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ANIMAL MODELS FOR SCREENING NEW AGENTS

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From knowledge of the aetiology and pathophysiology of the clinical condition, and the existence of clinically-active drugs with known pharmacological properties, the experimental pharmacologist creates model conditions in animals from which new agents may be discovered and brought to clinical trial. This is a well-documented procedure summarized in Figure 1.

It is therefore a moot point whether medicinal chemists create, or pharmacologists discover, new drugs—clearly, both men are essential and are complimentary to each other.

More important, however, in the field of psychotropic drugs, has been the role of the clinician. He has played an important part in the initial discovery of activity in several important classes of psychotropic agent, for example the imipramine-like and monoamine oxidase (MAO) inhibitor antidepressants. The reasons for this are fairly clear: very small changes in chemical structure have produced, unpredictably, marked qualitative changes in the central pharmacological activity of many compounds. Furthermore, so little is known about the aetiology and pathophysiology of the mental conditions we are attempting to combat, that the neuropharmacologist is unable to create typical, specific, model conditions in animals suitable for the detection and evaluation of new agents. Instead, often the neuropharmacologist is handed back from the clinic some agent with hitherto unsuspected properties; with only hazy ideas about the underlying cause of the condition for which he now has an active agent, the neuropharmacologist must set out to create model conditions in animals from which the mechanism of action of this compound might be determined, and with which other, more potent or specific agents might be detected.

This sounds a simple enough task for the experimental neuropharmacologist to perform, and indeed the number of such clinical 'breakthroughs' has been sufficient to help the neuropharmacologist produce a range of experimental techniques from which other discoveries have been made. However, the typical centrally-acting drug is by no means a specific agent, and frequently possesses several quite distinct pharmacological properties. As a consequence, there is often quite considerable overlap of properties between adjacent classes of psychotropic drug, and the pharmacologist has little idea which one, or which combination of two or more, of a drug's properties are responsible for the clinical activity already observed. The simple classification or spectrum of centrally-acting drugs in Figure 2 demonstrates this phenomenon of overlap.

This spectrum of drugs shows how activity moves from frank, unspecific stimulant effects at the top, to (in direct contrast) the unspecific depressants such as general anaesthetics, at the bottom of the list. There is considerable overlap of properties in certain adjacent groups. For example, many properties are shared by the amphetamines and certain MAO inhibitors; and this is true of the imipramine-like antidepressants and the phenothiazines, and by the benzodiazepines and anticonvulsants.

The initial study, 'profiling'

As a result, most classes of psychotropic drug are recognized or classified not by a single, but instead by a range, of central pharmacological actions in experimental animals, in much the same way that a human face is not recognized by nose or eyes alone, but by the whole face. By analogy, the pharmacologist should build up a 'profile' of pharmacological properties for each existing and potential psychotropic drug. A new drug's potential usefulness, its placement into an existing or new class of drugs, is then determined by the characteristic profile that has been created. Identical profiles in two drugs suggest identical clinical activities, whereas small changes in profile may indicate the loss or enhancement of an agent's desirable properties, or the loss of an important side-effect.

An important feature, and a disadvantage, emerging from the pharmacologist's use of standard drugs for modelling experimental techniques is that 'like tends to beget like'. Good examples in psychiatry are the vast range of phenothiazines which possess antipsychotic activity, or the imipramine-like compounds with antidepressant activity. By the use of profiling techniques, it should be possible to take potential drugs forward

