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Effects Of Analgesics On Centrally-induced "pain" In Rats

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LIST OF TABLES

EFFECTS OF ANALGETICS ON CENTRALLY-INDUCED "PAIN" IN RATS

by

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ABSTRACT

Few studies have been conducted on the effects of analgesic compounds on the behavior elicited by stimulation of brain structures implicated in pain mechanisms. It was the main purpose of the present investigation to use the technique of intracranial aversive stimulation in order to determine whether brain areas which play different functional roles in central pain mechanisms have a different pharmacological sensitivity to analgesic agents. The aversive thresholds of rats with electrodes permanently implanted into one of the following areas: mediodorsal, ventrodorsal or parafascicular - paraventricular thalamic nuclei, dorsal hippocampus, anterior or lateral hypothalamus, dorsal midbrain, medial lemniscus or optic tract, were determined by means of a titration schedule in a two-way shuttle-box. Dose-response curves for the depressant effects of five narcotic analgetics, morphine, heroin, fentanyl, propoxyphene, tilidine and of the phenothiazine derivative, levomepromazine, on the escape response elicited by suprathreshold stimulation of these areas were obtained. The intensity of stimulation used was always a constant function of the aversive threshold determined for each animal prior to each drug trial. In this manner, the effects

of the analgetics were studied against a behavioral response which was similar for all animals. When the effects of each drug in the various brain areas were compared, it was found that each narcotic analgetic was associated with a family of parallel dose-response curves whereas levomepromazine produced two sets of parallel dose-response curves. The shifts in the parallel dose-response curves obtained with each drug therefore provided an estimate of the differential sensitivity of the brain areas to the effects of that particular drug. There was some commonality of action among all of the analgetics investigated. On the basis of the differential sensitivity exhibited by the various brain areas, it was concluded that analgesia of the morphine type involves, first an alteration in the integrating activities of the non-specific, associational and specific-sensory thalamic nuclei; second, an influence upon the structures of the limbic system; third, an action upon the perceptual and behavioral responses mediated by the dorsal midbrain; and, fourth, an impairment of sensory impulse transmission within the main somesthetic conduction pathway. The observation that the thalamic nuclei were the most sensitive to the effects of the analgetics suggests that these drugs influence both the sensory and behavioral components of pain at the thalamic level. However, both within the opiate class of compounds and between this group of drugs and the phenothiazine derivative, some dissimilarities among the

actions of the drugs were also observed. This difference in drug action reflected the extent with which each drug influences the two components, sensory perception and behavioral reaction, of pain. Neuroanatomical correlates were obtained which were consistent with the findings that heroin and morphine predominantly influence the reaction component while fentanyl has a proportionately greater effect upon the perceptual mechanisms. This study also yielded results consistent with the hypothesis that heroin may not only act following a conversion to morphine. It was concluded that most of the analgesic actions of propoxyphene are mediated by the thalamic nuclei but that it also can significantly affect the behavioral response to pain mediated by limbic structures if given in sufficiently large doses. Since very high, toxic, doses of tilidine were required to produce a depression of the escape response, no definite conclusions could be made with respect to the sites involved in the analgesic action of this compound. It was concluded that the phenothiazine derivative produces an analgesia similar to that of morphine but mediated by receptor sites which are different from those of the opiates. It is suggested that the technique employed in the present study may serve as a basis for the design of other relevant approaches in studies on the central actions of analgesic compounds.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GLOSSARY OF ABBREVIATIONS FOR FIGURES 6-11

AA	Area amygdala anterior
BCI	Brachium colliculi inferioris
C	Cingulum
CAA	Commissura anterior, pars anterior
CAI	Capsula interna
CC	Crus cerebri
CFV	Commissura Fornicis Ventralis
CI	Colliculus inferior
CP	Commissura posterior
CS	Colliculus superior
DMB	Doral midbrain
F	Columna fornicis
FH	Fimbria hippocampi
FL	Fasciculus longitudinalis
FMP	Fasciculus medialis prosencephali
FMT	Fasciculus mamillo thalamicus
FMTG	Fasciculus mamillotegmentalis
FOR	Formatio reticularis
FR	Fasciculus retroflexus
GD	Gyrus dentatus
GP	Globus pallidus
HI	Hippocampus
ML	Medial lemniscus
S	Subiculum
SAM	Stratum album mediale colliculi superioris
SG	Substantia grisea
SGPV	Substantia grisea periventricularis
SM	Stria medullaris thalami
SPCC	Splenium corporis callosi
ST	Stria terminalis
TCC	Truncus corporis callosi
TO	Tractus opticus
V	Nucleus and tractus nesencephalici n. trigemini
cp	Nucleus caudatus putamen
h	Nucleus habenulae
ha	Nucleus anterior hypothalami
hl	Nucleus lateralis hypothalami
ip	Nucleus interpeduncularis
mn	Nucleus mamillaris
pf	Nucleus parafascicularis
pv	Nucleus paraventricularis
pvs	Nucleus periventricularis stellatocellularis

tav	Nucleus anterior ventralis thalami
tl	Nucleus lateralis thalami
tm	Nucleus medialis thalami
tpm	Nucleus posteromedianus thalami
tpo	Nucleus posterior thalami
tv	Nucleus ventralis thalami

INTRODUCTION AND PURPOSE OF PRESENT INVESTIGATION

The major drawback of many of the investigations dealing with the site of action of the opiates lies in their implicit assumption that responses to noxious stimuli can be explained in solely physiological terms. Pain, however, represents the result of at least three neuropsychological processes: (1) a sensory-discriminative process whereby stimuli are localized in space, time and along an intensity continuum; (2) a motivational-affective component which provides the powerful drive and unpleasant affect that trigger the organism's protective mechanisms; and (3) cognitive influences such as anxiety, anticipation or memory of past experiences (Melzack and Wall, 1965, 1968; Casey and Melzack, 1967; Melzack, 1973). That pain is comprised of both sensory and affective dimensions was clear to Sherrington as early as 1906 (cit. Casey and Melzack, 1967) when he stated that "the mind rarely, probably never, perceives any object with absolute indifference, that is, without 'feeling'.. affective tone is an attribute of all sensation." Despite this observation, historical emphasis on neurophysiological techniques has stressed the sensory mechanisms of pain and virtually ignored the contributions of motivational and cognitive processes. These latter factors may, in fact, play the

predominant role in the genesis of pain (Beecher, 1959).

Many of the investigations were also limited to only anesthetized, immobilized or decerebrate animals in which the responses to noxious stimuli may be affected by the drugs or surgery. Interactions between the analgetic studied and the paralyzing or anesthetic agents are also likely to occur. The potentiation of the depressant effects of morphine by anesthetic agents, such as the barbiturates, is well known and a possible central antagonism between morphine and curare-like compounds has also been suggested (Wikler, 1950, pg. 468; Routtenberg and Kramis, 1968).

With the advent of techniques for probing the brain with chronic, indwelling electrodes[1] and more sophisticated psychological testing methods for the objective measurement of motivation, the opportunity arose for correlating the effects of the narcotic analgetics with behavioural responses evoked by direct stimulation of brain structures involved in the transmission or elaboration of nociceptive sensations. The work of Delgado, Roberts and Miller (1954) clearly demonstrated that electrical stimulation of certain rostral brain stem structures, like peripherally administered pain, can motivate the learning and performance of escape and avoidance habits. Since then a number of reports have appeared in the literature which indicate that

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[1]Following the pioneer work of W. R. Hess (see "The Functional Organization of the Diencephalon", Grune & Stratton, New York, 1957).

similar behavioural responses can be elicited from structures lying at all levels of the neuraxis (Olds and Olds, 1963): for example, in the medulla, the Gasserian ganglion (Delgado, Rosvold and Looney, 1956; Weitzman and Ross, 1962) and the nucleus giganto-cellularis (Casey, 1971 a-e); in the mesencephalon, certain tectal (Spiegel, Kletzkin and Szekely, 1954; Delgado, et al., 1954; Spiegal and Wycis, 1961; Valenstein, 1966) and tegmental areas (Delgado, 1955; Abrahams, Hilton and Zbrozyna, 1960; Valenstein, 1966), the periaqueductal gray (Delgado, 1955; Delgado, et al., 1956) and the medial lemniscus (Spiegel, et al., 1954; Roberts, 1958a); in the diencephalon, the hypothalamus (Roberts, 1958a and b; Abrahams, et al., 1960; Wasman and Flynn, 1962) and the posteroventral (Delgado, 1955; Delgado et al., 1956; Roberts, 1962; Benton and Mefferd, 1967), mediodorsal (Roberts, 1962) and center median (Benton and Mefferd, 1967) nuclei of the thalamus; in the telencephalon, the fornix (Delgado, 1955), hippocampus (Delgado, 1955) and amygdaloid areas (Delgado et al., 1956; Hilton and Zbrozyna, 1963). Electrical stimulation of all of these structures produces strong aversive drive and behaviour typical of responses to naturally-occurring painful stimuli.

With the use of a titration schedule, an objective method is provided whereby the animal can communicate to

the observer the level of stimulus intensity it is willing to tolerate before and after the administration of analgetics, in a manner akin to the verbal reporting of the pain experience in man (Weis and Laties, 1958, 1964; Weitzman and Ross, 1962; Ross, 1966).

The technique consists of training the subject to make a response which reduces the intensity of an aversive stimulus in small steps. When the subject stops responding, the intensity rises in small increments until the subject again responds, thus completing a cycle in which the subject adjusts the intensity around a value which can be described as an aversive threshold. This technique therefore provides a neurophysiological tool for the direct study of the effect of narcotic analgetics on aversive systems within the brain. Since it is likely that the contribution of structures mediating pain varies along the neuraxis, it is logical to assume that different anatomical sites would have different pharmacological sensitivities to the narcotic analgetics. It is the aim of the present investigation to study the effects of different analgetics on the aversive responding elicited by electrical stimulation of various brain areas implicated in the genesis of pain with the titration technique to see whether such differential sensitivity does, indeed, exist. It is hoped that the data to be presented will yield additional infor-

mation concerning the central site of action of the analgetic compounds and that the results of this study, in context with those obtained by other methods, will assist in our achieving a clearer understanding of the basis of action of the narcotic analgetics. To date, only one such study has been reported (Vernier, Boren and Knapp, 1961).

HISTORICAL REVIEW OF STUDIES ON THE SITE OF ACTION OF THE NARCOTIC ANALGETICS

A great deal of research has been done on the site of action of the narcotic analgetics and this has been extensively reviewed by a number of investigators (Krueger, Eddy and Sumwalt, 1941; Wikler, 1950, 1958; Domino, 1962, 1968; Martin, 1963; Winter, 1965; Valdman, 1967). However, it is as yet impossible to relate the many facts which have been uncovered (many of which appear incongruous) into a single cohesive theory of drug action. The results have been as various as the investigators. The field is bedeviled by the necessity of frequently having to use non-physiological doses in order to produce visible effects, by intra- as well as interspecies variation, by the use of innumerable kinds of anesthetics and animal preparations (spinal, decerebrate, decorticate, etc.), by the unavailability of a single specific nociceptive stimulus, and, most important, by the uncertainty as to the course of the pain pathways and the implications arising from the reciprocal excitatory and inhibitory nature of central somesthetic innervation. It is important that these factors always be kept in mind when surveying the proposed neural mechanisms of action of the analgetic compounds.

In order to localize the supraspinal site of action

of the narcotic analgetics, investigators have commonly employed one of the following three techniques: (1) Investigation of the influence of analgetics on the direct or evoked excitability of nervous structures involved in the transmission and elaboration of pain. (2) Comparison of the effects of morphine before and after sectioning, partial removal or lesioning of various parts of the central nervous system. (3) Introduction of the drug directly into some particular site of the central nervous system via inplanted micro-cannulae, with or without concomitant localization of drug concentration by autoradiographic techniques.

Another line of approach which has a direct bearing on this problem is a study of the effects of analgetics on the aversive behaviour elicited by electrical stimulation of central nervous system structures implicated in pain processes. At present, however, only one group of researchers (Vernier, et al., 1961) has used this technique in an attempt to identify the central site of action of the narcotic analgetics. The results obtained by these four avenues of research and the conclusions made with respect to the central sites of action of the narcotic analgetics are discussed in the following sections.

1. Electrophysiological techniques

Studies on potentials evoked by tooth pulp stimulation

Regardless of the type of stimulus applied to the tooth, be it heat, cold, touch, pressure, chemical or electrical, it is interpreted centrally as pain, provided the stimuli are of sufficient intensity and confined to the pulp cavity. For this reason, the afferent system of dental stimulation has generally become to be accepted as a relatively pure nociceptive system, comparable to pathological pain in that deep nociceptors are primarily involved. Detailed quantitative studies of the somatomotor reaction threshold to tooth pulp stimulation in animals indicated that the behaviour evoked by dental stimulation was a pain response and not merely a simple reflex reaction (Radouco-Thomas, Nosal and Radouco-Thomas, 1962) while verbal reports of pain have been obtained in man under similar experimental conditions (Heng-Chin and Domino, 1961).

Electrical impulses evoked by suprathreshold tooth pulp stimulation were found to be transmitted along at least five distinct neural routes (Kerr, Haugen and Melzack, 1955): (1) a short-latency, dominantly contralateral projection of the trigeminal lemniscus; (2) a short-latency, bilateral trigemino-bulbo-thalamic pathway; (3) a secondary bilateral trigeminal pathway of medium

latency in the ascending portion of the central tegmental fasciculus; (4) a medium latency, bilateral projection pathway ascending in the intermedio-lateral area of the central gray; and (5) a long latency, bilateral pathway within the central core of reticulated cells.

Heng-Chin and Domino (1961) found that morphine in doses of 1 to 3 mg/kg, given intravenously, had no significant effect on the short latency components of the cortical evoked potential recorded in the primary afferent pathway (contralateral medial lemniscus, nucleus ventralis posteromedalis and coronol-gyrus) of dogs from single shock stimulation of the tooth pulp but reduced the longer latency negative components of the secondary pathway. These effects of morphine were nonspecific, however. A similar effect was seen on the longer latency components of the potentials evoked by stimulation of the median nerve, which subserves mainly touch and proprioception, while large doses of pentobarbital (20 to 30 mg/kg) produced the same phenomenon.

The actions of morphine on the secondary pathways in the diencephalon, midbrain and medullary reticular formation were too complex and variable for the authors to make any conclusive generalizations with respect to the effects of morphine at a given site. Potentials were usually enhanced in the areas surrounding the central gray (although areas within the central gray were characteristically

depressed), in many of the diencephalic nuclei and in most of the medullary reticular areas but occasionally they were depressed or remained unaffected. The responses in some thalamic nuclei, however, particularly in the centralis lateralis, centralis medialis and medialis dorsalis, tended to be suppressed in the majority of cases. The effects of nalorphine were equally variable, but, in general, antagonized the effects of morphine.

In contrast, Mizoguchi (1964) found some short latency responses in the ipsilateral spinal nucleus of the trigeminal nerve and the contralateral nucleus ventralis posterior of the thalamus which were selectively blocked by morphine (2 to 6 mg/kg iv.). When the spinal nucleus of the trigeminal nerve itself was stimulated, the potentials evoked in the nucleus ventralis posterior of the thalamus and the cerebral cortex were also depressed. On the other hand, the short-latency potentials evoked in the main sensory nucleus of the trigeminal nerve by stimulation of the tooth pulp or gingiva were unaffected by the same doses of morphine, as were the potentials elicited in the nucleus ventralis posterior medialis and coronal gyrus in response to stimulation of the main sensory nucleus itself. Thus Mizoguchi concluded that the major site of action of morphine was the nucleus of the spinal tract of the trigeminal nerve. This site was not

affected by 20 mg/kg doses of pentobarbital.

On recording single unit discharges in both the nucleus sensorius superior and the nucleus tractus spinalis n. trigemini in the spinal cat, Sasa (1969) found that similar doses of morphine (1-6 mg/kg iv.) depressed the firing rate of only relatively long-latency neurons in both nuclei. However, the drug was shown to have some differential effects upon the two nuclei: morphine increased the spontaneous discharge of the neurones in the nucleus sensorius superior but decreased the spontaneous firing rate of those in the nucleus tractus spinalis.

While most investigators limited their study to the effects of morphine alone or in comparison with other strong analgetics, Mitchell and co-workers (Straw and Mitchel, 1964; Nakamura and Mitchell, 1971) compared the effects of morphine with other nonanalgesic central nervous system depressants upon potentials evoked both by noxious (tooth pulp) and by non-noxious (gingival) stimulation. These authors felt, and quite rightly so, that if these effects on evoked potentials have any relevance to morphine analgesia, then there should be a qualitative and/or quantitative difference in these responses depending upon whether or not the compound in question possesses analgesic properties and the stimulus applied is a noxious

one.

Mitchell and his associates recorded potentials in the nucleus ventro-posteromedialis and caudal portion of the spinal trigeminal tract (primary short-latency pathways), in the periaqueductal gray matter and central tegmental fasciculus (medium latency secondary pathways) and in the dorsal and ventral tegmentum of the mesencephalic reticular formation (true secondary long-latency pathways) in cats immobilized with gallamine triethiodide. The agents studied by these investigators were morphine (1-4 mg/kg), nalorphine 2-8 mg/kg), pentazocine (2-8 mg/kg), pentobarbital (2.5-10 mg/kg) and chlorpromazine (1-4 mg/kg) and given intravenously.

The responses in the primary and central tegmental pathways were depressed by chlorpromazine and pentobarbital but were not affected by the narcotic analgetics. All drugs, except for chlorpromazine and the lower doses of nalorphine, depressed evoked potentials in the central gray. These results are in agreement with those of Heng-Ching and Domino (1961), and with the observation of Haugen and Melzack (1957) that an analgesic mixture of nitrous oxide and oxygen (4:1) consistently depressed the central gray response to tooth pulp stimulation.

Mitchell and colleagues also found the effects of

morphine on the reticular formation to be highly complex and dependent upon electrode location. All doses of all drugs except the lowest dose of nalorphine depressed the potentials evoked in the dorsal and ventral mesencephalic tegmentum. The effects of morphine were not dose-related and both the responses to noxious and to non-noxious stimulation were suppressed. Pentobarbital also significantly depressed both types of responses but in a dose-related manner. Chlorpromazine had no effect on the response elicited by gingival stimulation (non-noxious) but depressed that by tooth pulp stimulation (noxious). Mitchell and Killam (1964), on the other hand, observed no effect of morphine, in doses up to 2 mg/kg, on the evoked potential to tooth pulp stimulation in the dorsal tegmentum lateral to the periaqueductal gray. This area was, however, more medially and caudally located than the one studied by Mitchell and co-workers.

Except for the similarity in the effects of morphine on the primary pathways and central gray, the investigations of Mitchell and colleagues showed depressant effects of morphine in areas where Heng-Chin and Domino (1961) found enhancement. With regard to the effects of morphine on the mesencephalic reticular formation, Schimmerl and Stumpf (1958) found neither an enhancement nor a suppression of the spontaneous activity

of neurons within the red nucleus of rabbits in doses up to 120 mg/kg iv. That variability in electrode position is of crucial importance in studying the effects of morphine on the reticular formation was clearly demonstrated by Valdman (1962) who showed that morphine can have either a depressant, a facilitatory or no effect at all, depending upon the morphological peculiarity of the particular structure within the reticular formation that is being activated. Collectively, therefore, these data suggest that the action of morphine is very highly localized to specific regions of the reticular formation, in contrast to that of the barbiturates which depress all reticular responses, regardless of area.

The relevance of many of the investigations cited above with respect to analgesic mechanisms is open to question. Although an almost purely nociceptive afferent system is being stimulated, nonanalgesic agents were often equally or even more effective than morphine in blocking responses thought to be specific for pain. Conversely, morphine occasionally exerted an effect on responses not ordinarily implicated in pain processes (e.g., depression of evoked responses within the visual system) while leaving those associated with pain mechanisms intact (Mitchell and Killam, 1964). The above data, therefore, do not allow one to make any definite conclusions with respect to the

central site or sites mediating the antinociceptive actions of morphine-like compounds.

The most recent studies concerned with the effects of narcotic analgetics on the potentials evoked by tooth pulp stimulation at various levels of the central nervous system (Schmidt and Ruthrich, 1972; Ruthrich, Schmidt and Matthies, 1972) are perhaps the most valid since they alone were done on unanesthetized, unrestrained animals. The narcotic analgetics (morphine, 5-10 mg/kg, hydromorphine, 1-10 mg/kg, hydrocodone, 5-10 mg/kg and pethidine, 10 mg/kg), given subcutaneously to rabbits, were shown to produce a dose-dependent depression of the amplitude of the primary potential as well as a delay in the latency of the response in all the brain areas studied (trigeminal nerve, ventrolateral thalamus, sensorimotor cortex and dorsal hippocampus).

In these investigations, only the depression of the negative wave component of the primary potential was correlated with the raise in threshold for the jaw opening reflex; no relationship was found between the influence upon the surface-positive wave of the primary potential and the stimulus intensity necessary to elicit the jaw reflex. This correlation was specific for the analgetics. Other compounds, such as pentobarbital, pernocton, urethane and chlorpromazine, which also raised the threshold of the

motor reaction to tooth pulp stimulation, did not show such a relationship. Nalorphine selectively antagonized the changes in potentials caused by the narcotic analgetics. The authors felt that the differential action of the analgetics on the negative component of the primary potential was indicative of an action on inhibitory interneurones. The inhibitory effects of morphine were greater at low frequency of stimulation than at high and the greatest effect was observed in the trigeminal nucleus. In any event, their data is strongly in favour of the suggestion that morphine acts not at a single site but at many levels along the neuraxis.

From the above discussion it may be concluded that the neural mechanisms of morphine analgesia of tooth pulp pain are complex. It does not appear that a simple depression or blockade of single afferent impulses arising from the peripheral tooth pain receptors can explain the analgesic action of morphine. Earlier experiments seemed to indicate that the primary afferent tracts were resistant to the actions of morphine but later studies, perhaps because of an increasing emphasis on more sophisticated electrophysiological techniques and a decline in the use of anesthetic and curare-like agents, have implicated these pathways (particularly the nuclei of the trigeminal nerve) as a possible central site of action of morphine as

well.

The effects of the narcotic analgetics on the secondary pathways are variable. The question arises whether these discrepancies could be explained entirely on the basis of drug dosage, species variability and position of stimulating and recording electrodes.

It is interesting to note, in this respect, that the intra-arterial or intraperitoneal injection of bradykinin, which is also considered to be a purely nociceptive stimulus (Lim, 1967, 1968; Conseiller, Wyon-Maillard, Hamann and Besson, 1972), produced a marked and selective increase in the activity of neurons in the dorsal horn of the spinal cord (Satoh, Nakamura and Takagi, 1971), particularly of those located in lamina V of Rexed (Besson, Conseiller, Hamann and Maillard, 1972) and in the extralemniscal pain pathways (medullary and midbrain reticular formation, centromedian-parafascicular complex, dorsomedial and posterior nuclei of the thalamus and nucleus of the posterior commissure) of the cat and rabbit (Lim, Krauthamer, Guzman and Fulp, 1969; Krauthamer, Lim, Guzman and Fulp, 1970; Conseiller, et al., 1972). Little or no change in the firing frequency was observed from the neurones in the ventral posterolateral nucleus of the thalamus. Small doses of morphine (1-2 mg/kg iv.) completely abolished these effects of bradykinin (Lim, et al.,

1969; Krauthamer, et al., 1970).

Studies on potentials evoked by stimulation of somatic and visceral nerve afferents

The effects of the narcotic analgetics of the potentials evoked at different levels of the CNS by stimulation of exteroceptive and enteroceptive nerve afferents is another commonly employed method for the assessment of the site of action of these compounds. Their validity with respect to analgetic mechanisms, however, is less clear cut, for these techniques cannot differentiate between potentials elicited by pain from those produced by the stimulation of other cutaneous sensory modalities.

The most frequently quoted studies utilizing this line of investigation are those of Fujita, Tasuhara and Ogiu (1953), Fujita, Tasuhara, Tamamoto and Ogiu (1954), Sinitsin (1961, 1964) and McKenzie and Beechey (1962).

The first of these investigations (Fujita, et al., 1953, 1954) comprises an exhaustive study comparing the effects of narcotics, analgetics and hypnotics on the potentials evoked in various parts of the central nervous system by stimulation of somatic (sciatic and radial) and visceral (phrenic, inferior cardiac, vagus) nerves, as well as on potentials evoked by stimulation of structures within the brain. These experiments were carried out in

barbiturate anesthetized cats and rabbits. The parameters investigated consisted of primary responses (effects related to the conduction along specific afferent pathways, i.e., the lemniscal system), secondary responses originating through the complicated polysynaptic pathways (non-specific extralemniscal system), suppression of spontaneous activity, after-discharge and the absolute refractory period. The authors satisfied themselves that these responses were, in most cases, related to pain mechanisms. For the present, the discussion will be confined to the results of the evoked potential studies; the other actions of the narcotic analgetics will be described in the following section.

In their first study, Fujita and co-workers (1953) reported that large doses of morphine (10 mg/kg iv.) depressed, but did not abolish, the secondary response evoked in both sides of the cortex by sciatic or radial (somatic) nerve stimulation, whereas similar doses had no effect on the primary somesthetic cortical response. These effects on somatic nerve stimulation were confirmed by Valdman, 1967). In contrast to the relative lack of effects on somatic nerve stimulation, lower doses of morphine (6 mg/kg iv.) completely suppressed the primary and secondary cortical evoked potentials to splanchnic nerve (visceral) stimulation. Furthermore, the visceral

potentials evoked in the thalamus, medial lemniscus and medulla were abolished at the same time as the cortical potentials. When recording electrodes were placed at various levels of the spinal cord (i.e., in ipsilateral spinothalamic tract from T_2 to T_{10}), this same dose of morphine (6 mg/kg iv.) blocked the responses to splanchnic but not to sciatic, stimulation at all locations. Since morphine did not affect the action potentials recorded from the dorsal roots, the authors concluded that morphine selectively depresses the afferent pathways for visceral pain at the level of the spinal cord beyond the first order neuron. This conclusion was supported by a subsequent study (Fujita, et al., 1954), where it was shown that morphine (6 mg/kg iv.) had the same effect on the afferent pathway of other visceral nerves (phrenic, inferior cardiac) except for that of the vagus, which appeared to be blocked only at the medullary level (Fujita, et al., 1953).

However, since the somatic nerves carry only a smaller proportion of pain fibers relative to the visceral afferents, the absence of an effect of narcotic analgetics on somatic evoked potentials may, in fact, reflect their lack of an effect on sensory modalities other than pain sensation. Fujita and co-workers (1954) showed that this was indeed the case. Thus, the potentials evoked in the

contralateral medial lemniscus by ipsilateral stimulation of the sciatic nerve was completely suppressed by small doses of morphine (6 mg/kg iv.) when the dorsolateral region of the spinal cord on the same side as the sciatic nerve was ablated. This procedure eliminated the uncrossed, tactile and mechanosensitive division of the sciatic afferent pathway, but left the crossed, pain and temperature component intact. Similar effects were observed following ipsilateral splanchnic or phrenic nerve stimulation. These authors also showed that evoked potentials from other sources of tactile stimulation were not altered even by large doses of morphine (15 mg/kg). It was therefore concluded that one of the principal sites of action of the narcotic analgetics is on the synapses between receptor neurones and connector neurones of pain afferents (visceral and somatic), most of them being located in the spinal cord.

At the midbrain level, morphine (6 mg/kg) depressed the cortical response to repetitive, but not to single shock stimulation (8 cps) of the medial lemniscus, whereas larger doses of morphine (8 mg/kg) had little effect on the somesthetic (sciatic) potential recorded in the medial midbrain reticular formation (Fujita, et al., 1954). It was therefore concluded that morphine and related analgetics inhibit the conduction of repetitive impulses in the lateral

specific sensory pathway to the cortex, but do not impair impulse transmission within the medial multisynaptic pathway. The responses evoked in the posterior hypothalamus by stimulation of the medial lemniscus were also inhibited by morphine (6 mg/kg).

Similar results with respect to sensory nerve stimulation were obtained by Satoh, Yamatsu and Takagi (1970). These authors recorded the potentials elicited in the cerebral cortex, ventral posterolateral nucleus of the thalamus, midbrain reticular formation and ventrolateral funiculus of the spinal cord by sciatic or splanchnic nerve stimulation. The experiments with evoked potentials were carried out in cats anesthetized with a barbiturate or immobilized by curare-like agents. Morphine (6 mg/kg) suppressed the potentials evoked at all levels of the neuroaxis to splanchnic nerve stimulation; potentials evoked by sciatic nerve stimulation were suppressed only in animals with unilateral transaction of the half of the spinal cord ipsilateral to the stimulated side. Nalorphine and a new morphine antagonist, RAM-302, reversed the affects of morphine in all cases. In addition, although the antagonists themselves did not affect evoked potentials in the anesthetized cat, even when doses were raised to 20 and 30 mg/kg, 4-5 mg/kg of RAM-302 and 5-10 mg/kg of nalorphine suppressed potentials in the

spinal cord and thalamus evoked by splanchnic nerve stimulation in the unanesthetized preparation. These authors suggested that the antagonistic actions of RAM-302 and nalorphine occurred at the level of the spinal cord, while their inhibition of evoked responses per se might be mediated through a facilatory action on the midbrain reticular formation which forms inhibitory connections with the spinal afferent pathways. It was speculated that these latter effects of the narcotic antagonists might be related to the analgesic properties of these compounds. However, the authors warned that no definite conclusions could be made in this respect until a more specific pain response is found.

Matsumura, Takaori and Inoki (1959), using barbiturate-anesthetized, decerebrate and spinal cats, partially confirmed and extended these observations with morphine alone and in combination with methamphetamine. They were able to show that morphine and methamphetamine depressed the cortical and dorsal funicular spinal cord potential due to splanchnic nerve stimulation.

The main criticism of the work of Fujita, et al. (1953, 1954) is that, apart from inducing anesthesia with barbiturates, which have been shown to profoundly influence the functioning of brainstem afferent systems, even at subanesthetic doses (Killam, Killam and Shaw, 1957), the

doses of morphine used (6-8 mg/kg) were well above those required for analgesia. The effects on evoked potentials reported may therefore not be related to analgesia but to side effects. For these reasons, McKenzie and Beechey (1962) were careful to correlate the effects of morphine and pethidine on the midbrain-evoked potentials to supra-maximal, single shock tibial nerve stimulation with analgesic doses of these compounds in cats immobilized with gallamine. Since lightly-restrained, conscious dogs acted as if in pain under similar conditions of stimulation, the authors felt that they were dealing with a mixed sensory stimulus which was interpreted as pain. In these studies, potentials were evoked in large areas of the midbrain: medial lemniscus, paralemniscal area, spino-bulbothalamic tract, central gray, central tegmental fasciculus, dorsal and ventral tegmentum, superior colliculus, red nucleus and perirubral areas.

McKenzie and Beechey (1962) found that both morphine and pethidine, in low doses giving rise to analgesia with minimal side effects (1-2 mg/kg and 2-4 mg/kg iv. respectively) preferentially depressed the responses evoked in the lateral and ventral regions of the midbrain. The lateral mesencephalic areas most sensitive to the analgetics were regions contiguous with the medial lemniscus complex on its lateral and medial aspects. The morphine sensitive

ventro-lateral region was located ventral to the red nucleus and contains fibers ascending to subthalamic, hypothalamic and septal areas. Higher doses of morphine (4-6 mg/kg iv.) were necessary to depress the responses recorded from the medial lemniscus itself. The authors felt that at least part of morphine's effects on lemniscal responses, especially at the higher doses, may have been through an indirect action on the more sensitive para-lemniscal neurones. Structures lying in the medial core of the mesencephalic reticular formation, usually subsumed under "reticular arousal system", were not generally affected until higher doses (4-6 mg/kg) were given. Of these areas, the red nucleus and perirubral areas were the most resistant, whereas the central gray was the most sensitive to the effects of morphine. The least consistent action of morphine was that on the areas classed as spinothalamic and spino-bulbo-thalamic tracts. Doses of 2 to 4 mg/kg depressed the responses in only half of the animals. At 6 mg/kg, depression of response amplitude without block, or complete block, occurred for recording locations in medial and lateral sectors respectively.

McKenzie and Beechey found the actions of pethidine to be essentially the same as those of morphine except for its effects on the deep tectal responses in the superior colliculus. These responses were depressed by morphine

(2 mg/kg) whereas they remained unaffected by even the highest dose (7 mg/kg) of pethidine used. Since bilateral ventrobasal thalamic responses were not depressed by analgesic doses of morphine, the depression by morphine of spinothalamic responses at midbrain levels were felt by these authors to be due, at least in part, to morphine's effect upon the more sensitive tectopetal pathways which overlap the spinothalamic fibers in this region.

