locomotor patterns. Therefore, statistical analysis of these effects had to be performed differently from the procedure outlined above for opiate agonists. First, decreases in locomotor activity can only be determined in animals showing at least some locomotor activity during the pre-injection period. Hence, only animals with a minimum number of 10 locomotor patterns were used to analyze the behavioural effects elicited by the opiate antagonists. Furthermore, the magnitude of decreases in locomotor activity is dependent on the pre-injection X_{pre} values, which differed considerably between animals. Accordingly, the relative change in the number of locomotor patterns was determined per animal, i.e. the difference between pre- and post-injection values expressed as a percentage of the summated pre- and post-injection values:

$$\Delta_{rel} = \frac{X_{post} - X_{pre}}{X_{post} + X_{pre}} \times 100\% \qquad (X_{pre} > 10)$$

The values thus obtained per test group were compared with the corresponding values for the appropriate control group (Mann-Whitney U-test).

The difference should be noted between this definition of a relative change and the more conventional definition of a relative change, $\frac{X_{post} - X_{pre}}{X_{pre}} \times 100\%$.

The time-pattern of the behavioural effects elicited by the 2 opiate antagonists naloxone and naltrexone on locomotor activity differed considerably from each other. To show this difference, the time-dependent development of these effects is illustrated in Fig. 4.10. Because of inter-individual differences in locomotor activity, a particular procedure was followed to realize this figure:

1. Use was made of frequencies of locomotor patterns scored per min during the 10 min immediately preceding the intraseptal injection and during the 19 min immediately following the intraseptal injection.
2. For each individual animal these frequencies were normalized, i.e. the scores per min were divided by the total score over the 10 min preceding and the 19 min following the intraseptal injection.
3. Next, the values obtained in this manner were averaged over the test group.
4. The post-injection values expressed as percentages relative to the pre-injection level are presented.

Behavioural analysis in non-morphinized animals

After a habituation period of a quarter of an hour the inner cannulae were unscrewed and a 10 min observation period started. The injections were given immediately after this reference period and the animals subsequently observed for a period of 20 min. Since these experiments served as controls for drug effects in morphine-pretreated cats, particular attention was paid to the elements used in the analysis of morphine-induced behaviour.

4.4.4 Results

Intraseptal injections 15-16 min after intraperitoneal morphine

Intraseptal injections of naloxone (2 x 5 µg; 2 x 15 nmol; n = 8) caused a decrease in the frequencies of head and limb movements, when compared with control injections of distilled water (2 x 0.5 µl; n = 7) as indicated in Fig. 4.9. The remaining movements were still staccato-like and poorly co-ordinated. Naloxone did not affect the nature of the stereotyped behaviour patterns. The decrease in frequencies of head and limb movements reached its maximum level about 4 min after the injections.

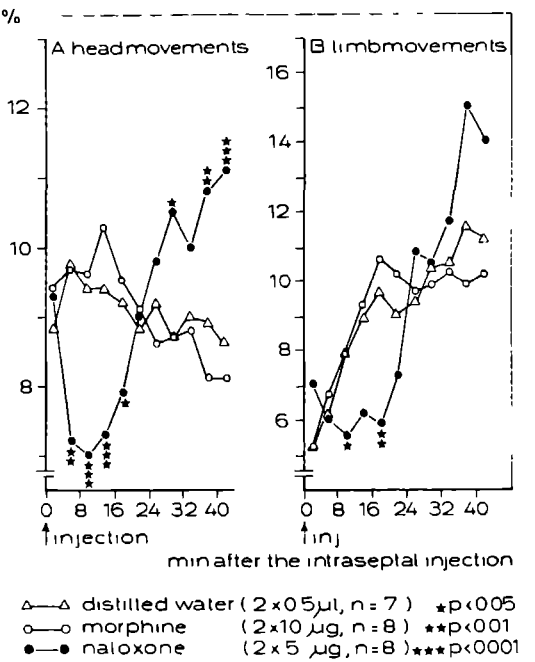

Fig. 4.9 Behavioural effects caused by intraseptal administration of distilled water, morphine, and naloxone 15-16 min after i.p. administration of morphine. The number of head and limb movements scored per animal per 4 min-period is expressed as a percentage of the total number of movements scored for each individual animal during the whole observation period of 44 min immediately following the intraseptal injection. Presented are mean values averaged over each test group. The values obtained for each test group were compared per 4 min-period with those obtained for the control animals (2-tailed Mann-Whitney U-test). For more details see section Behavioural analysis.

About 16-20 min after the administration of naloxone the frequencies of head and limb movements increased again whereas these frequencies in the control animals had already reached their maximum level at this time (Fig. 4.9). Intraseptal injections of morphine (2 x 10 µg; 2 x 31 nmol; n = 8) did not affect the behaviour: the frequencies of head and limb movements after the intraseptal injections were similar to those of the controls (Fig. 4.9).

Intraseptal injections 40-41 min after intraperitoneal morphine

Opiate agonists. Intraseptal injections of the opioid peptide β-endorphin (2 x 10 µg; 2 x 3 nmol; n = 14) caused an increase in the frequencies of locomotor patterns when compared with control injections of distilled water (2 x 0.5 µl; n = 15) as indicated in Table 4.8. β-Endorphin did not affect the nature of the stereotyped behaviour patterns: patterns already present before the injections remained present after the injections. The overall effect reached its maximum level about 3 min after the injections and was still present 16 min later. The β-endorphin-induced increase was suppressed by intraseptal injections of naltrexone (2 x 5 µg; 2 x 15 nmol; n = 8) given immediately before the β-endorphin injections (Table 4.8). In an additional experiment, in which 4 animals received the naltrexone/β-endorphin combination 24 hours after β-endorphin alone, a similar result was obtained (Table 4.8). It may be worth mentioning, however, that these 4 animals showed a remarkably reduced sensitivity for the second intraperitoneal administration of morphine, i.e. the values for the number of locomotor patterns displayed during the 10 min before the intraseptal injections (X_{pre}) were only 24±14% (mean ± S.E.M.) of those obtained in the first trial.

Intraseptal injections of fentanyl (2 x 5 µg; 2 x 9 nmol; n = 10) also caused a statistically significant increase in the frequencies of locomotor patterns (Table 4.8). Although the dose of fentanyl was higher than that of β-endorphin, the magnitude of the fentanyl effect was smaller than that of β-endorphin. Fentanyl did not affect the nature of the stereotyped behaviour patterns. The fentanyl effect appeared immediately after the injections and gradually decreased in the course of the 19 min following the intraseptal injections. The fentanyl effect was suppressed by simultaneous intraseptal injection of naloxone (2 x 5 µg; 2 x 15 nmol; n = 8).

Neither intraseptal injections of morphine itself (2 x 10 µg; 2 x 31 nmol; n = 11) nor intraseptal injections of the opioid peptide Met-enke-

phalin (2 x 10 µg; 2 x 17 nmol; n = 10) did affect the behaviour; the drug-induced changes in the frequencies of locomotor patterns were similar to those following control injections (Table 4.8).

TABLE 4.8

Behavioural effects elicited by intraseptal administration of opiate agonists 40-41 min after intraperitoneal morphine. Frequencies of locomotor patterns are used as dependent variables

	drug treatment		n	Δ_{abs} (mean±SEM)
1	distilled water	2 x 0.5 µl	15	6 ± 3[c]
2	morphine	2 x 10 µg	11	14 ± 7[ns]
3	fentanyl	2 x 5 µg	10	21 ± 7[a]
4	Met-enkephalin	2 x 10 µg	10	21 ± 12[ns]
5	β-endorphin	2 x 10 µg	14	31 ± 8[b]
6	fentanyl naloxone	2 x 5 µg 2 x 5 µg	8	-1 ± 1[ns,d]
7	β-endorphin naltrexone	2 x 10 µg 2 x 5 µg	8	11 ± 7[ns,e]
8	β-endorphin naltrexone	2 x 10 µg 2 x 5 µg [f]	4	14 ± 17[ns]

Mean values ± S.E.M. averaged over the whole testgroup for Δ_{abs} (defined as $X_{post} - X_{pre}$) are given for each drug treatment; for details see section Behavioural analysis.

a = p < 0.05
b = p < 0.02
ns = not significant
c = not tested

(drug vs. distilled water, 2-tailed Mann-Whitney U-test)

d = p < 0.01 (fentanyl/naloxone vs. fentanyl, 1-tailed Mann-Whitney U-test)

e = p < 0.05 (β-endorphin/naltrexone vs. β-endorphin, 1-tailed Mann-Whitney U-test)

f = same drug treatment as for group 7 with the difference that these animals were tested with an intertrial interval of 24 hours instead of the usual interval of 14 days

Opiate antagonists. Intraseptal injections of naloxone (2 x 5 µg; 2 x 15 nmol; n = 5) produced a decrease in the frequencies of locomotor patterns when compared with control injections of distilled water (2 x 0.5 µl; n = 9) as indicated in Table 4.9. The remaining behaviour was still stereotyped. Intraseptal injections of naloxone did not affect the nature of the stereotyped behaviour patterns. The overall effect

TABLE 4.9

Behavioural effects elicited by intraseptal administration of opiate antagonists 40-41 min after intraperitoneal morphine. Frequencies of locomotor patterns are used as dependent variables

drug treatment			n	Δ_{rel} (%) (mean±SEM)
1	distilled water	2 x 0.5 µl	9	13 ± 8[c]
2	naloxone	1 x 5 µg	9	-1 ± 9[ns]
3	naloxone	2 x 5 µg	5	-38 ± 15[b,d]
4	naltrexone	2 x 1 µg	5	-15 ± 13[ns]
5	naltrexone	2 x 5 µg	8	-29 ± 10[b,e]
6	naloxone morphine	2 x 5 µg 2 x 10 µg	7	-16 ± 8[a,f]

Mean values ± S.E.M. averaged over the whole test group for Δ_{rel} *(defined as* $(X_{post}-X_{pre})/(X_{post}+X_{pre})$ *x 100%) are given for each drug treatment; for details see section Behavioural Analysis.*

a = p < 0.05
b = p < 0.02
ns = not significant
c = not tested
(drug vs. distilled water, 2-tailed Mann-Whitney U-test)

d = not significant (unilateral naloxone vs. bilateral naloxone, 1-tailed Mann-Whitney U-test)

e = not significant (5 µg naltrexone vs. 1 µg naltrexone, 1-tailed Mann-Whitney U-test)

f = not significant (naloxone/morphine vs. naloxone, 1-tailed Mann-Whitney U-test)

of naloxone reached its maximum level (about 73% inhibition relative to the pre-injection level) about 3 min following the intraseptal injections and was still present 16 min later (Fig. 4.10A). Unilateral injections of naloxone (1 x 5 µg; 1 x 15 nmol; n = 9) elicited a less pronounced effect being just not significantly different from that following the control injections (Table 4.9; Fig. 4.10A). Intraseptal injections of morphine (2 x 10 µg; 2 x 31 nmol; n = 7) did not significantly affect the naloxone-induced decrease when administered immediately before naloxone (2 x 5 µg; 2 x 15 nmol) (Table 4.9).