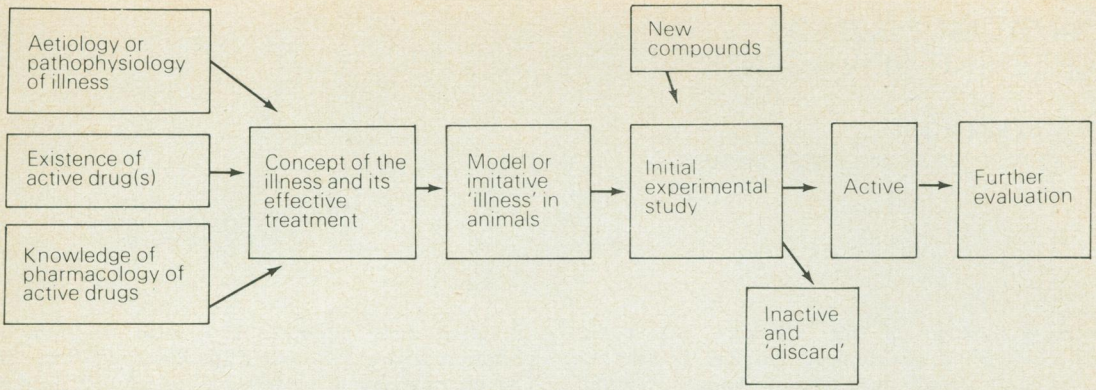

Figure 1 Normal series of events leading to the detection and further evaluation of new, potential drugs.

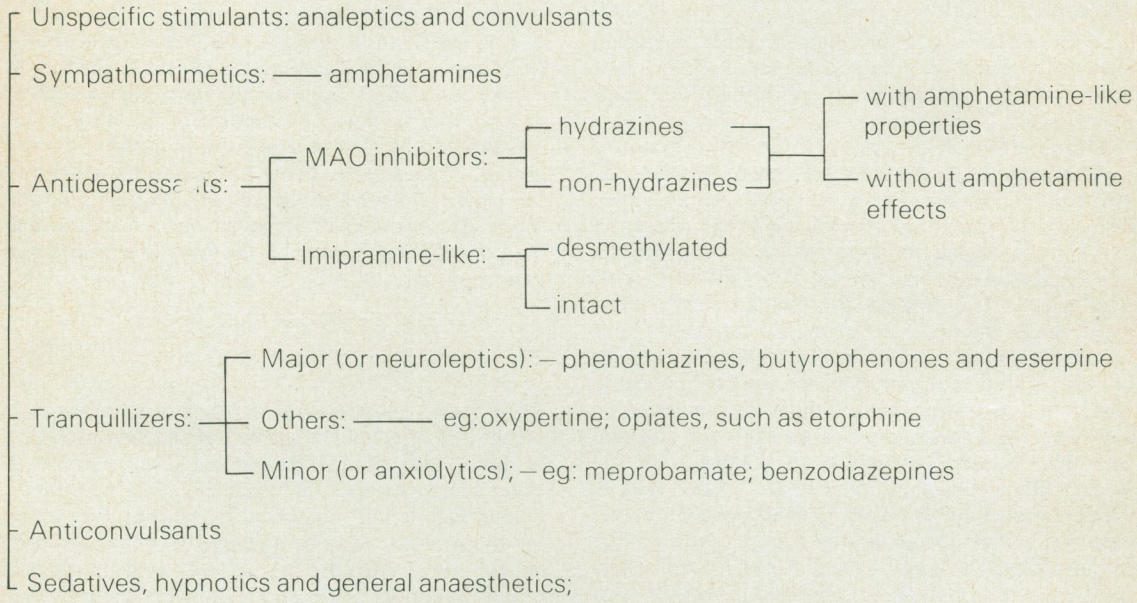

Figure 2 Classification of centrally-acting drugs into a spectrum, with a progression starting at the top, of unspecific stimulant and convulsant activity, through to the converse, general anaesthetic agents, at the bottom. The spectrum is drawn so that agents with some common properties possess adjacent positions in the spectrum.

into further study, on the basis of small but clear-cut changes in the pharmacological profile.

The construction of a neuropharmacological profile of activity is the first priority in the development of any new agent. The experimental procedures that can be used to profile an agent may be specific (to the class of agent sought), or general in nature (see Figure 3).

It is not important in which order (*a* or *b* first) these experiments are performed. It is possible to profile a wide range of centrally-acting drugs, using a few *in vivo* tests in small laboratory animals. Figure 4 gives a typical (but not exhaustive) list of such techniques, using only the mouse as experimental species.

The profiles of three well-known psychotropic drugs, constructed from these techniques, are set out for comparison in Figure 5.

