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INTEROCEPTIVE STIMULI AS TOOLS OF DRUG DEVELOPMENT*

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TOOLS OF DRUG DEVELOPMENT

Drug development is the foremost objective of most pharmacologists in the pharmaceutical companies and some pharmacologists in the other research institutions. To meet this objective, thousands of new chemical compounds must be evaluated in order to discover one which can be safely employed in the treatment of human illness. Such evaluation requires an enormous number of experimental subjects specially prepared for this purpose. Since use of human patients for initial screening of new chemicals is not feasible one must depend on laboratory animals that can be used repeatedly and extensively.

The art of screening drugs was only recently developed and, therefore, remains mostly empirical. We are not yet sure as to which method of evaluation yields clinically relevant information. We spend a great deal of time and effort continuously to devise new research tools with the following characteristics.

Simplicity: The tools should employ readily available laboratory facilities and animal species, minimum technical information

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required on the part of laboratory technicians, and minimum supervision by the senior scientists. The parameters to be observed should be objectively defined so that they can be recorded automatically and with minimum involvement of the human observers.

Reproducibility: The results of drug evaluation should be expressed in digital numbers which are reproducible. The same drug dosage tested repeatedly and across laboratories should produce results which fall within a narrow range. The previously established statistical methods should be directly applicable to the data obtained.

Efficiency and Speed: A minimum input of effort should provide maximum yield. A large number of new chemicals must be rapidly screened for activity classification and biological activity-spectrum as well as prediction of clinical efficacy by using a small number of experimental subjects in which reliability of data may be increased by repeated measure designs.

TWO RECENT DEVELOPMENTS

In recent years there have been two scientific developments which are worth mentioning, in that they possess the potential of virtually revolutionizing the pharmacological procedures that are currently employed in developing new drugs. One centers on the receptor binding methodology. A number of drugs have been found to serve as ligands of specific binding sites in the body. The influence of new chemicals on ligand-receptor binding is proving to be a good estimate of their *in vivo* potency in producing a therapeutic action and/or a side effect.

Although the receptor binding methodology meets a number of criteria considered desirable for drug development application, it possesses serious shortcomings. It is entirely an *in vitro* procedure which does not take into account the pharmacokinetic properties of drugs. Moreover, only a very limited number of receptor-specific ligands have been found that are useful in drug development.

Whereas the receptor binding methodology is an exclusive contribution of of biochemical pharmacologists, behavioral pharmacologists have come up with their contribution in providing a more versatile tool for drug development. In recent years, methodology has been developed to objectively measure, in the laboratory animals, the interoceptive stimuli specifically produced by various classes of drugs (Lal, 1976). This development constitutes the second break-through that provides a new tool widely applicable in drug development.

Interoceptive stimuli are the quantifiable subjective experiences that result from physiological changes occuring within the body. They are frequently induced by drugs and can now be reliably measured as discriminative stimuli just like the discriminative stimuli of sound and light which originate in the external environment of the organism.

INTEROCEPTIVE STIMULI

Stimuli are conventionally defined as environmental events that are perceived by a living organism to produce a behavioral response. The quality and quantity of the behavioral emission is different in the presence of those stimuli than in their absence. The experimental characterization of a stimulus is exclusively based upon the behaviors that are either emitted or elicited in the presence of the stimulus. The type of functional relationship between a stimulus and a behavioral response determines a functional category of the stimulus. At present many stimulus categories are recognized (for discussion see Lal, 1977a).

A stimulus may originate from either outside of from within the organism. Stimuli occuring only in the external environment are considered as the extroceptive stimuli. However, there are many events that are primarily initiated from within the body that can also act as stimuli. They are known as the interoceptive stimuli. Both interoceptive and extroceptive stimuli can be

divided into many functional categories, (for description see Lal, 1977a) one of which is a category of discriminative stimuli.