Since the actions of anesthetic substances (nitrous oxide, diethyl ether and pentobarbital) were in direct contrast, anatomically, to those of morphine and pethidine, McKenzie and Beechey concluded that the depression of peripheral midbrain areas was related to the analgesic effect of the opioids, whereas the depression of the medially located pathways of the ascending reticular system was related to their narcotic effect. Fujita, et al. (1953, 1954) and McKenzie and Beechey (1962) therefore reached the same conclusions, namely, that morphine and its derivatives exert their analgesic action through an inhibition of the lateral, specific pathways subserving discriminative sensory function.

The results of McKenzie and Beechey (1962) are also in general agreement with the tooth pulp data obtained by Mitchell and co-workers (Straw and Mitchell, 1964; Makamura and Mitchell, 1971). On the other hand, they are contrary

to those of Heng-Chin and Domino (1961), who, except for a depression of responses in the central gray, observed an enhancement of the response amplitudes in the midbrain reticular formation following morphine (1-2 mg/kg iv.).

Sinitsin (1961, 1964) testing the specificity of analgetics on afferent sensory input corroborated the earlier clinical and experimental observations that these drugs selectively depress pain reactions without affecting consciousness or other somesthetic modalities (Wikler, 1950), but his conclusions contradict those of Fujita, et al. (1953, 1954) and McKenzie and Beechey (1962). Sinitsin studied the influence of various narcotic analgetics on potentials evoked in specific and non-specific pathways by sciatic nerve, auditory, visual and subcortical stimulation in cats immobilized with succinylcholine. Evoked potentials were recorded in the somesthetic I and II, auditory I and II, and visual I and II specific projection areas of the cortex, in the motor cortex, in frontal and parietal associative areas, in the specific relay, associative and nonspecific nuclei of the thalamus, in the specific projection system of the medial lemniscus, and in the midbrain reticular formation.

Sinitsin showed that analgesic doses (1-3 mg/kg iv.) of morphine, trimeperidine and methadone did not affect potentials evoked by sciatic, auditory or visual stimulation

in the appropriate primary sensory areas of the cortex, specific thalamic nuclei or medial lemniscus. Larger doses of morphine (5-10 mg/kg), however, enhanced the primary cortical response in most sensory areas (except for a depression of the auditory and somatic evoked potentials in areas VisI and VisII) and in the motor cortex. Although small doses of the analgetics greatly potentiated (in the associative cortex), slightly enhanced, or did not effect (in the brainstem association areas) associative responses to acoustic and photic stimuli, somesthetic potentials were markedly depressed in all association areas. The depression of somatic responses was less pronounced in the diffuse thalamic nuclei than in the associative nuclei of the thalamus.

In contrast to the observations of McKenzie and Beechey (1962), Sinitsin found that the response to somatic stimulation in the midbrain reticular formation was only slightly reduced. Although morphine and its derivatives did not decrease the primary response in cortical area SI to electrical stimulation of the ML, these agents did depress the association response recorded from the parietal lobe. Only the long-latency sensorimotor cortical component in response to stimulation of the associative and diffuse thalamic nuclei was depressed by these analgetics. From these data, Sinitsin concluded that

the morphine group of analgetics do not fully depress the associative and reticular structures of the brain but that they selectively block the connections of the classical afferent pathways with the associative and non-specific projection systems of the brain, especially those concerned with somesthetic stimuli.

However, the narcotic analgetics do not always have the same effect upon somesthetic evoked potentials. Millan and Besson (1964), for instance, have demonstrated that morphine depresses the responses evoked in the centre median and sensory cortex (area SI) by stimulation of somatic afferents in high spinal or gallamine immobilized cats whereas its synthetic derivatives, meperidine, fentanyl and phenoperidine enhanced them.

Domino (1968) has extended these evoked potential studies to human subjects. He found that progressive destruction of the centre median and ventral postero-lateral nuclei of the thalamus reduced the size of the late negative component of the potential evoked in the somesthetic cortex to median nerve stimulation; meperidine, in intravenous doses of 50-100 mg, had similar effects suggesting a depressant action on both specific and diffuse thalamic nuclei.

Studies on the electrical activity evoked by stimulation of structures within the central nervous system

The EEG synchronization produced by low frequency stimulation of the diffuse thalamic nuclei is characterized by a wide dispersion of long-latency negative waves across much of the cortex which tend to grow or "recruit" with each succeeding stimulus. Since the recruiting response occurs following stimulation almost anywhere within the non-specific thalamic projection system, elicitation of cortical recruitment phenomena has become the paradigm of non-specific thalamic nuclear activity. In humans, such synchronized EEG activity is accompanied by a loss of awareness of, and in attention to, specific sensory stimuli (Skinner and Lindsley, 1967). An effect of the narcotic analgetics on such neurophysiological phenomena may therefore be related to their analgesic mechanism of action. In the following paragraphs, the effects of the narcotic analgetics on these and other cortical responses elicited by stimulation of various structures within the central nervous system will be examined in the light of such a proposal.

During their study on the influence of analgetics on the various components of the lateral and medial afferent pain pathways, Fujita, et al. (1954) noted that morphine (6 mg/kg) did not alter the cortical response

to single shock stimulation of the medial lemniscus but greatly reduced the cortical potentials upon repetitive stimulation (8 Hz) of the same structure; on the other hand, these drugs had no significant effect on the cortical responses following repetitive stimulation of the nucleus ventralis posterior lateralis of the thalamus. These results were taken to indicate that morphine and related drugs prolong the refractory period of the neurons synapsing in this nucleus. It is interesting that, according to the data of Carroll and Lim (1960), this nucleus is necessary for the development of the entire complex of the pain response in rats receiving nociceptive electrical stimuli to the tail. Matsumura, et al. (1959) and Satoh, et al. (1970) confirmed the fact that the augmenting response recorded from the sensory cortex to repetitive stimulation of the medial lemniscus is depressed by 6 mg/kg of morphine.

Fujita, et al. (1954) also noted that morphine, in doses of 8 mg/kg iv., decreased the cortical recruiting response elicited by repeated stimulation (10 Hz) of the nucleus centralis lateralis and centre median, but enhanced the cortical response to repetitive stimulation of the nucleus ventralis anterior in amytal anesthetized cats. A depression of recruitment from stimulation of the centre median was also reported by Matsumura, et al.

(1959) in the high spinal cat. This suppression of the intralaminal thalamic nuclei was thought to be related to the reduction in consciousness produced by the narcotic analgetics, rather than to their primary analgesic action. The observation of Fujita, et al. (1954) that very large doses of morphine (200 mg/kg), given intraperitoneally, produces EEG responses similar to those elicited by repetitive stimulation (7-20 Hz) of the medial thalamic structures supports this suggestion.

In contrast to the above findings, an enhancement of recruitment due to stimulation of the thalamic intralaminar system was obtained by Gangloff and Monnier (1957) with large doses of morphine (20-40 mg/kg iv.) and levorphanol (10 mg/kg iv.) in unanesthetized rabbits. Similar results were obtained by Heng-Chin and Domino (1961) with much smaller doses of morphine (1-2 mg/kg iv.) in dogs immobilized with decamethonium. Gangloff and Monnier (1957) were able to distinguish between the action of analgetics and phenothiazine tranquillizers on the basis of their effects on the reticular formation and intralaminar thalamic systems. According to these authors, the narcotic analgesic agonists and antagonists act upon brain structures which are functionally antagonistic; the agonists activate the medial thalamic system and depress the midbrain reticular formations, whereas

the antagonists stimulate the ascending reticular formation; phenothiazine tranquillizers do not affect the reticular formation but suppress the thalamic system.

The doses of morphine used by Gangloff and Monnier (1957) greatly exceeded the maximum analgesic intravenous dose required for the rabbit (2.5 mg/kg, Radouco-Thomas et al., 1962). These experiments were, therefore repeated, and confirmed, by Monnier and co-workers (Monnier, Nosal and Radouco-Thomas, 1962, 1963; Monnier and Nosal, 1968) in unanesthetized rabbits with smaller, analgesic dose levels of pethidine. They observed that pethidine, at doses which markedly increased the threshold of the nociceptive reaction to electrodental stimulation (5-10 mg/kg iv.), transitorily (30 minutes) increased the amplitude of the cortical recruiting response elicited by low frequency (3 Hz) stimulation of the unspecific medio-central thalamus and concomitantly depressed the cortical arousal reaction to high frequency stimulation (150 Hz) of the midbrain reticular system and postero-ventral hypothalamus.

On the contrary, the antipyretic analgetics (aminopyrine, acetophenetidin, acetylsalicylic acid), at doses which also raised the nociceptive threshold (though much less markedly than pethidine), either facilitated the arousal reaction and inhibited the unspecific

thalamic system or failed to alter these responses. Thus, the depression of the reticular and hypothalamic activating systems and the simultaneous stimulation of the medial thalamic projections could not be related to analgesia per se. The authors therefore concluded that the effects of the narcotic analgetics on these systems were a reflection of their hypnotic rather than their analgesic action.

However, in view of the well-documented evidence that these neurophysiological mechanisms play a significant role in central pain processes (Melzack and Wall, 1965, 1968; Lim, 1966; Albe-Fessard, 1968) and of the fact that the narcotic and antipyretic analgetics have a different mechanism of action, this conclusion is not necessarily justified. Therefore, while recognizing that there may be other varieties of analgesia, it would not be unreasonable to assume that these neurophysiological mechanisms, and the effects of narcotic analgetics thereon, are related to some of the processes involved in analgesia, at least of the morphine type.

These workers (Monnier, et al., 1962, 1963), like Fujita, et al. (1953, 1954), also reported that the somesthetic cortical responses elicited by low frequency repetitive stimulation (3 Hz) of the specific postero-ventro-lateral thalamus was not altered by the narcotic

analgetics. At the same time, however, the potentials evoked in the somesthetic cortex by stimulation of dental "pain" receptors were suppressed. Their data was therefore taken as supportive evidence that the narcotic analgetics act preferentially upon unspecific polysynaptic than upon specific oligosynaptic afferent systems.

While it appears likely that the narcotic analgetics have some action on the unspecific thalamic nuclei, it must be pointed out that not all investigators observed an effect of morphine on cortical recruiting responses (Deneau and Takaori, cit. Domino, 1962; Straw and Mitchell, 1964; Mizoguchi and Mitchell, 1969). The precise site and mechanism whereby the narcotic analgetics exert the effects described above therefore remain to be elucidated.

In view of the evidence which firmly establishes the importance of limbic structures in central pain mechanisms (Delgado, 1955; Delgado, et al., 1956; Akert, 1961; Brady, 1961; Spiegel and Wycis, 1961; Melzack and Wall, 1965, 1968; Runnels and Thompson, 1969; Poncy, Bernard and Chernov, 1972; Melzack, 1973) and of the suggestion made by a number of authors that the analgesic drugs exert their principal, if not entire, effect upon the behavioural reaction to pain (Wikler, 1958; Beecher,

1959; Soulairac, Gottesmann and Charpentier, 1967; Charpentier, 1968), it is not unlikely that certain limbic structures play a role in the antinociceptive action of the narcotic analgetics. Studies prior to 1950 that have dealt with the effects of the narcotic analgetics on the limbic system have been extensively reviewed by Wikler (1950). The electrophysiological investigations on the limbic actions of the narcotic analgetics that have been done during the past two decades are described in the following pages.

Monnier, et al. (1962) found, as did Gangloff and Monnier (1957), that the hippocampo-cortical projections were stimulated by the narcotic analgetics. Electrical excitation of the hippocampus, under morphine (20 mg/kg iv.), produced an increased response reactivity in the sensorimotor and parietal-occipital cortex, in the diffusely projecting thalamic nuclei, in the hippocampus itself and in the midbrain reticular formation; hippocampal afterdischarge was unaltered. In view of the fact that the rhinencephalon plays an inhibitory role in affective behavior, the activation of this region of the brain by the narcotic analgetics was considered to be related to the anti-anxiety effects of these compounds.

However, as noted previously, the doses used by these investigators were far in excess of the maximal

analgesic dose required for the rabbit. An entirely different view was presented by Soulairac, et al. (1967) who investigated the effects of various narcotic analgetics (morphine, 8-20 mg/kg, pethidine, 20-40 mg/kg, dextromoramide, 1-2 mg/kg, given intraperitoneally) on hippocampal activity in unanesthetized rats. According to these authors, the narcotic analgetics produced the high voltage, "spiking" activity within the hippocampus that was observed by Monnier and co-workers only at toxic doses which also induced epileptic seizures. On the other hand, smaller, analgesic doses of these compounds (8 mg/kg of morphine, 20 mg/kg of pethidine, 1 mg/kg of dextromoramide) inhibited -- rather than stimulated -- the hippocampal synchronization elicited by a painful shock to the tail of rats with chronic, indwelling electrodes; the cortical arousal reaction was much less depressed than the hippocampal response, while effects on the reticular formation of the upper brain stem were minimal. Furthermore, hippocampal arousal (synchronization) was associated only with the complex affective component (crying, biting of electrodes) of the pain response evoked by suprathreshold intensities of stimulation; stimuli at, or just above, the threshold level produced a startle or escape reaction, but did not alter the neural activity of the hippocampus. It was

therefore concluded that analgetics such as morphine alter the emotional reaction to pain by reducing the "affective vigilance" mediated by rhinencephalic structures.

These findings are consistent with those of McKenzie (1964) who demonstrated that morphine (2 mg/kg iv.) and pethidine (4 mg/kg iv.) depressed or inhibited somatic evoked potentials in the hippocampus of the cat, but did not modify the potentials elicited at the level of the cortex. In subsequent studies, Monnier, et al. (1963) and Monnier and Nosal (1968) also reported that low doses of pethidine (1-10 mg/kg iv.) did not alter the potentials evoked in the cortex by low frequency (3 Hz) stimulation of the hippocampus.

In agreement with the results obtained by Sinitsin (1964) and McKenzie (1964), Nakamura and Mitchell (1972) found that morphine reduced the sensory input into limbic structures, without altering the bioelectrical transmission within these areas. In their investigation, performed on halothane anesthetized cats, morphine, in doses of 2-4 mg/kg iv., depressed the entorhinal responses evoked by stimulation of the radial nerve or mesencephalic reticular formation but not those recorded from pathways within the limbic system (septum to hippocampus, hippo-

campus to septum and entorhinal cortex, entorhinal cortex to hippocampus, and pyriform cortex to entorhinal cortex). McKenzie (1964) also reported no significant change in the hippocampal activity following septal stimulation after 2 mg/kg morphine or 4 mg/kg meperidine in the cat.

Morphine, in a dose of 6 mg/kg, was also shown to abolish the potentials elicited in the posterior hypothalamus by stimulation of the medial lemniscus (Fujita, et al., 1954). These results are in agreement with those of Deneau and Takaori (cit. Domino, 1962) who demonstrated that morphine increased the threshold of the posterior hypothalamus for arousal and startle in the monkey. Other investigators have reported a direct depressant effect of morphine (3-5 mg/kg iv.) on the firing frequency of single cells in the anterior hypothalamus in urethane anesthetized rats (Eidelberg and Bond, 1972) while Wikler (1950) has extensively reviewed the mixed depressant and stimulant effects of morphine upon the hypothalamus.

Cumulatively, the data presented above strongly implicate the limbic system as one of the central sites of action of the narcotic analgetics. This view is supported by the recent investigation of Hiller, Pearson and Simon (1973) in which it was demonstrated that most

of the areas within the human brain which exhibited the highest level of stereospecific opiate binding were components of, or associated with, the limbic system.

In this and the previous sections, the actions of morphine on afferent pathways have been emphasized. It should be noted, however, that morphine and related drugs also exert an effect on the descending pathways within the brain stem. Fujita, et al. (1954), for example, have demonstrated that morphine abolishes the corticofugal projection responses produced in the intralaminar nuclei and midbrain reticular formation by strychnization or high frequency (100 Hz) electrical stimulation of the cortex. These results have been confirmed by Gangloff and Monnier (1957) and by Valdman (1967).

Studies on pain-induced EEG activation

The relative effectiveness of the narcotic analgetics in suppressing the pain-induced electrical excitability of the various functional brain structures is another common approach used in an attempt to localize the site of action of these compounds. Various afferent stimuli have differential effects in producing neocortical activation (EEG desynchronization). In the barbiturate anesthetized animal, the order of effectiveness in

eliciting the EEG arousal reaction is nociception, proprioception, auditory and visual stimulation (Gellhorn, 1953, cit. Domino, 1968).

Fujita, et al. (1953) have shown that the suppression of spontaneous cortical activity produced by stimulation of the sciatic nerve, midbrain reticular formation or medial thalamic nuclei is abolished by small doses of morphine (6 mg/kg iv.) in the cat and rabbit. Small doses of barbiturates (2 mg/kg) inhibited the response to stimulation of the sciatic nerve and mesencephalic reticular system but not that to stimulation of the medial thalamus. Since the spontaneous cerebral activity which was abolished by large doses of barbiturates reappeared following the administration of morphine, while arousal signs were not observed in animals, it was concluded that morphine stimulated the medial thalamic nuclei, but did not affect the reticular structures. The authors suggested that this effect of morphine might be the mechanism whereby the narcotic analgetics exert their selective inhibition of pain perception.

Silvestrini and Longo (1956) have confirmed earlier reports (Wikler, 1950) that morphine selectively depresses EEG arousal to painful stimuli. These authors noted that morphine, at doses of 5-10 mg/kg iv., blocks

the activation reactions to nociception in unanesthetized rabbits whereas the response to other sensory stimuli (tactile, acoustic, optic or stimulation of the midbrain reticular formation) was preserved. In addition, the threshold to excitation of the anteromedial thalamus was elevated, but not that to stimulation of the mesencephalic reticular formation. This was in direct contrast to the action of other narcotic drugs, such as scopolamine and pentobarbital, which blocked EEG activation to non-painful stimuli more than to nociception and which produced an increase in the threshold for excitation at both the thalamic and mesencephalic levels. Silvertrini and Longo therefore concluded that morphine produced a specific blockade of EEG activation to nociceptive stimuli at the thalamic level.

Radouco-Thomas and co-workers (1962) extended these studies by recording, in conscious rabbits, the activating response not only from the cortex, but also from various other subcortical structures as well. The characteristic cerebral activity after morphine--an alteration of alert (low voltage, fast frequency) and drowsy (spindles 12-14 Hz) states--was also typical of recordings from the rhinencephalon, the medial and ventrolateral thalamus and the midbrain reticular formation. Morphine, at a dose of 2.5 mg/kg iv., the maximal anal-

gesic dose for the rabbit, selectively blocked the reaction to tooth pulp stimulation, only slightly reduced the arousal reaction produced by auditory stimulation and did not alter the EEG activation due to stimulation of the midbrain reticular formation.

Although Killam (1962) found no evidence for a selective action of morphine on the reticular formation, Valdman (1967) reported that different analgetics suppressed the EEG activation in response to various afferent stimuli to varying degrees. Promedol, a meperidine derivative, and phenadone (methadone), in doses of 1-2 mg/kg, strongly and equally suppressed EEG activation to sound, light and painful stimuli. Morphine, in doses of 2-3 mg/kg blocked at first the reaction to light, and then to pain (5-6 mg/kg), while only slightly decreasing EEG arousal to sound. Low doses of all of these analgetics (1-2 mg/kg), however, suppressed completely the activation reaction to enteroceptive excitation, supporting the conclusions of Fujita, et al. (1954) that morphine and related narcotics act preferentially at the second or third order neurones for visceral pain.

Albus and Herz (1972), although they too found that analgesic doses of morphine equally inhibited the EEG activation produced by nociceptive and non-nociceptive stimuli, believe these effects of morphine to be mediated

by structures in the vicinity of the fourth ventricle. They also postulated that the EEG synchronization observed after the systemic administration of morphine is not due to a direct action upon the reticular core of the brain-stem, but rather, is the result of a selective inhibition of the activating mechanisms originating in the medial hypothalamus and midline thalamic nuclei.

From the above discussion, it becomes obvious that the relationship between the analgesic action of morphine and its effect on pain-induced EEG arousal is unclear. It is difficult to quantify a self-regenerative reaction such as EEG activation and to equate the intensity of the different stimuli with respect to their capacity to elicit such a reaction. Many of the reported discrepancies in the actions of morphine may be related to differences in techniques, species, doses and the intensities of the sensory modalities employed. The EEG is sensitive to environmental and homeostatic changes, so much so, that artefacts are often considered part of the effect. High doses of the narcotic analgetics, for instance, produce respiratory and cardiovascular effects which, in themselves, affect the EEG; the neuromuscular blocking agents, alone, can cause EEG synchronization due to reduced proprioceptive drive. The exact neurophysiological mechanism whereby morphine produces alterations in the EEG (impediment

of summation capacity, prolongation of positive after-discharge, etc.) still remains to be elucidated. The complete explanation must await more information on both the brain systems involved and the specific actions of morphine on these systems.

2. Neurosurgical Studies

A comparison of the effects of the analgetics before and after the selective destruction of specific brain structures or by sectioning the neuraxis at various levels also provides information, albeit of a cruder type, regarding the site of action of these compounds.

The reports of Irwin, et al. (1951) and Bonnycastle, et al. (1953) have indicated that analgetics are capable of raising the threshold to radiant heat stimulation of the tail in both acute and chronic spinal rats. The response of the spinal animal was undistinguishable from that of the intact rat except that the potency of the analgetics was reduced by about 70% after spinal transection. Since the tail flick response used by these investigators is a spinal reflex, physiologically resembling the nociceptive flexor and crossed-extensor reflexes, the locus of the depressant action of the narcotic analgetics in the spinal cord was considered to be the internuncial neuron system. The larger doses necessary

to depress the tail flick response in the spinal animal could not be explained entirely on the basis of the release of spinal cord centers from tonic central inhibitory control, thus requiring higher concentrations of the drug to override these effects. It was therefore concluded that the analgetics augmented the supraspinal inhibitory mechanisms, in addition to having a direct influence upon the spinal cord interneurones. This assumption was later proved to be correct by the electrophysiological studies of Jurna (1966).

An actual stimulatory action of morphine on the bulbar inhibitory area of the cat was demonstrated by Takagi, Matsumura, Yanai and Ogiu (1955). After ipsilateral destruction of the bulbar inhibitory area of the brainstem, morphine (7-14 mg/kg iv.) enhanced both mono- and polysynaptic discharges whereas, after destruction of the ipsilateral facilatory areas, morphine completely diminished polysynaptic response. It was concluded that morphine had stimulant actions on both the bulbar inhibitory and facilitatory areas but that the action on the inhibitory system was predominant.

Since vocalization is the result of the recruitment of higher CNS processes, the animal's cry or squeal is considered to be a better indication of the perception of pain than the reflex responses. In an attempt to disso-

ciate the reflex responses from those motivated by the perception of pain, Carroll and Lim (1960) sectioned the neuraxis of rats at millimeter intervals from the rostral spinal cord to the tip of the frontal pole. They demonstrated that the intact animal responds to increasing intensities of electric shock to the tail first, with a somatic motor response (0.3 ± 0.2 volts), then with vocalization during the stimulation in addition to the motor response, (0.6 ± 0.3 volts) and finally with vocalization after the stimulation (1.2 ± 0.7 volts); morphine blocked these responses in the reverse order. Thus vocalization after-discharge, which required the participation of the highest centers, was selectively blocked with the lowest dose of the drug and remained blocked even when the stimulus was raised to four times the threshold value. Rats with cortical ablations above the diencephalic level, or with brain transections rostral to the thalamus, deviated little from normal. With transection of the brain between the thalamus and midbrain, vocalization after-discharge was abolished and, with transection caudal to the medulla, vocalization during nociception was also eliminated. Noting the similarity of the effects of descending transections of the neuraxis and of increasing doses of morphine on both the nociceptive and postural responses, the authors

attributed morphine analgesia as being due to the blockade of first, the synapses in the thalamus (presumably in the nucleus ventralis posterolateralis) and later, in the brainstem and spinal internuncial.

Although Hoffmeister (1968) was able to distinguish between the analgesic and non-analgesic tranquilizers on the basis of their effects on these two types of vocalization in the rat, a recent report (Weller and Sulman, 1970) raises the question of whether even this type of response is relevant to analgesia. In this study, vocalization to electric stimulation of the tail in mice was unmodified following decortication and neural transection down to the pons. It therefore appears, at least in the mouse tail shock test, that the inhibition of vocalization results from pharmacological actions which are only contingently associated with analgesia. However, the potency of strong narcotic analgetics (i.e. morphine and methadone) was reduced by about 75% following transection of the brain caudal to the anterior commissure. The activity of less potent analgetics (codeine) and of the phenothiazine tranquilizer analgetic (methotrimeprazine) was unaltered. It was therefore concluded, in agreement with Carroll and Lim (1960), that the integrity of the thalamus or its connections with higher structures is essential for the full effect of the strong narcotic analgetics.

Studying the reactions of rats to painful electric stimulation via electrodes implanted at the base of the tail, Charpentier (1968), like Carroll and Lim (1960), postulated that the sequence of reactions observed with increasing intensities of simulation were mediated by different levels of the neuraxis, becoming progressively more complex and integrated as the stimulus intensity was raised. He demonstrated that the narcotic analgetics (morphine, pethidine, dextromoramide) as well as atropine, amphetamine, and lesions in the anterior thalamic nuclei, amygdaloid nucleus and frontal cortex selectively reduced the more complex affective and co-ordinated reactions (vocalization and biting of the electrodes) whereas acetylsalicylic acid, imipramine, reserine, dibenamine and lesions of the mesencephalic reticular formation preferentially diminished the simpler, non-specific, startle and flight reactions. The rhinencephalic areas, mediating vocalization, and the cortical and diencephalo-cortical connections, which are responsible for the spatio-temporal analysis resulting in the biting of the electrodes, were therefore excited only by strong nociceptive stimuli and selectively depressed by the narcotic analgetics.

These neurosurgical techniques therefore support the view that the narcotic analgetics act preferentially upon frontal-thalamic-limbic circuits. On the other hand,

Wikler, Norrell and Miller (1972) found that bilateral lesions in the cingulum, dorsomedial thalamic nucleus, amygdaloid complex, ventral hippocampus and septum had no effect on the development of tolerance to the analgesic effects of morphine in rats, or on the abstinence syndrome consequent to its withdrawal. It therefore appears that the neural sites involved in the development of tolerance and physical dependence are subcortical, perhaps at the hypothalamic level (Kerr and Pozuelo, 1971; Wei, 1973). Thus, while analgesic and addictive mechanisms may share many neuronal substrates in common, they are not, by any means, identical.

3. Drug Localization Studies

Although morphine is considered to be the prototype for the narcotic analgetics, its low lipid solubility forms a blood-brain barrier which does not exist for the majority of compounds in this class (Way, 1967; Cube, Teschemacher, Herz and Hess, 1970; Herz and Teschemacher, 1971; Oldendorf, Hyman, Braun and Oldendorf, 1972). Direct introduction of drugs into the CNS obviates the pharmacokinetic differences due to variations in chemical structure and thus provides a more accurate estimate of the actual efficacy of the drugs at active central receptor sites (Herz and Teschemacher, 1971). To demarcate the site

of action, substances in solution or crystalline form were applied to discrete areas of the brain by microinjection or introduced into segregated parts of the ventricular system.

Most of the experiments performed with the microinjection technique point to the periventricular gray matter of the third ventricle (belonging mainly to the hypothalamus and anterior thalamus as the main site of the antinociceptive action of morphine (Tsou and Jang, 1964; Lotti, Lomax and George, 1965; Foster, Jenden and Lomax, 1967; Herz, Metys, Schondorf and Hoppe, 1968; Buxbaum, Yarbrough and Carter, 1971) but the relative importance of the various thalamic nuclei and mesencephalic reticular formation remains ambiguous. Tsou and Yang (1964) for instance, obtained only an incomplete blockade of the nociceptive response when morphine was injected into the periaqueductal gray or mediodorsal thalamic nucleus and no analgesia after injection into the midbrain reticular formation, whereas Herz, et al. (1968) observed a strong analgesic effect from these areas. The former also reported no analgesia following injection into the ventral posteromedial nucleus of the thalamus and dorso-medial region of the medial geniculate body. Data obtained by Buxbaum, et al. (1971) are in general agreement with those of Tsou and Jang (1964), but indicate that the

nucleus ventral posterolateralis and reticularis of the thalamus should also be considered as possible sites of action. The analgesic site was not localized to any specific structure in the experiments of Lotti, et al. (1965), for he noted analgesia following microinjection of morphine into all of the thalamic and hypothalamic areas tested. The periventricular hypothalamic region is also considered to be the site through which morphine exerts its hypothermic (Lotti, et al., 1965; Foster, et al., 1967) and some of its endocrine effects (George and Way, 1959).

In contrast to the importance placed upon the hypothalamus by the previous investigators, Wei, Loh and Way (1972), using a different approach, found that severe abstinence syndromes were precipitated in morphine-dependent rats when the narcotic antagonist, naloxone, was injected into the medial thalamus and rostral portions of the midbrain but not when applied to tegmental or hypothalamic areas. Perhaps, as has been pointed out previously, the mechanisms of dependence and analgesia involve different neural structures, but their observations are also contradictory to those of Kerr and Pozuelo (1971) who noted that destruction of the ventromedial nucleus of the hypothalamus markedly reduced the withdrawal syndrome. The discrepancy in the results might be due to the fact

Wei, et al. (1972) applied their chemicals in crystalline rather than liquid form. Despite first impressions in favor of the application of crystals, the spread of material is actually greater than when applied in liquid form and the results are far less reproducible (Routtenberg, 1972).

All reports concurred, however, that injection of morphine into the hippocampus, septum, olfactory bulbs or caudate, as well as application directly onto the somatosensory cortex or sub-arachnoidally to the spinal cord, did not have any appreciable antinociceptive effect. However, the degree of diffusion from the site of injection limits the accuracy of such localization techniques.

The rostral site of the antinociceptive action of morphine implied by the above investigators is in direct contrast to the findings of Herz and his co-workers (Herz, Albus, Metys, Schubert and Teschemacher, 1970; Herz, Teschemacher, Albus and Zieglgansberger, 1972; Albus, Schott and Herz, 1970; Herz and Teschemacher, 1971; Vigouret, et al., 1973; Teschemacher, et al., 1973). Their experiments in rabbits, with morphine injected both intraventricularly into restricted parts of the ventricular system and intracerebrally, point to structures lying in the vicinity of the fossa rhomboides and, according to autoradiographic techniques (Schubert, Teschemacher,

Kreutzberg and Herz, 1970), at a depth of 1 to 2 mm from the ventricular wall as the site of action of morphine. Even with the use of very high doses, only a minimal inhibition of the nociceptive response was obtained when the analgetics (morphine and fentanyl) were restricted to the third ventricle, whereas the response was unaltered when the drugs were limited to the lateral ventricles. Supportive evidence was obtained by observations that the narcotic analgesic antagonists, nalorphine and levalorphan, were most effective in either antagonizing the antinociceptive action of morphine (Albus, et al., 1970; Vigouret, et al., 1973; Teschemacher, et al., 1973) or in precipitating the abstinence syndrome in morphine-dependent rabbits (Herz, et al., 1972) when they had access to structures in the immediate surroundings of the fourth ventricle. A similar caudal site of action for the hyperglycaemic effect of morphine has recently been reported by Feldberg and Shaligram (1972).

Herz, et al., (1970) consider the extralemniscal neurone chains ascending from the pars decendens of the trigeminal nucleus (their nociceptive response was the licking reaction to electrical stimulation of the tooth pulp) as the most likely site of action of morphine but effects on the efferent parts of the reflex arc, such as the hypoglossus nucleus, and the possibility that morphine

might be acting through mechanisms coming from outside the reflex pathway could not definitely be excluded.