The profile of neuropharmacological activity obtained for any new drug by this technique may

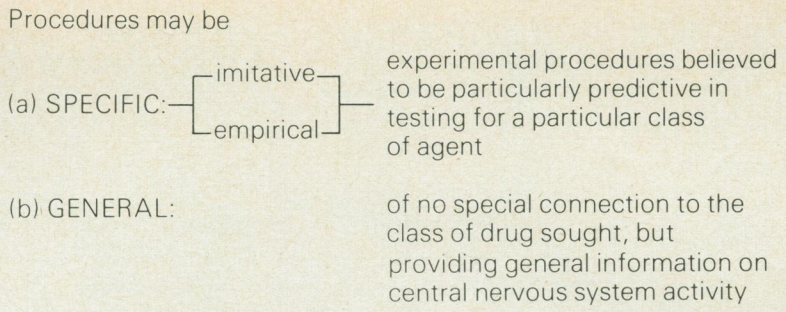

Figure 3 Initial neuropharmacological evaluation.

Acute (approximate) toxicity
Effects on general, spontaneous behaviour
Effects on body temperature
Ability to prevent and/or reverse effects of reserpine
Analgesic (anti-nociceptive) activity in;
- phenylquinone-writhing test
- tail-flick test
- hot-plate test

Ability to antagonize effects of tremorine or oxotremorine:
- especially, tremor
- and hypothermia

Anti-convulsant activity against:
- maximal electroshock
- leptazol convulsions

Ability to antagonize amphetamine toxicity under crowded (or aggregated) conditions

Figure 4 A list of common neuropharmacological techniques that may be used to profile the activities of new, potential psychotropic agents.

show marked similarity to an existing group of psychotropic drugs. Subtle differences between existing and potential drugs may provoke the further and continuing study of the new agent. The choice of procedures for further study will then be determined by the drug group (or groups) into which the new agent has been 'placed' after profiling.

Specific techniques

For each group of psychotropic drugs, a number of experimental techniques exist which are believed are fairly specific to that group. This means they are procedures in which the demonstration of pharmacological activity may be particularly indicative of a certain type of clinical activity. These specific techniques form the main part of the secondary or further evaluation stage, and (as indicated in Figure 3) may be *imitative* (that is, show marked similarity in design to the clinical condition to be treated) or be *empirical* (that is, show no overt similarity to the clinical condition, but to have shown consistently that a particular group of drugs exerts a positive effect in this experimental procedure). In the detection and evaluation of antidepressant compounds, the depressive syndrome in rats and mice produced by reserpine (or tetrabenazine) is an example of a specific, imitative test, and all known clinically-useful antidepressants are known to antagonize this reserpine-induced depressive syndrome (Spencer, 1967). By contrast, an example of an empirical technique is the ability of all known antidepressants to potentiate the convulsant activity of picrotoxin, despite the apparent lack of correlation between depressive illness in man and the pharmacological actions of picrotoxin.

The role or function of these imitative and empirical techniques is to confirm the picture emerging from the original profiling, that there is evidence for believing that a new agent can be classified into a particular group of psychotropic agents, and furthermore to examine its likely potency (by comparison with existing drugs) and the incidence of side-effects and drug interactions attributable to the new agent's overt biological properties. In Table 1 is set out a list of imitative and empirical tests which are commonly used during the secondary evaluation of potential antidepressants.

This list of experiments is not exhaustive, and different investigators will favour different techniques, or even perform similar techniques in different ways. In addition to confirming the classification emerging from the original profiling exercise, a compound's activities may be further sub-classified (for example, imipramine-like, monoamine oxidase inhibitor (MAOI) or amphetamine-like in the case of a potential antidepressant), on the evidence obtained from these