Interoceptive discriminable-stimuli (IDS) are biological events induced within the body to serve the basis of discriminative overt responding. In order to bring behaviors under the control of IDS a response is reinforced in the presence of the IDS but not in its absence. Often two alternating stimuli are employed and one response is reinforced in the presence of one stimulus and a different response is reinforced in the presence of the second stimulus. Thereafter the animal seeks reinforcement by differential responding to different stimuli, thereby indicating presence or the absence of a test stimulus. This is illustrated diagramatically in Figure 1.

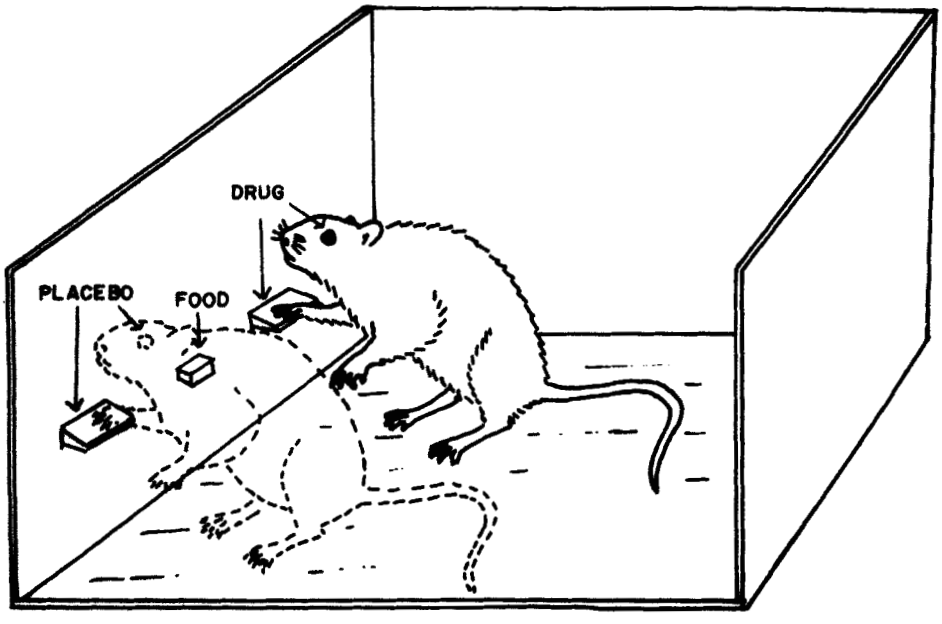

FIGURE 1

Selected drugs can be employed to produce specific IDS. The drug thus selected produces biochemical changes in the body that form a stimulus complex. When the nervous system is excited through this stimulus, a new state is organized within the nervous system and certain components of that state are consciously perceived, some dominantly and others minimally. In the beginning of the training, the organism responds to the dominant components of the stimulus complex. The other components are effectively ignored as irrelevant at that time. Subsequently, if the dominant stimulus component (cue) is found reliably associated with the response-consequence, it is adopted for discrimination learning. However, if a reliable association is not found with the dominant event, one of the other cues is selected.

The animal does not usually respond to the IDS at the beginning of drug-discrimination training. Rather, the subject first responds to more dominant stimuli present in its environment. The obvious ones include position of the operandum, sequence of the training sessions, or many other sensory cues which have been previously relevant for most organisms in their natural environment. It is only when the external stimuli do not work, that the distinct stimulus association with the drug action within the body becomes dominant.