Intraseptal injections of naltrexone (2 x 5 µg; 2 x 15 nmol; n = 8) caused an effect similar to that of naloxone, viz. a decrease in the frequencies of locomotor patterns (Table 4.9). The drug affected the behaviour of the majority of the animals within 3 min; the effect reached its maximum level (about 84% inhibition relative to the pre-injection level) about 11 min following the injections (Fig. 4.10B). A lower dose of naltrexone (2 x 1 µg; 2 x 3 nmol; n = 5) caused a smaller decrease, which also reached its maximum level (about 67% inhibition relative to the pre-injection level) about 11 min following the injections (Fig. 4.10B).

Intraseptal injections in non-morphinized cats

Neither intraseptal injections of morphine (2 x 10 µg; 2 x 31 nmol; n = 6) nor intraseptal injections of naloxone (2 x 5 µg; 2 x 15 nmol; n = 5) affected the behaviour of non-morphinized cats.

Localization of the injection sites

All injection sites were localized within the anterior part of the septal nuclei (i.e. between the stereotaxic co-ordinates A 15.0 and A 18.5; cf. Snider and Niemer (1964)). Table 4.10 shows in which parts of the septal nuclei the injection sites for the effective agents, i.e. for naloxone, naltrexone, fentanyl and β-endorphin, were localized: 66% of the injection sites were localized within the nucleus septalis medialis pars anterior (MA), and a further 26% with 1 mm of it. Furthermore, the ratio effective/ineffective sites was the highest for this part of the septal nuclei.

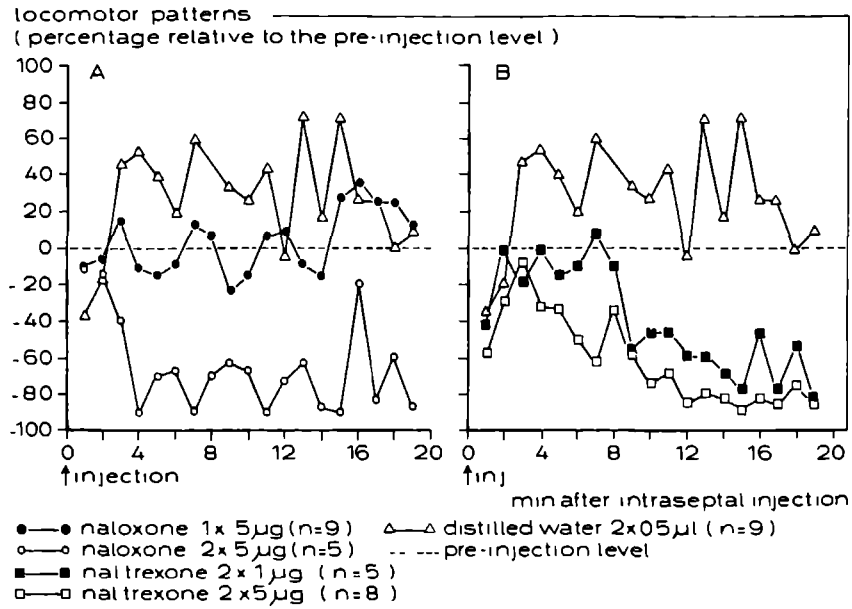

Fig. 4.10 Behavioural effects caused by intraseptal injection of distilled water, naloxone, and naltrexone at 40-41 min after intraperitoneal administration of morphine: time-course of the drug-induced effects. The scores per min of locomotor patterns were normalized for each individual animal and averaged over each test group. Presented are the mean post-injection values expressed as percentages relative to a pre-injection level of 10 min. For more details see section Behavioural Analysis.

TABLE 4.10

Distribution throughout the septal nuclei of injection sites into which naloxone, naltrexone, fentanyl or β-endorphin were injected. The ratio between the number of effective sites and ineffective sites is given per nucleus.

brain region	number of effective sites (a)	number of ineffective sites (b)	ratio (a/b)
BD	16	6	2.67
DA	2	1	2.00
DM	2	0	-
H	2	1	2.00
IC	0	1	0.00
LI	1	0	-
MA	69	12	5.75
(MA + 1 mm)	(94)	(19)	(4.95)
MP	8	2	4.00
Total	100	23	

Abbreviations:

BD = nucleus of the diagonal band of Broca pars dorsalis; DA = nucleus septalis dorsalis pars anterior; DM = nucleus septalis dorsalis pars intermedius; H = anterior continuation of the hippocampus; IC = islands of Calleja; LI = nucleus septalis lateralis pars interna; MA = nucleus septalis medialis pars anterior; MP = nucleus septalis medialis pars posterior. (Nomenclature according to Andy and Stephan (1964))

4.4.5 Discussion

Opiate agents injected into the septal nuclei can affect the behaviour of cats: behavioural effects of intraperitoneal administration of morphine are diminished by intraseptal injections of opiate antagonists (naloxone and naltrexone) and potentiated by intraseptal injections of some opiate agonists (β-endorphin and fentanyl). The behavioural effects reported here are most probably due to interactions of the drugs with opiate receptors since 1) opiate agonists and antagonists elicited mutually opposite effects, i.e. agonists increased and antagonists decreased the intensity of the behaviour, and 2) the effects elicited by the opiate agonists could be suppressed by opiate antagonists.

The effects under discussion are most probably mediated via septal structures:

1. In contrast to manipulation of structures adjacent to the septal nuclei such as the caudate nucleus, manipulation of the septal nuclei themselves only causes changes in the frequency of behavioural patterns induced by intraperitoneal morphine, without affecting the nature of the stereotyped behaviour patterns (Cools et al., 1978; Megens and Cools, 1979, 1981; Cools and Van Beek, unpublished results). Again, in the present study, only changes in the frequency of morphine-induced behaviour patterns have been found after manipulation of the septal nuclei.
2. At least the effect of β-endorphin is not mediated via diffusion into the adjacent ventricular system: in the present study, excitatory behavioural effects were seen within 3 min following intraseptal administration of β-endorphin whereas similar effects have been reported to occur after a latency of 15-30 min following intraventricular injection of a similar dose of the peptide (Feldberg and Smyth, 1977; Meglio et al., 1977).
3. The majority of the injection sites were localized within or near a restricted part of the septal nuclei, viz. the nucleus septalis medialis pars anterior. As the ratio effective/ineffective sites was highest for this part of the septal nuclei, a foremost conclusion is that, especially, receptors within this particular part, rather than septal structures adjacent to it, are essential for the display of the observed phenomena. It is just this anterior part of the septal nuclei which has been implicated earlier in the behavioural effects of morphine in rats (Kerr and Pozuelo, 1971).

A noticeable observation was that naltrexone produced its greatest effect about 11 min after intraseptal injection; the particular time-course of this effect may be related to the relatively low lipophilicity of this agent (cf. Herz and Teschemacher, 1971; Kaufman et al., 1975).

A further point of discussion is the differential effectiveness of the intraseptally injected opiate agonists. Although factors such as differences between drugs in dosage, pharmacokinetics, or localization of the injection sites cannot be excluded, a major factor appears to be the ability of the opiate agonists to interact differentially with various types of opiate receptors (see 4.4.1) as will be explained below.

The most effective opiate agonist in the present study was β-endorphin, although this agent was injected in the lowest dose (on a molar base). This argues for the involvement of the ε-type opiate receptor (cf. Schulz et al., 1979; Wüster et al., 1979, 1980). Although the presence of δ-receptors and enkephalins within the septal nuclei has been demonstrated before (Duka et al., 1981; Hong et al., 1977; Rossier et al., 1977; Sar et al., 1978; Simantov et al., 1977; Watson et al., 1977a), intraseptal injection of the δ-agonist Met-enkephalin was ineffective in the present study. Accordingly, the effects of the other opiate agents are probably not due to δ-receptors. Intraseptal injection of the μ-agonist morphine was also ineffective, although it was injected at a maximum dose (on a molar base). The effectiveness of higher doses was not assessed as increasing doses would have resulted in a higher spread of the drugs injected (cf. Bondareff et al., 1970). On the other hand, the potent μ-agonist fentanyl was effective in the present study. Although the dose used for fentanyl was 3 times as high as that for β-endorphin, the effect of fentanyl was however smaller than that of β-endorphin. Moreover, as fentanyl also has some potency on ε-type opiate receptors in contrast to morphine and Met-enkephalin, which were ineffective in the present study, these data argue for the involvement of ε-type receptors rather than μ-type receptors (cf. Wüster et al., 1979).