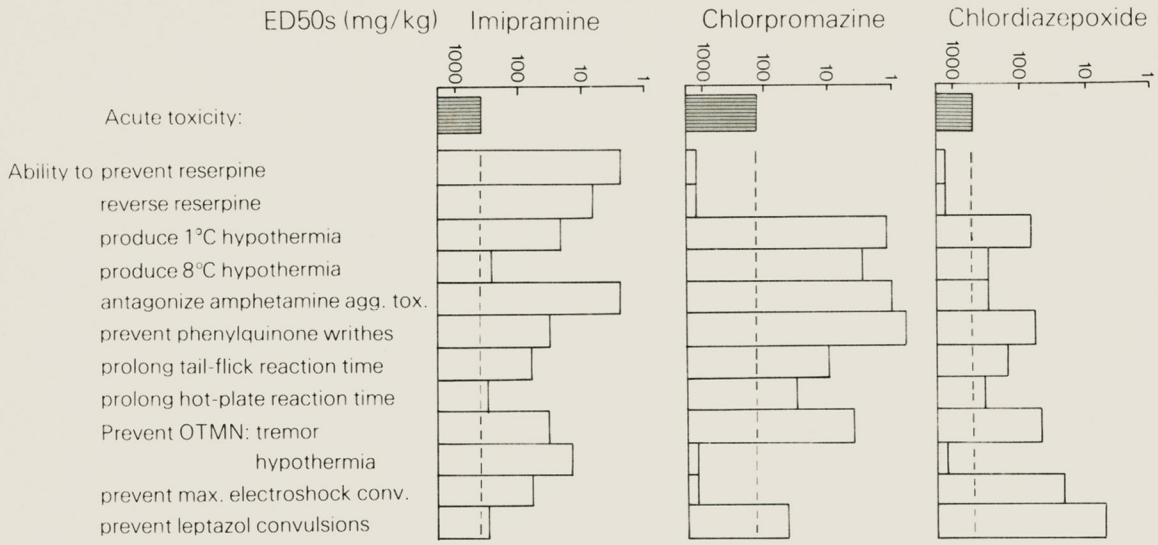

Figure 5 Simple neuropharmacological 'profiles' of imipramine, chlorpromazine and chlordiazepoxide.
All experiments were performed in the mouse. The data have been arranged so the potency of each agent in individual tests is indicated by the length of the relevant histogram.
All data obtained by a common (oral) route of administration.

simple *in vivo* experiments in laboratory animals. Nevertheless, the final concept of a subcellular, biochemical mechanism of action is not possible from this type of study, and the next stage in any investigation would be a full examination of the potential drug's biochemical-pharmacological properties (involving in the case of a potential, imipramine-like antidepressant, a study of the drug's effects on biogenic amine turnover and metabolism in the brain).

Table 1 Neuropharmacological techniques commonly used to evaluate antidepressant activity in new agents

'Imitative'	Prevent reserpine's effects (active: IL, MAOI)
	Reserve reserpine's effects (IL, AL)
	Prevent effects of icv-injected NA or ouabain
'Empirical'	Potentiate leptazol (AL)
	Potentiate picrotoxin (IL, MAOI, AL)
	Antagonize electroshock (IL)
	Enhance amphetamine's effects on spontaneous locomotor activity (IL, MAOI, AL)
	Prevent or reverse, wholly or in part, effects of tremorine or oxotremorine (IL, AL)
	Ability to enhance (MAOI) or antagonize (IL, AL) the head-twitch syndrome elicited in mice by 5-HTP

IL, Imipramine-like, AL, amphetamine-like

Second-order parameters

The use of so-called empirical techniques has already been referred to. Nevertheless, whether general or specific in nature, considerable use is made of experimental techniques which, on the surface at least, bear little resemblance to the mental condition for which an active drug is being sought. Their relevance lies in the fact that a drug may be exerting a pharmacological effect on some structure believed to be important or at least involved in the clinical condition, and the consequence of this pharmacological effect in animals may be some bizarre change in behaviour otherwise unrelated to the human condition. We are thus concerned about the pharmacological site of action (relevant to man), but may assess the intensity of drug effect by a second-order effect not obviously related to the clinical situation. The importance of this task is best explained by an example.

The ability of anti-psychotic drugs to interfere with hypothalamic function may be fundamental to their clinical usefulness. The degree to which hypothalamic function is affected by an agent can be reflected in the second-order phenomenon of temperature control, particularly easy to measure in a small animal species such as the mouse because it has a low mass and high