DRUGS AS TOOLS TO PRODUCE INTEROCEPTIVE DISCRIMINABLE STIMULI

Many drugs are known to form primary IDS; a detailed description of the IDS produced by various drugs can be found in a recently published monograph (Lal, 1977). The drug classes which have been experimentally employed to study the specific interoceptive stimuli are given in Table 1. It can be well appreciated, from the proposed nature of the interoceptive stimuli produced by each class of drugs, that a very wide variety of interoceptive stimuli can be generated in order to evaluate new drugs that either mimic or antagonize primary stimuli. From these illustrations, it can also be seen that drugs through their own drug-specific changes in

TABLE 1

Drugs Known to Produce Interoceptive Discriminable Stimuli

Drug	Proposed nature of interoceptive stimuli
Amphetamines (low)	Arousal
Amphetamines (high)	Psychotomimetic euphoria
Antidepressants	Not known
Apomorphine	Central dopamine stimulation
Aspirin	Peripheral analgesia
Barbiturates	Sedation
Benzodiazepines	Anxiolytic, anticonvulsants (?)
Cannabinoids	Euphoria, dysphoria (?)
Clonidine	Central adrenergic activity, dopamimergic (?) activity
Cocaine	Euphoria, anxiety (?)
Ethanol	Sedation
Hallucinogens	Psychotomimetic effects
Muscarinic -antimuscarinics	Central muscarinic stimulation/inhibition
Narcotics	Euphoria, analgesia (?)
Nartocotic Antagonists	Dysphoria, analgesia (?)
Neuroleptics	Not known
Nicotine	Central nicotinic stimulation
Pentylenetetrezole	Pre-convulsive and anxiety states
Phenylbenzoquinone	Visceral pain
Quipazine	Psychotomimetic

the CNS produce specific and discriminable sensations. These changes are qualitatively different that the conventional sensory experiences and are often characteristics of a drug class (for a detailed discussion see Lal, 1977). Here it may be sufficient to stress that the drugs are capable of initiating physiological changes in the CNS which are perceived as unlike those produced by normal life processes. Although, it is conceivable that the drug

action on sensory apparatus can provide a stimulus capable of being discriminated under appropriate experimental conditions, they are not the usual events which are the basis for drug discrimination.

METHODOLOGICAL CONSIDERATIONS

Laboratory procedures which are employed to measure interoceptive stimuli are many. The usual one was discussed in two recent reviews (Lal et al., 1977; Lal, 1977a) and will not be elaborated. It is sufficient to say that IDS can be easily established in a very wide variety of experimental subjects. Usually rats are trained in Skinner boxes to press one lever when injected with drug and another lever when injected with placebo. If responses are emitted on the appropriate lever, a food pellet is delivered after each 10 responses. In the trained rats, a test drug is injected before the rat is allowed to select the appropriate lever and no other cue is allowed for that selection. The selection of the appropriate lever is treated as an all or none response to calculate the effective dose of the test chemical according to the established procedures of drug evaluation and statical analyses.

There are three types of discrimination currently employed in the drug-discrimination experiments. The most often used discrimination employs a drug versus saline distinction which has been found very useful for defining specific IDS associated with any drug class. The second type of discrimination utilizes two doses of the same drug. This discrimination can provide information on the quantitative and qualitative aspects of the IDS produced by different drug doses. The third type of experiment involves discrimination between two drugs. This type is particularly useful in obtaining information on the differences between the pharmacological perperties of two drugs belonging to the same class. For example, many neuroleptics are also anticholinergics. If an animal can discriminate between two neuroleptics such as haloperidol and clozapine, one of which is devoid of anticholinergic properties, the discrimination between the two may provide a measure of

the presence or absence of this undesirable property. However, because there has been very little work done on drug-drug discrimination it is yet difficult to precisely evaluate its usefulness in drug development.

In addition to the above, one can conceive of several other discrimination strategies which can be used for specific purposes. They usually involve drug combinations at different doses. More than two DS may also be used. Generally the availability of the number and complexity of possible combinations if virtually unlimited.

APPLICATIONS IN DRUG DEVELOPMENT

The ways in which IDS can be employed in drug development are numerous. Some of them are illustrated below but this is not intended to be an exhaustive list. As the research in this field is progressing rapidly, new applications are continuously becoming apparent.