Accordingly, it may be concluded that the present effects are mediated via ε-like type of opiate receptors. This is corroborated by the findings of other authors: the high levels of β-endorphin in the septal nuclei (Bloom et al., 1978; Law et al., 1979; Rossier et al., 1977), the only moderate level of enkephalins (Hong et al., 1977;

Rossier et al., 1977; Sar et al., 1978; Simantov et al., 1977; Watson et al., 1977a) and the small density of μ-receptors (Atweh and Kuhar, 1977; Pert et al., 1976). In addition, it has been reported that intraseptal injection of β-endorphin is far more effective than intraseptal injection of morphine in decreasing the hippocampal turnover rate of acetylcholine (Moroni et al., 1977, 1978a).

The conclusion that the present effects are mediated via morphine-insensitive, ε-like types of opiate receptors has the following implications: 1) systemically administered morphine does not act directly on the opiate receptors involved in the present effects, and 2) the effects elicited by the intraseptally applied opiate antagonists are due to their ability to antagonize the action of endogenous β-endorphin on ε-like receptors and not to their ability to antagonize the action of systemically administered morphine on μ-receptors.

In order to explain the finding that intraseptally administered naloxone was effective only in morphinized cats but not in non-morphinized animals, the following is of relevance. Hypothalamic neurons containing both β-endorphin and adrenocorticotrophic hormone (ACTH) course to the septal nuclei (Bloom et al., 1978; Rossier et al., 1977; Watson et al., 1977b, 1978b). β-Endorphin can be released concomitantly with ACTH (Allen et al., 1978; Guillemin et al., 1977; Vale et al., 1978). Morphine administration results in an increased release of ACTH from the pituitary via an action on the hypothalamus (French et al., 1979; Guaza et al., 1979; Meites et al., 1979; Sloan, 1971). Accordingly, the hypothesis is put forward that systemic administration of morphine causes an increase in the release of β-endorphin from the hypothalamic-septal neurons, which, in turn, activates septal, ε-like opiate receptors. Indeed, it has been demonstrated that chronic morphine administration affects the level of β-endorphin within the septal nuclei (Przewlocki et al., 1979). This hypothesis offers a simple explanation of 1) the differential effectiveness of the intraseptally administered opiate agonists, and 2) the observation that intraseptally injected opiate agonists and antagonists respectively potentiate and diminish the effects of systemically administered morphine, although they do not act on morphine's receptors.

As a final remark, which already has been mentioned in section 4.4.4, intraseptal β-endorphin apparently reduced the sensitivity of the animals

to a second intraperitoneal injection of morphine 24 hours later, suggesting that septal opiate receptors of the ε-type are not devoid of a possible role in the development of tolerance (cf. Kerr and Pozuelo, 1971; Przewlocki et al., 1979).

It is concluded that 1) systemically administered morphine does not affect behaviour via a direct action on septal opiate receptors, and 2) the receptors mediating the effects of the intraseptal injections are most probably ε-type, opiate receptors. The hypothesis is put forward that systemically administered morphine causes an increase in the release of β-endorphin from hypothalamic-septal neurons.

4.5 GENERAL COMMENTS

Analysis of the effects of intraseptally injected drugs on the behaviour of morphine-treated cats was used in the present study as a method to get more insight into the pharmacological and functional properties of septal neurotransmission processes. This approach is only valid when the intracerebrally-evoked effects are mediated via septal structures. Indeed, the reported effects are most probably mediated via septal structures since:

1. Manipulation of structures adjacent to the septum, such as the caudate nucleus, causes changes in the nature of morphine-induced behaviour patterns (Cools et al., 1974; Cools and Van Beek, unpublished results; section 3.2). In contrast, the drugs injected into the septum caused only changes in the frequency and not in the nature of the morphine-induced behaviour patterns. Hence, it can be stated that the effects observed in this study are characteristic for septal structures.
2. The latencies for the intraseptally-evoked effects were, in general, rather small. These short latencies and the small injection volume (0.5 µl) argue against the possibility that the observed effects might have been mediated via a spread of the intraseptally injected drugs to extraseptal regions of the brain.
3. The injection sites were localized within a restricted part of the septum, i.e. mainly within the nucleus septalis medialis pars anterior (Fig. 4.1). Although mediation of the present effects via

more lateral parts of the septum cannot be excluded, this restricted localization of the injection sites, together with the short latencies and the small injection volume, indicates that the effects are most probably mediated via this nucleus septalis medialis pars anterior and not via caudal or ventral parts of the septum or via the lateral ventricles. In this context, it is worth mentioning that this rostral part of the septum has already previously been implicated in the behaviour effects of morphine and vasopressin in rats (Kerr and Pozuelo, 1971; Wimersma Greidanus et al., 1972).

The specificity of the effects seems established: 1) the effects are dose-dependent, 2) the effects are mimicked by those of other agents of the same drug-class, and 3) the effects are sensitive for agonist/antagonist interactions. The effects are certainly not due to the local anaesthetic properties of the drugs, since intraseptal injections of the local anaesthetic lidocaine (2 x 5 µg) did not affect the frequencies of morphine-induced locomotor patterns: $\Delta_{abs} = 10 \pm 7$ (n = 9) and $\Delta_{rel} = 14 \pm 13\%$ (n = 5) for lidocaine vs. $\Delta_{abs} = 6 \pm 3$ (n = 15) and $\Delta_{rel} = 13 \pm 8\%$ (n = 9) for distilled water (unpublished results).

The present method appeared to be suitable for the study of septal neurotransmission processes since it enabled us to get more insight into several aspects of these processes, e.g.

1. The functional activity of septal neurotransmission processes at the behaviour level

   The present study shows that septal cholinergic, dopaminergic, noradrenergic and endorphinergic processes are functionally active at the behaviour level. This study is the first report indicating a definite role for septal dopaminergic and endorphinergic systems in behaviour. Septal cholinergic processes have already previously been implicated in avoidance behaviour, anti-nociception, food- and water-intake, emotionality and urinary excretion (see section 4.1.4). Septal noradrenergic processes have been reported previously to play a role in avoidance behaviour, food- and water-intake, and urinary excretion (see section 4.1.4). The present study shows that these neuronal systems are also involved in morphine-induced behaviour of cats. Septal glutamatergic processes may also be functionally active at the behaviour level since intraseptal injections of glutamate (2 x

5 µg) caused a tendency towards decreased frequencies of morphine-induced locomotor patterns: $\Delta_{rel} = -21 \pm 15\%$ (n = 6) for glutamate vs. $\Delta_{rel} = 13 \pm 8\%$ (n = 9) for distilled water. The present results are considered as insufficient for determining the nature of septal function in behaviour although it is noticeable that the intraseptal injections affected only the frequencies, and not the nature of the morphine-induced behaviour patterns. More sophisticated test situations are required to investigate septal function in behaviour in more detail.

2. The pharmacological character of septal receptor sites

Taking advantage of the specificity of the intraseptally injected drugs, it has been possible in the present study to determine the pharmacological character of those receptor sites, within the septum, that were involved in the intracerebrally-evoked effects: the acetylcholine receptors are of the muscarinic type, the dopamine receptors of the DAi type, the noradrenaline receptors of the α-type, and the opiate receptors of the ε-type.

3. The functional interactions between septal neurotransmission processes

The intraseptally injected drugs produced a common effect, i.e. changes in frequencies of morphine-induced locomotor patterns, which implicates that the various effects are mediated via a common neuronal pathway. In other words, it is quite probable that the present effects are mediated via a direct or indirect action on a common output system of the septum. Intraseptal injections of cholinergic agonists, endorphinergic agonists or noradrenergic antagonists produced increased frequencies of morphine-induced locomotor patterns, whereas intraseptal injections of dopaminergic agonists, endorphinergic antagonists or, presumably, glutamatergic agonists produced decreased frequencies of morphine-induced locomotor patterns. This data indicates that septal cholinergic and endorphinergic systems on the one hand and septal dopaminergic, noradrenergic and, presumably, glutamatergic systems on the other hand exert opposite actions on a common output system of the septum. Several arguments are available to consider the cholinergic, septo-hippocampal pathway as the most probable candidate for this common output system: a) the injection sites were

located in the nucleus septalis medialis, which is the origin of the septo-hippocampal neurons (section 4.1.3), b) the intraseptal injection of opiate agonists or noradrenaline antagonists, which produced increased frequencies of morphine-induced locomotor patterns, have been reported to decrease the activity of the septo-hippocampal neurons (Moroni et al., 1977, 1978a; Robinson et al., 1978), c) the intraseptal injection of cholinergic agonists, which also produced increased frequencies of morphine-induced locomotor patterns, may also decrease the activity of the septo-hippocampal neurons since intraseptal injection of acetylcholine has been reported to decrease the activity of medial septal cells (Segal, 1974). On the basis of the present data, however, the (additional) involvement of other septal projections cannot be excluded. The functional interactions between septal dopaminergic and cholinergic processes are investigated in more detail in Chapter 5.

4. The action of morphine on septal neurotransmission processes
   The present results show that the morphine-induced behaviour is affected by changes in septal neuronal activity. Moreover, as septal cells respond to systemically administered morphine (Robinson and Wang, 1979), it is demonstrated that septal neuronal systems are intermediates between target sites of morphine and the behaviour consequences of morphine administration.

5. The action of morphine on septal opiate receptors
   The present results show that morphine does not act directly on septal opiate receptors but that it may act indirectly via an increased release of β-endorphin from hypothalamic-septal neurons.