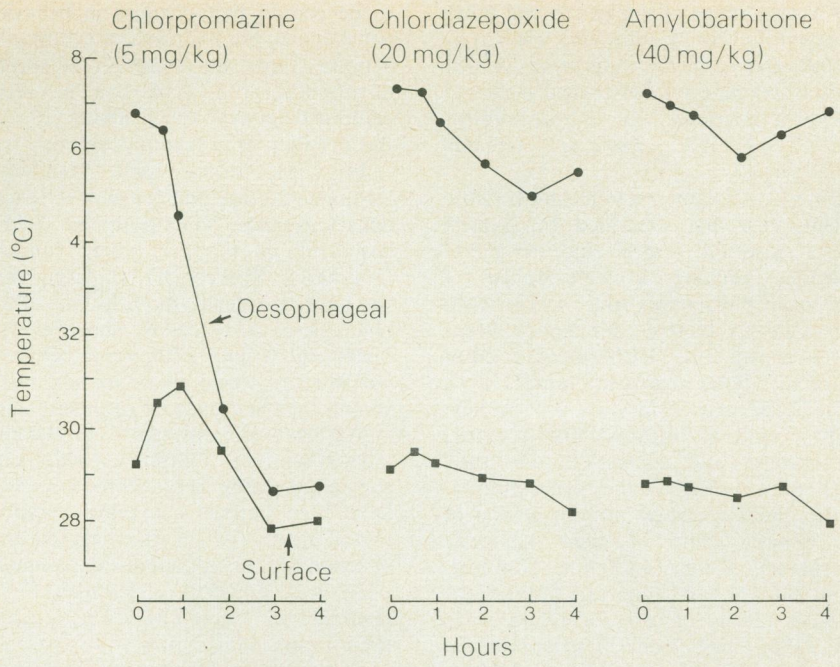

Figure 6 Effects of central nervous depressants on oesophageal and skin temperatures in the mouse.
Temperatures were recorded from the oesophagus and the ventral surface of the hind paw, using the method of Brittain & Spencer (1964), immediately before and at intervals after the subcutaneous injection of chlorpromazine (5 mg/kg), chlordiazepoxide (20 mg/kg) and amylobarbitone (40 mg/kg).

surface area, leading to a labile body temperature even in normal conditions. Thus, although of little clinical value, the depth of hypothermia induced by a depressant drug can be highly informative about the drug's actions on hypothalamic activity. In Figure 6, a comparison of the effects of three centrally-acting depressants is made.

All three agents cause a significant hypothermia, but the severity of the hypothermia is much greater in the case of chlorpromazine, a feature shared by all major tranquillizers. Occurring simultaneously, there were changes in skin temperature (increases being indicative of vasodilation and loss of sympathetic tone), and only in the case of chlorpromazine was there evidence of a vasodilation which would contribute to the rate at which hypothermia would develop. The vasodilation would also indicate a more substantial interference with hypothalamic function than was produced by either the amylobarbitone or chlordiazepoxide at the doses used. All three agents exert other, characteristic, pharmacological effects in various other neuropharmacological techniques, yet this single study of their effects on mouse body temperature shows clearly a fundamental difference in their basic central properties.

In a similar way, the exploratory activity of rats might appear to have little connection with psychoneurotic conditions in man. Yet, it can be shown that, under certain conditions, the minor tranquillizers or anxiolytics (for example chlordiazepoxide, or meprobamate) will markedly increase the exploratory activity of rats. Using a Y-shaped runway, Marriott & Spencer (1965) showed that whereas minor tranquilizers increased exploration, the major tranquillizers produced a marked reduction of exploratory activity; and one can argue that this indicates fundamental differences not only in pharmacological activity, but also in clinical usefulness.

Tool drugs

Many of the behavioural or other changes induced in experimental animals may be brought about by the administration of specific chemical agents which, although themselves possessing well-documented pharmacological and biochemical properties, are of no apparent direct clinical use. Instead, they are used primarily in experimental animals to help in the study of behaviour or help elucidate the actions of other agents with more likely clinical usefulness. This is what is meant by 'tool' drugs, and examples include leptazol (used in the evaluation of minor tranquillizers and certain anticonvulsants); phenylquinone (which induces a writhing syndrome in rodents, which can be

ameliorated by a wide range of analgesics and other drugs, see Brittain, Lehrer & Spencer, 1963); α-methyl-*m*-tyrosine and α-methyl-*p*-tyrosine (which interfere with catecholamine synthesis); and *p*-chlorophenylalanine which interferes with 5-hydroxytryptamine synthesis). The list is a long one, and increases daily.