Primary Screening of New Chemicals: An important application of drug induced IDS is to evaluate new compounds for pharmacological activity. For this purpose many classes of drugs have been employed to date as standards. They are listed in Table 1. Also are given the expected bases of IDS produced by those standard drugs. The IDS are usually produced by very low doses of the CNS active drugs and are associated with very specific drug actions. For example, the ED_{50} values for the narcotics in producing IDS are markedly lower than those obtained by measuring analgesia in the same animals (Colpaert et al., 1976; Lal, et al., 1977; Neimegeers, et al., 1976; Miksic and Lal, 1977). In the case of narcotic antagonists doses as low as 0.1 mg/kg are readily detected (Gianutsos and Lal, 1976). The discrimination procedure has been proven useful for screening analgesics, narcotic-antagonists, CNS stimulants, hypnotics, anxiolytics, cannabinols, serotonin agonists, dopamine antagonists and muscarinic drugs. The potential

for screening antidepressants, antihypertensives, antidiarrheals, anorexants, gabamimetics and anticonvulsants by this method is currently being investigated.

Drug Classification: The IDS produced by drugs can be used to classify new chemicals into various pharmacological categories or classes as well as to establish their mechanism of action. Several illustrations of this use have been published in literature. They suggest that there are sufficient data now available to demonstrate that IDS can be reliably used for predicting drug classifications, particularly in the case of the CNS stimulants, anxiolytics, narcotic analgesics, psychotomimetics, nicotinics, muscarinics, dopamine agonists, antianxiety drugs, anticonvulsants and anti-inflammatory analgesics. To date, various other drug classes have not been extensively investigated.

Screening for Specific Actions: An area in which IDS methodology may be particularly valuable is in providing a screening procedure by which a drug can be evaluated for specific therapeutic action without interference from side effects. To illustrate, animals may be trained to discriminate an anxiolytic drug from a mildly depressant drug and then used for testing new anxiolytics with no sedative properties (Lal and Fielding, 1978). In another situation an undesirable action of a drug may be blocked prior to its use as a training drug. As long as an antidote of a side effect is available it can be combined with the drug. The drug action in the presence of the antidoted side effect is then used as the training drug. From this, new drugs can be sought that generalize to the antidoted action. The need for such differentiation among drugs at the time of screening exists is a wide variety of pharmacological areas. Narcotic antagonists without agonist properties, neuroleptics without sedation or extrapyramidal side effects, anticonvulsants without sedation, anorexants without other CNS effects, and antidepressants without autonomic side effects are only a few examples of the CNS drugs which could be screened in drug discrimination tests.

Development of New Antagonists: New chemicals can be sought to antagonize the stimuli produced by specific drugs. For example, whereas no other drug is known to block narcotic stimuli, narcotic antagonists do that very well. Comparable antagonists which are specific to barbiturates, nicotine, amphetamines, cocaine and alcohol are not yet available. The discrimination procedure can be employed to discover and evaluate such antagonists. Whereas other procedures can be used to detect antagonists at the biochemical or physiological levels, the discrimination technique specifically determines if the potential antagonist is useful in blocking the subjective effects of the psychoactive drugs. Drug discrimination procedures may be critical where other procedures are not available. Where other procedures are available, the discrimination techniques provide more economical and reliable alternatives, as once trained, the same animals are used repeatedly throughout their life span.

New Drug Property: Induction of IDS produced by a drug is a new addition to the list of various actions of drugs. In as much as it is related to euphoria or dysphoria produced by the drugs, it lends itself to their quantitative and qualitative characterization in laboratory animals. This is a real breakthrough as these properties of drugs in the past have defied all attempts of their controlled measurement. Full potential of this property of drugs has yet to be realized.