Frequencies of morphine-induced locomotor patterns were used in this study as experimental variable for the drug-induced effects. No difference was observed between male and female cats in the degree of the morphine-induced locomotor activity as indicated by the number (mean $\pm$ S.E.M.) of locomotor patterns displayed in the period of 30-39 min after administration of morphine to naive cats, i.e. 25 $\pm$ 6 locomotor patterns for male cats (n = 87) vs. 27 $\pm$ 5 locomotor patterns for female cats (n = 99). Table 4.11 gives values for the number of locomotor patterns (displayed 30-39 min after morphine injection), ordered according to the time of injection during the year. This data - which

TABLE 4.11

Possible seasonal rhythm in the locomotor response of cats to morphine administration (5 mg/kg, i.p.)

month	number of cats	number of locomotor patterns
January	19	24 ± 8
February	14	12 ± 6
March	14	67 ± 21
April	9	4 ± 1
May	3	7 ± 4
June	23	44 ± 17
July	7	17 ± 10
August	26	21 ± 10
September	8	14 ± 12
October	16	31 ± 15
November	19	26 ± 11
December	28	18 ± 5

Given are values (mean ± SEM) for the number of locomotor patterns displayed in the period 30-39 min after morphine administration (5 mg/kg, i.p.) to naive animals.
(The values are averaged over 4 years)

is averaged over a period of 4 years - is not sufficient to prove the existence of a seasonable rhythm in the locomotor response of cats to morphine administration. Nevertheless, it may be a warning for investigators working in this field. An important observation considering the relationship between septal damage and the occurrence of hyperreactivity (Fried, 1973) is that no relation was found between the anatomical localization of the cannula-placement and the degree of morphine-induced locomotor activity (not shown).

In general, the cats were used in more than one experiment in the present study. An intertrial interval of 2 weeks was used in those cases. Using this schedule of morphine administration, no signs of tolerance or abstinence were observed, apart from an increasing salivation. As all drug-induced effects reported in this chapter were also observed in naive animals, it can be stated that these were not secondary effects, due to previous drug-treatment. Apart from carbachol, the intraseptally injected drugs produced no long-term effects. Intraseptal injections of carbachol (2 x 1 µg) at 40-41 min after morphine administration resulted in an enhanced locomotor response of the cats to morphine, not only immediately following the intraseptal injection of carbachol but also when morphine was injected once more 2 weeks later. This is illustrated by the following sets of data: 1) When comparing the number of locomotor patterns displayed during the 10 min immediately before the carbachol injection, i.e. 30-39 min after morphine injection, with that displayed by the cats during the corresponding period after the morphine injection 2 weeks later, there is an increase of 25 $\pm$ 11 locomotor patterns ($n = 16$; $p < 0.05$, two-tailed Wilcoxon matched-pairs signed ranks test). 2) When distilled water (2 x 0.5 µl, $n = 11$) was injected instead of carbachol the corresponding value was only 0 $\pm$ 5 locomotor patterns.

In conclusion, it can be stated that the analysis of the effects of intraseptally injected drugs on the morphine-induced behaviour of cats appeared to be a suitable method for the study of pharmacological and functional properties of neurotransmission processes in the septum.

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MORPHINE-INDUCED BEHAVIOUR OF CATS: A TOOL TO STUDY FUNCTIONAL INTERACTIONS BETWEEN NEUROTRANSMISSION PROCESSES WITHIN SEPTAL AND STRIATAL NUCLEI

5.1 THE SEPTAL NUCLEI AND INVOLVEMENT IN CENTRAL DOPAMINE-ACETYLCHOLINE BALANCE: A BEHAVIOURAL STUDY ON CATS

5.1.1 Summary

An imbalance between dopaminergic and cholinergic interactions within the basal ganglia, particularly the caudate nucleus, is generally thought to be one of the etiological factors in Parkinson's disease. In the present study, we call attention to a definite role of the septal nuclei in the central dopamine-acetylcholine balance. Functional interactions between the septal nuclei and the caudate nucleus were studied by analysis of the behavioural effects elicited by intracerebrally injected drugs in cats pretreated with intraperitoneal morphine (5 mg/kg). Changes in frequencies of morphine-induced locomotor patterns were used as experimental variables. Simultaneous activation of the so-called inhibition-mediating dopamine (DAi) receptors within the septal nuclei and those within the caudate nucleus by means of the DAi-agonist (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) resulted simply in an effect characteristic of activation of the septal DAi receptors. This effect is a significant decrease in the frequencies of morphine-induced locomotor patterns; the effect characteristic of activation of the caudate DAi receptors, i.e. a significant potentiation of the morphine-induced behaviour, was fully suppressed. Analogous results were obtained in experiments in which the activity at the level of the DAi receptors within the septal nuclei and that within the caudate nucleus were simultaneously inhibited by the DAi-antagonist ergometrine. Finally, it was found that the behavioural effect elicited by intraseptal injections of the cholinergic agonist carbachol, i.e. a significant increase in the frequencies of morphine-induced locomotor patterns, was suppressed either by activa-

tion of the DAi receptors within the septal nuclei or by inhibition of the DAi receptors within the caudate nucleus. In the latter case, the behavioural effect characteristic of inhibition of caudate DAi receptors, i.e. the replacement of stereotyped behaviour patterns by normal ones, remained absent. The present study shows a functional relationship between dopaminergic activity within the caudate nucleus and a dopamine-acetylcholine balance within the septal nuclei. The hypothesis is put forward that changes in DAi activity within the caudate nucleus trigger the activity of a feed-forward system coursing from the caudate nucleus to the septal nuclei. The significance of the present findings is discussed in the context of the central dopamine-acetylcholine balance.

5.1.2 Introduction

Studies on the functional interaction between cholinergic and dopaminergic mechanisms have demonstrated that there exists a so-called dopamine-acetylcholine (DA-ACh) balance within the brain (Arnfred and Randrup, 1968; Colpaert et al., 1975; Corrodi et al., 1972; Costall and Olley, 1971; Gianutsos and Lal, 1977; Mennear, 1965; Scheel-Krüger, 1970). Effects elicited by activation of the dopaminergic system are respectively attenuated or potentiated by activation or inhibition of the cholinergic system, and vice versa. In other words, the overall interactions between cholinergic and dopaminergic neuronal systems are mutually antagonistic.

A disturbance of this DA-ACh balance is thought to be one of the etiological factors in Parkinson's disease; indeed, degeneration of dopaminergic, neostriatal neurons has been demonstrated in Parkinson patients (Bernheimer et al., 1973; Calne, 1970; Hornykiewicz, 1975; Spehlmann and Stahl, 1976). Biochemical and electrophysiological studies provide evidence in favour of the hypothesis that the DA-ACh balance has its origin within the basal ganglia, in particular the caudate nucleus (Agid et al., 1975; Connor, 1968; Consolo et al., 1974; Ladinski et al., 1975; McLennan and York, 1966; Sethy and Van Woert, 1974; Stadler et al., 1973; Stoof et al., 1979; Trabucchi et al., 1975). Behavioural studies however point in the opposite direction: dopamine and acetylcholine appear to co-operate with rather than counteract each other within this brain region (Cools, 1974; Cools et al., 1975; Costall

and Naylor, 1972; Poirier et al., 1974; Wolfarth and Kolasiewicz, 1977). Accordingly, the DA-ACh balance has to be considered as a result of a functional interaction between dopaminergic activity within the caudate nucleus and cholinergic systems outside the caudate nucleus.

To make a first move in that direction, we investigated whether the septal nuclei are important in this respect. Besides the fact that the septal nuclei are vulnerable links in various central, cholinergic systems (Bajgar et al., 1977; Dudar, 1975; Gottesfeld and Jacobowitz, 1978, 1979; Kataoka et al., 1977; Mellgren and Srebro, 1973; Sethy et al., 1973; Smith, 1974), there is in any case already some indirect evidence that the caudate nucleus is functionally connected with the cholinergic, septo-hippocampal system (Baker and Kratky, 1975; Baker et al., 1979; Fish, 1976 (referred in Colbern et al., 1977); Glick et al., 1974, 1980).

In the present study, functional relationships were investigated between cholinergic and dopaminergic activity within the septal nuclei and dopaminergic activity within the caudate nucleus. The intracerebral injection technique was used for changing the activity of these neurotransmitter processes. The resulting behavioural effects were investigated in cats pretreated with morphine (5 mg/kg, i.p.), since the morphine-induced behaviour of cats has been shown to be a reliable and valid model for assessing the efficacy of experimentally induced changes in the neurotransmission activity within either the septal nuclei or the caudate nucleus (Cools et al., 1978; Megens and Cools, 1979, 1981). As both the septal nuclei and the anterodorsal part of the caudate nucleus contain so-called inhibition-mediating dopamine (DAi) receptors (Cools and Van Rossum, 1976, 1980; Cools et al., 1976; Megens and Cools, 1981), we chose the DAi-agonist (3,4-dihydroxyphenylamino)-2-imidazoline (DPI) and the DAi-antagonist ergometrine as tools to modify dopaminergic activity within the brain structures mentioned; the cholinergic agonist carbachol was chosen as a tool to change cholinergic activity within the septal nuclei.

5.1.3 Materials and methods

For a detailed description of the experimental conditions and procedures, the reader is referred to previous reports (Megens and Cools, 1979, 1981); only details of the experimental conditions and procedures relevant to the present experiments will be described below. Adult cats (2.0-3.5 kg) of both sexes were used. Each animal was bilaterally equipped with a cannula directed to the septal nuclei and/or one aimed at the anterodorsal part of the caudate nucleus (r-CRM region; cf. Cools et al., 1978).

Drugs were administered intracerebrally by means of Hamilton injection syringes. The volume injected was 0.5 µl for target sites within the septal nuclei and 5.0 µl for target sites within the caudate nucleus (Cools et al., 1978; Megens and Cools, 1979, 1981). All the drugs were administered bilaterally. The following agents were used: carbamylcholine hydrochloride (carbachol; Sigma), (3,4-dihydroxyphenylamino)-2-imidazoline hydrochloride (DPI; Wander), ergometrine maleate (Halewood Chemicals), and morphine hydrochloride (De Onderlinge Pharmaceutische Groothandel). All the drugs were dissolved in distilled water apart from morphine, which was dissolved in saline (0.9% NaCl). The doses mentioned in the text refer to the salts.