One such agent of special interest to me is tremorine (TREM). A substance first described by Everett (1956), it exerts powerful central and peripheral muscarinic actions, eliciting a wide range of behavioural and other phenomena which are believed to be due to the massive cholinergic stimulation. Some years later, it was shown that TREM is converted *in vivo* to an active metabolite oxotremorine (OTMN), and today both TREM and OTMN are used widely. Their principal central effects are tremor, hypothermia, analgesia and depression, and their primary (or specific) value to the neuropharmacologist is in the initial detection and evaluation of anti-Parkinson drugs. Some of their analgesic properties also resemble those of morphine. However, both TREM and OTMN have important interactions with a much wider variety of centrally-acting drugs (Spencer, 1965, 1966) and Table 2 illustrates the potencies of a number of drugs in reversing TREM-induced tremor and hypothermia in the mouse.

A study of this table shows again how the individual groups of psychotropic drugs exert different yet characteristic effects in a neuropharmacological technique which seemingly has little relevance to the mental states for which these drugs are commonly used, but the information thus obtained is useful in predicting possible mechanisms of action.

Special techniques

A fundamental problem for the neuropharmacologist is the need to separate central from peripheral effects, to be certain that changes in behaviour elicited by some agents are the result of central pharmacological actions. There are a number of ways in which the distribution of a drug can be restricted to the cerebrospinal axis, and Feldberg (1963) has described the technique of intracerebroventricular (icv) injection.

In 1967, Brittain and Handley described a technique for injecting drugs directly into the brains of conscious mice, while a similar technique has been described in the rat, using implanted cannulae (Sparkes & Spencer, 1971). Using these techniques, we have examined recently the central actions of uncouplers of oxidative phosphorylation (Doggett, Spencer & Waite, 1970), which behave very much like barbiturates when given by icv injection (yet are stimulants when given by peripheral injection).

We have also attempted to elucidate the central interactions of morphine with certain biogenic substances, using these techniques, and to date studies have been carried out using narcotic agonist and partial agonist/antagonist agents in animals given icv injections of acetylcholine, dopamine, noradrenaline (NA) and 5-hydroxytryptamine (5-HT). Figure 7 summarizes some of our results with these experiments in the mouse.

Our tentative conclusions from these experiments are that the analgesic activity of narcotic agonists and partial agonists may be determined by a change they induce in the brain in the balance of NA and 5-HT metabolism, and we hope subsequently that this type of technique may prove to be a simple, acute method of detecting narcotic agonist-like activity in any potential analgesic. Studies with known, non-narcotic agents are currently in progress, as are experiments involving long-term administration of these agents in tolerance and dependence studies. These interactions between the biogenic substances and the opiates are not observed when the amines are administered peripherally. This suggests that they are central interactions which we are observing, and furthermore might not have been detected without these special techniques.

Table 2 Ability of some centrally-acting and other drugs to reverse established effects of tremorine in the mouse:

	Dose (mg/kg) to reduce by 50%	
	Tremor	*Hypothermia*
Atropine	4.3	5.6
Benzhexol	7.5	8.1
Methylatropine (quat.)	>50	>50
(+)-Amphetamine	12	5.0
Tranylcypromine	12	7.3
Imipramine	21	15
Desipramine	24	12
Chlorpromazine	5.5	>25
Chlordiazepoxide	25	>150
Pethidine	> 50	>50

(All orally administered)

Summary

In a situation in which little definite is known about the aetiology or pathophysiology underlying most mental illness, it is not surprising that the current state of affairs shows that: several major breakthroughs have occurred through clinical rather than laboratory observations; drugs discovered in the laboratory frequently possess disappointing clinical effects; and new drugs too frequently offer only marginal improvement over almost identical existing drugs which they are supposed to replace.