Mechanism of Drug Action: A critical question in pharmacology centers on the establishment of the biochemical mechanisms underlying each of the specific drug actions. Because the drug produced IDS is a specific action that can be reliably measured, it is a potentially good tool in establishing mechanisms of drug actions. It is particularly useful where the objective is to determine the mechanisms underlying the elicitation of subjective feelings by the drug since no other procedure lends itself to the study of this aspect of drug actions in animals. The biochemical mechanisms underlying the various actions of the same drug are not

necessarily the same. For example, whereas the drugs which alter brain neurotransmitters are known to modify the analgesic actions of narcotics, no such alteration is produced in the IDS produced by narcotics, (Gianutsos and Lal, 1976; Miksic et al., 1978, Lal, et al., 1977). Similarly, biochemical mechanisms of IDS produced by metrazol seem to be different than those underlying its convulsant properties (Shearman and Lal, 1978). These are some of the concrete instances in which subjectively perceived actions of drugs are mechanistically separated from other actions of the same drug.

Animal Models: Due to considerations of safety, human subjects cannot be used for drug screening. Therefore we must depend upon laboratory animals to evaluate the new chemicals thought to be effective against any one of the vast number of diseases affecting namking. A limiting factor is that animal models of certain human diseases are difficult to find. IDS produced by drugs provide an approach which could be utilized to approximate relevant aspects of several human disease conditions in animals for the purpose of drug screening and research. This is particularly valuable in situations where other animal models are not available. For example, there is no good model of headache to screen antiheadache drugs. Generalization with IDS produced by aspirin or serotonin-like drugs may offer a new approach. Similarly, generalization with clonidine in genetically hypertensive animals may serve as a procedure of screening antihypertensive agents.

Predictability of Abuse Potential: Acquisition and continuation of drug abuse is usually based upon the specific actions of the drug on the CNS. Not enough is known to define this CNS activity precisely. It is said to be some sort of euphoric effect which is described as a feeling of satisfaction or a "high" (for a discussion, see Lal, 1976a). According to Eddy et al., (1970), "this mental state is the most powerful of all the factors involved in chronic intoxication with psychotropic drugs, and with certain types of drugs it may be the only factor involved even in most

intense craving and perpetuation of compulsive abuse". The most basic requirement for a drug to be considered as euphorogenic or dysphorogenic is that the drug must cause CNS action which can be clearly perceived subjectively. It is reasonable to assume that if the CNS effects of a drug cannot reach the threshold of perception, then they will not be regarded as euphorogenic or dysphorogenic. If the CNS effect of a drug is readily perceived (i.e. discriminated), and if that effect generalizes to the euphoric effect of a training drug which is abused, in all likelihood, the new drug will possess high abuse potential. On the other hand, if the perceived (or discriminated) IDS generalize to those produced by dysphoric training drugs, the test drug would not be expected to be abused. Also if the drug action cannot be perceived, as shown by a failure to form IDS, this drug is not likely to be abused. According to Frazer et al., (1961), "if one were to select, on the basis of single doses, the most important single subjective response identifying a drug as being subject to morphine-like abuse property this measure would be whether the former opiate addicts identify this drug as an opiate". Therefore, generalization to saline IDS may predict non-abusability and generalization to IDS of a drug having proven abuse potential would predict an abuse potential of the drug class.

Disease Models: The learning of internally produced discriminable stimuli is not limited to drugs alone. It is quite conceivable that other changes due to disease states can also be perceived and demonstrated by using the tool of drug discrimination. Thus, this is a new approach to bioassay a disease state which can be best described through illustrations at present. If is hoped that in the future these application will be experimentally tested to establish their potentials.

A drug may be selected to produce hyperthermia which can be discriminated by a laboratory animal. If it is established that the drug discrimination was based upon the hyperthermia produced by this drug, this would provide a paradigm in which a disease

state (fever) could be behaviorally discriminated. Whenever there is a disease condition which either produces hyperthermia or relives an already elevated body temperature, it could be behaviorally detected by the trained animals. Fever production would be generalized to the training drug and fever elimination would be discriminated from it. This is one illustration of how a disease state can be bioassayed with drug discrimination technology. Recently, we tested discrimination of IDS produced by phenylbenzyoquinone, an agent known to produce visceral pain in the peritoneal region (unpublished data). In this model of visceral pain analgesic drugs may be tested. Demonstration of anxiety-states induced by metrazol as measured by specific IDS (Shearman and Lal, 1978) is certainly another advance with great potentials for measuring anxiety states in the animal. Such procedures can be readily applied in the development of new drugs (Fielding and Lal, 1978).