An interesting phenomenon arose out of the work of Herz and colleagues: whereas the peak antinociceptive effect usually occurs within 30 minutes following intraperitoneal or subcutaneous injection of analgesic doses of morphine, the maximum effect was not observed until 60 minutes after intraventricular injection; such differences were not observed with fentanyl, the peak effect occurring within a matter of minutes regardless of the route of administration. The authors felt that the large difference in the lipid solubility of the two compounds adequately explained this phenomenon. In connection with these observations, it is surprising to note that bradykinin, a potent algesic agent when applied peripherally, mimics many of the actions of morphine when injected intraventricularly (Ribeiro, Corrado and Graeff, 1971; Ribeiro and Rocha E. Silva, 1973). The antinociceptive action of bradykinin was also localized to the periventricular structures. What is more interesting, however, is that the onset and duration of the central analgesic effect of bradykinin was similar to that of fentanyl. The explanation by Herz and colleagues that the delayed onset with morphine and rapid start with fentanyl is due to differences in lipid solubility does not account for the

quick onset after bradykinin for it, like morphine, is also sparingly soluble in lipids. Perhaps, then, the differences in the onset of action between morphine and fentanyl, might actually reflect dissimilar sites of action rather than differences in the physico-chemical properties of the two compounds.

Such studies as these make the interpretation of intraventricular injection techniques hard to follow and justifies the clinically more relevant route of administration (subcutaneous) employed in the present study.

Investigation on the selective distribution of the narcotic analgetics within the central nervous sytem (as determined by autoradiographic techniques) in an attempt to correlate the physiological disposition with the pharmacological response also have provided little information concerning the central site of action of these compounds. Such studies are complicated by the fact that the drug may be very highly concentrated in an area which plays no functional role in its mechanism of action or conversely, that a low concentration of the drug within a particular area does not necessarily exclude it as a possible site of action for the area may either be very sensitive to the drug or part of a critical circuit of neurones responsible for the drug's effects.

With the exception that morphine tended to have a higher affinity for gray matter, most investigators have also found little tendency for analgetics to localize in any particular area of the CNS, in either the non-tolerant (Miller and Elliot, 1955; Chernov and Woods, 1965; Lomax, 1966) or tolerant animals (Mule and Woods, 1962). To avoid the more general labelling consequent to parenteral administration, Adler (1964) injected radioactive morphine and codeine intraventricularly in mice, and reported a localization of radioactivity in the hypothalamic and pontile areas and in the hippocampus. In accordance with these results, Schubert, et al. (1970) also found a high concentration of morphine in the hypothalamus and hippocampus in the rabbit but there was no evidence for a specific localization as could be found after the application of noradrenaline.

However, according to Cube, et al. (1970), the concentration of the narcotic analgetics (including morphine) found in the various areas of the brain following intraventricular injection differs by no more than a factor of two. The differential distribution of morphine may therefore be attributed to its different physicochemical properties rather than providing evidence for binding to specific receptor sites. Because of its hydrophilic nature, morphine tends to remain in the plasma

fluid compartment and diffusion into brain tissue, which contains very little or no extracellular fluid space, is greatly hindered. This factor, together with the greater vascular density of the gray matter and the exceedingly faster circulation rate in comparison with white matter, most likely play a major role in the differential distribution of morphine.

4. Behavioural Studies

In the study of the action of analgetics it is important to recognize that the response to pain consists of learned reflex and behavioural reactions which are integrated at all levels of the nervous system (Melzack and Scott, 1957; Beecher, 1959; Charpentier, 1968). This factor has not always received due consideration for, in many of the investigations cited above, the relationship between the response measured and pain is not always clear. Moreover, the literature on the effects of narcotic analgetics on the behaviour elicited by stimulation of brain structures implicated in pain mechanisms, particularly as it relates to the problem of delineating the central site of action of these compounds, is not large.

All of the investigations that have dealt with the effects of analgetics on operant behaviour maintained by

intracranial aversive stimulation (Boren and Malis, 1961; Vernier, et al., 1961; Weitzman and Ross, 1962; Halpern and Alleva, 1964; Ross, 1966) were based upon a "titration schedule" first described by Weiss and Laties in 1958. This technique, which is similar to the one used in the present study, consists of an arrangement whereby increments in the intensity of an aversive stimulus are automatically programmed and decrements in intensity follow designated responses (bar-pressing) of the subject. By continually recording the stimulus intensity, the threshold level of shock necessary to maintain lever pressing can be determined. Such operant control procedures provide an objective, quantitative assessment of an animal's behavioural response to pain and have shown a sensitivity superior to that of the more conventional algesimetric techniques (Weiss and Laties, 1961, 1964; McConnell, 1962; Malis, 1962, 1964).

Most investigations, however, have been confined to an examination of the effects of narcotic analgetics on the behaviour elicited by the stimulation of only one brain structure. The area most commonly explored in these studies was either the trigeminal (Gasserian) ganglion (Weitzman and Ross, 1962; Halpern and Alleva, 1964; Ross, 1966) or the midbrain reticular formation adjacent to the lateral spinothalamic tract (Boren and Malis, 1961). Only

one study has, as yet, been reported in which the effects of narcotic analgetics on the aversive thresholds elicited by the electrical stimulation of various brain structures involved in pain mechanism were compared (Vernier, et al., 1961). In this study, electrodes were chronically implanted in the ventralis postero-lateralis, ventralis postero-medialis and center median nuclei of the thalamus, in the midbrain reticular formation and in the trigeminal ganglion of Rhesus monkeys. Evidence of a differential nuclear sensitivity to the narcotic analgetics (morphine and anileridine) was noted, with the ventralis postero-lateralis nucleus of the thalamus and the trigeminal ganglion being the most affected and the centre median nucleus of the thalamus being the least affected. The above studies therefore suggest that, under behavioural operant conditions, the narcotic analgetics preferentially suppress the specific sensory-discriminative pain pathways over those of the non-specific extra-lemniscal system.

SUMMARY OF HISTORICAL REVIEW AND SCOPE OF THESIS

In the above discussion, three basic methods have been employed in an attempt to differentiate the action of the opiates at various levels of the central nervous system; (1) electrophysiological investigations of afferent

and efferent pathways, (2) transection of the neuraxis and/or electrolytic lesions at various levels of integration, and (3) drug localization by autoradiographic techniques or direct application of the drug into discrete regions of the central nervous system.

With all three techniques, there appeared to be an equal number of investigators favoring a selective depression of the primary sensory-discriminative (lemniscal) pathway by the narcotic analgetics as there were authors upholding the view that these drugs preferentially inhibited structures belonging to the motivational-affective (extra-lemniscal) pathway. Others found no evidence for a selective inhibitory action of morphine on either pathway, nor was suppression of pain pathways always characteristic of analgesic compounds.

There are a number of reasons which would account for the many conflicting views in the literature dealing with the central site of action of the narcotic analgetics; the most important of these are summarized in the following paragraphs.

Sectioning of the cerebrospinal axis or ablation of structures at various levels of the central nervous system involves drastic surgical procedures which undoubtedly alters the activity of the remaining brain structures.

Remembering that the balance of tonic inhibitory and excitatory influences varies at different levels of the neuraxis, it is quite likely that, with neurosurgical techniques, mechanisms are activated which are of minor importance in the intact animal. There are alternative ways of impairing function and, depending upon the time allowed for recovery, alternate ways of restoring function. A distorted view of the presumed site of action might thus be obtained.

The intracranial chemical injection technique was at first thought to provide a degree of localization comparable to that of electrical stimulation of the brain (Lomax, 1966) but, in a recent review, Routtenberg (1972), clearly demonstrated that the behavioural effects induced by the microinjection of a drug into a particular brain area are not necessarily related to an action of the chemical at that point. The possibility of the spread of the drug to some more distant site, either through the ventricular system or cerebral vasculature or along axonal fibers, has not always been sufficiently considered in such experiments. Factors, such as the size of the cannula, implantation-microinjection interval, vehicular form (crystal or liquid), pH, osmolality and volume, as well as the rate and number of injections per animal, also play an important role with respect to the amount of brain

tissue damage incurred, the pathological response to the presence of a foreign substance, the degree of spread of the chemical and, ultimately, the type of behavioural response elicited; these factors have varied widely from one investigator to another and, until more standard microinjection techniques are used, reliable conclusions concerning the central site of action of the narcotic analgetics cannot be made.

The results obtained by researchers using autoradiographic techniques have also proved to be inconclusive. It is well known that the number of grains seen is a direct function of the length of exposure, the development time and the thickness of the emulsion coating, all of which varied with the individual investigators. Other factors are of significance, the most important of which is that high concentrations of radioactivity probably represent, in a large part, drug association with non-specific receptors. The relative specificity of the ^{14}C and ^{3}H label and diffusion factors also play a determining role. In the latter case, substances, especially highly lipophilic ones such as fentanyl, may diffuse so rapidly that, at the time of sampling, the penetration front has already reached the maximum point and is in the process of withdrawal; the true penetration front would therefore lie somewhere beyond the actual point of highest radio-

activity. It is therefore likely that autoradiographic techniques provide, at best, only a crude estimate of the specific site of action of the applied drug.

Electrophysiological techniques remain among the most accurate and reliable, although, they too, are not without drawbacks. Perhaps the greatest oversimplification in this field is the commonly held belief that electrical stimulation of a nerve or brain "center" closely resembles normal neuronal stimulation, which is far from being the case. Although stimulus parameters may be independently controlled and monitored with great accuracy, the innumerable combinations that are possible and that have been used by different investigators makes comparison of the data difficult. It is a well established fact that entirely different effects can be obtained by electrical stimulation of the same preparation if just one of the stimulus parameters is altered. Then too, electrophysiological methods are beset by difficulties such as temporary effects of the operation, varying states of the preparation, stimulation artifacts, destruction of brain tissue, formation of scar tissue and pathological reactions in chronic studies, and distal effects due to spread of current or activation of polysynaptic mechanisms. The importance of remote responses should be emphasized because the explored part of the brain is only a small

fraction of a large mass of functionally active neurones with varying thresholds.

Observations concerning the central site of action of the narcotic analgetics have also been complicated by marked intra- and interspecies variation, not only in terms of drug responses, but also in the anatomy of the pain pathways and brain complexity. Furthermore, it was not always clear whether the effects described were related to pharmacological or toxicological amounts of drug and dose-response parameters were rarely determined.

Thus, in view of the non-physiological nature and limitations of each investigational procedure and of the complex interaction, both anatomically and functionally, between the many components of the central nervous system involved in sensory transmission, it is likely that a consideration of the data from all of the methods outlined above would come nearer to explaining what goes on in the intact, normally functioning brain than the application of any one technique. Collectively, the information presented in the foregoing sections supports the concept that afferent information signalling pain ascends the brainstem in multiple pathways and that analgesia may be effected through an action on any one of these components. It is therefore not likely that any one structure of the brain is responsible for mediating the antinociceptive

actions of the narcotic analgetics, but rather, that the analgesia obtained with these compounds is the result of a combined action at many different sites throughout the central nervous system.

In the present study, a modification of the titration technique (see part 4 of previous section) was used to examine the effects of various analgesic agents on the escape reaction elicited by the electrical stimulation of a number of different brain areas which have been implicated in central pain mechanisms. The technique used in this study therefore represents a combination of electrophysiological and behavioural methods.

Although the technique of exploring the brain of conscious, freely moving animals with chronic, implanted electrodes shares many of the limitations of electrophysiological methods, such as the problem of stimulus parameters (Mickle, 1961) and distant effects, its advantages are many: (1) with the use of the titration schedule, the experimental design is freed from the bias of the investigator, adding to the analysis of the data a greater degree of objectivity--a quality which is difficult to attain in the assessment of pain in both man and animals; (2) the stimulus characteristics can be accurately monitored and variables due to tissue impedance can therefore be taken into account; (3) although some

trauma as a result of electrode insertion is inevitable, the present day materials used for electrode construction are well tolerated by the animal and pathological reactions are minimal; (Delgado, 1961); (4) repeated stimulations over long periods of time do not produce permanent anatomical or functional modifications (Delgado, 1961); control measurements can therefore be repeatedly determined and different doses of the same drug as well as different drugs can be studied in the same animal; consequently, both the within and between treatment variability is greatly reduced; (5) modifications of behaviour may be studied at the moment a cerebral structure is stimulated; and (6) the lack of adjunctive medication and bodily restraints allow for a more normal display of overt behavioural responses. As Huxley (1952) noted: "Captivity cages minds as well as bodies and rigid experimental procedures limit the range of performance, while freedom liberates the creatures capacities and permits the observer to study their fullest developments".

The brain areas explored in the present investigation consisted of the ventrodorsal, dorsomedial, parafascicular and ventral anterior nuclei of the thalamus, caudate nucleus, dorsal and ventral hippocampus, fimbria hippocampi, anterior amygdaloid area, anterior and lateral hypothalamus, medial lemniscus, optic tract and a dorsal

midbrain region containing the dorsolateral central gray and its suprajacent structures (deep layers of the superior colliculus). An abundant literature attests to the importance of thalamic, limbic (hippocampus, amygdala, hypothalamus and dorsal midbrain area) and caudate structures in the transmission and/or elaboration of the neural responses to pain (see Historical Review and also Yoss, 1953; Melzack, Stotler and Livingston, 1958; Sweet, 1959; Poggio and Mountcastle, 1960; Melzack, 1961; Mark, Ervin and Takovlev, 1962; Bowsher and Albe-Fessard, 1962; Perl, 1963; Rubinstein and Delgado, 1963; Albe-Fessard and Krauthamer, 1964; Mehler, 1966; Sutin, 1966; Kuypers and Lawrence, 1967; Hassler, 1968; Passouant, 1968; Poirier, Bouvier, Olivier and Boucher, 1968; Runnels and Thompson, 1969; Westner, 1971; Wilburn and Kesner, 1972; Sinnamon and Schwartzbaum, 1973; Keene, 1973). However, little is known about the relative importance of these structures in the analgesic action of morphine-like compounds.

Basic to our understanding of the role played by various brain areas in the mode of action of analgesic drugs is the observation that there are two components involved in the pain response; first, a conscious perception of the pain as a specific sensation without any appreciable emotional involvement, and, second, the reaction to pain with a concomitant change in behaviour and affect which may or may not be related to the nature

or intensity of the stimulus (Wikler, 1958; Beecher, 1959). Behavioural and physiological studies give reason to believe that the two components of the pain response are served by at least two distinct conduction systems with quite different characteristics. Thus, it has been proposed that the specific, paucisynaptic neo-spinothalamic system provides the neurological basis of the sensory-discriminative dimension of pain while the diffuse, multisynaptic paleo-spino-reticulo-thalamic pathways subserve the motivational drive and unpleasant affect that trigger the organism into action (Melzack and Wall, 1965, 1968; Casey and Melzack, 1967; Melzack, 1973). These two systems project to different regions of the brain: the former to the somatosensory areas of the thalamus and cerebral cortex, the latter to various areas of the brainstem reticular formation, limbic system and medial thalamic nuclei (Poirier, et al., 1968).

With the exception of the caudate nucleus and optic tract, the brain structures investigated in this study are therefore a part of either the neo-spinothalamic (ventrodorsal nucleus of the thalamus, medial lemniscus above the level of the midbrain) or paleo-spino-reticulo-thalamic (dorsomedial, parafascicular and ventral anterior nuclei of the thalamus, hippocampus, amygdala, hypothalamus and mesencephalic reticular formation) pathways. In view of the commonly-held belief that analgesic drugs of

the morphine type exert their principal, if not entire, effect upon the reaction component of pain, (Wikler, 1958; Beecher, 1959) the major question of the present study was to what extent morphine-like drugs affect, differentially or non-differentially, the escape responses elicited by the electrical stimulation of brain structures subserving these two different modalities of pain.

In this study, five narcotic analgetics (morphine, heroin, fentanyl, propoxyphene and tilidine) and one phenothiazine derivative (levomepromazine) were tested for their effects on disparate aversive brain stimulation.

Morphine, a naturally occurring phenanthrene alkaloid of opium is the prototype for all narcotic analgetics and is the standard reference substance by which other analgesic drugs are judged. In animals, the analgesic potency of morphine varies with the species and algesimetric technique employed. For the rat, using the subcutaneous route of administration, ED_{50} values ranging from 0.8 to 15.4 mg/kg have been reported (Eddy, Friebel, Hahn and Halbach, 1968). The major drawback limiting the usefulness of morphine as an analgetic is the development of tolerance, physical and psychic dependence (ie., addiction) that follows repeated administration of the drug.

Heroin (di-O-acetylmorphine), a semi-synthetic derivative of morphine, is approximately three times as

potent and analgetic as morphine in both man and animals (May and Sargent, 1965). The current crisis in heroin abuse tends to reinforce the commonly-held belief that it is more euphorigenic than morphine. Heroin was, therefore, included in the present study in order to determine whether the more intractable addiction to heroin is the result of a differential action of heroin on central nervous system structures or whether, as has been suggested by some investigators (Oldendorf, Hyman, Braun and Oldendorf, 1972), it is merely due to differences in the pharmacokinetic properties of these compounds.

Fentanyl (Sublimaze) is a highly potent synthetic analgetic structurally related to both the diphenylpropylamines (methadone family) and the 4-phenylpiperidines (meperidine family). In animals, fentanyl is approximately 125 to 400 times as potent an analgetic as morphine (Janssen, Niemegeers and Dony, 1963; Gardocki and Yelnosky, 1964; Blane, 1967). On the basis of his studies on the verbal reports of pain induced by applying increasing amounts of pressure to the tibial nerve of human subjects, Morrison (1970) has postulated that the narcotic analgetics used in neuroleptanalgesia (eg., phenoperidine, fentanyl) influence mainly the perceptual mechanisms of pain whereas those more commonly used in the clinic (eg., morphine, heroin) act mostly upon the affective experience of pain. It was therefore of interest to compare the mode of action of

fentanyl, on the one hand, with that of heroin or morphine, on the other, by the present technique in order to see whether the brain structures subserving these two aspects of the pain experience exhibit a differential sensitivity to the effects of these drugs.

Propoxyphene, generally marketed as dextropropoxyphene (Darvon), the dextrorotary isomer of this compound, and tilidine (Valoron) are weak narcotic analgetics with a very low addiction liability. Although structurally related to the potent narcotic analgetic, methadone, propoxyphene is only 0.1 to 0.4 times as potent an analgetic as morphine when given subcutaneously to rats (Eddy, Friebel, Hahn and Halbach, 1969). Tilidine (Valoron) is a recently synthesized meperidine derivative whose analgesic potency, when administered subcutaneously in rats, is approximately 0.02 times that of morphine (Herrmann, 1970; Herrmann, Steinbrecher and Heldt, 1970). Although propoxyphene is a weak narcotic analgetic, it retains many of the adverse side effects of the more potent opioids. Tilidine, on the other hand, is apparently devoid of these side effects and appears to have a spectrum of activity which is different from that of the other narcotic analgetics (Herrmann, et al., 1970). These compounds were, therefore, included in the present study in order to determine whether their low potency and addiction liability, as well as the different pharmacological profile of tilidine, is related

to a differential effect, or lack of effect, upon brain structures involved in the genesis of pain.

The phenothiazine derivative, levomepromazine (methotrimeprazine, Nozinan) was also included in the present investigation in order to determine whether the effects of the narcotic analgetics, as a whole, could be differentiated from the effects of an analgesic drug which does not belong to the opiate class of compounds. A number of studies (Courvoisier and Leau, 1959; Maxwell, Palmer and Ryall, 1961; Bloomfield, Simard-Savoie, Bernier and Tetreault, 1964; Beaver, Walkenstein, Houde and Rogers, 1966) have shown that levomepromazine possesses potent analgesic properties in both man (one-half to two-thirds as effective an analgetic as morphine) and animals (1-7 times the analgesic potency of morphine). Its most valuable attribute lies in the complete absence of addiction liability. However, it should be noted that levomepromazine was not found to be an effective analgetic in all animal algesimetric assays, even when very high dose levels were used (Janssen, et al., 1963; Blane, 1967).

Thus it was hoped that an investigation of analgesic drugs with such widely different pharmacological profiles by the present technique would lead to a better understanding of not only the mode of action of morphine-like compounds, but also of the neural structures related to the mechanisms of analgesia and addiction.

MATERIALS AND METHODS

Subjects

The experiments were performed with male albino rats, of a specific pathogen-free Sprague-Dawley strain (Biobreeding, Ottawa) and weighing between 250 and 350 grams at the start of the study. The rats were housed in individual cages located in environmentally controlled (20°C, twelve hour light-dark cycle) animal quarters. They were maintained on an ad lib food (Purina rat chow) and water schedule throughout the entire study.

Apparatus

The apparatus employed was designed specifically for the purpose of this experiment and built by Mr. J. Klaase of the Department of Pharmacology, University of Western Ontario.

The two way shuttle-apparatus was constructed of stainless steel rods, 3 mm in diameter, imbedded in plexiglass, and spaced 1 cm apart center to center. The metallic grid floor consisted of two halves, each 29 cm long by 18 cm wide. The outer 18 cm edges were hinged while the corresponding inner edges of each half of the floor rested upon a central axis which was connected to a SPDT microswitch located to one side of the grid floor.

Movements of the animal back and forth across the two halves of the grid tilted the central axis and activated the microswitch, which in turn, operated the appropriate control and recording devices. A rectangular plexiglass hood, 55 cm long, 17 cm wide and 19 cm high, with a 2 by 38 cm slit along the top, restricted the animal to the grid floor.

The shuttle apparatus was housed in a wooden box, 76 cm long, 51 cm wide and 58 cm high, which was lined with an insulating layer of foam rubber and aspenite, except for the floor, which was constructed of formica in order to facilitate cleaning. The inside dimensions of the wooden box were 65 x 40 x 47 cm.

For foot stimulation, grid shock was scrambled through a twelve-relay, two-position consecutive triggering device. For intracranial stimulation, the rat was attached via male leads (303-.018" - 302 receiver cords, Plastic Products Co., Roanoke, Virginia) and connecting plugs to a four-channel, mercury-pool commutator (Model MC4, Scientific Prototype Manufacturing Co., N.Y., N.Y.) fastened to the roof of the wooden box, in order to allow free movement of the animal.

Several of these wooden boxes, each containing a shuttle apparatus, were housed in a larger, sound-proofed chamber equipped with two shielded 60-watt lights and

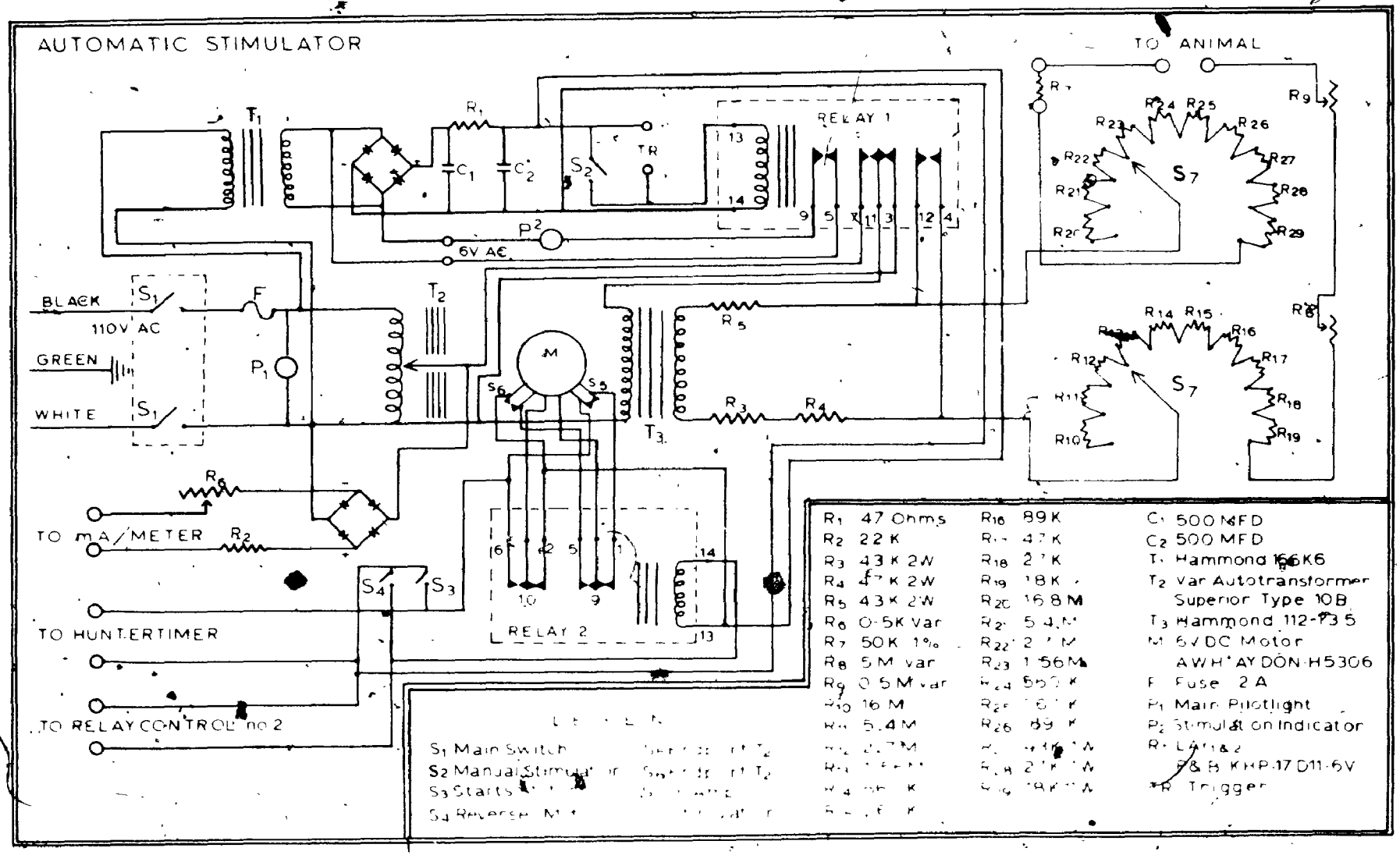
exhaust fan. This chamber, also fully shielded, was located in a room adjoining the one containing the stimulating and recording apparatus.

The shock source consisted of an AC stimulator whose circuitry is outlined in Figure 1. The output (60 Hertz sine wave) of a variable transformer (superior type 10B, T2, Figure 1) is fed into a second, nonvariable, step up transformer (Hammond 112-1:3.5, T3, Figure 1) and then via relay 1 to various resistors (R3-R5, R7, R10-29, Figure 1) before reaching the animal.

Ten second shock intervals were alternated with randomized rest periods ranging from 3 to 10 seconds and were regulated by a Lehigh Valley Electronic (L.V.E.) timer and Gerband clock respectively; the timers triggered each other and also a Hunter timer which controlled the length of time an electric motor (M, Figure 1) was driven. If the rat responded to the stimulus by crossing to the opposite side of the grid during the interval of shock, relay 2 (Figure 1) activated leads 5 and 6 which, in turn, negatively polarized the motor, causing it to run backwards. If the rat did not respond to the shock, the subsequent activation of leads 1 and 2 by relay 2 reversed the polarity of the motor which then ran forwards. Since the motor regulated the variable transformers through which the stimulus output had to pass before reaching the animal, its

Figure 1

Circuitry of Automatic Stimulator


AUTOMATIC STIMULATOR
TO ANIMAL
RELAY 1
RELAY 2
BLACK
110V AC
GREEN
WHITE
TO mA/METER
TO HUNT-ERTIMER
TO RELAY CONTROL no 2
6V AC
S1 Main Switch
S2 Manual Stimulator
S3 Starts
S4 Reverse
R1 47 Ohms
R2 22 K
R3 43 K 2W
R4 47 K 2W
R5 43 K 2W
R6 0-5K Var
R7 50K 1%
R8 5 M var
R9 0 5 M var
R10 16 M
R11 5.4 M
R16 89 K
R17 47 K
R18 2.7 K
R19 18 K
R20 16 8 M
R21 5 4 M
R22 2.7 M
R23 1 56 M
C1 500 MFD
C2 500 MFD
T1 Hammond 166K6
T2 Var Autotransformer Superior Type 10B
T3 Hammond 112-73 5
M 6V DC Motor AWHAYDON-H5306
F Fuse 2A
P1 Main Pilotlight
P2 Stimulation Indicator
R+ L A 1 & 2
P & B KHP-17 D11-6V
TR Trigger

excursions, either forward or backward, led to a stepwise increase or decrease in the intensity of the stimulus.

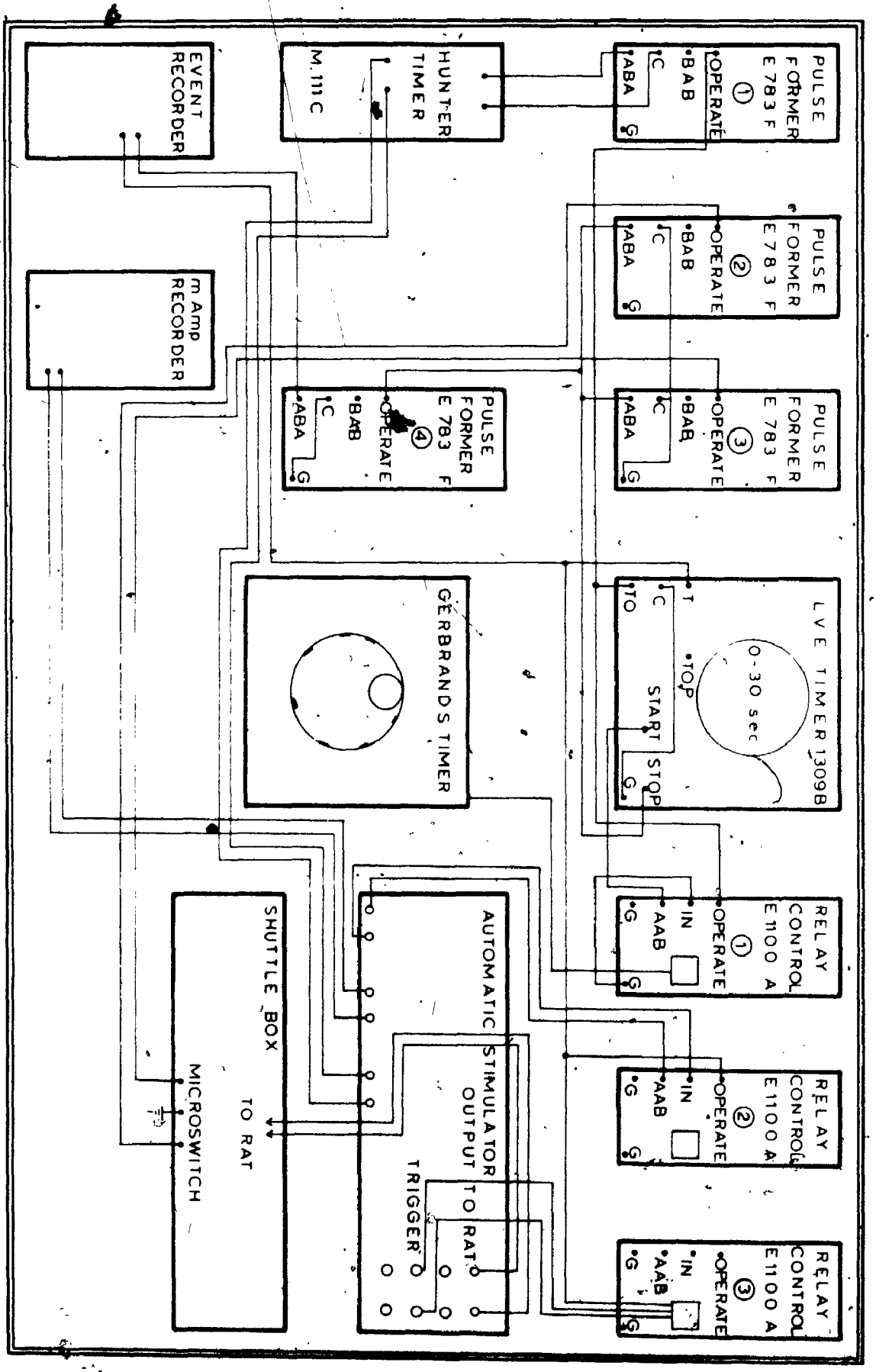
The size of the increment or decrement in shock intensity were regulated by a Hunter timer and the position of a multiplier switch (S7, Figure 1) which were operated manually. The total number of steps between zero and maximum current intensity was inversely proportional to the pulse duration setting of the Hunter timer. This latter parameter was usually adjusted to such a value so that maximum current levels were attained in twenty-five equal steps. If the multiplier switch was at position 4, for example, the stimulus intensity varied between 0 to a maximum of 100 μA, with a step size of 4 μA. The current being delivered to the animal was continuously monitored across a 50 KΩ resistor placed in series with oscilloscope terminals and recorded on an Esterline-Angus milliampere recorder. The resistors within the circuitry were of a large enough denomination to make any variations in the tissue impedance of the rat negligible. The total current delivered to the animal at any particular instant could therefore be directly determined from the recorder tracings. A block diagram of the apparatus is shown in Figure 2.