The pharmacologist's role is to produce model, imitative, illnesses in which both established and potential new agents are effective. In those fields where the underlying pathophysiological condition is

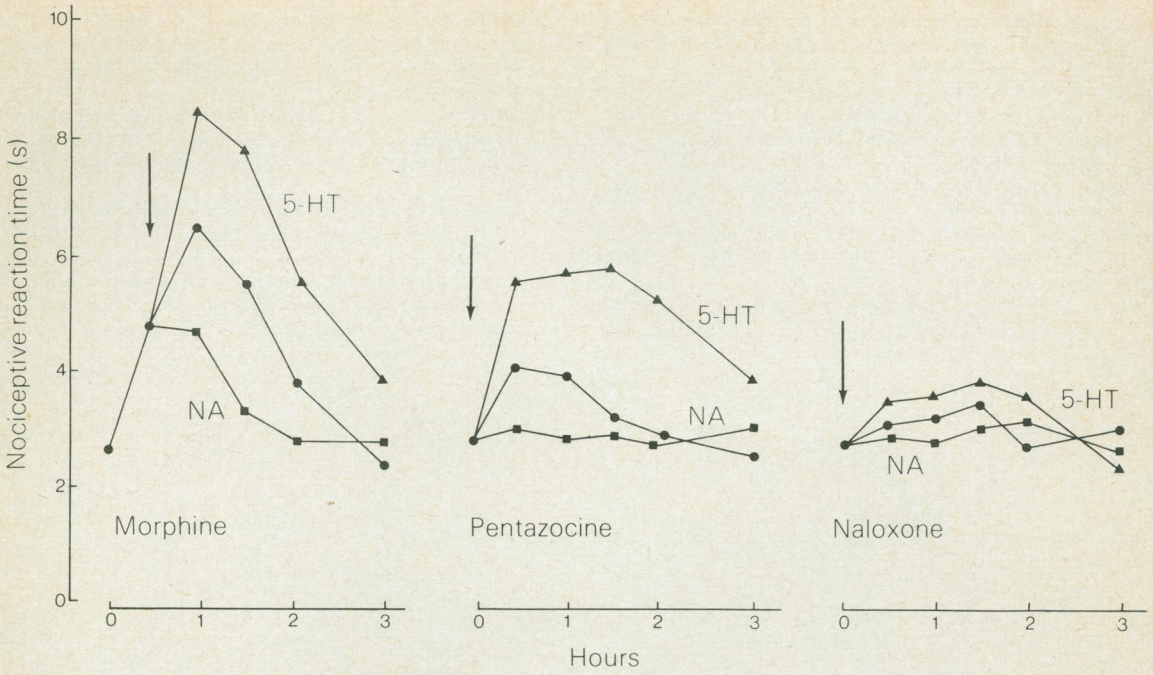

Figure 7 Anti-nociceptive activity of morphine, pentazocine and naloxone in the mouse, after intracerebroventricular injection of NA or 5-HT.

Nociceptive sensitivity was determined at intervals, using a tail-immersion test. Intracerebroventricular injections of NA (10 μg/mouse) or 5-HT (10 μg), were made 15 min (arrowed) before the maximum activity of morphine (3.5 mg/kg, sc), pentazocine (5 mg/kg) and naloxone (5 mg/kg).

relatively well understood (such as hypertension), an army of effective combative agents has now been discovered.

By contrast, each significant major new discovery in the treatment of mental illness has occurred either through astute clinical observation, or after painstaking animal studies spread over a decade or more. In the absence of specific knowledge about the cause of the human condition, the pharmacologist's model illness can show only passing imitation, and consequently the predictive value of the pharmacologist's observations are suspect. Therefore, reliance is not placed on individual observations, but on the results of wide-ranging neuropharmacological studies.

The initial studies should work to produce a comprehensive profile of central actions for each potential drug, and the drug's allocation to an existing or postulated new class of drugs is determined by this profile, as also must be decisions to cease or continue further work. The secondary pharmacological evaluation can take a variety of forms, but the purpose is clear: to confirm that certain central actions exist, what the level of potency may be, and what are the drug's likely advantages (or disadvantages) when compared with existing drugs. The use of imitative and empirical techniques, the use of second order parameters, tool drugs and special techniques, illustrate the great variety of ways in which the neuropharmacologist obtains his information.

The data and views expressed in this paper are the result of discussions and investigations over a number of years with the following present or former colleagues: R.T. Brittain; C.R. Calcutt; N.S. Doggett; S.L. Handley; A.S. Marriott; H. Reno; R.D.E. Sewell; C.G. Sparkes and R. Waite.

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