If we find ways to produce different diseases in experimental animals but no way of characterizing those diseases without sacrificing the animals, the drug discrimination procedure may be found handy. For example, there is no experimental method to directly detect headache in experimental animals. Also, in normal animals aspirin-saline discrimination is difficult. We know that aspirin is an effective drug against headache. If one can train animals to discriminate aspirin in the presence of and not in the absence of a potential headache, an experimental procedure capable of detecting headache in the animal has been devised. Here discrimination of aspirin will be employed to bioassay headache. Similar procedures can be developed to bioassay several other disease conditions. As long as there is a drug known to provide relief from a symptom which is specific of that disease, the presence or absence of this disease can be determined by the use of drug discrimination. It is very likely that this approach can be applied to bioassay anorexia, constipation, pain, inflammation, hypertension, depression, anxiety and even paranoid schizophrenia. We certainly do not mean that the diseases will be real, but we are talking

about animal models for purpose of drug development. Some of these ideas have already been tried. Weissman (1976) found that normal animals do not readily discriminate action of aspirin. However, they readily learned to discriminate aspirin in the presence of chronic pain. Similarly it was found that specific neuroleptics such as haloperidol are very difficult to discriminate because they do not produce subjective effects. However, animals treated with amphetamine rapidly learn to discriminate small doses of haloperidol. Another example is that of naloxone. This narcotic antagonist is usually not discriminable. However the animals experienced with morphine can readily discriminate from saline (Lal, et al., 1978). We are currently investigating discrimination of antidepressant drugs in reserpine treated animals and of clonidine in genetically hypertensive rats, in order to establish experimental models of depression and hypertension as they are applicable to drug screening.

Reminder Drug: If a drug is found to be potent eliciter of IDS but lacking other pharmacological action, it may be useful as a reminder drug (Weissman, 1976). For example, such a drug may be dispensed with a vitamin pill or birth control pill. For a busy individual the need to remember these pills were taken on a particular day will be minimized. One would know the answer because of the presenc or absence of the subjective feeling produced by the reminder drug. Such a drug also provides a unique tool for the study of subjectively perceived mood alterations in the absence of other pharmacological actions.

Drugs as Warning Stimuli: IDS induced by drugs may be employed in training of the patients to become aware of the subjectively perceived physiological signals often produced prior to the onset of certain overt symptoms. For example, if it is true that convulsive drugs can produce IDS at subconvulsive doses, those IDS may generalize to the physiological effects of a pre-convulsive state. If so, sub-convulsive doses of selected drugs may be used to train patients to clearly discriminate the stimuli usually produced prior to an onset of an epileptic seizure. Whenever those stimuli

become manifested, the patient may be treated with pharmacological or other interventions.

Delayed Report of Drug Effect or Disease State: When patients are seen by a physician they are asked how they feel with regard to a particular time reference. An insomniac patient may be asked for a verbal report of sleep condition during the previous night. Here the patient must rely on his/her memory of the body condition during a particular time span. Can an experimental animal be trained to report, through a discriminative behavioral paradigm, a physiological condition that existed several hours earlier. There is some evidence to suggest that it can be done. We have recently found that a rat can be given morphine sulfate and trained to discriminate naloxone given several hours later (Lal et al., 1978). Once trained, this subject can discriminate naloxone from saline readily. During generalization testing, conducted 24 hours after morphine administration, we found that this rat when given naloxone for generalization will select naloxone appropriate lever only if pretreated with morphine 24 hours earlier. If morphine treatment is not given naloxone will be generalized with saline. If similar paradigms can be established using other drug treatments, a way may be found to obtain an objective report of when a subject "remembers" of a past biological event that was interoceptive. when imagination is stretched one can think of asking questions about autonomic symptoms, stomach ache, convulsive states, etc., several hours or days after they were experienced.