2 3

OF/DE

Figure 2

Block diagram of apparatus.
L.V.E.: Leighigh Valley Electronics


PULSE FORMER E 783 F ① OPERATE
PULSE FORMER E 783 F ② OPERATE
PULSE FORMER E 783 F ③ OPERATE
PULSE FORMER E 783 F ④ OPERATE
BAB
C
ABA
G
EVENT RECORDER
HUNTER TIMER M.111 C
m Amp RECORDER
LVE TIMER 1309B
0-30 sec
T
TO
TOP
START
STOP
GERBRANDS TIMER
RELAY CONTROL E1100 A ① OPERATE
RELAY CONTROL E1100 A ② OPERATE
RELAY CONTROL E1100 A ③ OPERATE
IN
AAB
AUTOMATIC STIMULATOR
OUTPUT TO RAT
TRIGGER
SHUTTLE BOX
TO RAT
MICROSWITCH

Electrode placement and localization

Rats were chronically implanted with bipolar, Nichrome electrodes (Plastic Products Co., Roanoke, Va.), 254μ in diameter, and insulated with Formvar except for the cross-section of the tips. The female amphenol connector of the electrode was firmly affixed to the skull by means of cranioplastic cement. Four stainless steel jeweller's screws, positioned around the electrode and screwed to a depth of 1 mm into the bone, served to anchor the cement. Before insertion, electrodes were tested with an ohmmeter to check for defects in the insulation. Prior to electrode implantation, the animals were anesthetized with a mixture of phencyclidine (50 mg/kg) and diazepam (10 mg/kg). Supplementary doses of chloral hydrate (30-60 mg) were administered when necessary. At least two weeks were allowed for recovery from surgery. A system of stereotaxic coordinates similar to those described by Olds and Olds (1963) was employed.

Following the completion of the experiment, animals were anesthetized with nembutal (100 mg/kg) and perfused intracardially with physiological saline followed by 10% buffered formalin (pH 7.0-7.1). Each brain was then dissected from the cranium and stored in the fixative for at least five days. Frozen sections, parallel to the frontal plane were taken at 50μ and stained with cresyl violet according to the method of Kluever and Barrera (1953)

for the localization of the electrode tracts. The deepest point of penetration was taken to be the area stimulated by the electrode tip.

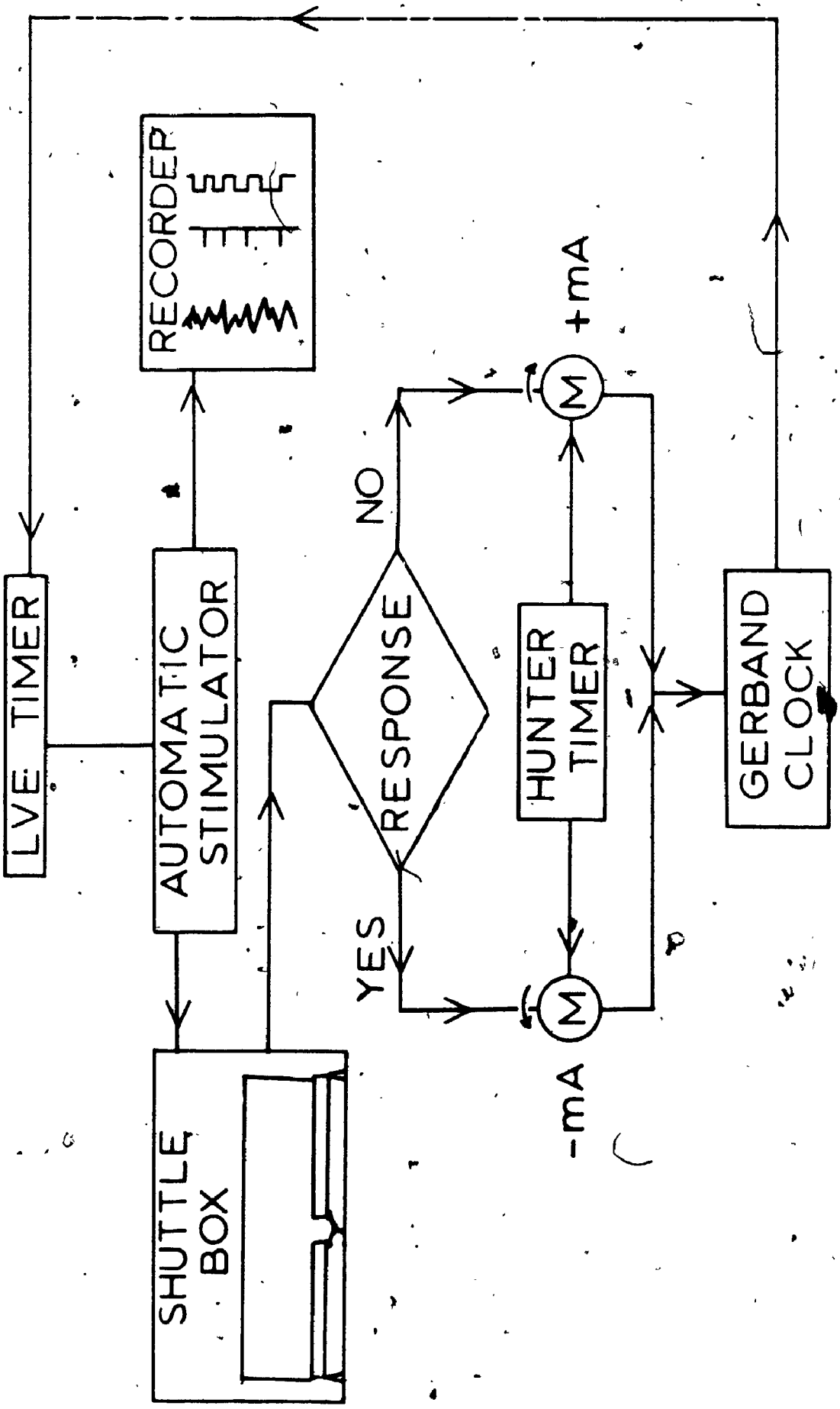
Measurement of the response

For both peripheral and central stimulation, the animal's aversive threshold was determined by means of a titration schedule (Figure 3) whereby the intensity of the stimulus decreased in stepwise fashion if the rat responded to the stimulus by crossing to the opposite half of the grid floor during the shock interval, or increased in similar steps if it did not respond. In the absence of a response, the stimulus intensity rose in small increments until a response was once again elicited, thus completing the cycle, or until a ceiling level of stimulation was reached. To limit the contribution of stimulus spread, a ceiling of 100 μA was used as the criterium of effectiveness for central stimulation. Any rat not responding to an intracranial stimulation of 100 μA was eliminated from the experiment. For peripheral stimulation (foot shock), the maximum stimulus intensity used was 2.0 mA, beyond which damage to the foot pads was occasionally observed.

Prior to each drug trial, animals were accustomed to the titration schedule for a period of 5 to 10 minutes, then removed from the suttle-box, injected with saline and tested for a further 30 minutes. The performance of the animal during the last fifteen minutes of this interval

Figure 3

Flow chart for automatic determination of aversive threshold: + ma sign indicates a stepwise increase in current intensity; - ma sign indicates a stepwise decrease in current intensity.


RECORDER
+mA
NO
LVE TIMER
AUTOMATIC STIMULATOR
RESPONSE
HUNTER TIMER
GERBAND CLOCK
YES
SHUTTLE BOX
-mA

was used as the control value. The aversive threshold was determined by averaging the peak intensities with which the animal allowed itself to be stimulated over a period of fifteen minutes.

In order to study the escape response of rats to fixed intensities of stimulation, the current level was raised to the desired value (as determined by the reading on the milliampere recorder) and the pulse duration of the Hunter timer set to zero. The total number of electric shocks presented to the animal, the number and latency of the escape responses and the amount of crossing during the rest periods were recorded by an Esterline-Angus event recorder. All investigations concerning the effects of drugs on central aversive stimulation employed the escape response to fixed suprathreshold intensities of stimulation.

Once the control threshold for each animal had been obtained, the current was raised to approximately twice this value and maintained at this level so that each stimulus evoked escape responses of fairly short latency. Each rat was then subjected to a fifteen-minute control period under saline and the mean and standard deviation of the response latencies determined. Since the low doses of the analgetics sent few rats to the long cut-off time of 10 seconds, reaction times greater than two standard

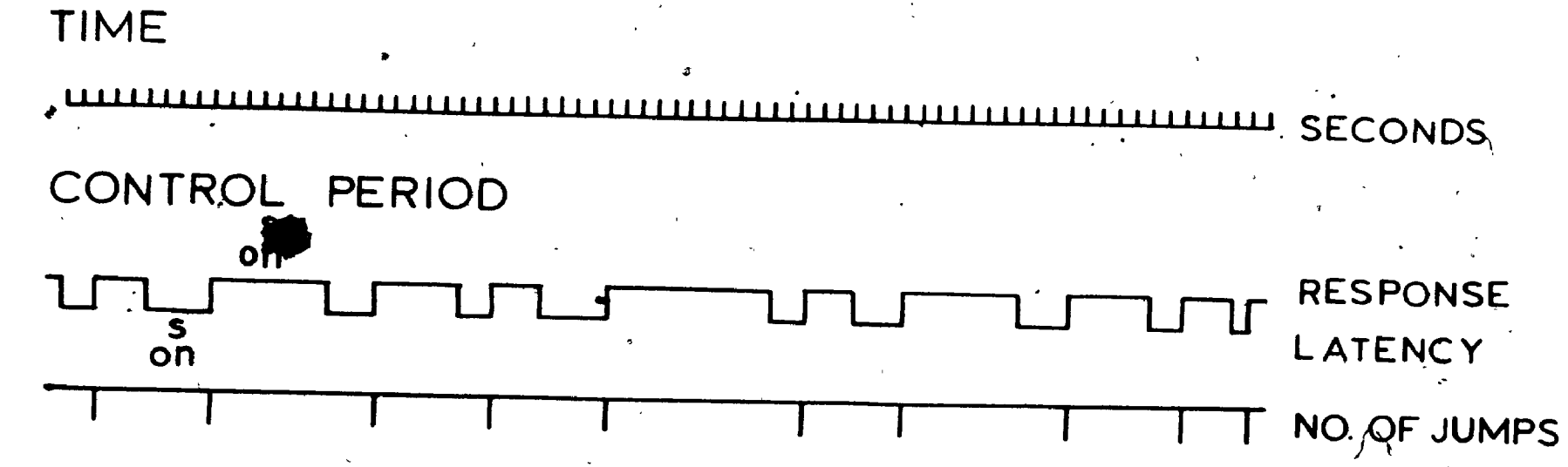
deviations from the control mean response latency were considered to be indicative of analgesia. Drug effects were therefore expressed as the number of stimuli eliciting an escape response latency of less than, or equal, to the upper 95% confidence limit of the control mean response latency in terms of the percentage of the total number of shocks presented to the animal over a fifteen-minute interval during the time of peak drug action (Figure 4). All animals received all three dose levels of each drug administered by random allocation. The percent analgesic effect was converted to probits and probit-log dose regression lines were calculated by an IBM 7400 computer using a probit analysis program based on Finney's (1952) methods. Tests for the parallelism of the dose-response curves and the calculation of relative potencies was also done with the aid of a computer program.

Determination of psychophysical response functions

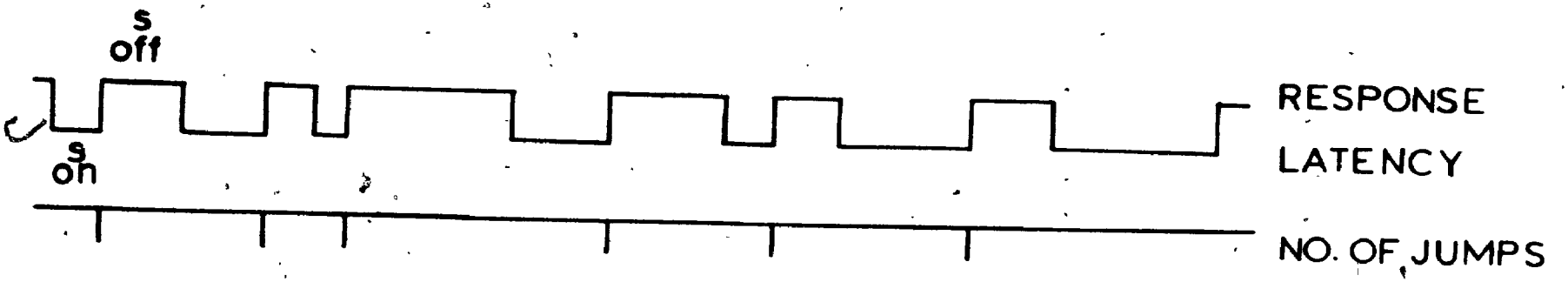
Because of the nature of the threshold procedure, low levels of shock, which are discriminable but not in themselves aversive, might come to occupy the status of warning signals for the higher amplitudes (Boren and Malis, 1961; Weis and Laties, 1963). In order to estimate the degree to which conditioned aversive stimuli influenced the thresholds being measured, the frequency of the escape response (using, in this case, the end-point of 10 seconds) as a function of stimulus intensity was determined for ten rats in the foot shock situation. The rate of responding

Figure 4

An example calculation of the percent escape response before and after drug administration. Although the animal jumped 6 out of 7 times after drug administration, the percent response calculated was only 3 out of 7, or 43%, since, in 3 of the jumps, the animal exceeded the response latency chosen as the cut-off time (mean control response latency + 2 standard deviations) for the analgesic effect.



Eg. Mean Response = 2.6 ± 0.3 sec.
Cut off = 2.6 + 2(0.3) = 3.2 sec.
% Response = 10/10 = 100%



Eg. % Response = 3/7 = 43%

at fixed levels of current was measured over fifteen minute intervals, with stimulus intensities ranging from zero milliamperes to values where animals responded to every shock.

Since preliminary studies revealed that the amount of escape behaviour generated at a particular fixed level of current depended upon the order in which the stimulus intensities were presented (animals tended to respond only at much higher values if an ascending series was used and vice versa), threshold determinations were alternated with each response rate trial and the order of presentation of the fixed stimulus intensities was randomized. With this type of procedure, low intensity levels which might have served as warning signals during the threshold measurements would be incapable of motivating a response since they were no longer being reinforced by the truly aversive stimuli.

Drugs studied

The salts of the following compounds were investigated:

morphine hydrochloride - British Drug Houses Ltd.

fentanyl citrate - McNeil Laboratories

diacetylmorphine hydrochloride - National Drug and Chemical Co.

dextropropoxyphene hydrochloride - Merck-Frosst Laboratories

tilidine hydrochloride - Warner-Lambert Laboratories

levomepromazine hydrochloride - Poulenc Ltd.

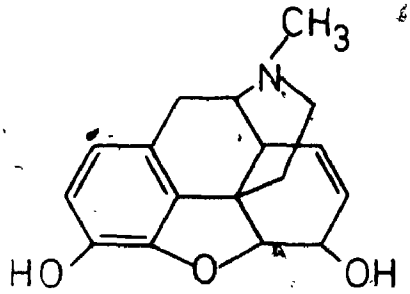
The structural formulae of these drugs and their corresponding molecular weights are shown in Figure 5. All drugs except for levomepromazine were dissolved in physiological saline and administered subcutaneously in volumes of 1.1 to 0.5 mg/kg. The pH of the drug solutions was adjusted as close as possible to neutrality with 0.1 N NaOH. All drug solutions were prepared just prior to each drug trial. Levomepromazine (methotrimeprazine) was obtained in commercially prepared ampoules, in a concentration of 5 mg/ml and injected intraperitoneally. All doses presented in this paper are in terms of the salts of these compounds. Equivalent volumes of saline were injected during the control trials.

Determination of the temporal features of the drug effect

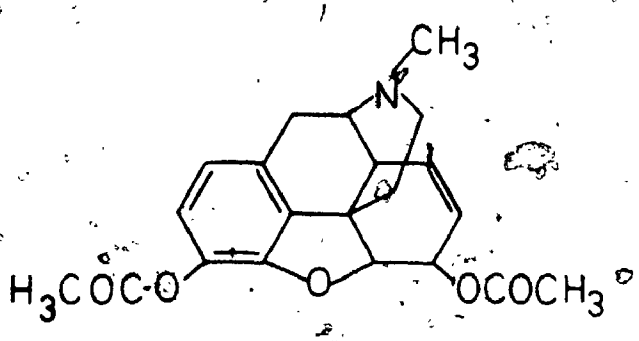
In order to determine the onset and duration of drug activity, response (percent escape at suprathreshold intensities of stimulation, cut-off time of $\bar{x}$ + 2SD) duration curves were obtained for each compound using animals with chronically implanted electrodes. Since the temporal features of drug effect were similar regardless of the location of the electrode, the values were grouped and the mean response plotted against time. After the control escape latency for each rat had been obtained, the animal was removed from the apparatus, medicated and immediately replaced. The escape response for three dose levels was determined at fifteen

Figure 5

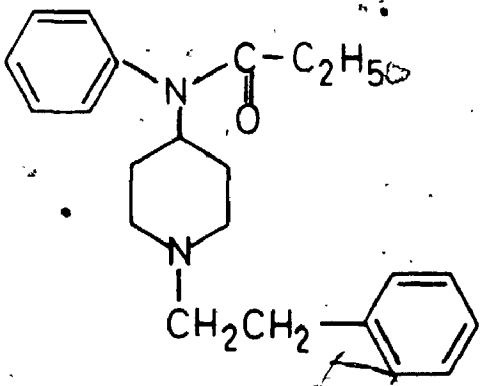
Structural formulae and molecular weights of the analgetics tested.



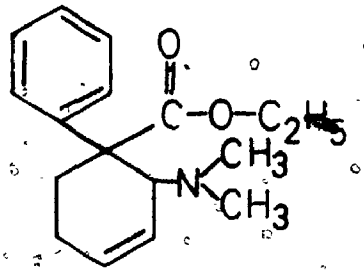
Morphine 286



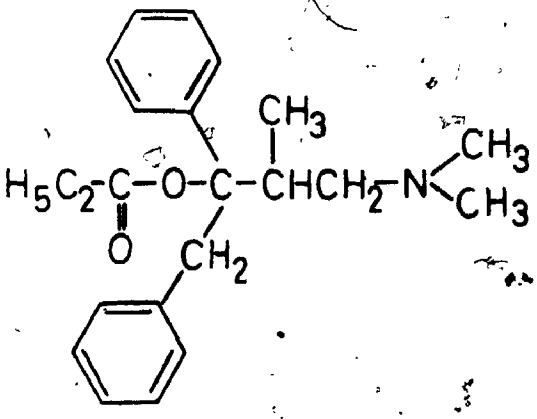
Heroin 370



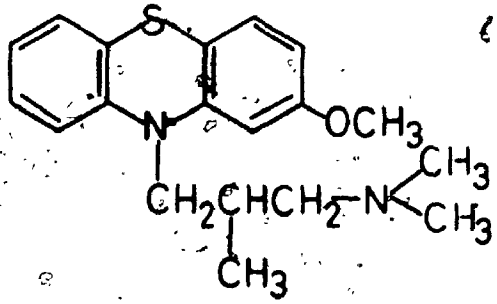
Fentanyl 337



Tilidine 273



Propoxyphene 340



Levomepromazine 329

minute intervals from the time of injection until the response had returned to at least 60% of the control value.

Estimation of dose-response curves

For the estimation of dose-response curves, a protocol similar to the one described above was used, except that the animal was returned to its home cage after medication and replaced in the shuttle-box only at the time of onset of peak drug action. Drug effects were monitored at fifteen minute intervals for a period of up to one hour after this time. The maximum change in response, irrespective of the time interval in which it occurred, was used. Dose-response curves consisted of three logarithmically-spaced doses with each dose level representing the mean escape response of at least seven animals. At least one to two weeks intervened between drug administration to any one animal in order to avoid any tolerance effects. It should be noted that, in these studies, the analgesic action of the drugs is expressed in terms of the dose causing a 50% reduction in the escape responses of seven or more animals rather than in the more usual terms of the dose producing a desired effect in 50% of the total population of animals investigated. This value, which is analogous to the ED_{50} determination, will be referred to as the analgesic dose 50, or AD_{50}, in the present investigation.

Test for motor deficit

Concurrent with the estimation of dose-response curves, rats underwent the rotating drum test as described by Collier, Hall and Fieller (1949) to ensure that a lack of response was a true indication of antinociception and not due to a motor insufficiency.

Tail flick assay

For purposes of comparison, the analgesic properties of morphine, heroin and levomepromazine were also determined by one of the more conventional algesimetric techniques. The D'Amour-Smith test, as modified by Davies, Raventos and Walpole (1946) was used. In this method, which is considered to be highly specific for the opiate analgetics, the complete inhibition of the rat tail flick reflex in response to radiant heat stimulation is the criterion of the analgesic effect. To control the duration of stimulation, so that tissue damage will not occur, the normal reaction time for each rat in an experimental group was determined three times at twenty minute intervals or until the last two readings did not differ significantly.

Three experimental groups (one group per dose level) were used, each consisting of five to seven animals. Saline was then injected and the mean response and standard deviation of the group calculated. The stimulus intensity

was such that normal reaction times varied from 3 to 5 seconds. After drug administration, the animals were tested at 1/4, 1/2, 1 and 2 hour intervals. The analgesic effect was scored as the relative number of rats having a response time greater than two standard deviations above the control response time. The percent analgesic effect was converted to probits and probit-log dose regression lines calculated as described previously.

RESULTS

Histology

The brain areas investigated, their coordinates (based upon those of Olds and Olds, 1963), the number of rats implanted per area and the mean aversive threshold and escape latency obtained from each of these regions are listed in Table 1. Figures 6 to 10 are schematical diagrams of transverse sections of the brain stem taken from the atlas of König and Klippel (1963) which show the location of the brain areas from which aversive thresholds and escape responses were obtained. Each of these figures represents the transverse plane in which the majority of electrodes implanted in each brain area were found. For all areas, the rostro-caudal extent with which electrode placements deviated from these planes never exceeded 0.5 mm.

The brain areas from which aversive thresholds and escape responding were obtained and which were subsequently employed in the evaluation of the effects of analgetics on centrally-elicited aversive behaviour consisted of the anterior hypothalamus and optic tract (Figure 6), mediodorsal nucleus of the thalamus and the lateral hypothalamus (Figure 7), parafascicular-paraventricular complex and ventral nucleus, pars dorsalis, of the

TABLE 1

The brain areas studied, their abbreviations and coordinates, the number of rats implanted per area and the mean aversive threshold and escape latency obtained from each of these regions.

Brain Area	Abbreviation	Coordinate	No.of Rats	Mean Aversive Threshold (UA)	Average Escape Latency (mm)
Fimbria Hippocampi	FIH	-125	10	Not obtained	- - - - -
Ventral Hippocampus	VHP	-327	10	Not obtained	- - - - -
Dorsal Hippocampus	HPC	-515	40	32 ± 2.3	1.5 ± 0.24
Anterior Hypothalamus	AHA	-118	20	30 ± 2.3	1.6 ± 0.25
Lateral Hypothalamus	LH	-3,1.5,8.5	37	15 ± 1.0	3.9 ± 0.39
Anterior Amygdaloid Area	AAA	+129	10	Not obtained	- - - - -
Dorsal Midbrain	DMB	-715	40	20 ± 1.6	1.1 ± 0.19
Caudate Nucleus	CPU	025	10	Not obtained	- - - - -
Medial Lemniscus	ML	-518	41	19 ± 1.2	2.2 ± 0.36
Optic Tract	OT	-129	12	14 ± 1.5	2.4 ± 0.41
Thalmic Nuclei					
Parafascicular Paraventricular Complex	PVF	-416	30	53 ± 4.3	1.5 ± 0.21
Mediodorsal Nucleus	MD	-316	40	46 ± 5.5	2.2 ± 0.34
Ventrodorsal Nucleus[1]	VD	-426	40	42 ± 3.1	2.0 ± 0.41
Ventral Anterior Nucleus	VA	-227	10	Not obtained	- - - - -

[1]This is the ventral nucleus, pars dorsalis of the thalamus which, for the sake of brevity, will be referred to as the ventrodorsal nucleus in the body of the text.

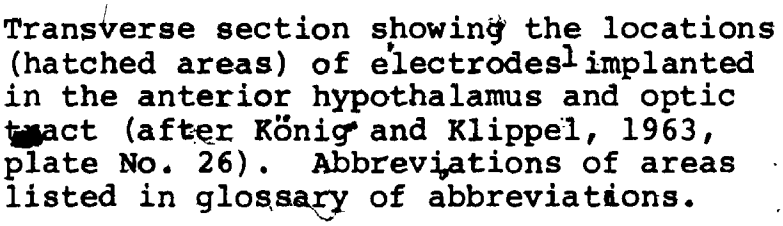
thalamus (Figure 8), dorsal hippocampus (subiculum) and medial lemniscus (Figure 9) and the dorsal mesencephalic reticular formation (Figure 10). In all cases, the stimulus intensities required to elicit the aversive behaviour were of the same order of magnitude as those reported by other investigators using similar experimental techniques (Delgado, et al., 1954, 1956; Delgado, 1955; Olds and Olds, 1963; Valenstein, 1966). Brain areas from which aversive thresholds and/or escape reactions could not be obtained but from which ipsilateral circling or cringing was elicited in response to the electrical stimulus included the anterior amygdaloid area, caudate nucleus, ventral anterior nucleus of the thalamus, fimbria hippocampi and the ventral hippocampus.

The relative anatomical positions of all the brain areas investigated in the present study are depicted in a composite sagittal section of the brain shown in Figure 11. Figure 12 shows photomicrographs taken of representative histological sections in which the electrode tract was located in either the mediodorsal nucleus of the thalamus (top), dorsal hippocampus (middle) or dorsal mesencephalic reticular formation (bottom).

Distribution of aversive thresholds

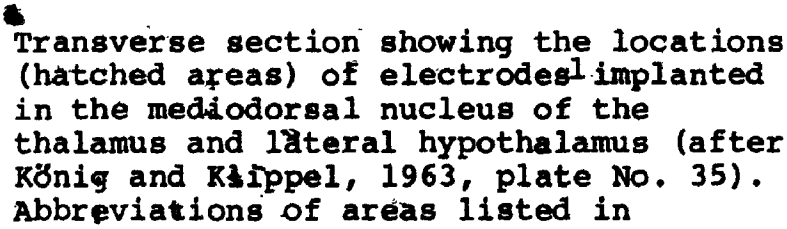
In order to ensure that the behavioural responses obtained with this type of experimental apparatus pro-

Figure 6



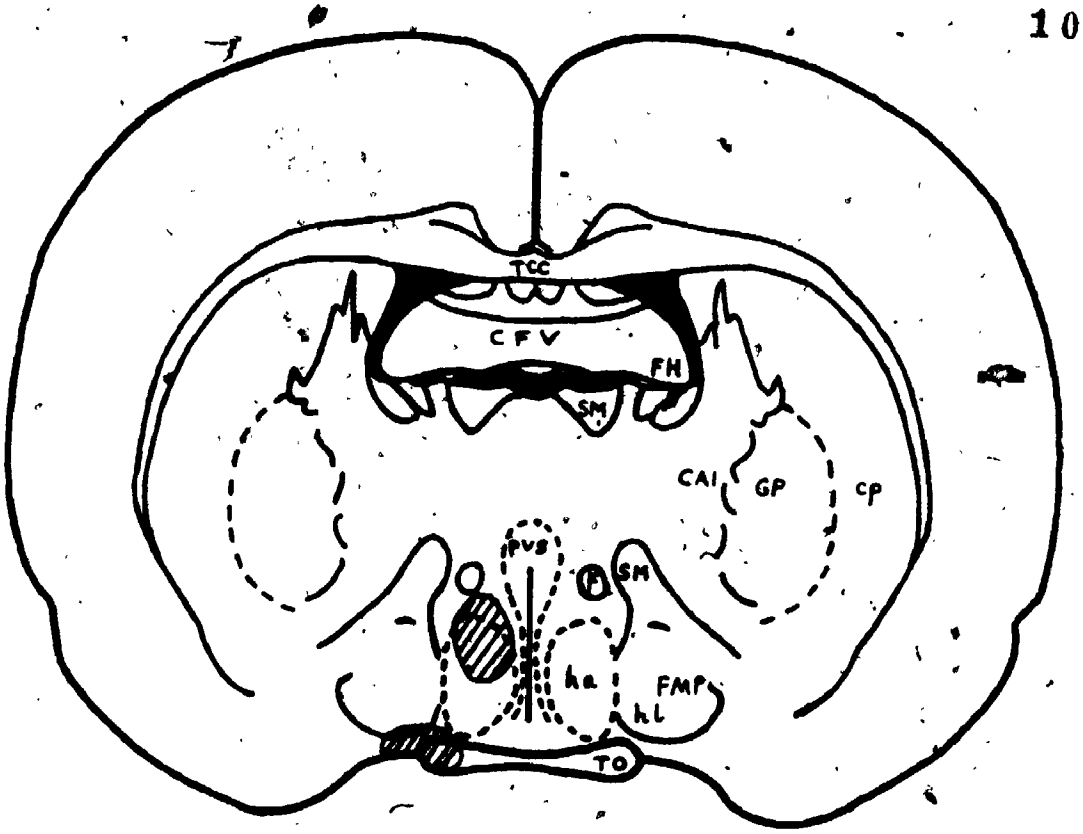
Transverse section showing the locations (hatched areas) of electrodes[1] implanted in the anterior hypothalamus and optic tract (after König and Klippel, 1963, plate No. 26). Abbreviations of areas listed in glossary of abbreviations.

Figure 7



Transverse section showing the locations (hatched areas) of electrodes[1] implanted in the mediodorsal nucleus of the thalamus and lateral hypothalamus (after König and Klippel, 1963, plate No. 35). Abbreviations of areas listed in glossary of abbreviations.

[1] The number of electrodes implanted into each brain area is indicated in Table 1.