When animals were tested more than once, an intertrial interval of 2 weeks was used. Furthermore, the experiments were performed under comparable experimental conditions. After the experiments the animals were sacrificed by means of an overdose of pentobarbital (Nembutal^{R} or Narcovet^{R}) and intracardially perfused with a formaldehyde solution (4-10%) containing the anticoagulant heparin (50 mg/l). The brains were cut open along the tracks of the guide cannulae. The injection sites were localized with the help of Snider and Niemer's atlas (1964).

Behavioural analysis

All the cats were pretreated with morphine (5 mg/kg, i.p.) 40 min before the intracerebral injections and were observed for a period of 60 min immediately following the morphine administration. The morphine-induced behaviour of cats has been extensively described elsewhere (Cools et al., 1974, 1977, 1978). In this study, frequencies of morphine-induced locomotor patterns (for definition, see Megens and Cools, 1981) were used for the statistical evaluation of the drug-induced effects. Both the totals of locomotor patterns over the 10 min immediately preceding the

intracerebral injections (X_{pre}) and those over the 10 min immediately following the intracerebral injections (X_{post}) were determined per animal. The behaviour was not analyzed during the 2 min injection period.

Drug-induced increases were assessed by measuring the absolute difference per animal in the number of locomotor patterns during the pre- and post-injection periods:

$$\Delta_{abs} = X_{post} - X_{pre}$$

The values obtained in this manner per test group were compared with the corresponding values for the appropriate control group.

As drug-induced decreases can only occur in animals showing at least some locomotor patterns during the pre-injection period, only animals with at least 10 locomotor patterns during the pre-injection period were used in the assessment of drug-induced decreases. Furthermore, the differences between pre- and post-injection values were expressed as percentages of the pre- and post-injection values summed, since a decrease in the number of locomotor patterns measured by the variable Δ_{abs} would be limited by the pre-injection value X_{pre}:

$$\Delta_{rel} = \frac{X_{post} - X_{pre}}{X_{post} + X_{pre}} \times 100\% \qquad (X_{pre} \geq 10)$$

The resulting values were compared with the corresponding values for the appropriate control group. Note the difference between this definition of relative changes and the more conventional definition $\frac{X_{post} - X_{pre}}{X_{pre}} \times 100\%$.

For assessing intra-individual differences, the Wilcoxon matched pairs signed rank test was used in contrast to the assessment of inter-individual differences, for which the Mann-Whitney U-test was used. All statistical tests were two-tailed.

Experimental set-up

Five experiments were conducted. In the first experiment, animals received simultaneous injections of the DAi-agonist DPI into the septal nuclei and the caudate nucleus; cats receiving single injections of DPI into the septal nuclei served as controls. In the second experiment, the

DAi-antagonist ergometrine was simultaneously injected into the septal nuclei and the caudate nucleus; controls in this case were animals receiving single injections of ergometrine into the septal nuclei. In the third experiment, single intraseptal injections of the cholinergic agonist carbachol were given in 2 separate trials, 2 weeks apart. In the fourth experiment, animals were also treated with intraseptal carbachol in 2 trials, 2 weeks apart; in the second trial, however, these animals received simultaneous intraseptal injections of DPI. In the fifth experiment, the animals were tested with intraseptal injections of carbachol in combination with intracaudate injections of distilled water; 2 weeks later, these animals received intraseptal injections of carbachol in combination with intracaudate injections of ergometrine.

5.1.4 Results

In the first experiment, the cats received simultaneous injections of DPI (2 x 10 µg) into the septal nuclei and the caudate nucleus; animals receiving single intraseptal injections of DPI served as controls. The intracaudate injections of DPI did not affect the decrease in frequencies of morphine-induced locomotor patterns elicited by the intraseptal injections of DPI (cf. Megens and Cools, 1981). No difference between the groups was found in the values for Δ_{rel} (mean $\pm$ SEM): $\Delta_{rel} = -29\pm9\%$ (n=8) for the experimental group vs. $\Delta_{rel} = -36\pm15\%$ (n=9) for the control group. Fig. 5.1 illustrates the time course of the drug-induced effects for both groups. On the other hand, the intraseptal injection of DPI fully suppressed the effect commonly observed after single intracaudate injections of DPI, as extensively described elsewhere (Cools et al., 1978), i.e. no potentiation of the morphine-induced behaviour occurred.

In the second experiment, the animals were simultaneously treated with injections of ergometrine (2 x 10 µg) into the septal nuclei and the caudate nucleus; animals receiving single injections of ergometrine into the septal nuclei served as controls. The injections of ergometrine into the caudate nucleus did not affect the failure of intraseptal ergometrine to change the frequencies of morphine-induced locomotor patterns, which has been previously reported in detail (cf. Megens and Cools, 1981). Neither the values for Δ_{abs} nor the values for Δ_{rel} differed between the groups: $\Delta_{abs} = -1\pm20$ (n=11) and $\Delta_{rel} = 6\pm15\%$ (n=8) for the experimental

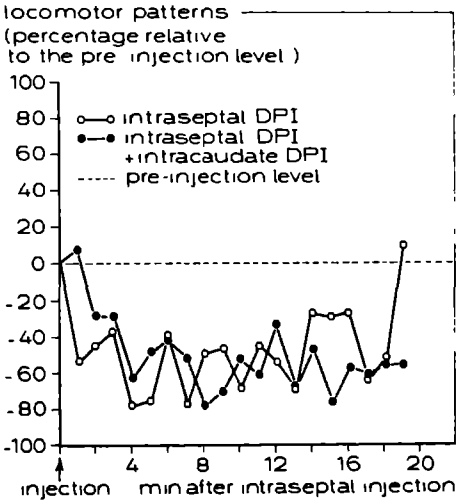

Fig. 5.1 Time-dependent development of changes in frequencies of morphine-induced locomotor patterns elicited by intracerebral injections of 1) DPI (2 x 10 µg, n=9) into the septal nuclei, and 2) DPI (2 x 10 µg, n=8) into the septal nuclei in combination with DPI (2 x 10 µg) into the caudate nucleus. The number of locomotor patterns scored per animal per minute is expressed as a percentage of the total number of locomotor patterns scored for each individual animal during the 10 min immediately preceding and the 19 min immediately following the local injection. Presented are the mean values of these percentages averaged over the test group and expressed as a percentage of the pre-injection level.

group vs. Δ_{abs} = 14±6 (n=10) and Δ_{rel} = 6±9% (n=4) for the control group. On the other hand, the intraseptal injection of ergometrine suppressed the behavioural effect that was commonly observed following single injections of ergometrine into the caudate nucleus as described elsewhere in detail (Cools et al., 1978), i.e. the stereotyped behaviour patterns were not replaced by normal behaviour patterns.

The results of the last 3 experiments are illustrated in Fig. 5.2. In all 3 experiments, the animals received intraseptal injections of

carbachol (2 x 1 µg) in 2 separate trials, 2 weeks apart. In the first trial, the magnitude of the carbachol effect, i.e. an increase in the frequencies of morphine-induced locomotor patterns (cf. Megens and Cools, 1979) was comparable for the 3 groups of animals as indicated by the values for Δ_{abs} in Fig. 5.2A; simultaneous injection of distilled water (2 x 5 µl) into the caudate nucleus does not apparently affect the effect of intraseptal carbachol. Furthermore, the carbachol effect was consistent in magnitude when single intraseptal injections of carbachol were given in the second trial (Fig. 5.2B). However, when

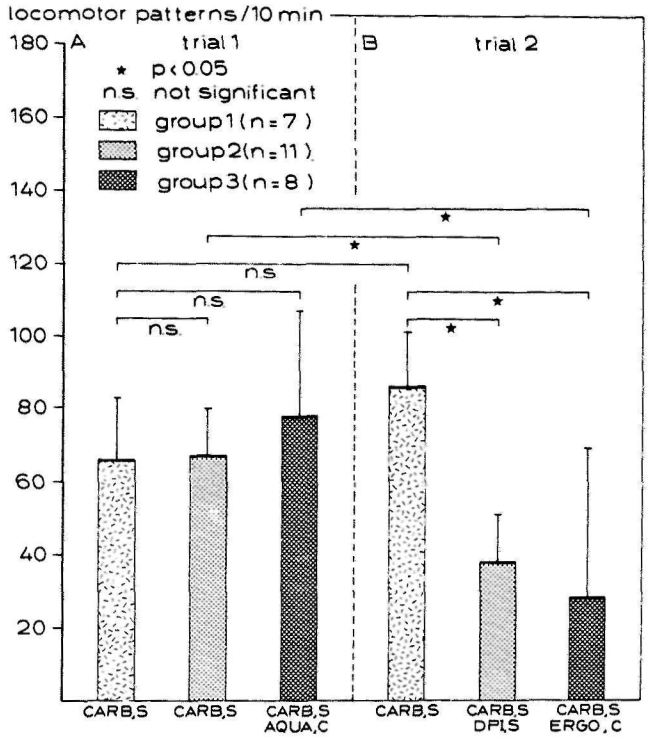

Fig. 5.2 Behavioural effects elicited by intraseptal (s) and/or intracaudate (c) injections of carbachol (CARB, 2 x 1 µg), DPI (2 x 5 µg), ergometrine (ERGO, 2 x 10 µg), and distilled water (AQUA, 2 x 5 µl) in cats pretreated with morphine (5 mg/kg). Drugs were administered in 2 separate trials, 2 weeks apart. Values for Δ_{abs} (mean ± SEM) are given for each drug treatment.

in the second trial the DAi-agonist DPI (2 x 5 µg) was simultaneously injected into the septal nuclei, the carbachol effect was significantly reduced (Fig. 5.2B). When in the second trial the DAi-antagonist ergometrine (2 x 10 µg) was simultaneously injected into the caudate nucleus, the carbachol effect was also significantly reduced (Fig. 5.2B); moreover, in this latter case no behavioural effects occurred that are commonly observed following single injections of ergometrine into the caudate nucleus as previously reported (Cools et al., 1978), that is to say the stereotyped behaviour patterns were not replaced by normal ones. As a final remark, no differences were observed in the results obtained for male and female animals.