REFERENCES

F.C.Colpaert, C.J.E.Neimegeers and P.A.J.Janssen. The narcotic discriminative stimulus complex: relation to analgesic activity. J. Pharm. Pharmacol. 28, 183-187 (1976).

N.B.Eddy, H.Hallach, H.Isabell and M.H.Seevers. Drug dependence: its significance and characteristics. In: Drug Abuse, Data and Debate, P.H.Blacky, ed., Springfield Thomas, 1970.

H.F.Frazer, G.D.Van Horn, W.R.Martin, A.B.Wolback and H.Isabell. Methods for evaluating addiction liability. (A) Attitude of opiate addicts towards opiate-like drug, (B) A short-term direct addiction test. J. Pharmacol. Exp. Therap. 133, 371-387 (1961).

S.Fielding and H.Lal. Future prospectives on anxiolytic drugs. In: The Anxiolytics Vol. 3, S. Fielding and H. Lal, eds., Futura Publishing Co., 1978.

G.Gianutsos and H.Lal. Selective interaction of drugs with a discriminable stimulus associated with narcotic action. Life Sciences 19, 91-98 (1976).

H.Lal. General characteristics of discriminative stimuli produced by drugs. Psychopharmacol. Comm. 2, 205-309 (1976).

H.Lal. Facts and falacies of addiction liability and abuse potentials. In: Synthetic Antidiarrheal Drugs, W.Van Beuer and H. Lal, eds., New York, Marcel Dekker Inc., 1976a, pp. 235-251.

H.Lal. Discriminative Stimulus Properties of Drugs. Adv. Behav. Biol., Vol. 22, New York, Plenum Press, 1977.

H.Lal. Drug induced discriminable stimuli: Past research and future perspectives. In: Discriminative Stimulus Properties of Drugs. H.Lal, ed., New York, Plenum Press, 1977a, pp. 207-231.

H.Lal, G.Gianutsos and S.Miksic. Dsicriminable stimuli produced by analgesics. In: Discriminative Stimulus Properties of Drugs, H.Lal, ed., New York, Plenum Press, 1977, pp. 23-45.

H.Lal and S.Fielding. Discriminative stimuli produced by anxiolytic drugs, a new procedure for screening of antianxiety drugs. In: Anxiolytic Drugs, S.Fielding and H.Lal, eds., Futura Publishing Co., 1978.

H.Lal, S.Miksic and M.McCarten. A comparison of discriminative stimuli produced by naloxone, cyclazocine and morphine in the rat. Psychopharmacology 58, 8 (1978).

S.Miksic and H.Lal. Tolerance to morphine produced discriminative stimuli and analgesia. Psychopharmacology 54, 217-222 (1977).

S.Miksic, G.Shearman and H.Lal. Generalization study with some narcotic and non-narcotic drugs in rats trained for morphine-saline discrimination. Psychopharmacology, in press.

C.Niemegeers, F.Lenaerts and F.Awouters. In vivo pharmacology of antidiarrheal drugs. In: Synthetic Antidiarrheal Drugs, W.Van Beuer and H.Lal, eds., Marcel Dekker, Inc., 1976, pp. 65-114.

G.Shearman and H.Lal. Differential responding controlled by the discriminative stimuli produced by convulsant drugs in the rat. Psychopharmacology 58, 10 (1978).

A.Weissman. The discriminability of aspirin in arthritic and non-arthritic rats. Pharmacol. Biochem. Behav. 5, 583-586 (1976).

A.Weissman. Future perspectives in research in drug discrimination, Paper presented at symposium on "Research Applications of Drug-induced Discriminable Stimuli," 1976a.