TCC
CFV
FH
SM
CAI
GP
cp
IPVS
SM
hL
FMP
TO

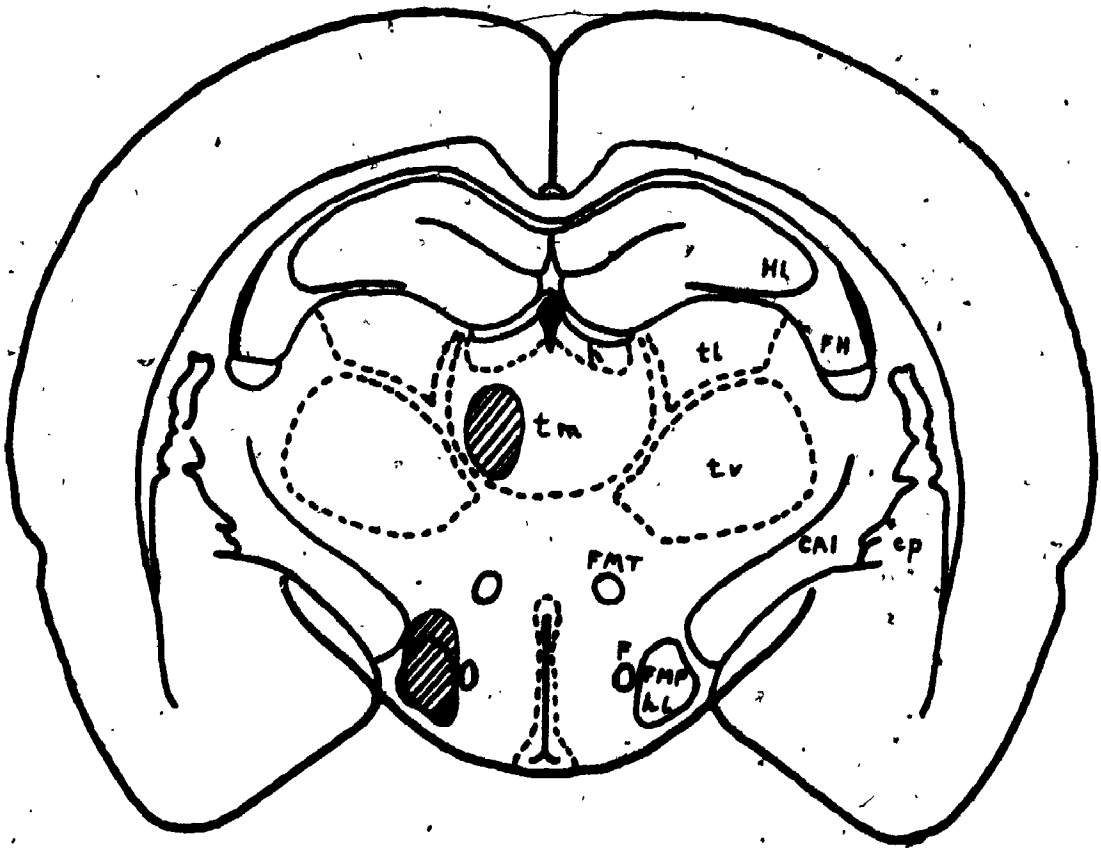
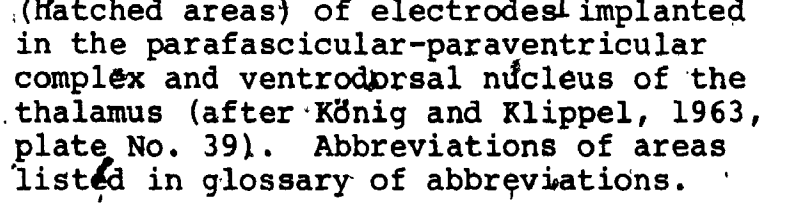
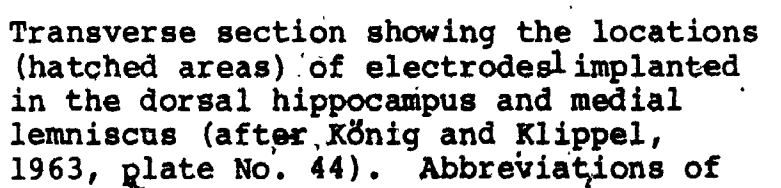

HL
FH
tl
tm
tv
CAI
cp
FMT
FMP
hL

Figure 8



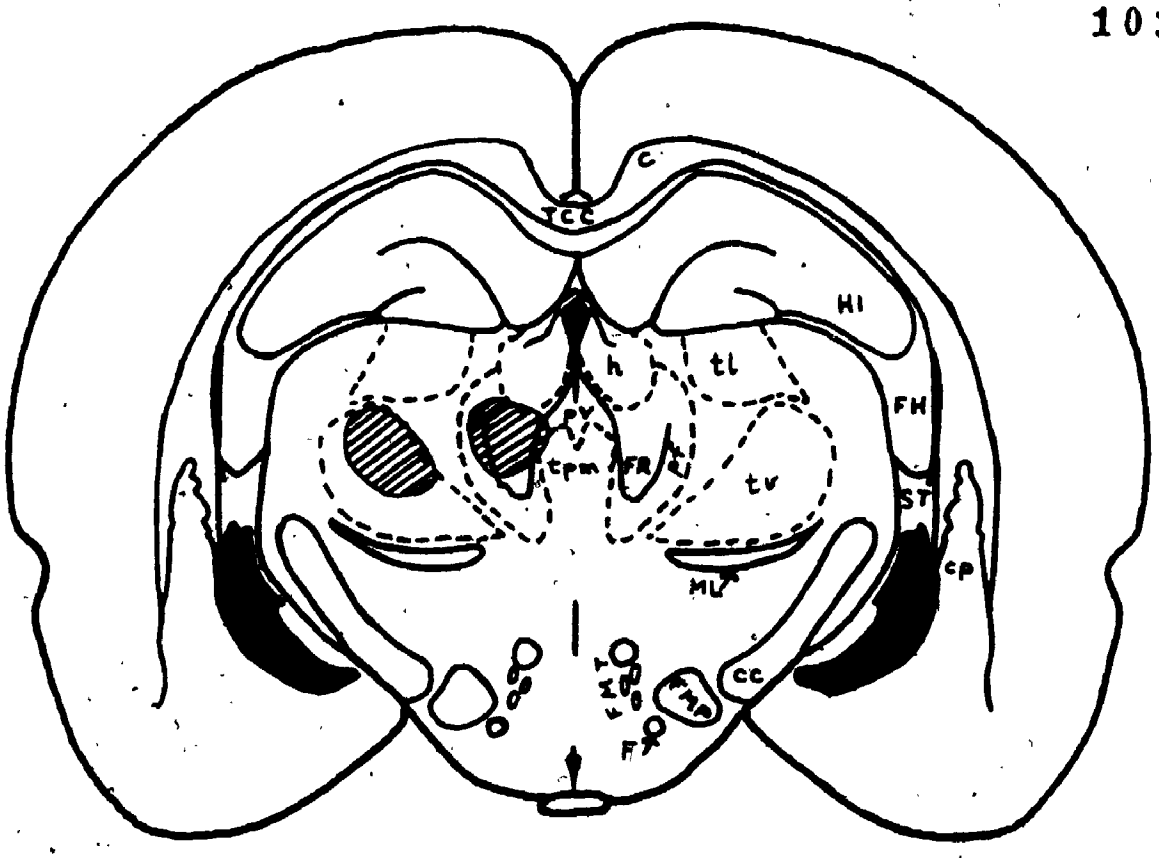
Transverse section showing the locations (hatched areas) of electrodes[1] implanted in the parafascicular-paraventricular complex and ventrodorsal nucleus of the thalamus (after König and Klippel, 1963, plate No. 39). Abbreviations of areas listed in glossary of abbreviations.

Figure 9



Transverse section showing the locations (hatched areas) of electrodes[1] implanted in the dorsal hippocampus and medial lemniscus (after König and Klippel, 1963, plate No. 44). Abbreviations of areas listed in glossary of abbreviations.

[1]The number of electrodes implanted into each brain area is indicated in Table 1.


C
TCC
HI
h
tl
FH
tpm
FR
tv
ST
CP
ML
CC
FMP
FR

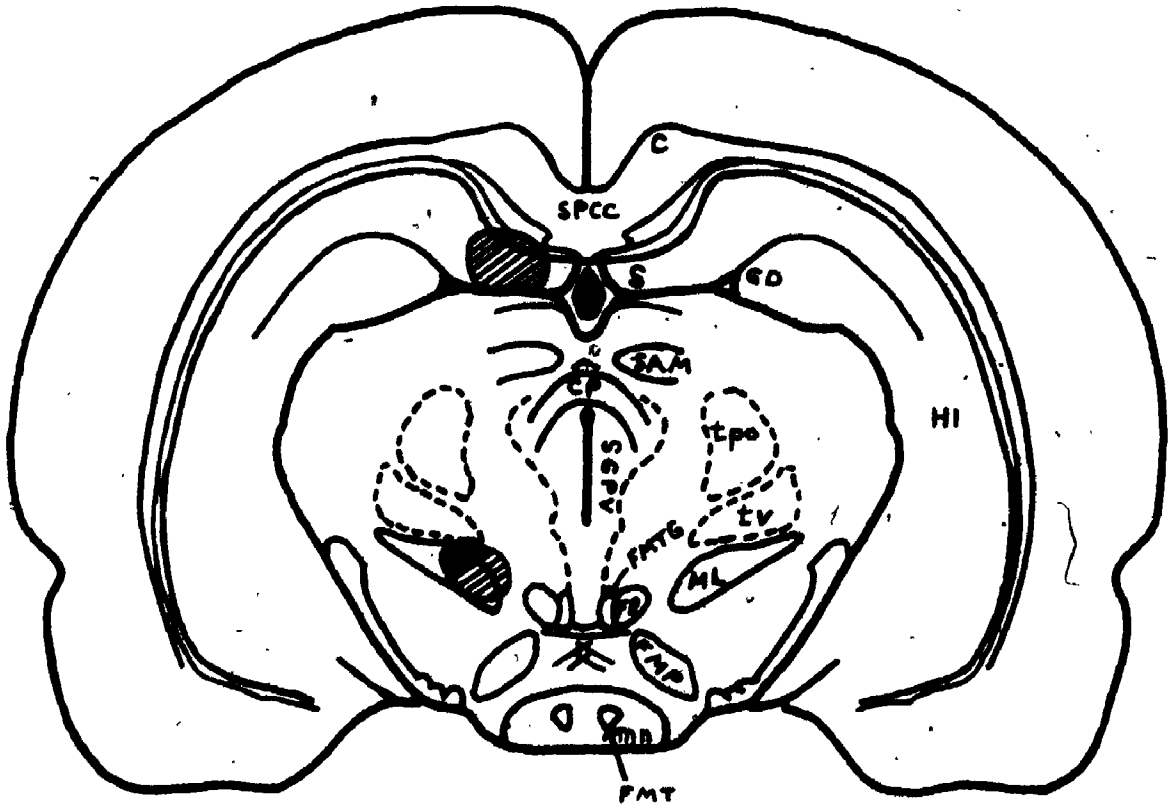
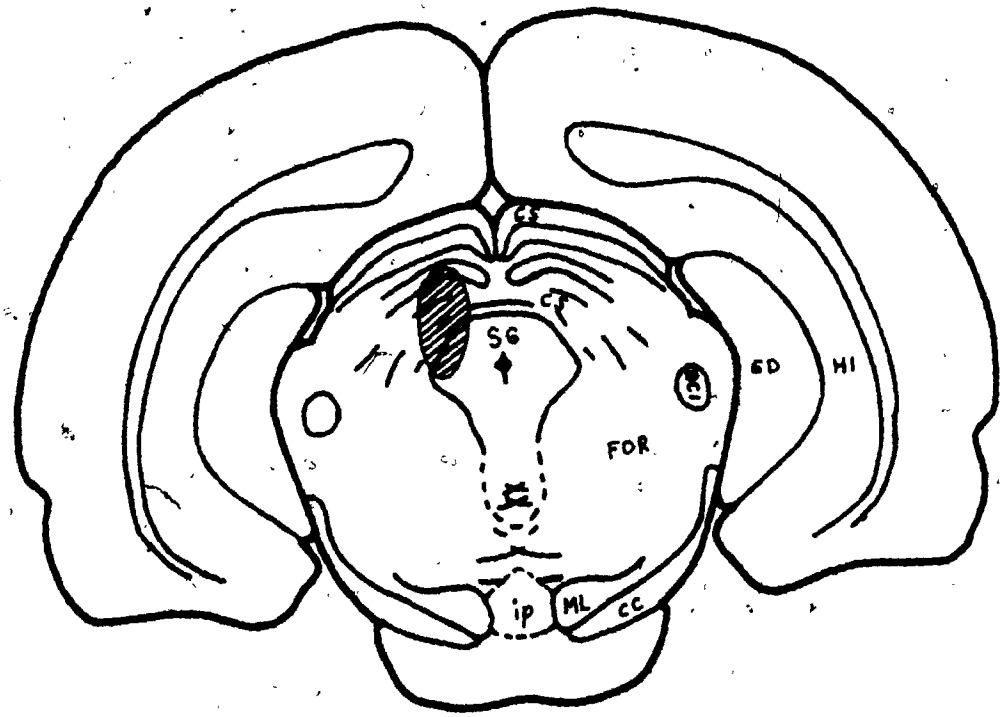

C
SPCC
S
GD
SAM
HI
tpo
tv
FMTG
ML
FMP
FMT

Figure 10

Transverse section showing the locations (hatched area) of electrodes implanted in the dorsal mesencephalic reticular formation (after König and Klippel, 1963, plate No. 50). The number of electrodes implanted into this brain area is indicated in Table 1. Abbreviations of areas listed in glossary of abbreviations.

Figure 11

Composite saggital section depicting the brain areas stimulated in the present study. Abbreviations of areas listed in glossary of abbreviations.


CS
CF
SG
GD
HI
FOR
ip
ML
CC

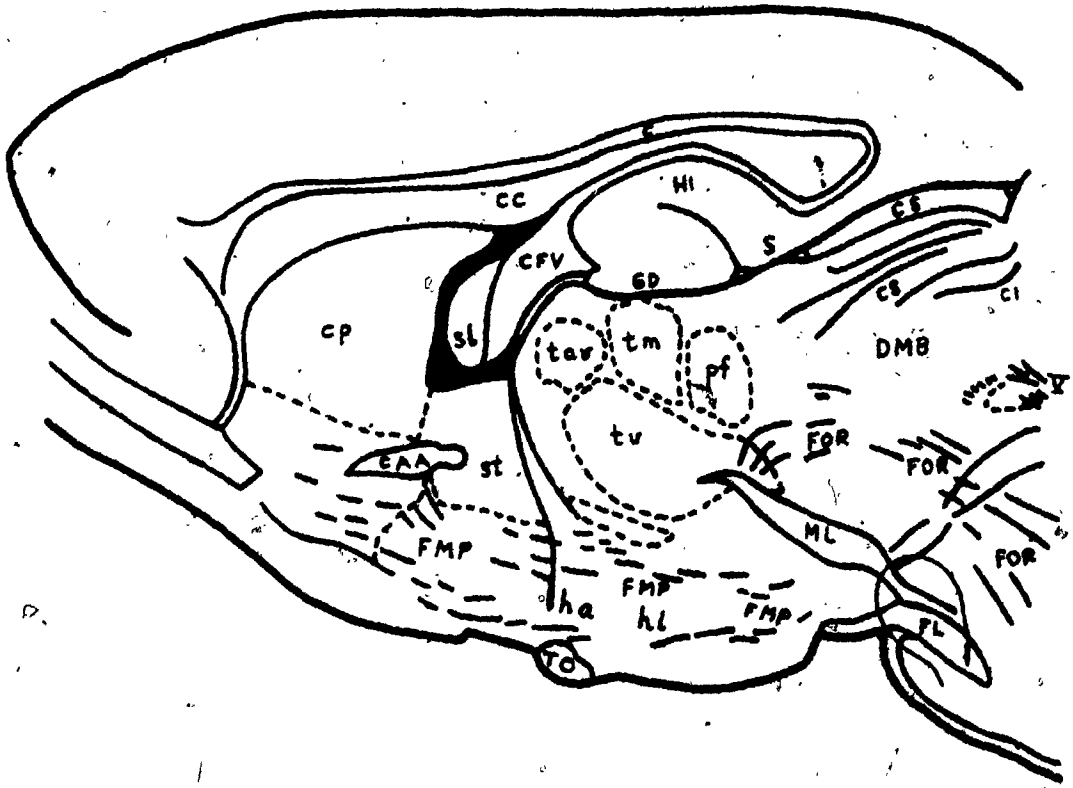

CC
HI
CS
CFV
S
GD
CS
CI
CP
SL
tar
tm
pf
DMB
V
tv
FOR
FOR
EAA
ST
ML
FMP
FOR
FMP
ha
hl
FMP
FL
TO

Figure 12

Photomicrographs of representative histological sections in which the electrode tract was located in either the mediodorsal nucleus of the thalamus (top), dorsal hippocampus (middle) or dorsal mesencephalic reticular formation (bottom). The deepest point of penetration was taken to be the area stimulated by the electrode tip.

vides meaningful statistical data, the aversive thresholds of 80 naive and 48 "stabilized" rats were determined in the foot shock situation. The latter group was included since it was found, as did others (Shaklee, 1957), that rats with previous shuttle-box experience consistently attained lower thresholds than rats without prior exposure to the experimental apparatus. As is common with most biological phenomena, the distributions of the aversive thresholds were found to be normal for both groups, and slightly skewed to the right (Figure 13). Some of the more important characteristics of the two normal frequency distributions were as follows:

	N	Mean	SD	Mode	Median	Skew
Naive rats	80	.603	.14	.560	.587	+ .29
Stabilized rats	48	.434[1]	.07	.331	.337	+ .17

The stabilized rats produced a narrower, more symmetrical frequency distribution, but, in both cases, the skew was insignificant. These data therefore indicated that the assumptions of normality and homogeneity required for the use of parametric statistics were justified.

Escape performance - shock intensity functions

The functional relationship between escape performance and shock intensity for ten "stabilized" rats was found to be typically sigmoid (Figure 14). As has

[1] Significantly different from the mean of the naive rats ($P<0.01$).

Figure 13.

Distribution of the aversive thresholds to foot-shock of 80 naive and 48 "stabilized" rats. "Stabilized" rats are those animals which have had prior shuttle-box experience and whose threshold values had attained steady baseline levels.

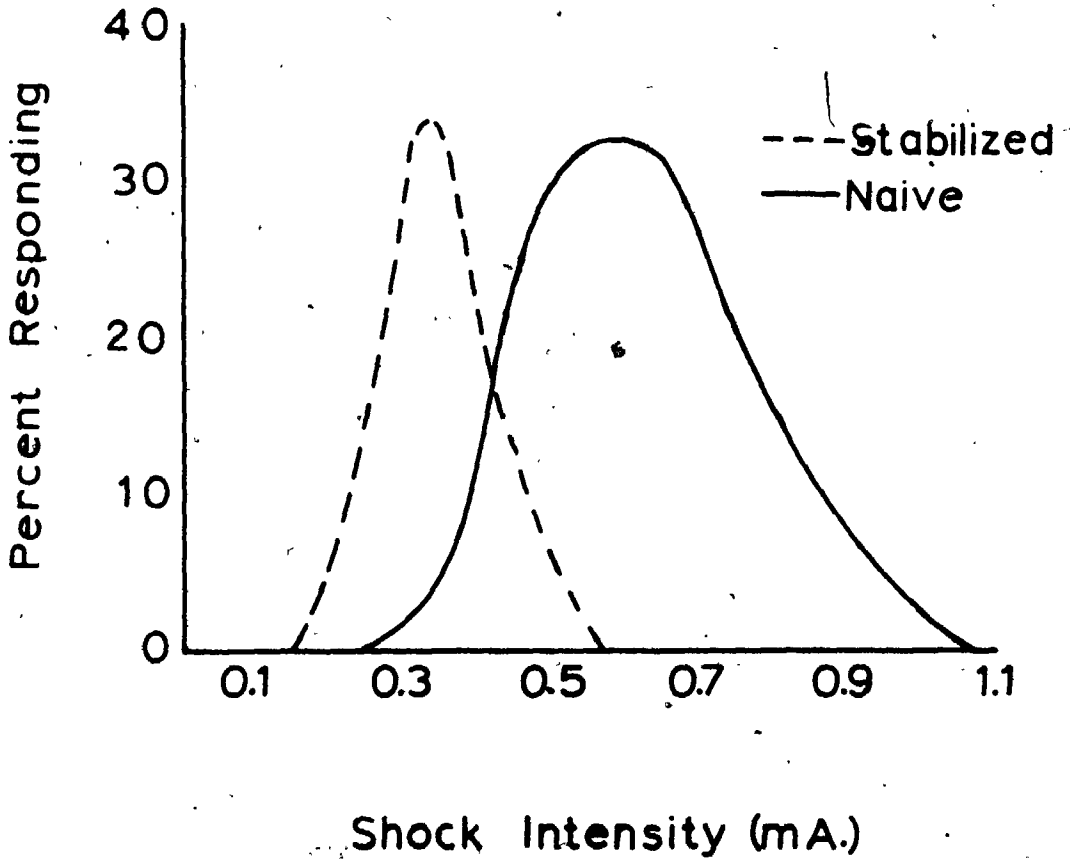
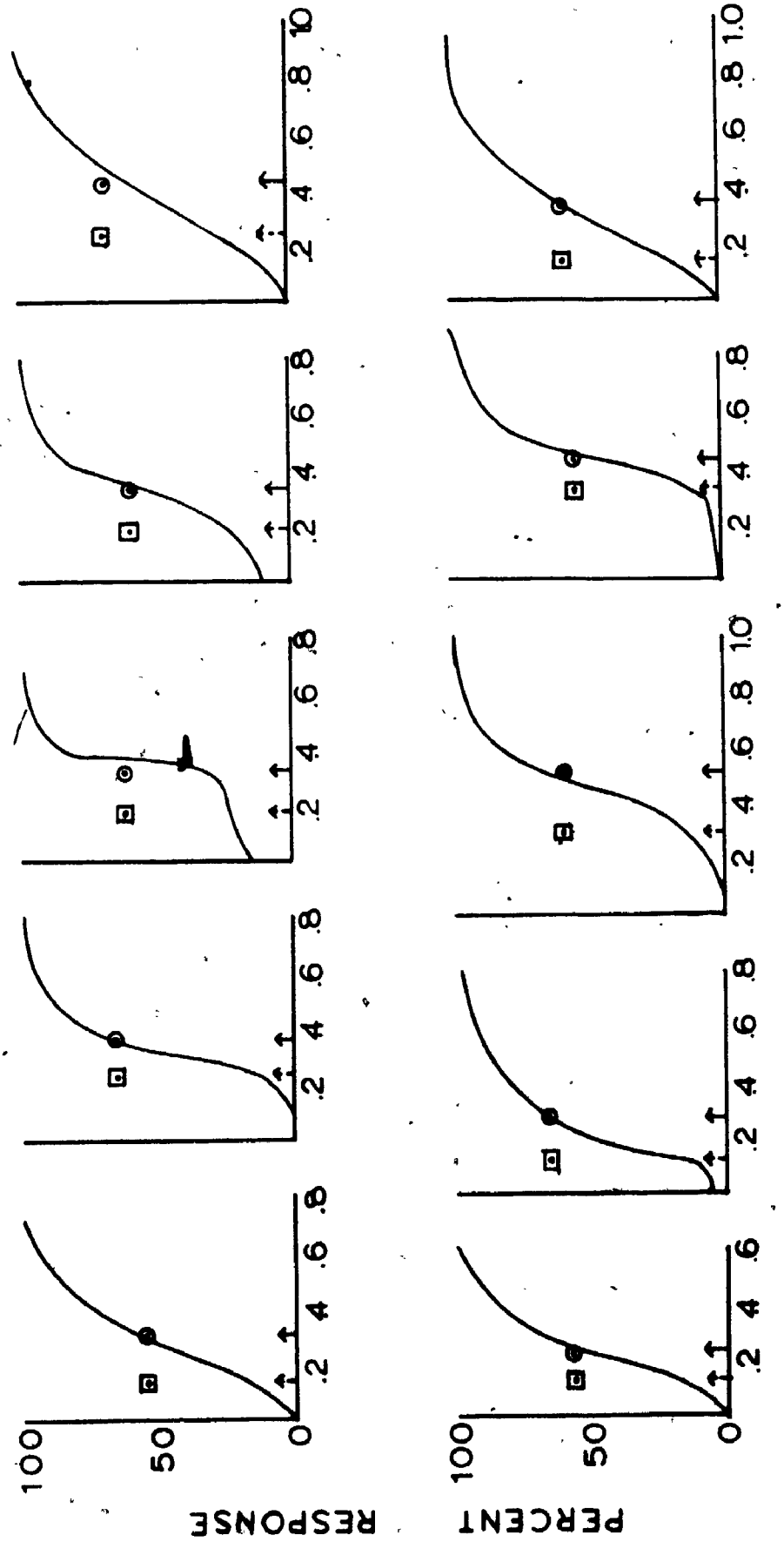

40
30
20
10
0
Percent Responding
Stabilized
Naive
0.1
0.3
0.5
0.7
0.9
1.1
Shock Intensity (mA.)

Figure 14

Functional relationship between percent escape response (abscissa) and shock intensity (ordinate) for 10 "stabilized" rats.


PERCENT RESPONSE
100
50
0
.2
.4
.6
.8
1.0
SHOCK INTENSITY (mA.)

been thoroughly demonstrated by other investigators (Dinsmoor and Winograd, 1958; Boren, Sidman and Herrnstein, 1959; Hutchinson, Azrin and Renfrew, 1968; Domjan and Rowell, 1969), the rates of responding were roughly proportional to the level of current and approached 100% responding asymptotically as the stimulus intensity neared one milliampere. The current levels generating a consistent escape response were, on the average, 2.2 times the threshold values.

The results of an analysis of variance of these data showed that the percent response was significantly related to shock intensity ($P<0.01$). A trend analysis within these data by orthogonal polynomial comparisons indicated that the functional relationship between response frequency and current level contained a significant linear component ($P<0.01$), with the F value for the residual variance after extraction of this linear component being less than 1.

Because the electrical stimulation is gradually increased from zero to some maximal value, the titration technique arranges that low-level stimuli, not in themselves aversive, are presented before the really aversive levels are reached. If the low-level stimuli are discriminable, they might well come to occupy the status of warning signals which precede the truly aversive stimulus

(Boren and Malis, 1961). One would therefore expect the animal to respond to the warning signals rather than waiting for the shock to appear, in a manner similar to a conditioned avoidance procedure. For this reason, thresholds were quantified in two ways, one taking into account the low stimulus intensities (average median current) and one in which only the highest stimulus intensities which elicited a response (average peak current) were considered. These threshold values were superimposed on the response rate-shock intensity curves shown in Figure 14. The solid line arrows represent the threshold values measured in terms of the average peak current observed over a fifteen-minute interval (a peak being defined when the current had previously risen by at least three steps and when the animal then drove the current down three steps or more), whereas the broken arrows indicate the threshold values when expressed in terms of the average median current (average of the peaks and valleys of the threshold tracing) obtained over the same time period (Figure 14). The corresponding rates of responding at these two threshold values are indicated by the circles and squares respectively (Figure 14).

When threshold values were expressed in terms of average peak current, all ten rats responded with the same frequency as would be expected if the stimulus

intensities were fixed at this level, i.e., the response rate lay directly upon the escape performance-shock intensity continuum. This was taken as evidence that, at these thresholds, animals were responding to stimulus intensities that were aversive to them. On the other hand, when thresholds were quantified in terms of average median current, the response rates were much higher than would be expected from the escape performance-shock intensity curves, indicating that, at these values, the response was due, in a large part, to conditioned avoidance. Hence, for the purpose of this study, thresholds were always measured in terms of average peak current, and this value was doubled when the effects of the narcotic analgetics on centrally-elicited escape were evaluated.

Endpoint for analgesia

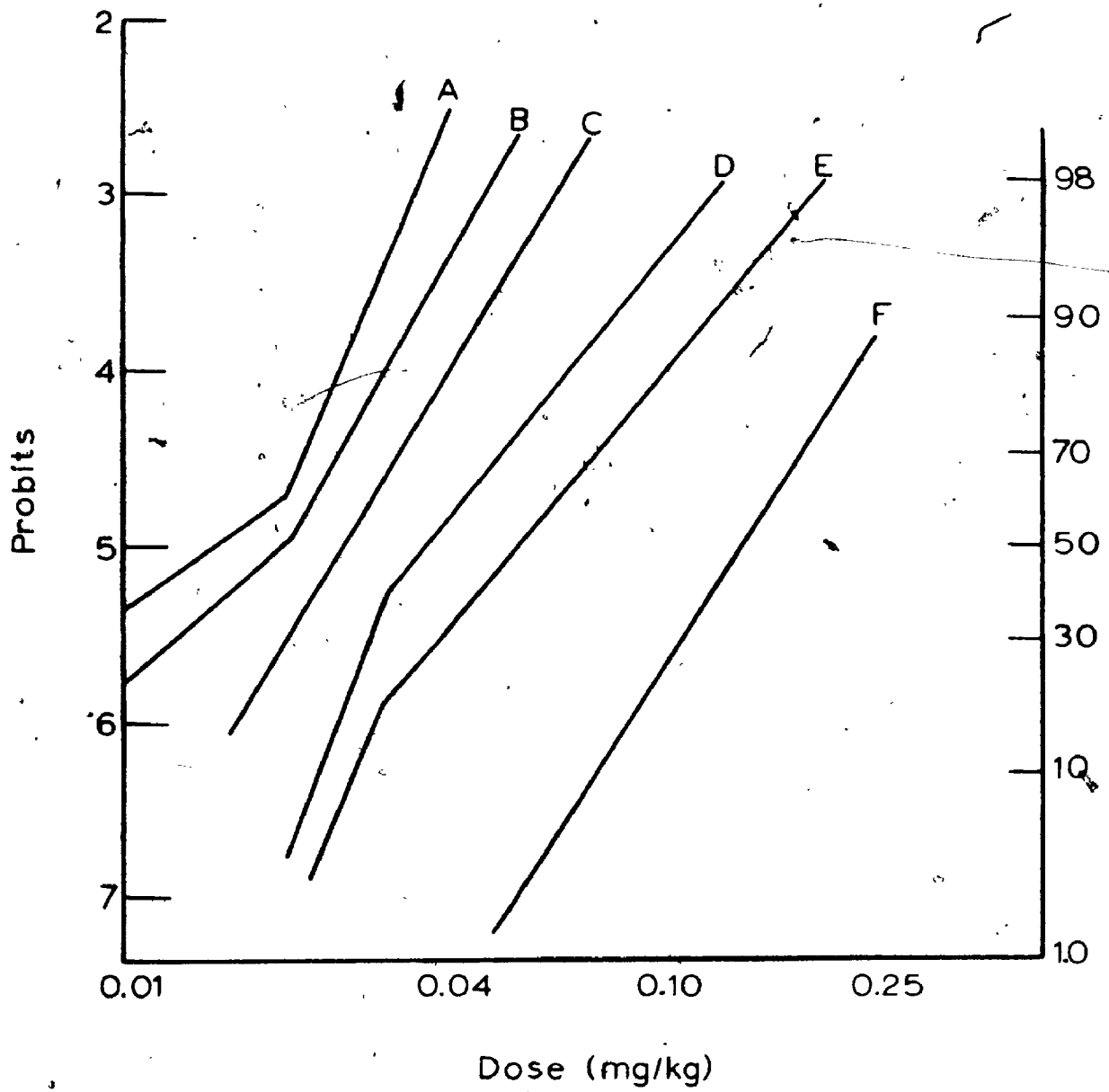
In the evaluation of escape responses following drug administration, several endpoints were investigated as criteria for analgesia in order to determine which would yield the best dose-response data. This study was conducted with fentanyl in rats with electrodes chronically implanted in the hippocampus. The dose-response curves generated by the following five criteria: (1) escape latencies less than, or equal to, the mean control response latency, (2) escape latencies less than,

or equal to, the mean control response latency plus one or (3) two standard deviations of the mean, (4) escape latencies less than, or equal to, the longest response latency observed over a fifteen-minute control period, and (5) escape latencies equal to, or less than, the duration of the shock interval (10 seconds), are shown in Figure 15, lines A to E, respectively.

As one might expect, the selection of different criteria of drug effectiveness led to different AD_{50} values, with the longer response latencies yielding the higher AD_{50} values. Of the various endpoints used, numbers (3) and (5) yielded linear dose-response curves when the percentages of escape responses were plotted against log-dose on probit paper. However, the dose required to completely abolish the escape response using the latter as the criterium induced "lead pipe rigidity" in over 50% of the animals (line F, Figure 15), whereas complete inhibition of escape, with the former as the endpoint, occurred at dose levels which produced adverse side effects in only 10% of the animals. This suggested that some of the rats allowed to go to a cut off time of 10 seconds (the duration of the shock period) may have failed to escape because they had been temporarily incapacitated in their performance of the motor response. The AD_{50} value obtained with response latencies less than,

Figure 15

Dose-response curves obtained by using various functions of the control response latency as the end-point for analgesia. The different criteria of analgesic effectiveness used were: escape latencies less than, or equal to, the mean control response latency (line A), escape latencies less than, or equal to, the mean control response latency plus one (line B) or two (line C) standard deviations of the mean, escape latencies less than, or equal to, the longest response latency observed over a fifteen-minute control period (line D), and escape latencies less than, or equal to, the duration of the shock interval (ten seconds, line E). Line F indicates the percent of animals (right-hand abscissa) showing "lead-pipe" rigidity at the higher dose levels.


2
3
4
5
6
7
Probits
A
B
C
D
E
F
98
90
70
50
30
10
1.0
Percent of animals showing 'lead pipe' rigidity
0.01
0.04
0.10
0.25
Dose (mg/kg)

or equal to, the upper limit of the 95% confidence interval of the control mean response latency therefore provided the best criterium for expressing the dose required to induce analgesia uncomplicated by untoward side effects.

Reproducibility of the aversive behaviour

Once the threshold values had become stabilized, they remained constant and relatively characteristic for each brain area for periods up to several months (Table 2). Since escape responding was always studied at stimulus intensities twice that of the threshold values, these too remained stable over long periods of time (Table 2).

Temporal features of the drug effect

Quite often the magnitude of a drug effect is measured at a uniform, rigidly pre-set time interval after drug administration which may not necessarily coincide with the time of the peak effect. The intensity measured may then be an intensity of effect reached anywhere during the rise to, or fall from, the peak effect of the dose examined. Unless ED_{50} values are determined at the time of peak effect, they are virtually meaningless as a measure of potency of the drug, particularly when they are to be compared to the ED_{50} of other compounds in a relative potency analysis (Loewe, 1952). The time-effect curves for the various analgetics were therefore determined in pilot experiments and are shown in Figure 16.