Localization of the injection sites

The septal injection sites were localized within the anterior part of the septal nuclei (stereotaxic co-ordinates: A 15.0 - A 19.0, L 0.0 - L 2.0, H 1.0 - H 5.5; cf. Snider and Niemer, 1964); the caudate injection sites were localized within the anterodorsal part of the caudate nucleus (stereotaxic co-ordinates: A 17.5 - A 19.5, L 2.8 - L 4.5, H 3.0 - H 7.0; cf. Snider and Niemer, 1964). It might be useful to note that for the behavioural effects elicited both by intraseptal injection of carbachol and by intraseptal injection of DPI the effective sites were localized within or near (= within 1 mm of) the nucleus septalis medialis pars anterior (cf. Andy and Stephan, 1964).

5.1.5 Discussion

The present results indicate the existence of a functional relationship between the septal nuclei and the caudate nucleus: 1) simultaneous activation of DAi receptors within the septal nuclei and those within the caudate nucleus results in an effect characteristic of activation of septal DAi receptors and suppresses the effect characteristic of activation of caudate DAi receptors; 2) simultaneous inhibition of DAi receptors within the septal nuclei and those within the caudate nucleus results in an effect characteristic of inhibition of septal DAi receptors and suppresses the effect characteristic of inhibition of caudate DAi receptors; 3) simultaneous activation of acetylcholine receptors within the septal nuclei and inhibition of DAi receptors within the caudate nucleus inhibits both the effect characteristic of inhibition of caudate DAi receptors and the effect characteristic of activation of

septal acetylcholine receptors. Such interactions between the septal nuclei and the caudate nucleus are remarkable since no direct connections between these two brain regions have yet been demonstrated. Before discussing these interactions in more detail, the interactions between dopamine and acetylcholine within the septal nuclei themselves will be discussed in detail.

The finding that the behavioural effect elicited by intraseptal injection of the cholinergic agonist carbachol is inhibited by intraseptal injection of the DAi-agonist DPI indicates the existence of a functional antagonism between cholinergic and dopaminergic neuronal systems within the septal nuclei themselves. This is not surprising, as it has been previously reported that intraseptal injection of DAi agonists results in behavioural effects opposite to those elicited by intraseptal injection of cholinergic agonists (Megens and Cools, 1979, 1981). This antagonism between septal cholinergic activity and septal DAi activity cannot be due to a cholinergic-induced inhibition of septal DAi activity since the behavioural effect of the cholinergic agonist carbachol is not mimicked by that of the DAi antagonist ergometrine (Megens and Cools, 1979, 1981). Nor can it be due to a dopaminergic-induced inhibition of septal cholinergic activity since the behavioural effect of the DAi agonist DPI is not mimicked by that of the cholinergic antagonists atropine or scopolamine (Megens and Cools, 1979, 1981). Consequently, it is argued that the septal DAi system and the septal acetylcholine system are not serially connected, but that both neuronal systems converge on a common output system upon where they exert mutually opposite actions.

The mechanism for the interactions observed between the septal nuclei and the caudate nucleus is less evident. The finding that intraseptal injection of the DAi-antagonist ergometrine, which is ineffective *per se* (Megens and Cools, 1981), suppresses the effect caused by intracaudate injection of ergometrine indicates that the latter effect is, at least partly, mediated via an enhanced DAi activity within the septal nuclei. This indication is further strengthened by the ability of intracaudate ergometrine to inhibit, just as intraseptal injection of the DAi-agonist DPI, the effect of intraseptal carbachol. Septal DAi activity is apparently inversely related to caudate DAi activity. Both the septal nuclei and the anterodorsal part of the caudate nucleus, in which the caudate

DAi receptors are concentrated (Cools et al., 1976; Cools and Van Rossum, 1976, 1980), receive their dopaminergic afferents from a common origin, the A-10 cell group in the mesencephalic area ventralis tegmenti (Deniau et al., 1980; Fallon and Moore, 1978; Simon et al., 1979). Consequently, it is quite possible that changes to caudate DAi activity trigger "feed-back" mechanisms coupling the caudate nucleus with the septal nuclei via the area ventralis tegmenti. Evidence for the existence of an analogous feed-back loop mechanism between two central dopaminergic pathways has been previously presented by Yehuda (1979). Indeed, such a mechanism, being in fact a "feed-forward" mechanism, offers a reasonable explanation of the observation that effects caused by alteration of DAi activity within the caudate nucleus are suppressed by manipulation at the level of the septal nuclei.

The existence of the interactions mentioned above suggests an important role for the septal nuclei in the central DA-ACh balance. The septal nuclei are the origin of several cholinergic projections, particularly to the hippocampal formation, but also to the habenula, the nucleus interpeduncularis, and the amygdala (Bajgar et al., 1977; Dudar, 1975; Gottesfeld and Jacobowitz, 1978, 1979; Kataoka et al., 1977; Mellgren and Srebro, 1973; Sethy et al., 1973; Smith, 1974). Based on the present results, septal neuronal activity and, presumably, the release of acetylcholine from septal, cholinergic projections are related to dopaminergic activity within the caudate nucleus. In other words, caudate dopamine controls the cholinergic output of the septal nuclei. This line of reasoning can be easily extended to include all mutual interactions between dopaminergic input of the caudate nucleus and cholinergic output of the septal nuclei. Furthermore, the output stations of the septal, cholinergic projections and the area ventralis tegmenti may also co-operate via mechanisms that are analogous to the above-mentioned feed-forward mechanism. Indeed, the existence of a hippocampo-septal pathway has been demonstrated (Chronister and De France, 1979; Meibach and Siegel, 1977b; Siegel and Edinger, 1976; Swanson and Cowan, 1977); moreover, there is evidence for a septal projection to the area ventralis tegmenti, which controls the dopaminergic output of this region of the brain (Krayniak et al., 1980; Maeda and Mogenson, 1981; Meibach and Siegel, 1977a; Phillipson, 1979; Swanson and Cowan, 1979).

In any case, the involvement of the septal nuclei in the central DA-ACh balance suggests a role for the septal nuclei in the etiology of Parkinson's disease. Indeed, two sets of data point in that direction: 1) The generation of hippocampal theta thythm critically depends upon the functioning of the cholinergic, septo-hippocampal neurons (Apostel and Creutzfeldt, 1974; Green and Arduini, 1954; Kramis and Vanderwolf, 1980; Petsche et al., 1962; Rawlins et al., 1979). This hippocampal theta rhythm has been correlated with the occurrence of voluntary movements or with transitions from one behavioural state to another (Arnolds et al., 1979; Vanderwolf, 1971). Such aspects of behaviour are disturbed in patients suffering from Parkinson's disease. 2) Behavioural effects caused by peripherally administered anticholinergics can be mimicked by manipulation at the level of the septal nuclei (Hamilton et al., 1968; Hamilton and Grossman, 1969). These peripherally administered anticholinergics are beneficial in the treatment of Parkinson's disease (Bernheimer et al., 1973; Calne, 1970; Hornykiewicz, 1975; Spehlmann and Stahl, 1976).

The existence of a DA-ACh balance within the brain is chiefly based on studies investigating the interactions between peripherally administered dopaminergic and cholinergic drugs (see 5.1.2). Such peripherally administered drugs spread throughout the brain and act on all drug-sensitive, neuronal systems. Most studies have focussed attention on the presence of DA-ACh interactions within the caudate nucleus (see 5.1.2). The present study suggests a functional relationship between dopaminergic activity within the caudate nucleus and a dopamine-acetylcholine balance within the septal nuclei. Moreover, the present findings indicate the existence of a feed-forward mechanism operating between the caudate nucleus and the septal nuclei. Consequently, dopaminergic activity within the caudate nucleus is related to septal neuronal activity and, presumably, to the release of acetylcholine from the various septal cholinergic projections. These data indicate a definite role for the septal nuclei in the central DA-ACh balance. This notion may stimulate further studies in this direction in order to elucidate the true nature of the central DA-ACh balance and to increase our basic insight into the etiology of Parkinson's disease.

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The work described in this thesis analyzes the effects of intracerebrally injected drugs on the morphine-induced behaviour of the cat. The aim of this work was to investigate whether this analysis would enable us to study the pharmacological and functional properties of neurotransmission processes within anatomically circumscribed regions of the brain. Attention is paid to several aspects of neurotransmission processes, e.g. 1) the functional activity of neurotransmission processes at the behaviour level, 2) the pharmacological character of receptor sites, 3) the flexibility of neurotransmission processes, 4) functional interactions between neurotransmission processes, 5) the action of morphine on neurotransmission processes, and 6) the action of morphine on opiate receptors.

The Preface deals with some introductory comments on the scope, general method and the purpose of the investigations. It states the research problems and explains the reasons for investigating them.

Chapter 1 is a general introduction to the use of morphine in the present experiments. In the first section, the history of the opiate agonist is briefly summarized. The remaining of the chapter is devoted to a survey of the various effects of morphine in man and cats. After giving a survey of the overt effects of morphine, the more specific effects are discussed in detail. A separate section deals with the central effects of morphine in the cat. Attention is paid to such subjects as specificity, dose-dependency, development of tolerance and dependence, effects of drugs, intracerebral injections, and effects of opioid peptides. It is concluded that the cat is a suitable experimental animal for the study of morphine's central action.