TABLE 2

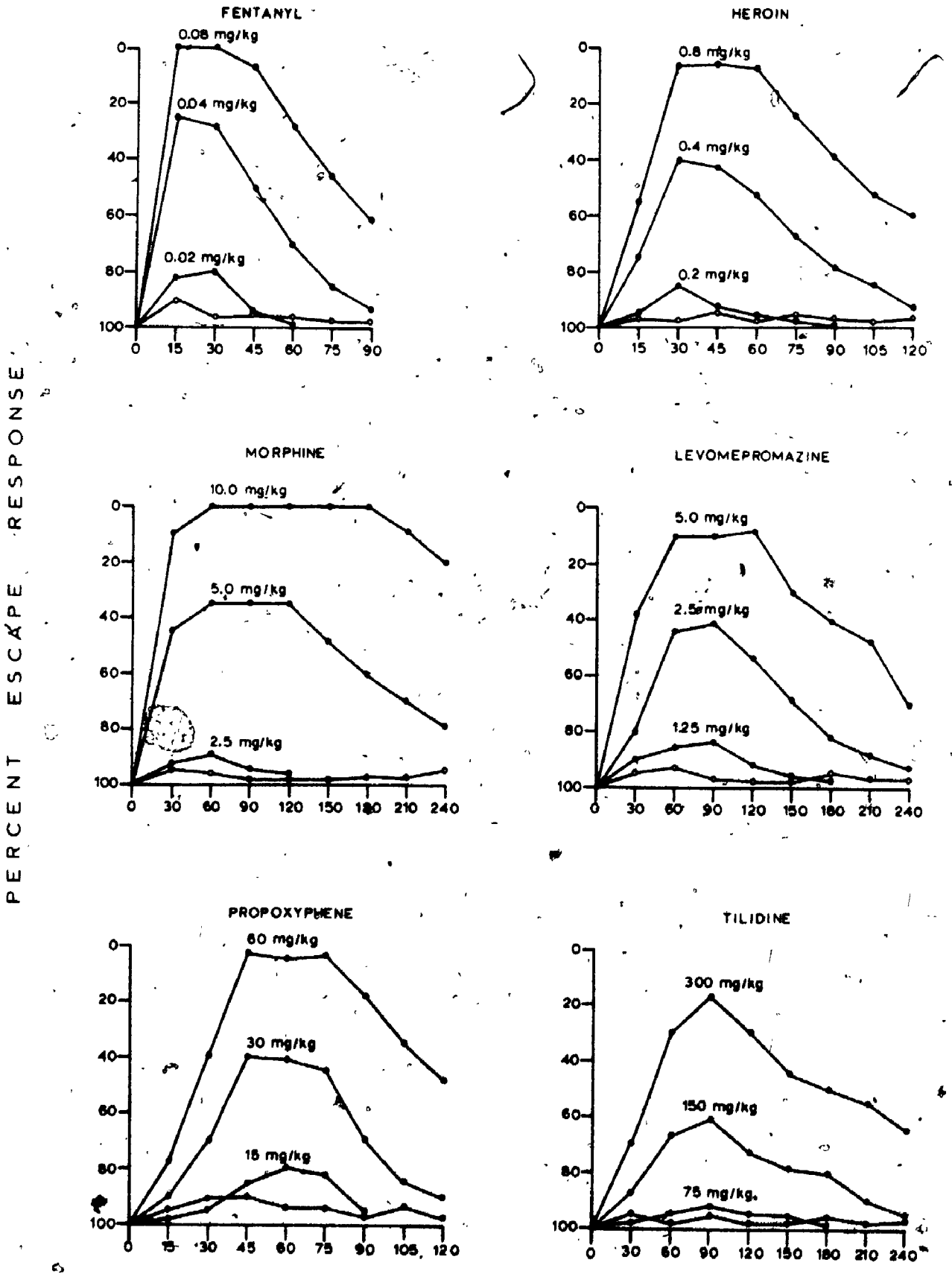
An example, for each brain area, of the aversive thresholds and escape latencies obtained from the same rat at monthly intervals for a period of six months.

BRAIN AREA	MONTH 1	2	3	4	5	6	MEAN ± S.D.
			AVERSIVE THRESHOLDS (μA)				
HPC	25	33	28	39	26	31	30 ± 4.7
AHA	30	26	24	28	35	29	29 ± 3.5
LH	15	21	17	12	16	19	17 ± 2.9
DMB	18	11	17	22	19	25	19 ± 4.1
ML	18	23	27	19	24	21	22 ± 3.1
OT	7	13	16	8	12	9	11 ± 3.1
PVF	50	48	53	55	59	48	52 ± 3.9
MD	48	50	44	39	46	41	45 ± 3.8
VD	35	39	45	41	49	36	41 ± 4.9
			ESCAPE LATENCIES (mm)[1]				
HPC	1.6	1.7	1.3	1.5	1.0	1.9	1.5 ± 0.29
AHA	1.8	2.2	2.0	1.4	1.6	1.3	1.7 ± 0.32
LH	3.9	3.3	4.1	3.5	4.2	3.8	3.8 ± 0.31
DMB	1.4	0.9	0.9	1.1	1.0	1.1	1.1 ± 0.17
ML	2.3	2.3	1.9	2.4	1.7	1.9	2.1 ± 0.26
OT	2.9	3.3	2.5	2.0	2.3	2.7	2.6 ± 0.42
PVF	1.4	0.9	1.5	0.9	1.2	1.3	1.2 ± 0.23
MD	2.7	2.1	2.9	2.7	1.9	2.2	2.4 ± 0.37
VD	1.6	1.7	2.2	1.6	2.4	2.5	2.0 ± 0.39

[1] 1 mm represents 1.5 seconds

Figure 16

Time-effect curves for fentanyl, heroin, morphine, levomepromazine, propoxyphene and tilidine. Each point represents the mean response of 10 animals.


FENTANYL
0.08 mg/kg
0.04 mg/kg
0.02 mg/kg
HEROIN
0.8 mg/kg
0.4 mg/kg
0.2 mg/kg
MORPHINE
10.0 mg/kg
5.0 mg/kg
2.5 mg/kg
LEVOMEPROMAZINE
5.0 mg/kg
2.5mg/kg
1.25 mg/kg
PROPOXYPHENE
60 mg/kg
30 mg/kg
15 mg/kg
TILIDINE
300 mg/kg
150 mg/kg
75 mg/kg
PERCENT ESCAPE RESPONSE
TIME (min)

The response-duration parameters of these compounds at doses near the AD_{50} value are presented in Table 3.

Table 3

The response-duration parameters of the various analgetics at doses approximately the AD_{50} value.

Drug	Dose mg/kg	Percent Response	Times (min) Peak	Duration
Morphine	5.0	35	30-60	240
Fentanyl	0.04	25	15	90
Heroin	0.4	40	30	120
Levomepromazine	2.5	41	60-90	240
Propoxyphene	36	40	45-60	120
Tilidine	175	61	60-90	240

The time-effect curves for morphine, levomepromazine, propoxyphene and tilidine were roughly similar; the first significant effects were observed, at AD_{50} levels, within 30 minutes after subcutaneous injection, the peak effects between 30 and 90 minutes and the last significant effects at approximately 4 hours after administration. Propoxyphene tended to be somewhat shorter acting (2 hours) than the other three compounds. Heroin and fentanyl were faster and shorter acting at AD_{50} levels than morphine. At higher dose levels, however, the duration of action of these drugs increased considerably.

Effects of analgetics on centrally-elicited escape

Table 4 lists the dose-response parameters, and the results of the relative potency analysis of these data, for the effects of fentanyl, heroin, morphine, levomepromazine, propoxyphene and tilidine on the escape response elicited by disparate aversive brain stimulation. These data are depicted in graphical form in Figure 17. For all drugs, a differential sensitivity of the brain areas was noted.

The dose-response curves obtained with each narcotic analgetic in the various brain areas did not differ significantly from parallelism ($P>0.05$), as was the case when the dose-response curves obtained with the different narcotic analgetics in any one brain area were compared ($P>.05$). Levomepromazine, on the other hand, produced two families of parallel dose-response curves — one with a shallow slope (areas MD, HPC, LH and OT) and one with a very steep slope (areas VD, DMB and ML) — neither of which was parallel to the corresponding dose-response curves obtained with the narcotic analgetics. Since the specific opiate antagonist, naloxone (10 mg/kg sc.), antagonized the effects of the narcotic analgetics but not those of levomepromazine, these results may be taken to indicate that the narcotic analgetics act at receptor sites, or have mechanisms of action, which differ from

TABLE 4

Dose-response parameters of the various analgetics in the different areas of the brain

FENTANYL

Brain Area	AD_{50} (mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No. of Trials	Relative Activity
VD	0.018	0.014-0.022	2.1	0.22	21	1.00[a]
MD	0.022	0.018-0.026	2.1	0.23	21	0.82[a]
PVF	0.023	0.014-0.032	2.1	0.40	18	0.78[a]
ML	0.042	0.035-0.048	2.2	0.31	21	0.43[b]
LH	0.052	0.049-0.056	1.9	0.20	21	0.35[c]
HPC	0.063	0.061-0.066	2.3	0.18	27	0.29[d]
DMB	0.073	0.071-0.076	2.0	0.18	30	0.25[e]

common slope = 2.1 ± 0.09

HEROIN

Brain Area	AD_{50} (mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No. of Trials	Relative Activity
MD	0.21	0.17-0.25	1.9	0.24	21	1.00[a]
VD	0.23	0.18-0.28	1.9	0.29	24	0.88[a]
LH	0.24	0.20-0.27	1.9	0.23	24	0.78[a]
HPC	0.30	0.27-0.33	2.0	0.22	27	0.70[b]
DMB	0.75	0.65-0.85	2.2	0.42	24	0.28[c]
AHA	1.12	1.08-1.17	2.0	0.26	15	0.19[d]
ML	1.18	1.12-1.25	2.0	0.32	21	0.18[d]

common slope = 2.0 ± 0.06

a, b, c, d, e AD_{50} values significantly different from each other, $P < 0.05$

TABLE 4: *Continued*

MORPHINE

Brain Area	AD_{50} (Mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No. of Trials	Relative Activity
MD	3.0	2.6-3.4	2.6	0.24	21	1.00[a]
PVF	3.2	2.6-3.8	2.6	0.29	15	0.94[a]
LH	3.4	3.0-3.9	2.6	0.26	24	0.88[a]
VD	3.6	3.2-3.9	2.6	0.21	21	0.83[a]
HPC	4.8	4.0-5.6	2.7	0.39	24	0.63[b]
OT	5.6	5.2-6.1	2.7	0.25	15	0.54[b]
DMB	7.1	6.2-8.0	2.8	0.41	27	0.42[c]
AHA	8.8	8.1-9.4	2.7	0.32	15	0.34[d]
ML	9.0	8.6-9.4	2.4	0.24	18	0.33[d]

common slope = 2.6 ± 0.11

LEVOMEPROMAZINE

Brain Area	AD_{50} (Mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No. of Trials	Relative Activity
MD	1.2	0.8-1.6	1.4	0.23	15	1.00[a]
HPC	2.0	1.6-2.5	1.2	0.25	15	0.60[b]
LH	2.2	1.4-3.1	1.2	0.39	15	0.55[b]
OT	3.6	3.3-3.8	1.6	0.13	15	0.33[b]

common slope = 1.3 ± 0.15

VD	5.2	3.9-6.4	3.8	0.52	15	1.00[a]
DMB	5.8	4.3-7.3	5.1	0.65	15	0.90[a]
ML	7.5	5.8-9.3	4.7	0.70	15	0.69[a]

common slope = 4.5 ± 0.45

a, b, c, d AD_{50} significantly different from each other, $P < 0.05$

TABLE 4: *Continued*

PROPOXYPHENE

Brain Area	AD_{50} (mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No.of Trials	Relative Activity
MD	24.7	12.9-36.5	2.3	0.47	24	1.00[a]
VD	28.4	20.4-36.3	2.1	0.38	24	0.87[a]
LH	57.1	49.8-64.5	2.1	0.35	21	0.43[b]
HPC	61.4	57.2-65.6	2.2	0.24	24	0.40[b]
DMB	71.5	67.5-75.5	2.2	0.24	21	0.35[c]
AHA	78.4	75.4-83.3	2.1	0.22	15	0.32[d]
ML	81.8	73.3-90.3	2.5	0.39	21	0.30[d]

common slope = 2.2 ± 0.12

TILIDINE

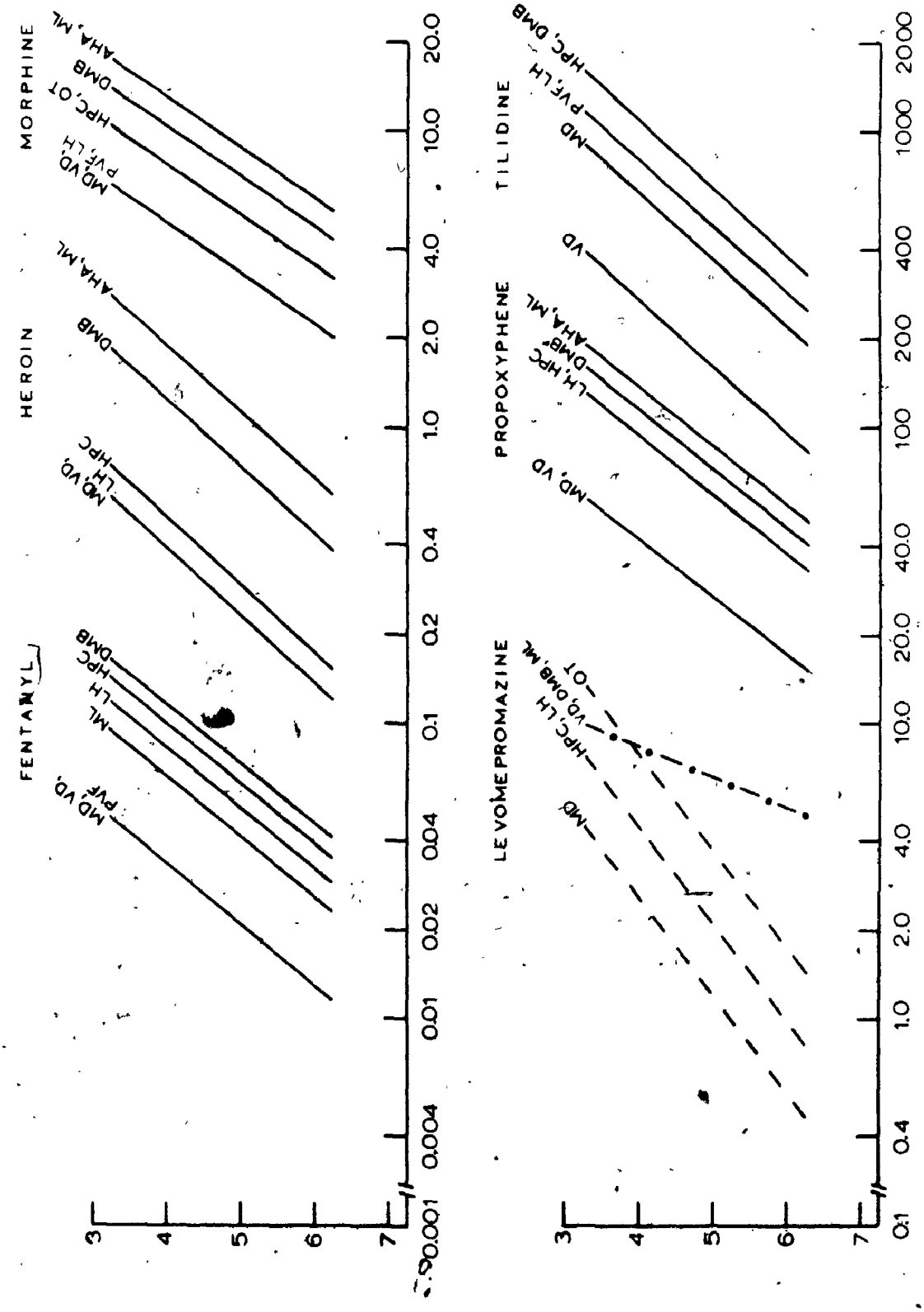
Brain Area	AD_{50} (mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No.of Trials	Relative Activity
VD	158.5	151.4-165.6	2.1	0.33	25	1.00[a]
MD	366.6	358.4-374.7	1.9	0.36	21	0.43[b]
PVF	469.9	459.8-479.9	1.9	0.42	24	0.34[c]
LH	481.6	470.1-493.0	1.8	0.46	21	0.33[c]
HPC	603.9	590.2-617.5	1.9	0.59	21	0.26[d]
DMB	632.1	615.3-648.8	2.0	0.68	21	0.25[d]

common slope = 1.9 ± 0.29

a, b, c, d AD_{50} significantly different from each other, $P < 0.05$

Figure 17

Dose-response curves for the various analgetics in the different areas of the brain. AHA, anterior hypothalamus; DMB, dorsal midbrain; HPC, dorsal hippocampus; LH, lateral hypothalamus; MD, mediodorsal nucleus of thalamus; ML, medial lemniscus; OT, optic tract; PVF, parafascicular-paraventricular complex of thalamus; VD, ventrodorsal nucleus of thalamus. If the AD_{50} values obtained for a particular drug in different areas of the brain were not found to be significantly different ($P>0.05$), the dose-response curves for these areas were drawn as a single line with a slope equal to the common slope derived from relative potency analysis. The family of dose-response curves associated with each drug therefore indicates statistically different AD_{50} values at the 5% level. The shifts in the parallel lines thus provides an estimate of the differential sensitivity ($P<0.05$) of the various brain areas to the effects of that particular drug.


FENTANYL
HEROIN
MORPHINE
LEVOMEPROMAZINE
PROPOXYPHENE
TILIDINE
PROBITS
DOSE (mg/kg)

that of the phenothiazine tranquilizers.

The sensitivity of the various brain areas to the effects of the different analgetics may be summarized as follows:

Fentanyl:
VD = MD = PVF < ML < LH < HPC < DMB

Heroin:
MD = VD = LH < HPC < DMB < AHA = ML

Morphine:
MD = PVF = LH = VD < HPC = OT < DMB < AHA = ML

Propoxyphene:
MD = VD < LH = HPC < DMB < AHA = ML

Tilidine:
VD < MD < PVF = LH < HPC = DMB

Levomepromazine:
(1) MD < HPC = LH < OT
(2) VD = DMB = ML

where VD = ventrodorsal nucleus, MD = mediodorsal nucleus, PVF = parafascicular-paraventricular complex, LH = lateral hypothalamus, HPC = dorsal hippocampus, DMB = dorsal mid-brain area, AHA = anterior hypothalamus, ML = medial lemniscus, and OT = optic tract. The equal sign in the above summary signifies statistically similar ($P>0.05$) AD_{50} values whereas the < sign indicates that the AD_{50} values for the preceding structure(s) were significantly less than ($P<0.05$) those obtained for the area(s) following this sign, as determined by the relative potency assay. The use of the AD_{50} as an estimate of the differential sensitivity of the various brain areas to the effects of

the analgetics (i.e., "relative activity", Table 4) is valid since no significant differences were observed among the corresponding slope functions.

The areas most sensitive to the effects of fentanyl were the mediodorsal, ventrodorsal and parafascicular-paraventricular nuclei of the thalamus; the AD_{50} values from these three areas did not differ significantly ($P>0.05$). The medial lemniscus was next in the declining order of sensitivity, followed by, in turn, the lateral hypothalamus and dorsal hippocampus. Least affected by fentanyl were the reticular structures of the dorsal midbrain area.

Morphine and heroin showed similar profiles of activity which were, however, different from that of fentanyl. The thalamic nuclei as well as the lateral hypothalamus were the most sensitive to the effects of morphine and heroin. The anterior hypothalamus and medial lemniscus were the least affected by these drugs, while the hippocampus and optic tract (in the case of morphine) exhibited an intermediate sensitivity.

The activity profile of propoxyphene was similar to that of morphine or heroin, except that the limbic structures (lateral hypothalamus and hippocampus) were less sensitive to the effects of this compound. As with morphine or heroin, the mediodorsal and ventrodorsal

nuclei were the most sensitive to the effects of propoxyphene. The lateral hypothalamus and hippocampus were next in the decreasing order of sensitivity, followed by, first, the dorsal midbrain area and second, the anterior hypothalamus and medial lemniscus.

The activity profile of tilidine differed from that of the other narcotic analgetics in that only one area — the ventrodorsal nucleus of the thalamus — was most affected by the drug. This was followed by a significantly lesser effect on the mediodorsal nucleus. The remaining groups of areas in which tilidine was equally active but significantly less effective were, in the decreasing order of sensitivity, (a) the parafascicular nucleus and lateral hypothalamus and (b) the hippocampus and dorsal midbrain area. However, it should be noted that very high doses of tilidine had to be used in order to produce an effect and that any effects of tilidine beyond those on the mediodorsal nucleus occurred at dose levels exceeding the $\bar{L}D_{50}$ value (400 mg/kg sc.) reported for tilidine in rats (Herrmann, 1970). The results obtained with tilidine are therefore likely to be more a reflection of its toxic effects rather than an indication of its analgesic action. However, even at very high dose levels, no deaths consequent to the administration of this drug were ever observed in the

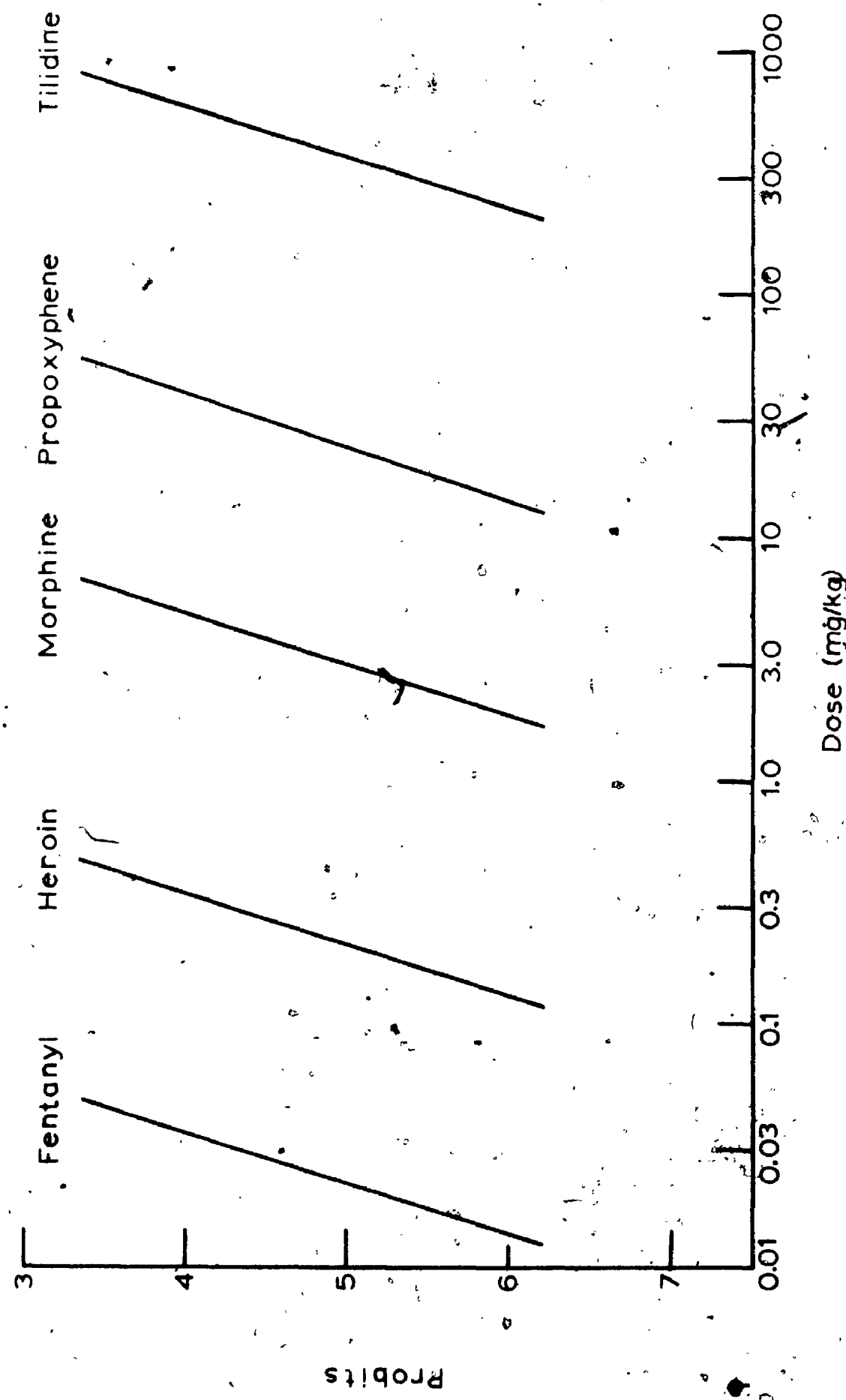
present study.

As has been mentioned previously, two sets of parallel dose response curves were obtained with levomepromazine, one with a shallow slope, the other with a very steep slope. The first group consisted of the dose-response curves obtained from the mediodorsal nucleus of the thalamus, the dorsal hippocampus, the lateral hypothalamus and the optic tract. Within this family of dose-response curves, the AD_{50} values from the hippocampus and lateral hypothalamus were statistically similar ($P>.05$) and intermediate between those from the mediodorsal nucleus (significantly greater, $P<.05$) and optic tract (significantly less, $P<.05$). The set of steep dose-response curves were obtained from stimulation of the ventrodorsal nucleus of the thalamus, the medial lemniscus and the mesencephalic areas; the AD_{50} values from these three areas did not differ significantly from each other at the 5% level. None of the dose-response curves obtained with levomepromazine were parallel to those of the narcotic analgetics when the slopes of these lines were compared according to brain area ($P<0.05$).

Figure 18 is representative of the type of dose-response curves that were obtained for the various narcotic analgetics in each of the brain areas; the example, in this case, is the mediodorsal nucleus of the

Figure 18

Representative dose-response curves obtained with the various narcotic analgetics from the mediodorsal nucleus of the thalamus.


Tilidine
Propoxyphene
Morphine
Heroin
Fentanyl
Probits
3
4
5
6
7
0.01
0.03
0.1
0.3
1.0
3.0
10
30
100
300
1000
Dose (mg/kg)

thalamus. Since the dose-response curves for the different narcotic analgetics in each brain area were parallel, relative potencies (using morphine as the standard) could be calculated. These values are shown in Table 5. The relative potency of each compound did not vary greatly from one brain area to another. The mean of these values was therefore determined for each compound and was as follows: fentanyl, 132; heroin, 12; propoxyphene, 0.10; tilidine, 0.01. Except for heroin, these values are of the same order of magnitude as those reported by other investigators (Table 6).

In the present study, the relative potency obtained for heroin was approximately three times greater than what would be predicted on the basis of other animal studies. However, the relative potency estimates of other investigators, particularly those obtained in clinical studies, should be interpreted with caution since it is not always clear whether complete dose-response curves had been constructed and tests of linearity and parallelism conducted.

Figure 19 summarizes the relative potency and relative activity of the narcotic analgetics in the various brain areas that was obtained in the present study.

For purposes of comparison, the analgesic activities of heroin, morphine and levomepromazine were also evaluated

TABLE 5

Relative potencies of the narcotic analgetics in each brain area

Drug/Area	VD	MD	PVF	LH	HPC	DMB	ML	AHA	Mean R.P.
Morphine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Fentanyl	200.0	136.0	139.0	64.0	76.0	97.0	214.0	–	132.0
Heroin	16.0	14.0	–	14.0	16.0	9.0	8.0	8.0	12.0
Propoxyphene	0.13	0.12	–	0.06	0.08	0.10	0.10	0.11	0.10
Tilidine	0.02	0.01	0.01	0.01	0.01	0.01	–	–	0.01
Common Slope	2.2	2.2	2.2	2.1	2.2	2.2	2.3	2.3	

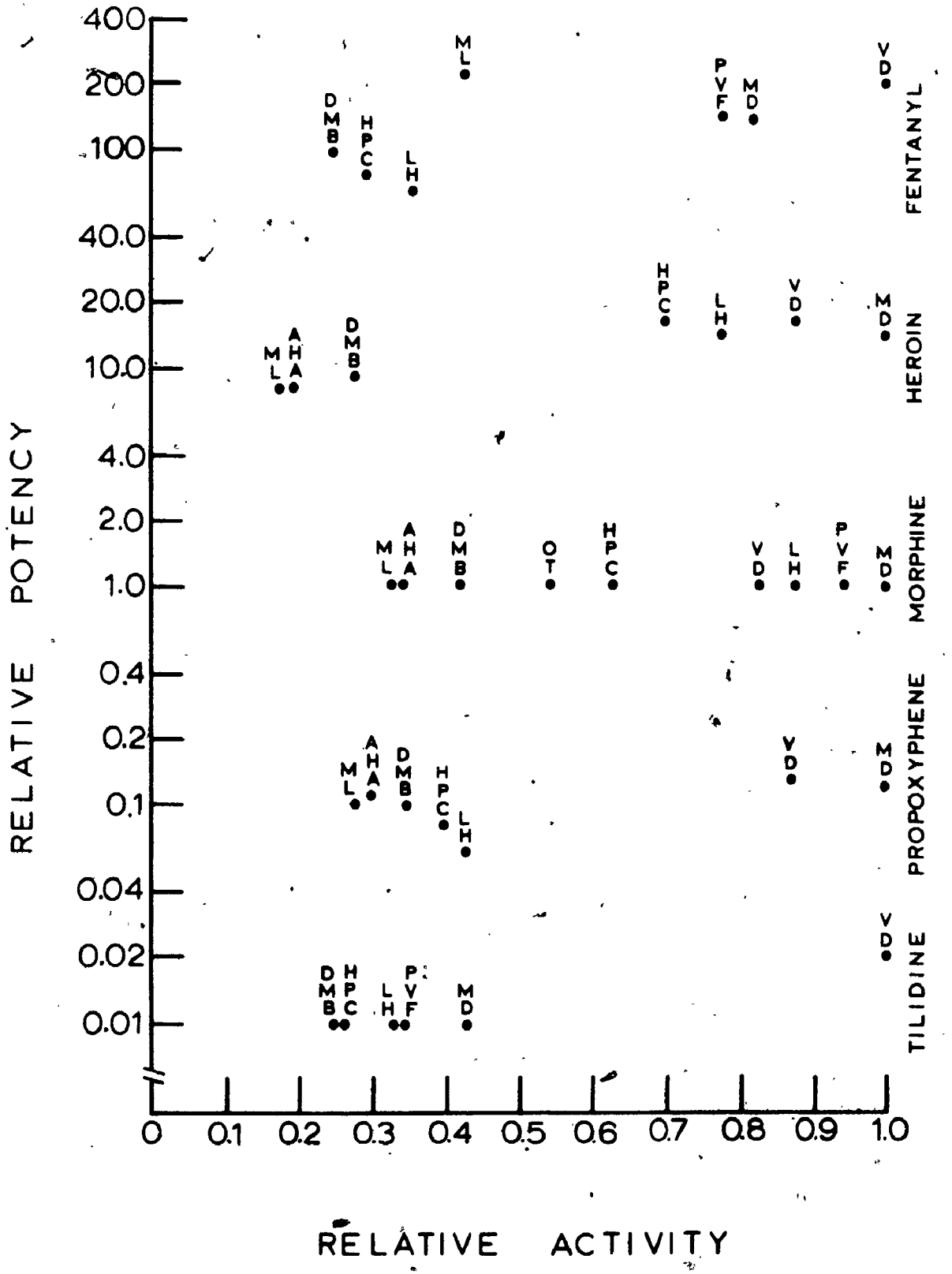
TABLE 6

Comparison of the relative potency values obtained for the narcotic analgetics in the present study with those reported by other investigators

Drugs	Present Study	Animal Studies	References	Clinical Studies	References
Morphine	1.0	1.0		1.0	
Fentanyl	132	188	Gardocki and Yelnosky, 1964	50	Halpern, 1973
Heroin	12	4	May and Sargent 1965	3	Halpern, 1973
Propoxyphene	0.10	0.14	Eddy, et al., 1969	0.04	Eddy, et al., 1969
Tilidine	0.01	0.02	Herrmann, 1970	0.05	Dekornfeld and Finch, 1971

Figure 19

Summary of the relative potency and relative activity of the narcotic analgetics in the various brain areas. Relative potency values for the different narcotic analgetics in each of the brain areas were calculated using the activity of morphine as 1.0. Relative activity values for each drug in the various brain areas were calculated as fractions of the AD_{50} value obtained from the brain area which was most sensitive to the effects of that particular drug. AHA, anterior hypothalamus, DMB, dorsal midbrain; HPC, dorsal hippocampus; LH, lateral hypothalamus; MD, mediodorsal nucleus of the thalamus; ML, medial lemniscus; OT, optic tract; PVF, parafascicular-paraventricular complex of the thalamus; VD, ventrodorsal nucleus of the thalamus.


400
200
100
40.0
20.0
10.0
4.0
2.0
1.0
0.4
0.2
0.1
0.04
0.02
0.01
RELATIVE POTENCY
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
RELATIVE ACTIVITY
FENTANYL
HEROIN
MORPHINE
PROPOXYPHENE
TILIDINE

by the rat tail-flick technique (Davies, et al., 1946). The results of this assay are shown in Table 7. For each drug, the dose-response curves obtained by this method were much shallower than those produced by central stimulation ($P<0.05$). In contrast to the dose-response curves obtained by brain stimulation, the dose-response curve for levomepromazine was found to be parallel ($P>0.05$) to that of heroin or morphine when determined by the rat tail-flick method.

Table 7

Dose-response parameters obtained for heroin, morphine and levomepromazine (LMPZ) by the rat tail-flick method.

Drug	ED_{50} (mg/kg)	95 Percent Fiducial Limits	Slope	S.E. of Slope	No. of Rats	Relative Potency
Morphine	4.1	1.5-6.6	1.0	0.68	15	1.0
Heroin	0.31	0.13-0.50	1.2	0.54	15	13
LMPZ	11.6	6.0-16.1	0.7	0.89	15	0.4
common slope = 1.0 ± 0.71						

However, since the fiducial limits about the regression lines obtained with this technique were very large as compared to those obtained with central stimulation, a parallelism of the dose-response curves was indicated which ordinarily might not have been the case. This view is supported by the observation that in the rat

tail-flick assay, as in the case of central stimulation, naloxone (10 mg/kg sc.) antagonized the effects of the narcotic analgetics but not those of levomepromazine.

Moreover, it is questionable whether the effects of levomepromazine on the rat tail-flick response is an accurate measure of its analgesic properties. In this assay, levomepromzine produced a very shallow dose-response curve with an extremely high ED_{50} value. Similar doses were found by other investigators (Gowdey, et al., 1960), to produce a large degree of motor incoordination in rats, and it is quite possible that the animals failed to respond because they were temporarily incapacitated. When the behaviour of the animals was observed, it was noted that, following levomepromazine, a "humping" of the tail occurred, even at very high doses, whereas after high doses of morphine or heroin, the animal kept its tail in place, without any movement whatsoever. Hence, only when the criterion of analgesia — namely, complete removal of the tail from the source of the noxious stimulus — was strictly applied did both types of drugs show analgesia.

DISCUSSION

The salient features of the present study may be summarized as follows:

(a) The relative potency of the various narcotic analgetics investigated in this study, morphine, heroin, fentanyl, propoxyphene and tilidine, and of the phenothiazine derivative, levomepromazine, in depressing the escape response elicited by aversive brain stimulation agreed well with those reported by other investigators using more conventional animal algesimetric techniques. This suggests that the response parameter being measured in the present study is a pain response and the technique of intracranial aversive stimulation is comparable to those employed in the more classical algesimetric procedures in that reproducible measurements of the relative analgesic potency of the different compounds tested were obtained. However, although brain structures are stimulated directly in this technique, whereas classical algesimetric techniques involve excitation of peripheral receptors, it is of interest the method did not provide any significant advantage over the more conventional procedures in terms of sensitivity to drug effects when the absolute potencies of these drugs were compared. On the other hand, the slope functions of the dose-response curves obtained in this study with the tail-flick assay were always significantly

less than those obtained with the same drug when central stimulation was employed. Thus, while the intracranial stimulation techniques may not be more sensitive to the absolute amounts of drug required to attenuate pain-related behavior, it does appear to be superior to the more commonly used algesimetric methods in so far as detecting the effects of smaller changes in dose interval are concerned.

(b) Brain areas which play different functional roles in the modulation of the sensory and behavioral response to pain displayed a differential sensitivity to the effects of the analgesic agents. There existed some commonality of action among all of the analgetics investigated in the present study. Broadly speaking, the thalamic nuclei (mediodorsal, ventrodorsal and parafascicular - paraventricular complex) were the most sensitive to the effects of the analgesic compounds, whereas the anterior hypothalamus and medial lemniscus tended to be the least affected by these drugs; the lateral hypothalamus, hippocampus and dorsal midbrain area usually exhibited an intermediate sensitivity. Intermediate doses of both morphine and levomepromazine also depressed the escape response elicited by stimulation of the optic tract, an area of the brain which is not ordinarily associated with central pain mechanisms. On the basis of this similarity of action, the drugs investigated in the present study may

be considered to form a pharmacological class of compounds whose common properties deserve further study.

On the other hand, both within the opiate class of compounds and between this group of drugs taken as a whole and the phenothiazine derivative, some striking dissmimilarities among the actions of the drugs were also observed. This difference in drug action may be related to the extent with which each of these drugs influences the two components (sensory perception and behavioral reaction) of the pain response.

(c) For the narcotic analgetics, both the intra- and inter-drug differences in the values of the slope functions of the dose-response curves obtained from the different regions of the brain were not statistically significant. Thus, each narcotic analgetic was associated with a family of parallel dose-response curves, the shifts in the parallel lines providing an estimate of the differential sensitivity of the various brain areas to the effects of that particular drug.

Levomepromazine produced two sets of parallel dose-response curves, one in which the slopes of the lines were significantly shallower (areas MD, LH, HPC and OT) and one where the slopes of the dose-response curves were significantly steeper (areas VD, ML and DMB) than those of the corresponding dose-response curves obtained with the narcotic analgetics. Again, the shift in the parallel

dose-response curves in each group provided a measure of the selective effects of levomepromazine on the different structures of the brain and reflected its relative activity on the perceptual and behavioral components of the pain response. Furthermore, the shallower slopes of the dose-response obtained with levomepromazine in the medial thalamus and limbic system may partly explain why these two classes of compounds -- the narcotic analgetics and the phenothiazines -- so closely resemble each other in their behavioral effects, yet are poles apart in terms of their addiction liability.

Before proceeding with a more detailed analysis of the latter two aspects of the present study, a number of questions which may conceivably have arisen from the preceding discussion should perhaps be considered initially, in order to place the discussion of these results in a more proper perspective. These questions will, therefore, be dealt with in the following pages and are, in their order of presentation: (a) What is meant by the terms "stimulation" and "depression" as used in the context of the present study? (b) Can it be assumed that the response elicited by the electrical stimulation of any one area in the intact brain of conscious animals is the direct consequence of a stimulus-induced alteration in neural activity which is confined to that site alone or are more distal structures also involved? (c) Is the

centrally-elicited escape response a reflection of an actual perception of pain or is it merely the result of internal cues which are not aversive and in no way related to pain? And finally, (d) What are the inherent advantages or disadvantages in the nature of the response parameter and apparatus employed in the present investigation which might reinforce, or detract from, the conclusions which will be made on the basis of the results obtained in this study?

(a) Definition of terms: Since either an activation or an inhibition of central nervous system structures can be elicited by electrical stimulation of the brain, the term "stimulation" in this study, is used only in the descriptive sense to refer to the application of an electrical current to a region of the brain without reference to the underlying mechanisms (such as depression or excitation of inhibitory or facilitatory systems) consequent to such a procedure. Similarily, when morphine is said to suppress or enhance the activity of a particular brain structure, it should be remembered that this could be due to an inhibition of facilitatory mechanisms or to an excitation of inhibitory neurons and vice versa.

(b) Effective radius of stimulating current: Basic to the consideration of the effect of electrical stimulation on a complex network is the question of which neuronal units are activated when an electric current is

passed through a region of the central nervous system. Indeed, one of the major difficulties inherent in stimulation studies of the intact brain is that no one area of the central nervous system can be stimulated without altering the neuronal activity of numerous other cerebral structures. Furthermore, it seems unlikely that many topographical regions of the brain are sufficiently homogeneous in structure or unitary in function to result in a single alteration of the integrative processes mediated by an area in response to a stimulating electrode. Thus, in considering the results of the present study, it is important to remember that the aversive behaviour elicited by stimulation of a brain area is a compound response, with other structures also contributing to the behaviour observed.

In the present study, it has been assumed that the response measured was mediated primarily by the site being stimulated. This view is substantiated by a number of investigators. Valenstein (1966) has reviewed several lines of evidence which indicate that the neural field activated by bipolar electrodes of the type used in this investigation is restricted to a relatively small spherical region around the electrode tips and does not involve massive activation of large brain areas, even with very high intensities of stimulation. Recent studies of effective current radius with monopolar stimulation (Stoney, Thompson

and Asanuma, 1968; BeMent and Ranck, 1969) also support this assumption, especially when it is considered that the radial extent of effective stimulating current is greater when monopolar, rather than bipolar electrodes are used. These studies provide a useful basis for estimating the distribution of stimulating current in the present experiments; an effective radius of less than 200μ was indicated for low threshold areas (14-15μA) whereas less than 400μ would apply to sites requiring up to 100μA of current intensity. Allowing for differences in stimulus parameters, stimulation of most of the aversive areas in the present study should not excite even the largest, lowest-threshold fibers 400μ away; higher threshold elements are likely to be unaffected beyond 200μ.

Also relevant to this issue was the finding that, in this investigation, significantly different thresholds were obtained from areas that were only one millimeter apart (for example, the thresholds from the parafascicular - paraventricular complex as compared to those from the mediodorsal or the ventrodorsal nucleus) and that the analgetics differentially affected such closely interconnected areas as the various structures of the limbic system or the ventrodorsal thalamic nucleus and medial lemniscus.

(c) Relationship of centrally-elicited escape to pain: Direct stimulation of the brain is a highly unnatural way of eliciting behaviour and the properties of shock-

motivated behaviour must therefore be interpreted with caution. In particular, one can never be certain of the significance of such stimuli so far as the sensory experience of the animal is concerned. Although there can be no direct verification that the stimulation of brain structures eliciting an escape response induces a mental awareness akin to the pain experience in man, a number of observations may be brought to bear upon this point.

First, the effect of central stimulation is immediately generalized so that it results in behaviour which had previously been associated with somatic pain. In the present study, electrical stimulation of the brain, like peripherally applied shock, motivated rapid learning of a complicated operative task in order to terminate the stimulus. In the cognitive avoidance theory, such a stimulus is therefore considered to be aversive or noxious to the animal (Roberts, 1958). This is further supported by a number of investigations (Delgado, et al., 1954; Roberts, 1962) which have demonstrated, in carefully controlled studies, that such stimuli are capable of producing the motivational drives and negative affect that are characteristic of pain.

Second, it should be noted that a stimulus-bound motor effect alone fails to elicit escape. In the present study, stimulation of the caudate nucleus, anterior amygdaloid area or anterior ventral thalamic nuclei elicited

circling and/or cringing but had little motivational effect, for not even prolonged response shaping led to the acquisition of an escape response. The autonomic responses evoked at some sites may also be aversive but this could not be tested since autonomic activity was not monitored in these studies. Siderhoff, Elster and Schneiderman (1972), however, have found that electrical stimulation of brain areas with both appetitive (self-simulation) and aversive properties elicited conditioned cardiovascular responses which were the same in both direction and magnitude, indicating that the autonomic changes associated with intracranial stimulation are not necessarily related to the motivational properties of the stimulus. Furthermore, these effects were observed only after high intensities of stimulation (100 to 500μA). Lower stimulus intensities, such as those employed in the present study, did not produce classical conditioning of the heart rate even though a reliable unconditioned response was always obtained. One may therefore assume that, in the present study, the aversive properties of central stimulation are not attributable to any changes in autonomic activity.

Third, at sites eliciting escape, vocalization, urination, defecation, piloerection, biting of the electrodes leads and other reactions suggestive of pain were frequently observed in this study. This is consonant with the pain behavior produced by peripheral noxious

stimulation at intensities sufficient to elicit escape.

The above observations attest to the unpleasant, if not noxious, nature of the central stimuli used to elicit escape responding in the present study. One may, therefore, conclude that the stimulation of the brain structures investigated in this study possessed a distinct aversive quality which was related to pain.

In closing, it should be noted that, with the exception of the lateral hypothalamus, stimulation of the brain areas investigated in the present study is aversive from its onset and remains so throughout the period of stimulation (Roberts, 1958b; Miller, 1961). A curiosity of lateral hypothalamus stimulation is that it motivates escape but not avoidance responding (Bower and Miller, 1958; Miller, 1961). In order to explain this apparently paradoxical behavior, it was postulated that stimulation at this site with the same stimulus intensity can produce dual rewarding and punishing effects; namely, the onset of the stimulation is rewarding, its continuation reverses the sign of reinforcement to motivate escape, and its termination reinforces escape (Roberts, 1958b; Mendelson and Freed, 1973). An animal thus stimulated will learn one task to initiate stimulation and another to turn the stimulus off. Unfortunately, the rats used in the present study were not tested for avoidance conditioning, but the observation that the rats stimulated in the lateral hypothalamus showed

statistically significant ($P<0.01$) longer response latencies than those with electrodes implanted in the other cerebral structures is an indication that similar effects were also operative in the present investigation.

(d) Advantages and disadvantages inherent in the present experimental procedure: A common feature of the investigations which have dealt with the effects of analgetics on operant behaviour maintained by aversive intracranial stimulation (Boren and Malis, 1961; Vernier, et. al., 1961; Weitzman and Ross, 1962; Halpern and Alleva, 1964; Ross, 1966) is that they all measured the effects of these drugs on the aversive threshold. In the present study, however, the escape response to suprathreshold brain stimulation was chosen as the most appropriate experimental response parameter for the initial evaluation of the analgesic activity of such compounds. This was done for the following reasons:

(1) An abundant literature on both the clinical and experimental assessment of the narcotic analgetics indicates that these drugs relieve pain primarily by modifying the reaction component while leaving the perception of the sensation relatively unaltered (Wikler, 1958; Beecher, 1959). In other words, the opiates do not appear to affect the sensory modality of pain as much as they influence the psychological factors involved in pain perception. Since the reaction component becomes increasingly

dominant as stimulus intensities are raised, the behavioral response at suprathreshold intensities, with its proportionately larger psychological component (Gelfand, 1964), is therefore likely to be more sensitive to the analgesic action of the opiates than the behavior maintained by threshold levels of stimulation.

(2) In humans, the results obtained by experimental measurements of the effects of morphine on the pain threshold are inconsistent, while studies in both man and animals have shown that firmer conclusions are tenable when stimuli which constantly evoke unequivocal and unalloyed pain are used (Beecher, 1959).

(3) A difficulty inherent in the titration technique is that the responses are confounded by conditioned avoidance behavior (Boren and Malis, 1961). Since in paradigms of this sort the stimulus intensity increases more or less insidiously from nonaversive to aversive levels, lower stimulus intensities, which are discriminable but in themselves not aversive, come to occupy the status of conditioning stimuli which precede the higher, aversive, shock levels. Under these conditions, a great deal of responding (66 - 80%) occurs at stimulus intensities which are essentially nonaversive. The thresholds thus obtained are therefore mainly the reflection of an animal's reaction to the anticipation of pain (conditioned avoidance) rather than to the pain experience itself. Obviously the use of

suprathreshold stimuli (where each stimulus is aversive) circumvents this difficulty and ensures that the behavioral response measured is directly related to pain.

The present method also differs from the more usual procedures (Weiss and Laties, 1958, 1961, 1964; Boren and Malis, 1961) in that responding terminates the electric stimulus instead of causing a decrement in the intensity of a continuous shock. This modification considerably reduced the amount of conditioned responding, probably by placing the behaviour more under the control of the immediate consequence of escaping the shock rather than the more distant consequence of avoiding future stimulation. Thus, in the present study, when animals were stimulated at current intensities held constant at their predetermined threshold values (thereby eliminating the effectiveness of these stimuli to act as warning signals) the rates of responding averaged around the 50% level. These response rates corresponded closely with those predicted by the functional relationship between shock intensity and escape performance. Using the same procedure, Boren and Malis (1961) observed a response rate of only 20-34% (ie., in their technique, approximately 30% of the responding at threshold levels was due to conditioned avoidance). The aversive thresholds obtained by the present method may therefore be considered to be a direct function of the intensity of pain experienced by the animal.

Although narcotic analgetics were not evaluated in terms of their effects on the aversive threshold, the determination of these response parameters formed an integral part of this study. Because of the varying sensitivities of neural structures to electrical stimulation, an arbitrarily chosen suprathreshold level of stimulation does not necessarily produce a stimulus intensity that is felt equally by all animals. A valid comparison between the behavioral responses of such animals therefore cannot be made. However, a common baseline is provided if the stimulus level employed is a constant function of the animal's aversive threshold. More importantly, this value represents an objective stimulus intensity which has been determined by the animal itself.

Since pilot studies had indicated that, in the foot shock situation, the mean current value which consistently evoked escape responding was 2.2 times the threshold value, all animals with indwelling electrodes were stimulated at intensities which were 2.2 times their aversive threshold. The application of foot shock data to brain stimulation is valid for it has been demonstrated that both peripherally and intracranially induced pain conform to Stevens' power law (Kestenbaum, Deutsch and Coons 1973).

Also, the sensitivity of neural tissue varies over time so that the maintenance of a constant current level throughout the experiment is no assurance that the effects

of the stimulus are remaining constant (Szeligo and Colavita, 1972). Consequently the threshold values were determined for all animals prior to each drug trial and the suprathreshold stimulus adjusted accordingly. In this way, a constant monitoring of (and the subsequent correction for) any fluctuations in neural sensitivity is obtained. In the present study, therefore, the effects of analgesic agents were always studied against a behavioral response intensity which was the same for all animals, regardless of their sensitivity to the stimulus. The comparison made between such animals are thus valid.

All of the above considerations reinforce the view that the effects of the analgetics on intracranial aversive stimulation as determined by the response parameter used in the present study are related to their analgesic mechanism of action. The conclusions drawn from the results obtained in this investigation regarding the cerebral structures involved in the antinociceptive action of the various analgesic agents tested are therefore tenable.

However, it may be argued that the nature of the apparatus employed in the present study may be a source of confusion in the intepretation of the results. A two-way shuttle-box was used in this investigation, since pilot experiments with lever pressing in a Skinner box lead to difficulties, such as bar-holding, which resulted in an indefinite postponement of stimulus. Even when the

lever was automatically returned to the "up" position once a response had been made, the animals continued to lean on the lever, making it difficult to ascertain whether the response to the subsequent stimulus was merely due to the weight of the animal resting on the bar or a true behavioral reaction. Similar difficulties had been encountered by other investigators (Dinsmoor and Hughes, 1956).

In the shuttle-box situation, there is no "safe" versus "dangerous" area; the subject receives an aversive stimulus on either side of the cage. For this reason, an animal may develop a passive avoidance tendency for either one side of the cage or the other (Vanderwolf, 1963). Since passive avoidance response develops fairly rapidly and completely, this type of behavior could clearly interfere with the performance of an active escape response. Thus, an inherent disadvantage of the shuttle-box technique is that some measure of behavioral conflict is always present. To what extent this factor may modify the conclusions reached in the present study cannot be stated until the identical experiment has been repeated in an apparatus where such passive avoidance responses do not occur.

This factor may possibly explain why the present method was not more sensitive to the effects of the analgesic agents, on a milligram basis, than the more

conventional algesimetric procedures, whereas other investigators, using intracranial aversive stimulation and bar-pressing as the experimental parameter, have reported evidence of the analgesic action of morphine in doses as low as 0.25 mg/kg im. (Halpern and Alleva, 1964). It may be speculated that in order to overcome the initial avoidance response tendencies in the shuttle-box situation, the intensities of noxious stimulation which elicit an escape response have to be higher than what would ordinarily be required. Since it is a well known fact that the greater the intensity of pain, the larger the dose of morphine required to abolish it, it would not be unreasonable to assume that this is the reason why larger doses of analgetics were needed in order to suppress escape responding in the shuttle-box.

The above consideration also argues in favour of the view that the behavioral conflict incurred in the shuttle-box is not a significant operative factor at higher intensities of stimulation since, implicit in the foregoing paragraph, is the assumption that as the stimulus increases in its aversiveness, the more the escape response predominates over the passive avoidance tendency. Since the effects of the analgetics were always tested at suprathreshold intensities of stimulation, it does not seem likely that this factor would greatly modify the results obtained in the present study.

However, it should be noted that stimulation of certain areas, notably the fimbria hippocampi, ventral hippocampus, anterior amygdaloidal area, caudate nucleus and the ventral anterior nucleus of the thalamus failed to elicit escape responding in the shuttle-box even though they were reported to be highly efficacious in establishing an escape response (lever-pressing) in the Skinner box (Olds and Olds, 1963). The reason why escape responding is obtained in the shuttle-box when certain aversive areas are stimulated, but not others, is not clear. It may be related to the fact that all of the sites which failed to elicit escape responding are associated with brain areas which mediate avoidance behavior (Ursin, 1965; Buchwald and Hull, 1967; Winocur and Mills, 1969; Van Hoesen, Willson, MacDougall and Mitchell, 1972). It is conceivable, therefore, that the effects of stimulation of these structures enhance the response inhibitions produced in the shuttle-box, thereby causing an impairment of escape responding which not even higher intensities of stimulation can overcome. Whatever the explanation may be, the failure of the shuttle-box technique to detect the aversive properties of certain brain structures by the commonly employed behavioral criteria suggests that the shuttle-box may not be the most appropriate apparatus to use if studies of drug effects on centrally-elicited aversive behavior are to be complete.

In returning to the discussion of the results of the present study, those obtained with morphine and its semi-synthetic congener, heroin, will be dealt with first. Most of the studies on the central actions of analgestic agents have been done with morphine. Hence, more is known about the central effects of this drug than any other analgetic. No data is available in the literature concerning the central site of action of heroin. However, in view of the similar profiles of activity exhibited by heroin and morphine, both in the clinic and in the present study, it would not be unreasonable to assume that many of the effects reported for morphine are equally applicable to herbin.

Nevertheless, it should be noted that, while the differential central effects of heroin and morphine obtained in this study resembled each other closely, they were not identical. The difference in the profiles of activity for heroin and morphine was that the hippocampus tended to be more sensitive whereas the dorsal midbrain area, anterior hypothalamus and medial lemniscus were less sensitive to the effects of heroin than to morphine. This difference in the action of these two drugs may be taken as evidence that the analgesic action of heroin is mediated by a drug-receptor interaction with the heroin molecule itself, and/or with its monoacetyl derivatives, and not through a prior conversion to morphine, as has been suggested by some investigators (May and Sargent, 1965; Way, 1967).

The central effects of morphine and heroin will be discussed in terms of the following three levels of activity: (1) thalamic action; (2) limbic action; and (3) mesencephalic action.

(1) Thalamic action.

In the present study, it was found that morphine and heroin were most effective in suppressing the escape behavior evoked by stimulation of the ventrodorsal, mediodorsal and parafasciular-paraventricular thalamic nuclei. Although these sites are respresentative of three functionally distinct systems within the thalamus -- the specific sensory, associational and nonspecific projection systems respectively -- the areas did not show a differential sensitivity to the effects of these two drugs.

The relation of the above three sites to pain may be briefly summarised as follows: the ventrodorsal nucleus forms part of the thalamic component of the specific, paucisynaptic, neospinothalamic pathway for pain, which relays directly received somesthetic sensory information from the spinal cord to the primary sensory areas of the somatosensory cortex with little delay or modification; the mediodorsal nucleus, which receives multisensory input from both the specific and nonspecific thalamic nuclei, and its projections to cortical association areas are important for the elaboration and integration of the affective tone of the various sensory inputs; the

parafascicular-paraventricular complex belongs to the nonspecific thalamic system which comprises the rostral extension of the diffuse, multisynaptic, paleospinoreticulothalamic division of the pain pathway. Because of the capacity of the nonspecific thalamic system to exert a dual control, one inhibitory, the other excitatory, over electrocortical activity (Skinner and Lindsley, 1967), the nonspecific thalamic nuclei are particularly important in the neural mechanisms underlying selective perception. Since these nuclei project diffusely by multisynaptic pathways to widespread regions of the neocortex but predominantly to the associational cortices (Chambers, et al., 1971), they also play a role in modifying the behavioral response to pain (Albe-Fessard, 1968).

Thus, it may be concluded from the results of the present study that the thalamus represents a major site for mediating the analgesic effects of heroin and morphine and that this action may be effected through either one of the following mechanisms: (1) by increasing the threshold of pain perception through an action on the specific thalamic sensory nuclei; (2) by decreasing the emotional response to pain through an action on the associational and nonspecific systems of the thalamus; and (3) by decreasing the attention paid to painful stimuli through an action on the nonspecific thalamic system. There is an abundant evidence in the literature which

supports each of the points made in the above statement. However, no study has been reported in the literature in which all these three actions of morphine were observed in the same investigation.

Evidence in support of the conclusions made in the present study comes from intracerebral injection studies, where it has been shown that strong antinociceptive effects are produced when morphine is injected directly into either the ventrobasal thalamus (Buxbaum, et al., 1968), dorsomedial nucleus (Herz, et al., 1968) or nonspecific thalamic system (Tsou and Jang, 1964; Lotti, et al., 1965; Buxbaum, et al., 1971).

The medial thalamic nuclei have also been implicated as the likely site of action whereby morphine decreases brain arousal to nociceptive stimulation (Fujita, et al., 1953; Silvestrini and Longo, 1965; Gangloff and Monnier, 1957; Radouco-Thomas, et al., 1962; Monnier, et al., 1962, 1963; Valdman, 1967; Monnier and Nosal, 1968). The enhancement of the cortical recruiting response (which is elicited by low frequency stimulation of the nonspecific thalamic system) that was observed by a number of investigators (Gangloff and Monnier, 1957; Heng-Chin and Domino, 1961; Monnier, et al., 1962, 1963; Valdman, 1967; Monnier and Nosal, 1968) with morphine-like compounds supports the proposal made in the present study that morphine decreases the attention paid to nociceptive stimuli by an

action on the nonspecific nuclei of the thalamus. In humans, the EEG synchrony produced by low frequency stimulation of these structures is accompanied by a loss of awareness of, and inattention to, specific sensory stimuli (Skinner and Lindsley, 1967).

However, these observations can not be directly compared to the results of the present study, since, in this investigation, the nonspecific thalamic system was stimulated at fairly high frequency rates (60 Hz). There is no data available in the literature which has dealt with the effects of morphine on the responses elicited by high frequency stimulation of this system. However, the observation that morphine elevates the threshold to nociceptive stimulation in the anteromedial thalamus (Silvestrini and Longo, 1956) is consistent with the depressant effects of morphine that were observed in this study. Domino (1968) has also shown that the narcotic analgetics have a depressant action on the nonspecific thalamic system in humans.

The conclusions made in the present study with respect to the effects of morphine on the mediodorsal nucleus are corroborated by a number of behavioral studies. In these investigations it was shown that morphine exerted its greatest inhibitory action on the complex affective responses to pain mediated by the thalamus (Hoffmeister, 1968) or the diencephalocortical projection systems

(Charpentier, 1968); simpler, reflex nociceptive responses mediated by rhombencephalic structures were either not affected (Charpentier, 1968) or suppressed only at higher dose levels (Hoffmeister, 1968).

In this respect, it should also be noted that removal of the frontal cortex or severing of the thalamocortical pathways in patients receiving prefrontal lobotomies for the relief of intractable pain results in a total retrograde degeneration of the dorsomedial nucleus (Rose and Woolsey, 1948) and in a clear dissociation of the emotional from the perceptual response to pain (Barber, 1959). Since morphine produces a somewhat similar psychic disregard for pain (Wikler, 1950, 1958), it has long been speculated that the analgesic action of the opiates may be related to a depression of impulse transmission in the dorsomedial nucleus (Wikler, 1950, 1958; Chambers, et al., 1971). The results of the present study substantiate this view.

A number of electrophysiological studies have also shown that morphine depresses the electrical activity evoked in the nonspecific and the associational thalamic nuclei by various noxious stimuli to the same degree (Fujita, et al., 1953; Heng-Chin and Domino, 1961; Lim, et al., 1969; Krauthamer, et al., 1970). These studies are consistent with the conclusions of the present study that morphine and heroin exert an equal action upon the

nonspecific and associational systems of the thalamus.

One study which is not in agreement with this view is that of Sinitsin (1964). In testing the effects of various opiates, this author reported that the responses to somatic nerve stimulation were depressed more markedly in the associational nuclei than in the nonspecific thalamic system. However, the relevancy of this observation to pain mechanisms is questionable, since the stimulus intensities employed (4 - 6 V) were much less than those required to elicit pain reactions in animals (50 V, McKenzie and Beechey, 1962) or verbal reports or aversive sensations in man (60 - 100 V, Domino, 1968) under similar experimental conditions.

On the other hand, with the exception of Fujita, et al., 1953, 1954), many of the above investigators (Sinitsin, 1964; Lim, et al., 1969; Krauthamer, et al., 1970), as well as others who have used comparable electrophysiological techniques (McKenzie and Beechey, 1962; Straw and Mitchell, 1964), have failed to find an effect of morphine on the potentials evoked in ventrobasal thalamus. On occasion, it was even reported that morphine actually enhanced the potentials evoked in this region of the thalamus at a time when the responses in the associational or nonspecific thalamic nuclei were depressed (Heng-Chin and Domino, 1961; Sinitsin, 1964). These results are contrary to those obtained in the present

investigation. There are several reasons which may account for this discrepancy:

(1) Since it has been shown that morphine preferentially depresses the responses to repetitive as opposed to single shock stimulation (Fujita, et al., 1954; Matsumura, et al., 1959; Jurna, 1965; Valdman, 1967; Satoh, et al., 1970), the use of single shock stimulation by many of the investigators who studied the effects of morphine on ventrobasal thalamic evoked potentials (McKenzie and Beechey, 1962; Straw and Mitchell, 1964; Sinitsin, 1964) may, therefore, account for the lack of an effect of morphine on these responses. This would not be applicable to single shock studies on potentials evoked in the associational and nonspecific thalamic systems since, by virtue of the diffuse, multisynaptic nature of the systems to which they belong, a single stimulus is capable of exciting reverberating circuits, thereby producing effects which are analogous to repetitive stimulation. An effect of morphine on these structures would therefore be more clearly and more consistently observed by these investigators. This would not be the case in the present study, since all brain areas investigated were stimulated repetitively.

(2) Unlike the present study, all the above investigations were performed in anesthetized or immobilized animals. Since the neurones of the ventrobasal thalamus

are entirely dependent upon somesthetic impulses for their activation, they are relatively quiescent due to the lack of input associated with anesthetic or paralying agents. Hence, when a large volley arrives as a result of the artificial electric stimulation of the neospinothalamic pathway, many neurons are receptive to excitation at the same instant. Under these circumstances, therefore, it is possible that doses of morphine larger than commonly used would be required before a depressant action on these responses would be observed. On the other hand, the nonspecific and associational thalamic nuclei receive a continuous input from the reticular structures of the brain, whose activity is constantly being re-activated, at all levels of the neuraxis, by sensory impulses not only from the somesthetic system, but from other sensory modalities as well.

(3) If one assumes that the paleospinothalamic division of the pain pathway and/or cortex maintain a tonic inhibitory influence on the somatosensory relay nuclei, as has been suggested by a number of authors (Bowsher and Albe-Fessard, 1962; Skinner and Lindsley, 1967; Hassler, 1968), morphine's enhancement of the potentials evoked in the ventrobasal complex may represent a "release phenomenon" due to the primary depression of thalamic or cortical inhibitory centers. It has already been noted that this enhancement occurs concomitantly with

a depression of potentials evoked in the nonspecific or associational thalamic nuclei (Heng-Chin and Domino, 1961; Sinitsin, 1964) and Fujita, et al. (1954), as well as Gangloff and Monnier (1957), have shown that morphine also has a depressant effect on corticofugal pathways. Thus, in the present study, the possibility must be considered that the effects of morphine on the escape response elicited by stimulation of the ventrodorsal nucleus may be through an indirect action on the more medial thalamic nuclei. It has been suggested that the nonspecific thalamic nuclei influence the specific relay nuclei via intrathalamic neurones, differentially inhibiting or exciting them in order to provide thresholds appropriate to the degree of attention required in any given sense mode (Skinner and Lindsley, 1967).