Chapter 2 deals with the experimental method used in the present investigations, i.e. the analysis of effects of intracerebrally injected drugs on the morphine-induced behaviour of cats. First, the rationale is given for using this method: it might enable us to study, in acute experiments, the pharmacological and functional properties of neurotransmission processes within anatomically circumscribed regions

of the brain, including the action of morphine on these processes. Next, after giving a general outline of the experimental procedure, both qualitative and quantitative aspects of the morphine-induced behaviour of the cat are discussed. Three behaviour phases are distinguished, i.e. depression, re-organization and ritualization, which appear in succession. It is argued that, on the basis of its highly structured organization and characteristic behaviour patterns, the morphine-induced behaviour of the cat is a suitable tool for the disclosure of experimentally evoked changes in behaviour.

Chapters 3, 4 and 5 deal with the experimental studies of the present thesis. For a detailed survey of the results obtained in these studies, the reader is referred to the summary presented at the beginning of each section. A general survey is given below.

Chapter 3 is devoted to two studies dealing with the action of morphine on central neurotransmission processes. In the first section, the analysis is described of both the acute- and the after-effects of noradrenergic agents intracerebrally injected into the nucleus raphe linearis. It is demonstrated that noradrenergic processes in this raphe nucleus adjust their properties to changed neurotransmission activity, and that this flexibility might play a critical role in the development of tolerance to morphine. The second section deals with a study concerning the effect of morphine on two pharmacologically distinct dopaminergic systems in the caudate nucleus. It is demonstrated that this effect is biphasic and that it depends on which dopaminergic system is studied. The data is discussed in the context of two diametrically opposite hypotheses concerning morphine's effect on central dopaminergic activity. Some general comments on the results obtained are given in the final section.

Chapter 4 deals with studies on neurotransmission processes within the septum. The first section is a general introduction to this subject and gives a survey of the morphology, the anatomical connections and the neurochemistry of the septum. It is argued that it is nearly impossible, because of the heterogeneity of the septum, to analyze the function of this brain structure by classic methods, such as electrical stimulation and lesions. The second section - dealing with the effects of intraseptally injected cholinergic agents - shows that the morphine-induced behaviour of the cat can be used as an variable for experimentally-evoked

changes in septal neuronal activity. It is suggested that intraseptally injected cholinergics and systemically administered morphine exert a synergistic action on a common output system of the septum, presumably the septo-hippocampal system. The third section deals with the effects of intraseptal injections of dopaminergic and noradrenergic agents. The results show that a subpopulation of dopamine receptors, i.e. the so-called DAi receptors, are present within the septum, and that septal dopaminergic and noradrenergic processes are involved in behaviour. The fourth section deals with the effects of intraseptally injected opiate agents. It is demonstrated that septal opiate receptors are most probably of the ε-type, and that these, although involved in behaviour, are not a site of action for systemically administered morphine to produce behaviour effects. It is suggested that morphine enhances the release of β-endorphin in the septum via an action on hypothalamic-septal, endorphinergic neurons. At the end of this chapter, some general comments on the results obtained are given. It is demonstrated that no difference exists between male and female cats in the effect of morphine on locomotor activity. Furthermore, data is presented which might indicate the existence of a seasonal rhythm in this morphine-induced locomotor response.

Chapter 5 deals with the effects of single and combined injections of dopaminergic and cholinergic agents into the septum and the caudate nucleus. The study demonstrates the existence of a functional relationship between 1) dopaminergic and cholinergic activity in the septum, and 2) dopaminergic activity in the caudate nucleus and dopaminergic and cholinergic activity in the septum. A possible mechanism for the observed interactions is proposed. Moreover, a possible role of septal structures in central dopamine-acetylcholine interactions and in the etiology of Parkinson's disease is discussed.

It is concluded that the analysis of the effects of intracerebrally injected drugs on the morphine-induced behaviour of the cat is a suitable method for the study of pharmacological and functional properties of central neurotransmission processes.

In de experimenten, die in dit proefschrift beschreven worden, worden de effecten geanalyseerd van intracerebraal toegediende farmaca op het morfine-geïnduceerde gedrag van de kat. Het doel van dit onderzoek was na te gaan of deze analyse ons in staat zou stellen de farmacologische en functionele eigenschappen te bestuderen van neurotransmissie processen in anatomisch omschreven hersengebieden. Aandacht wordt besteed aan verschillende aspecten van neurotransmissie processen, bijv. 1) de functionele activiteit van neurotransmissie processen op gedragsniveau, 2) het farmacologische karakter van receptoren, 3) de flexibiliteit van neurotransmissie processen, 4) functionele interacties tussen neurotransmissie processen, 5) het effect van morfine op neurotransmissie processen, en 6) het effect van morfine op opiate receptoren.

In het Voorwoord worden enkele inleidende opmerkingen gemaakt over het onderzoeksgebied, de gebruikte methode en het doel van het onderzoek. Verder wordt een uiteenzetting gegeven van de onderzoeksproblemen en van de redenen om deze problemen te onderzoeken.

Hoofdstuk 1 is een algemene inleiding op het gebruik van morfine in de experimenten. In de eerste sectie wordt in het kort de geschiedenis van de opiate agonist samengevat. De volgende secties worden besteed aan een overzicht van de verschillende effecten van morfine in mens en kat. Nadat een overzicht is gegeven van de direct zichtbare effecten van morfine, worden de meer specifieke effecten in detail beschreven. Een afzonderlijke sectie behandelt de centrale effecten van morfine in de kat. Aandacht wordt besteed aan onderwerpen als specificiteit, dosisafhankelijkheid, ontwikkeling van tolerantie en afhankelijkheid, effecten van farmaca, intracerebrale injecties, en effecten van opioïd peptiden. Als conclusie wordt betoogd dat de kat een geschikt proefdier is voor de bestudering van de centrale werking van morfine.

Hoofdstuk 2 handelt over de methode die gebruikt wordt in de experimenten, i.e. de analyse van de effecten van intracerebraal toegediende farmaca op het morfine-geïnduceerde gedrag van de kat. Op de eerste plaats wordt de rationale gegeven voor het gebruik van deze methode:

het zou ons in staat kunnen stellen om, in acute experimenten, meer inzicht te verkrijgen in de farmacologische en functionele eigenschappen van neurotransmissie processen binnen anatomisch omschreven hersengebieden, als ook in het effect van morfine op deze neurotransmissie processen. Nadat vervolgens in grote lijnen de experimentele methode is aangegeven, worden kwalitatieve en kwantitatieve aspecten van het morfine-geïnduceerde gedrag van de kat besproken. Drie opeenvolgende gedragsfasen worden onderscheiden, i.e. depressie, reorganisatie en ritualisatie. Het wordt betoogd dat, op basis van zijn zeer gestructureerde opbouw en karakteristieke gedragspatronen, het morfine-geïnduceerde gedrag van de kat een geschikt middel is om experimenteel opgeroepen veranderingen in gedrag te ontdekken.

Hoofdstukken 3, 4 en 5 beschrijven de experimentele studies van dit proefschrift. Voor een gedetailleerd overzicht van de resultaten, die in deze studies verkregen werden, wordt de lezer verwezen naar de samenvatting aan het begin van iedere sectie. Een algemeen overzicht wordt hieronder gegeven.

Hoofdstuk 3 beschrijft 2 studies naar het effect van morfine op centrale neurotransmissie processen. In de eerste sectie wordt de analyse beschreven van zowel de acute werking als de nawerking van noradrenerge farmaca die intracerebraal werden toegediend in de nucleus raphe linearis. Aangetoond wordt dat noradrenerge processen in deze raphe kern hun eigenschappen aanpassen aan veranderde neurotransmissie activiteit, en dat deze flexibiliteit een belangrijke rol zou kunnen spelen in de ontwikkeling van tolerantie voor morfine. De tweede sectie beschrijft een studie betreffende het effect van morfine op twee farmacologisch verschillende dopaminerge systemen in de nucleus caudatus. Aangetoond wordt dat dit effect bifasisch is en dat het afhangt van welk dopaminerg systeem bestudeerd wordt. De data worden bediscussieerd in de kontekst van twee lijnrecht tegenover elkaar staande hypothesen over het effect van morfine op centrale dopaminerge activiteit. In de laatste sectie worden enkele, algemene opmerkingen gemaakt over de verkregen resultaten.

Hoofdstuk 4 is gewijd aan studies betreffende neurotransmissie processen in het septum. De eerste sectie is een algemene inleiding tot dit onderwerp en geeft een overzicht van de morfologie, de anatomische verbindingen en de neurochemie van het septum. Het wordt betoogd

dat het, vanwege de heterogeniteit van het septum, bijna onmogelijk is de functie van deze hersenstructuur te analyseren met behulp van klassieke methoden zoals electrische stimulatie of lesies. De tweede sectie - die de effecten van intraseptaal toegediende cholinerge farmaca beschrijft - laat zien dat het morfine-geïnduceerde gedrag van de kat gebruikt kan worden als variabele voor experimenteel opgeroepen veranderingen in septale neuronale activiteit. De suggestie wordt gedaan dat intraseptaal geïnjecteerde cholinergica en systemisch toegediend morfine een synergistisch effect uitoefenen op een gemeenschappelijk output systeem van het septum, vermoedelijk het septo-hippocampale systeem. De derde sectie behandelt de effecten van intraseptale injecties van dopaminerge en noradrenerge farmaca. De resultaten laten zien dat een subpopulatie van dopamine receptoren, i.e. de zo genoemde DAi receptoren, aanwezig zijn in het septum, en dat septale dopaminerge en noradrenerge processen betrokken zijn bij gedrag. De vierde sectie betreft de effecten van intraseptaal toegediende opiaten. Aangetoond wordt dat septale opiate receptoren het meest waarschijnlijk van het ε-type zijn en dat deze, ofschoon betrokken bij gedrag, geen aangrijpingspunt zijn voor systemisch toegediend morfine om gedragseffecten te produceren. De suggestie wordt gedaan dat morfine de afgifte van β-endorfine in het septum verhoogt via een effect op hypothalamische-septale, endorfinerge neuronen. Ter afsluiting van het hoofdstuk worden enkele algemene opmerkingen gemaakt over de verkregen resultaten. Aangetoond wordt dat geen verschil bestaat tussen mannelijke en vrouwelijke katten in het effect van morfine op locomotorische activiteit. Verder worden data gepresenteerd, die erop zouden kunnen wijzen dat een seizoensritme bestaat in deze morfine-geïnduceerde locomotorische activiteit.