Despite these difficulties, there have been a number of electrophysiological investigations in which a specific effect of morphine in depressing ventrobasal thalamic responses was observed (Fujita, et al., 1953, 1954; Mizoguchi, 1964; Schmidt and Ruthrich, 1972; Ruthrich, et al., 1972). Moreover, investigators who have studied the effects of descending serial transections of the neuraxis on morphine analgesia (Carroll and Lim, 1960; Cahn and Herold, 1968) have attributed the actions of morphine as being due to the blockade of first, the synapses in the thalamus (presumed to be in the nucleus ventralis posterolateralis), and, later, in the lower brain stem structures. Furthermore,

Vernier, et al. (1961), who measured the effects of morphine and anileridine on the self-determined intracranial aversive threshold, also reported that the ventral posterolateral nucleus was the area most affected by these drugs. These observations, therefore, support the conclusion of the present study that the ventrobasal thalamus represents a main central site mediating the analgesic actions of morphine and heroin.

It should be pointed out, however, that Vernier, et al. (1961) reported the centre median (which belongs to the same group of thalamic nuclei as the parafascicular complex) as being the least sensitive to the effects of the opiates. This is in contrast to the present finding that the ventrodorsal nucleus and parafascicular-paraventricular complex were equally affected by morphine. This discrepancy in results is quite likely due to the different characteristics of the response parameters employed. As has been pointed out previously (see Historical Review and also Manning and Vierck, 1973), the perceptual component of the pain response (ie., the detection of the pain threshold) is mediated primarily by the neospinothalamic pathway (of which the ventrobasal nuclei are a part), whereas the paleospinothalamic system (which projects to the associational and nonspecific thalamic nuclei) becomes involved when pain becomes intolerable and a large affective component is present,

ie., at suprathreshold intensities of stimulation. Since Vernier, et al. (1961) studied the effects of morphine on the aversive threshold, the intensities of stimulation used were not sufficiently high enough for the medial thalamic nuclei to contribute significantly to the pain response being measured. Hence, a lesser effect of morphine on these structures than those obtained in the present study would be expected to be observed.

Thus, the results of Vernier, et al. (1961), taken together with those obtained by various other lines of experimental approach, strongly support the conclusions of the present study that the specific sensory, associational and nonspecific thalamic nuclei are among the areas first to become involved in mediating the analgesic actions of morphine and heroin.

(2) Limbic action.

Not much is known about the differential effects of the narcotic analgetics upon the structures on the limbic system. In the present study it was found that the lateral hypothalamus was as sensitive to the effects of morphine and heroin as were the thalamic nuclei. This was followed by a slightly less, though statistically significant, effect upon hippocampus. On the other hand, the anterior hypothalamic area (as well as the medial lemniscus) was found to be the least affected by these compounds.

The hippocampus and hypothalamus play an important role in the mechanisms of emotion and motivational behavior; the former serves mainly as a modulatory influence on these activities, whereas the latter is critical in the control of these processes (Leaton, 1971; Chambers, et al., 1971). Thus, it may be concluded from the results of the present study that, in addition to altering the integrative functions of the various thalamic nuclei, one of the first effects of morphine which contribute to its analgesic activity is an attenuation of the emotional response to pain by an action upon hypothalamic structures; a slightly less important, though still significant, effect of morphine is an alteration of these behavioral responses through an action upon the modulatory activities of the hippocampus.

The differential effect of heroin and morphine on the two regions of the hypothalamus may be taken as an indication that the narcotic analgetics affect aggressive behavior (essentially under the control of the more posterior regions of the hypothalamus) more so than defensive reactions (regulated by the more anterior regions of the hypothalamus). This is supported by the fact that the "personality profiles" of narcotic addicts consist mainly of individuals who "prefer to handle... anxieties and conflicts passively by avoidance rather than aggressive acts," and further ... "opiates seem to suppress the sources of these anxieties, thus permitting the user of

narcotics to make a passive adaptation to his inner tensions" (Jaffe, 1965, p. 287).

The inclusion of the lateral hypothalamus among the areas most sensitive to the effects of morphine and heroin (this effect was not observed with any of the other analgetics tested) and the lesser effect exerted by these drugs on the medial lemniscus might account for the clinical observations that these analgetics influence predominantly the reaction component of the pain experience (Wikler, 1958; Beecher, 1959; Morrison, 1970).

Since, in the present study, it was found that the hippocampus tended to be more sensitive to heroin than morphine (ie, the differences between the doses required to depress the responses elicited by stimulation of the lateral hypothalamus or thalamic nuclei and the hippocampus were not as great with heroin as they were with morphine), one would expect heroin to have a greater effect on the affective component of pain than morphine. This is borne out by the greater potency of heroin as a clinical analgetic and the more intractable addiction in heroin abuse. This observation also suggests that heroin possesses greater euphorogenic properties than morphine and that the more intractable addiction to heroin is a reflection of this differential action of heroin on limbic structures and not, as has been suggested by some investigators (Oldendorf, et al., 1972), merely due to differences in the

lipophilic properties of these compounds.

Investigations which have shown that the opiates depress: (a) the somatosensory input into hypothalamic (Fujita, et al., 1954; Deneau and Takaori, cit. Domino, 1962) and hippocampal (McKenzie, 1964; Nakamura and Mitchell, 1972) structures; (b) the EEG arousal response mediated by hypothalamic activating mechanisms (Monnier, et al., 1962; Albus and Herz, 1972); (c) hypothalamic neuronal activity (Eidelberg and Bond, 1972); and (d) hippocampal arousal elicited by nociceptive stimulation (Soulairac, et al., 1967) support the conclusions of the present study that the hypothalamus and hippocampus play an important role in the antinociceptive mechanism of action of heroin and morphine. Furthermore, the studies reviewed by Wikler (1950) and those of George and Way (1959) and Lotti, et al. (1969) strongly implicate the hypothalamus as a main site whereby morphine exerts, not only its analgesic action, but also its effects on endocrine systems and body temperature.

However, differential effects of the narcotic analgetics on the limbic system, such as those observed in this study were not stressed by other investigators. From the graphs published in the study of Charpentier (1968), the diencephalocortical projections responsible for the complex behavioural response to nociceptive stimuli appeared to be more sensitive to the effects of morphine than the emotional

behaviour mediated by rhinencephalic system. On the other hand, Cahn and Herold (1968) reported a reverse order for the sensitivity of these structures whereas the results of Hoffmeister (1968) indicated that the responses mediated by the thalamo-hypothalamo-rhinencephalic system were equally affected by morphine. None of the above investigators differentiated between the effects of morphine on hypothalamic and hippocampal structures as did this study.

Although morphine was found to be localized in both the hypothalamus and hippocampus following the intraventricular injection of radioactive morphine (Adler, 1964; Schubert, et al., 1970), only the direct injection of morphine into the hypothalamus produced strong antinociceptive effects (Tsou and Jang, 1964; Lotti, et al., 1965; Foster, et al., 1967; Herz, et al., 1970; Buxbaum, et al., 1971); no analgesia was observed when morphine was applied directly to the dorsal hippocampus (Buxbaum, et al., 1971; Herz, et al., 1970). Thus, as far as intracerebral injection techniques are concerned the hippocampus is not an important site for the antinociceptive action of morphine.

An explanation for this discrepancy in results is not immediately clear and requires further investigation. It may be due to differences in the characteristics of the response parameter being measured (ie., in the neural mechanisms which mediate a particular nociceptive response) and that structures which mainly have a modulatory influence

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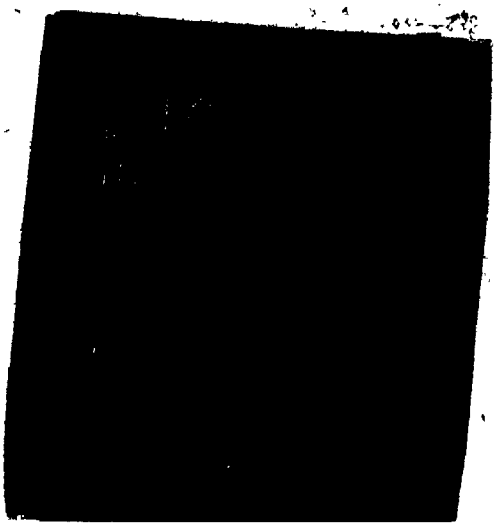

on pain-related behavior (eg., the hippocampus) are less affected by the direct injection of morphine than those which are primarily concerned in the control of such behavior (eg., the hypothalamus). However, recent studies on the stereospecific binding of opiates in both human and monkey brains (Hiller, et al., 1973; Kuhar, Pert and Snyder, 1973) have shown the greatest degree of binding to occur within the structures of the limbic system and medial thalamus. These observations therefore support the conclusion of the present study that thalamic and limbic structures contribute significantly to the analgesic mechanism of action of the opiates.

(3) Mesencephalic action.

In the present study, the dorsal midbrain area (dorsolateral central gray and deep layers of the superior colliculus) was found to be significantly less sensitive to heroin and morphine than the limbic structures. This was followed by a significantly lesser effect on the medial lemniscus, which was the area most resistent to the effects of these drugs.

Because of the close relationship of the dorsal midbrain area to the mesencephalic reticular formation and limbic system (Melzack, 1973), this area also plays a role in the mechanisms of sensory perception and behaviour. Furthermore, the observations that the central gray

represents a major conduction pathway for the transmission of pain impulses (Melzack, 1961, 1973) and that the mesencephalic tectum may function as a relay station along an ascending pathway for pain impulses en route to the hypothalamus (Spiegel, et al., 1954; Spiegel and Wycis, 1961) clearly implicates this area in central pain mechanisms. It has been suggested that the tectum mesencephali represents the lowest level of the neuraxis where the integration and conscious perception of pain could occur (Walker, cit. Passouant, 1968). From the results of the present study, it may therefore be concluded that a depression of the perceptual and behavioral responses to pain mediated by the dorsal midbrain area is less important to the analgesic mechanism of action of morphine and heroin than a suppression of those mediated by thalamic and limbic structures. Moreover, an impairment of sensory impulse transmission within the main somesthetic conduction pathway, the medial lemniscus, appears to be the least contributing factor in morphine analgesia.

The results of investigators who have used electrophysiological techniques to determine the effects of morphine on the mesencephalic structures were highly variable: either a facilitatory (Heng-Chin and Domino, 1961), a depressant (Heng-Chin and Domino, 1961; McKenzie and Beechey, 1962; Sinitsin, 1964; Straw and Mitchell, 1964; Nakamura and Mitchell, 1971), or no effect (Schimmerl

and Stumpf, 1958; Mitchell and Killam 1964) on the electrical activity (spontaneous or evoked) of the mesencephalic reticular neurones was observed following morphine. In many cases these effects of morphine could not be specifically related to its analgesic mechanism of action. For instance, morphine was found to block the responses to both noxious and non-noxious stimuli while nonanalgetic drugs, such as chlorpromazine or pentobarbital, were often equally or even more effective than morphine in blocking responses thought to be specifically related to pain (Heng-Chin and Domino, 1961; Straw and Mitchell, 1964; Nakamura and Mitchell, 1971). On occasion, morphine was even shown to exert an effect on responses not ordinarily implicated in pain processes (eg., depression of evoked responses within the visual system) while leaving those that were concerned with the transmission of pain intact (Mitchell and Killam, 1964).

However, there have been some studies reported in the literature which have shown that low doses of morphine selectively suppress the responses evoked in the medial lemniscus (Fujita, et al., 1953, 1954; Satoh, et al., 1970) and deep layers of the superior colliculus (KcKenzie and Beechey, 1962) by nociceptive stimulation. This is in agreement with the observations of the present study that morphine can also inhibit the escape responses elicited by stimulation of the dorsal midbrain and medial lemniscus.

There is some controversy as to whether the mesencephalic reticular formation and the nonspecific thalamic nuclei are equally sensitive to the effects of morphine (Gangloff and Monnier, 1957; Monnier, et al., 1962, 1963; Monnier and Nosal, 1968) or whether the diencephalic or rhinencephalic systems are affected by doses of morphine which have only a slight effect on, or do not alter, mesencephalic reticular activity (Silvestrini and Longo, 1956; Radouco-Thomas, et al., 1962; Sinitsin, 1964; Soulairac, et al., 1967; Charpentier, 1968). However, the consensus of most investigators (Krueger, et al., 1941; Carroll and Lim, 1960; Hoffmeister, 1968) is that increasing doses of morphine are required to block the responses mediated by succeedingly lower levels of the brainstem. This view is in general agreement with the rank order in the sensitivity of the brain areas that was observed in the present study.

In general, the conclusions made in this study with respect to the differential effects of heroin and morphine are also applicable to the other analgesic compounds tested. However, some dissimilarities in the central actions of these drugs did exist. The manner in which the effects of the other analgesic agents investigated in the the present study differed from those of heroin or morphine will be discussed for each drug in the following pages.

(a) Fentanyl

Although it has been suggested that the narcotic analgetics exert their principal, if not entire, effect upon the reaction component of pain (Wikler, 1958; Beecher, 1959), it would be an oversimplification to ascribe all of the narcotic-induced analgesia to such a mechanism of action. This is true particularly in view of the existence of neuroleptanalgesia in which conscious patients undergo major surgery with combinations of butyrophenones and meperidine derivatives as the sole anesthetic agents (Jaffe, 1965; Morrison, 1970). Since droperidol (the neuroleptic commonly used in combination with fentanyl) has no analgesic activity of its own (Ordy, et al., 1970), a depressant effect on the perceptual mechanisms of pain would be a more reasonable explanation to account for the profound analgesia produced by the analgetics used in neuroleptanalgesia. This hypothesis was tested by Morrison (1970) who found that this was indeed the case; all the analgetics used in neuroleptanalgesia (eg., phenoperidine, fentanyl) influenced mainly the perceptual mechanisms of pain, whereas the clinically used analgetics (eg., morphine, heroin) had a greater effect on the affective experience of pain.

This difference in the mechanism of action of these two types of analgetics was reflected in the profiles of activity obtained for fentanyl and morphine (or heroin) in

the present study. The escape responses elicited by stimulation of the ventrodorsal nucleus were depressed by doses of fentanyl which were consistenly lower (though not significantly different) than those required to suppress the responses evoked by stimulation of the mediodorsal or parafascicular-paraventricular nuclei; the reverse was observed with morphine. However, the most striking difference between the central actions of fentanyl and morphine was that the medial lemniscus was next to the thalamic nuclei in the order of decreasing sensitivity to the effects of fentanyl, whereas this area was the least affected by morphine. Furthermore, when the relative activity of these two drugs in the various areas of the brain were compared, it was found that the limbic structures (hypothalamus and hippocampus) were approximately only half as sensitive to the effects of fentanyl than to morphine. These results support the observation of Morrison (1970) that the neuroleptanalgesics predominantly influence the perceptual mechanisms of pain. From the data obtained in this study, it may be concluded that this action is due to the relatively greater and lesser effects exerted by fentanyl upon the specific somatosensory pathway and limbic system respectively.

From the study of Freeman and Ingvar (1967), it appears that fentanyl has an excitatory action on the mesencephalic reticular system which masks a less intensive

depressant action on cortico-thalamic pathways. This observation is in agreement with the present findings that fentanyl has a depressant action on thalamic systems and that the dorsal midbrain area was the least sensitive to the effects of this drug. In the present study, toxic symptoms were becoming manifest at the doses of fentanyl which were required to suppress the responses to dorsal midbrain stimulation. Thus, the effects of fentanyl on this region of the brain may partly reflect toxic effects which interfered with the performance of the escape response and may not be entirely related to its analgesic mechanism of action.

On the other hand, the present findings are at variance with those of Herz, et al. (1970) who used the method of injecting drugs into restricted parts of the ventricular system of rabbits. These authors felt that the most effective sites for the anti-nociceptive action of fentanyl (and morphine) were located in the fossa rhomboides and surrounding regions. Diencephalic and midbrain structures were considered to be of minor importance in the actions of the analgetics. The doses of fentanyl injected correspond to those used in the present study when body weight and the greater effectiveness of the intraventricular route of administration are taken into account.

One cannot explain this discrepancy in results on the basis that different nociceptive responses (licking reaction

to tooth pulp stimulation as compared to the escape (response) were used or by a lack of diffusion to effective receptor sites. According to Hoffmeister (1968), the licking reaction in rabbits is mediated exclusively by thalamic structures and Schubert, et al. (1970) have shown that fentanyl rapidly penetrates into periventricular structures following intraventricular injection. At present, therefore, it is difficult to reconcile the results of this study with those of Herz and his co-workers.

(b) Propoxyphene.

The profile of activity obtained with propoxyphene resembled that of morphine except that the hypothalamus and hippocampus were found to be much less sensitive to the effects of this drug when the relative activities of morphine and propoxyphene in these two areas of the brain were compared. The lesser effect of propoxyphene on limbic structures may be partly responsible for the lower addiction liability of this compound. This view is supported by Hoffmeister (cit. Eddy, et al. 1969), who demonstrated that morphine has a much greater effect upon the electrical activity (spontaneous or evoked) of the rhinencephalic structures than propoxyphene and related these differential effects to the differences in the euphorigenic potency of these two compounds.

It should be mentioned, however, that, except for the

thalamic nuclei, the doses which suppressed the escape responses elicited by stimulation of the remaining brain areas exceeded the minimal dose required to produce convulsions in the rat. Thus, it may be concluded that most of the analgesic actions of propoxyphene are mediated by thalamic structures. Nevertheless, the fact that the hypothalamus and hippocampus were next to the thalamic nuclei in the declining order of sensitivity, suggests that propoxyphene can also significantly affect the behavioral responses to pain mediated by the limbic system, if given in sufficiently large doses. In this respect, it should be noted that addiction to propoxyphene can, and does, occur following the administration of high doses in man (Halpern, 1973) and that this compound also shows evidence of some addiction liability in animals (Eddy et al., 1969).

(c) Tilidine.

The profile of activity of tilidine was unique among the narcotic analgetics in that the ventrodorsal nucleus was the only area found to be the most sensitive to the effects of this compound. These results suggest that the main action of tilidine is on the specific somatosensory pathway, at least at the thalamic level. In this respect, it resembles fentanyl, which is also a meperidine derivative.

Tilidine was significantly less effective in the mediodorsal and parafascicular-paraventricular nuclei of

the thalamus, with the former being more sensitive to the effects of this drug than the latter. However, it must be pointed out that very high doses of tilidine had to be used to produce an effect, and that any effects of tilidine beyond those on the mediodorsal nucleus occurred at dose levels exceeding the LD_{50} value (400 mg/kg sc.) reported for this compound in rats (Herrmann, et al., 1970). The results obtained with tilidine are, therefore, likely to be more a reflection of its toxic effects than an indication of its analgesic action. Thus, on the basis of the present study, no definite conclusions with respect to the sites and mechanisms involved in the analgesic action of tilidine can be made.

(d) Levomepromazine.

From an analysis of the dose response curves obtained in the present study, one would predict that levomepromazine, like morphine, influences primarily the reaction component to pain. At low doses this action is mediated by the mediodorsal nucleus, but, with increasing doses, the hippocampus and hypothalamus also become involved. This view is supported by Hoffmeister (1968) who has shown that both morphine and levomepromazine exert their greatest inhibitory effect on pain reactions mediated by the thalamo-hypothalamo-rhinencephalic system. Moreover, the analgesic activity of these compounds appeared to be

specifically related to a thalamic site, whereas their anti-anxiety effects were associated with an action on the limbic structures.

On the other hand, Weller and Sulman (1970), in studying the effects of serial neural transection on the analgesic potency of various compounds, have concluded that the integrity of the thalamus and/or its connections with higher structures are necessary for the full analgesic effect of morphine but not for that of levomepromazine. These results are contrary to those obtained in the present study.

The observations of Weller and Sulman (1970, do not necessarily preclude the importance of the thalamus in the analgesic activity of levomepromazine. The vocal response measured by Weller and Sulman was shown by these authors to be mediated by the medulla. Thus, if one assumes that levomepromazine inhibits this response by a direction action on the medullary centers (and the depressant effects of the phenothiazines on bulbar regions are well-known) whereas morphine acts mainly indirectly through an effect on the thalamus, then the results observed by Weller and Sulman may be accounted for, without having to exclude a possible thalamic stie of action for levomepromazine. Furthermore, since the vocal response was found to be mediated at the medullary level, the authors suggested that its inhibition by drugs may be the result of

pharmacological actions which are only contingently associated with analgesia.

On the basis of the dose-response curves obtained with levomepromazine in the ventrodorsal nucleus, medial lemniscus and dorsal midbrain, one would postulate that levomepromazine also impairs the perception of pain. However, these effects of levomepromazine occurred at dose levels which were beginning to show some of the sedative and cataleptic side effects of levomepromazine (Gowdey, et al., 1960). Beyond this level, even slight increments in dose result in a marked increase in the severity of the side effects. Thus, the effects of levomepromazine on ventrodorsal nucleus, medial lemniscus and dorsal midbrain area must be considered as being primarily due to toxic effects which interfered with the performance of the escape response.

The most striking feature of the present study was that the slopes of the dose-response curves obtained with levomepromazine were significantly different from those produced by the narcotic analgetics in the same brain area, whereas the dose-response curves obtained with the different narcotic analgetics in each of the brain areas investigated were all found to be parallel. These results are consistent with the hypothesis that narcotic analgetics act at the same receptor sites throughout the central nervous system (Grumbach and Chernov, 1965), which are, however, different

from those mediating the actions of levomepromazine (Maxwell, et al., 1961; Weller and Sulman, 1970; Kuromi, Satoh and Takagi, 1972).

Although the differential sensitivities of the various areas mediating affective behaviour (mediodorsal nucleus, lateral hypothalamus, and hippocampus) were very similar for heroin, morphine and levomepromazine, the slopes of the dose-response curves produced by levomepromazine were only half as steep as those obtained with heroin or morphine in these areas. Thus, if the intensity of affect (instead of escape responding) were plotted against dose and assuming that these two parameters conform to a linear log-log relationship (Charpentier, 1968), one can see that the alteration in affect produced by levomepromazine, both in absolute terms and in the degree to which the parameter is changed by identical shifts in doses, would be much less than that produced by morphine. Such a factor would greatly reduce the reinforcing effects obtained by an individual consequent to drug administration. These observations may therefore partly account for the fact that the narcotic analgetics and the phenothiazines resemble each other closely in their behavioural effects and yet are so far apart (the former comprising the most addicting drugs and the latter the least addicting drugs) in terms of their addiction liability.

In the present study, intermediate doses of both morphine and levomepromazine were also found to depress, in a dose dependent manner, the escape response elicited by stimulation of the optic tract. This area is not ordinarily implicated in central pain processes. These results, however, do not necessarily imply that the drugs are nonspecific in their effects upon various sensory system. As Olds and Olds (1963) have pointed out, a strong negative affect is probably attached to excessive stimulation of any sensory modality. Levomepromazine and morphine may therefore not have been judicious choices for investigating the effects of analgetics on optic tract stimulation, since both drugs relieve pain primarily by altering the affective component. Had an analgetic which acts mainly on perceptual mechanisms been used, it is likely that a clearer separation of effects would have been obtained.

This view is supported by the fact that the dose-response curve for levomepromazine in the optic tract belonged to the same family of curves which contained the mediodorsal nucleus, lateral hypothalamus and hippocampus and ranked last in terms of the differential sensitivity exhibited by this area. In the case of morphine, the dose-response curve for optic tract stimulation was parallel to those from the other areas; the sensitivity of the optic tract ranked between that of the hippocampus and dorsal

midbrain area. It may therefore be concluded that the effects of morphine and levomepromazine on optic tract stimulation were mediated through an indirect action on structures responsible for the creation and modulation of affective behavior.

If we are to accept the concept that the main action of morphine is upon behaviour (Dews, 1969), and, considering that the response to pain is a complex, learned behavioural reaction "involving the whole of the nervous system and psyche" (Charpentier, 1968), then only studies which use behavioural response measures as an index of drug action (such as the present investigation) can provide any relevant information about the central actions of morphine. However, a single measure of behaviour will usually not be adequate enough to differentiate the action of morphine from that of other drugs. It is necessary, therefore, that studies on the behavioural effects of morphine include a spectrum of behavioural criteria in a variety of species so that the effects on fundamental components of behaviour can be deduced. This is the line of approach that future research on the neuropharmacology of analgesic compounds should take if a clearer understanding of the neurological mechanisms underlying the fundamental mode of action of these drugs is to be obtained.

SUMMARY AND CONCLUSIONS

1. In a series of 360 rats, bipolar, Nichrome electrodes, 254μ in diameter and insulated with Formvar except for the cross-section at the tips, were permanently implanted into various regions of the brain which have been implicated in central pain mechanisms. The stereotaxic co-ordinates of the brain areas chosen for investigation - the ventrodorsal, dorsomedial, parafascicular - paraventricular and ventral anterior nuclei of the thalamus, dorsal and ventral hippocampus, fimbria hippocampi, anterior amygdaloid area, caudate nucleus, anterior and lateral hypothalamus, dorsal midbrain and medial lemniscus - corresponded to those described by Olds and Olds (1963) as being the most efficacious in establishing an escape response. Electrodes were also implanted in the optic tract in order to include a sensory system unrelated to pain mechanisms. All electrode placements were verified histologically.

2. Rats were placed in a two-way shuttle-box and tested for escape responding using a 60 Hz current delivered from an AC stimulator built specifically for the purpose of the present investigation. In order to ensure a minimal spread of current, rats not responding to a

current intensity of 100 μA were eliminated from the experiment.

3. Stimulation of a number of areas, notably the ventral hippocampus, fimbria hippocampi, anterior amygdaloid area, caudate nucleus and ventral anterior nucleus of the thalamus, did not elicit escape responding, even though prolonged response shaping and higher intensities of stimulation were tried. This may be due to the conflict of behaviour incurred in the shuttle-box situation and the role these areas play in passive avoidance behaviour.

4. Stimulation of the remaining sites motivated rapid learning of the escape response. The aversive thresholds of rats with electrodes implanted in these regions of the brain were determined by means of a titration schedule whereby the intensity of the stimulus decreased in a stepwise fashion if the animal responded to the stimulus by crossing to the opposite half of the cage during the shock interval or increased in similar steps if it did not respond.

5. The effects of a number of analgesic drugs - morphine, heroin, fentanyl, propoxyphene, tilidine and levomepromazine - on the escape response to suprathreshold stimulation of the areas eliciting aversive thresholds were then tested. The intensities of stimulation used were always a constant function of the aversive threshold

(2.2 times that of the aversive threshold) determined for each animal prior to each drug trial. In this manner, the effects of the analgesic agents were always studied against a behavioural response which was similar for all animals.

6. It was found that the technique of intracranial aversive stimulation was comparable to those employed in the more classical algesimetric procedures in that reproducible measurements of the relative analgesic potency of the different compounds were obtained. Comparative studies with the rat tail-flick assay indicated that, while the intracranial stimulation technique used in the present study was not more sensitive to the absolute amounts of drug required to attenuate pain-related behaviour, it was superior to the more commonly used algesimetric methods in so far as detecting the effects of smaller changes in dose interval are concerned.

7. The results of the present study indicate that brain areas which play different functional roles in the modulation of the sensory and behavioural response to pain have a different pharmacological sensitivity to analgesic agents. When the effects of each drug in the various brain areas were compared, it was found that each narcotic analgetic was associated with a family of parallel dose-response curves whereas the

phenothiazine derivative, levomepromazine, produced two sets of parallel dose-response curves. The shifts in the parallel dose-response curves obtained with each compound therefore provided an estimate of the differential sensitivity of the various brain areas to the effects of that particular drug.

8. The slopes of the dose-response curves obtained with levomepromazine were significantly different from those produced by the narcotic analgetics in the corresponding brain areas, whereas the dose-response curves obtained with the different narcotic analgetics in each of the brain areas investigated were all found to be parallel. Furthermore, the specific narcotic antagonist, naloxone, blocked the effects of the narcotic analgetics but not those of levomepromazine. These results are consistent with the hypothesis that narcotic analgetics act at the same receptor sites throughout the central nervous system which are, however, different from those mediating the actions of the phenothiazine derivative, levomepromazine.

9. There existed some commonality of action among all of the analgetics investigated in the present study. Broadly speaking, the thalamic nuclei (mediodorsal, ventrodorsal and parafascicular - paraventricular complex) were the most sensitive to the effects of the analgesic compounds, whereas the anterior hypothalamus and medial lemniscus tended to be the least affected by these drugs;

the lateral hypothalamus, hippocampus and dorsal midbrain area usually exhibited an intermediate sensitivity. Intermediate doses of both morphine and levomepromazine were also found to depress the escape response elicited by stimulation of the optic tract, an area of the brain which is not ordinarily associated with central pain mechanisms. This similarity of actions suggests that the drugs investigated in the present study form a pharmacological class of compounds whose common properties deserve further study.

10. On the basis of the differential sensitivity exhibited by the various brain areas to the effects of the analgesic drugs tested in the present investigation, it was concluded that analgesia of the morphine type involves first, an alteration in the integrating activities of the non-specific (selective perception), associational (emotional behavior) and specific somatosensory (sensory awareness) thalamic nuclei; second, an influence upon the structures controlling (hypothalamus) or modulating (hippocampus) emotional behavior; third, an action upon the perceptual and behavioral responses mediated by the dorsal midbrain; and, fourth, an impairment of sensory impulse transmission within the main somesthetic conduction pathway (medial lemniscus). The observation that the thalamic nuclei were the most sensitive to the effects of the

analgetics suggests that these drugs influence both the sensory and behavioral components of pain at the thalamic level.

11. On the other hand, both within the opiate class of compounds and between this group of drugs as a whole and the phenothiazine derivative, some striking dissimilarities among the actions of the drugs were also observed. This difference in drug action reflected the extent with which each drug influences the two components, sensory perception and behavioural reaction of the pain response.

12. The greater sensitivity of the lateral hypothalamus and hippocampus to heroin and morphine may account for the proportionately larger influence that these drugs have upon the reaction component of pain and may be related to their high addiction liability.

13. While the differential central effects of heroin and morphine obtained in this study resembled each other generally, they were not entirely identical. The hippocampus tended to be more sensitive while the dorsal midbrain, anterior hypothalamus and medial lemniscus were less sensitive to heroin than morphine. From these results it was concluded that heroin may have a greater effect upon the behavioural component of pain than morphine and that, contrary to the commonly

accepted theory, the analgesic action of heroin is mediated by a more efficacious drug-receptor interaction with the heroin molecule itself and not solely through a prior conversion to morphine.

14. The greater effectiveness of fentanyl on the specific somatosensory pathway (ie., ventrodorsal nucleus of the thalamus and medial lemniscus) and the lesser effect of this drug on the structures of the limbic system suggests that it, like the other analgesic agents used in neuroleptanalgesia, has a proportionately greater influence upon the perceptual mechanisms of pain than either heroin or morphine.

15. With the exception of the thalamic nuclei, the doses of propoxyphene required to suppress the escape responses elicited by stimulation of the remaining brain areas approached toxic levels. It was therefore concluded that most of the analgesic actions of propoxyphene are mediated by thalamic structures. Nevertheless, the fact that the hypothalamus and hippocampus ranked next to the thalamic nuclei in the declining order of sensitivity suggests that propoxyphene can also significantly affect the behavioral responses to pain mediated by the limbic system if given in sufficiently large doses.

16. Tilidine exhibited a profile of activity which was different from that of the remaining narcotic analgetics.

However, since very high doses of this compound had to be used to produce an effect, no definite conclusions with respect to the sites and mechanisms involved in the analgesic action of tilidine could be made.

17. The differential sensitivity of the brain areas mediating affective behaviour (mediodorsal nucleus of the thalamus, lateral hypothalamus and dorsal hippocampus) to the effects of levomepromazine were similar to those observed for heroin or morphine. However, the slope functions of the dose-response curves produced by levomepromazine were significantly less than those obtained with the narcotic analgetics in these areas. Moreover, as stated above, naloxone blocked the actions of the opiates but not those of levomepromazine and it was therefore concluded that the phenothiazine derivative produces an analgesia similar to that of morphine but mediated by receptor sites which are different from those of the narcotic analgetics. These observations may explain why the phenothiazine tranquilizers resemble each other closely in their behavioural effects despite being at the opposite extremes in terms of their addiction liability.

18. Although both morphine and levomepromazine also depressed the escape response elicited by optic tract stimulation, it was concluded that this data did not necessarily

imply a nonselective mechanism of action for these compounds.

19. Undoubtedly, a great many parallels in the effects of analgesic agents on centrally-elicited aversive behaviour must be established before any definite conclusions concerning the fundamental mode of actions of these compounds can be made. Nevertheless, it is suggested that the experimental techniques employed in the present study may serve as a basis for the design of other relevant approaches in studies on the central actions of analgesic compounds.

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