Hoofdstuk 5 behandelt de effecten van enkelvoudige en gecombineerde injecties van dopaminerge en cholinerge farmaca in het septum en de nucleus caudatus. De studie toont aan dat een functionele relatie bestaat tussen 1) dopaminerge en cholinerge activiteit in het septum, en 2) dopaminerge activiteit in de nucleus caudatus en dopaminerge en cholinerge activiteit in het septum. Een mogelijk mechanisme voor de waargenomen interacties wordt voorgesteld. Bovendien wordt een mogelijke rol van septale structuren in centrale dopamine-acetylcholine interacties en in de etiologie van de Ziekte van Parkinson bediscussieerd.

Als conclusie wordt gesteld dat de analyse van de effecten van intracerebraal toegediende farmaca op het morfine-geïnduceerde gedrag van de kat een geschikte methode is voor de bestudering van farmacologische en functionele eigenschappen van centrale neurotransmissie processen.

Antonius Adrianus Hendrikus Petrus Megens werd geboren op 4 april 1953 te Nijmegen. Hij bezocht het St. Gabriëlcollege te Mook en behaalde aldaar in 1971 het diploma Gymnasium-β. Vervolgens studeerde hij scheikunde aan de Katholieke Universiteit te Nijmegen, waar hij in 1974 het kandidaatsexamen en in 1977 het doctoraalexamen aflegde, met als hoofdvak farmacochemie en als bijvakken organische chemie en biofysische chemie. In 1977 behaalde hij tevens het C-diploma radioisotopen en verkreeg hij de eerste graads onderwijsbevoegdheid in de scheikunde. In augustus 1977 trad hij in dienst van de Katholieke Universiteit te Nijmegen, en vanaf die tijd is hij werkzaam geweest als wetenschappelijk medewerker op het Farmacologisch Instituut aldaar. Hij heeft als lid van de werkgroep psychoneurofarmacologie onder leiding van Dr. A.R. Cools en Prof.Dr. J.M. van Rossum onderzoek verricht naar de farmacologische en functionele eigenschappen van neurotransmissie processen in de hersenen; de resultaten van dit onderzoek zijn beschreven in dit proefschrift.

Uit gezamenlijk onderzoek zijn de volgende publicaties voortgekomen:

Cools, A.R., C.L.E. Broekkamp, L.C.M. Gieles, A.A.H.P. Megens and H.J.G.M. Mortiaux, Site of action of development of partial tolerance to morphine in cats, Psychoneuroendocrinology 2, 17-33 (1977).

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Cools, A.R., P.A.M. van Dongen, H.-J. Janssen and A.A.H.P. Megens, Functional antagonism between dopamine and noradrenaline within the caudate nucleus of cats: a phenomenon of rhythmically changing susceptibility, Psychopharmacology 59, 231-242 (1978).

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Megens, A.A.H.P. and A.R. Cools, Intraseptally injected opiate agents: effects on morphine-induced behaviour of cats. Submitted for publication.

Megens, A.A.H.P. and A.R. Cools, The septal nuclei and involvement in central dopamine-acetylcholine balance: a behavioural study on cats. Submitted for publication.

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Het Centraal Dierenlaboratorium heeft op uitstekende wijze zorg gedragen voor de levering, huisvesting en verzorging van de proefdieren, in het bijzonder J. Koedam, A.M. Peters, P.B. Spaan en hun medewerkers. W.J. Nagelvoort en zijn medewerkers van de Instrumentmakerij wil ik danken voor het vervaardigen en onderhouden van de stereotactische apparatuur en de injectie-cannules. Technische en algemene assistentie werd zonodig verleend door W.F.H. van Amerom, D. Heeren, W.B. Hoeboer en J.A.L. Janssen.

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For when was honey ever made
with one bee in the hive?

Thomas Hood

STELLINGEN

1

Analyse van gedragsveranderingen welke middels intracerebrale toediening van specifiek werkende farmaca in morfine behandelde katten worden opgeroepen is een geschikte methode om inzicht te verkrijgen in gedragsregulerende, neurochemische processen in het brein die anderszins niet of nauwelijks bestudeerd kunnen worden.

dit proefschrift

2

Antagonisme door intracerebraal toegediend naloxon of naltrexon van effecten die worden opgeroepen door systemisch toegediend morfine is een noodzakelijke, maar niet voldoende voorwaarde om de directe betrokkenheid van centrale morfine receptoren bij het tot stand komen van deze effecten aan te tonen.

dit proefschrift

3

Een mogelijke rol van septale, cholinerge projecties in het tot stand komen van het therapeutisch effect van anticholinergica bij de behandeling van de ziekte van Parkinson verdient aandacht.

dit proefschrift

4

De rol van centrale noradrenerge processen in de ontwikkeling van tolerantie voor morfine opent perspectieven voor een mogelijk effectief gebruik van noradrenerge farmaca ter preventie van verschijnselen als tolerantie en afhankelijkheid.

dit proefschrift

5

Bij de bestudering van morfine-geïnduceerde effecten dient ruime aandacht besteed te worden aan het tijdsverloop van deze effecten.

dit proefschrift

De stelling van Woodruff et al. (1976, 1978) dat ergometrine een agonist is van dopamine receptoren in de nucleus caudatus en de nucleus accumbens van de rat wordt onvoldoende gestaafd door hun electrofysiologische bevindingen.

Woodruff, G.N. et al., Brain Res. 115, 233-242 (1976)
Woodruff, G.N., Adv. Biochem. Psychopharmacol. 19, 89-119 (1978)

7

Ten onrechte concluderen Kilbey en Ellinwood (1980) dat katten weinig of geen fysiologische afhankelijkheid van morfine ontwikkelen.

Kilbey, M.M. en E.H. Ellinwood, Int. J. Addict. 15, 447-460 (1980)

8

De biochemische veranderingen door Moroni et al. (1978) gemeten op meer dan 20 min na intracerebrale toediening van farmaca zijn niet zonder meer te beschouwen als directe effecten van deze farmaca, aangezien het optreden van "rebound" verschijnselen niet kan worden uitgesloten.

Moroni, F. et al., Neuropharmacology 17, 191-196 (1978)

9

Bij het vaststellen van de mate waarin de metabolieten van paracetamol worden uitgescheiden door middel van actieve transport processen houden Duggin en Mudge (1975) onvoldoende rekening met metabolisme van paracetamol in de nier.

Duggin, G.G. en G.H. Mudge, Br. J. Pharmac. 54, 359-366 (1975)
Hart, S. et al., Clin. Sci. 58, 379-384 (1980)

Bij het interpreteren van de kinetiek van farmaca dient meer aandacht besteed te worden aan de centrale werking van deze farmaca aangezien deze een belangrijke bepalende factor in de kinetiek van farmaca kan zijn.

11

Het leggen van een causaal verband tussen plasmaconcentratie en therapeutisch effect van chlorpromazine is weinig zinvol.

Rivera-Calimbin, L. et al., Commun. Psychopharmacol. 2, 215-222 (1978)
Kolakowska, T. et al., Psychopharmacology 49, 101-107 (1976)

12

In de stelling van Alderson en Baum (1981) dat - indien steroïd deprivatie leidt tot degeneratie van mesolimbische dopamine neuronen - acute toediening van haloperidol minder gemakkelijk zou leiden tot verhoogde DOPAC en HVA concentraties in gecastreerde ratten zonder steroïd implantatie dan in gecastreerde ratten met steroïd implantatie dienen de woorden zonder en met vervangen te worden door resp. met en zonder.

Anderson, L.M. en M.J. Baum, Brain Res. 219, 189-206 (1981)

13

Aangezien het niet goed functioneren van neurochemisch verschillende processen in het brein kan leiden tot één en hetzelfde ziektebeeld, dient men er zich terdege van bewust te zijn dat patiënten met één en hetzelfde ziektebeeld mogelijk farmacotherapeutisch verschillend behandeld dienen te worden.

14

Overwogen dient te worden het gebruik van de LD_{50}, i.e. de dosis van een farmacon waarbij 50% van de proefdieren sterft binnen 24 uur na toediening van dat farmacon, als maat voor de toxiciteit van farmaca te vervangen door het gebruik van bijvoorbeeld de TD_{50}, i.e. de dosis waarbij 50% van de proefdieren de eerste toxische verschijnselen vertoont.

15

Speciaal schrijvers van boeken met de titel "Hoe schrijf ik een wetenschappelijke tekst?" dienen op de omslag van hun eigen publicatie de door hen gegeven voorschriften na te volgen.

Lamers, H.A.J.M., "Hoe schrijf ik een wetenschappelijke tekst?", ed. D. Coutinho, Muiderberg (1979)

16

Het op de markt brengen van zelfklevende postzegels zou de noodzaak van het bekende oro-linguale ritueel bij het verzenden van post wegnemen.

17

In het kader van de problemen rond de exploitatie van de ons ter beschikking staande energiebronnen, kunnen onze automobielen niet langer als "zelf voortbewegend" worden beschouwd en dient, derhalve, een andere naamgeving overwogen te worden.

Nijmegen, 18 december 1981
A.A.H.P. Megens