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November 26, 1965

Dear Doctor

Enclosed in quadruplicate is an unsolicited proposal to provide for the development of techniques and a facility for screening chemicals for their potential property of inducing temporary behavioral changes.

We have proposed a three-year program with the first year being devoted primarily to the development of techniques using known compounds as models. The following two years would be devoted to screening compounds with some provision for constantly improving techniques. We realize that such a project can be funded only a year at a time.

While we cannot be certain, we believe that by use of our electronic data processing equipment we may be able to analyze the activity profile of screened compounds in order to provide a more reliable guide to further compound development.

If we may clarify or amplify any point in the proposal, please do not hesitate to let us know.

Sincerely yours,

Enclosures

AN UNSOLICITED PROPOSAL

SCREENING OF COMPOUNDS EFFECTING BEHAVIOR

Date Submitted: November 30, 1965

INTRODUCTION

This proposal concerns the screening of chemical compounds for their ability to alter the functional integrity of the complex processes of the central nervous system. The ideal compound, in addition, would be effective orally at low doses, water soluble, and would have neither taste nor odor.

The drugs which are classified by various authors as hallucinogens, psychomimetic drugs, psychopharmacological agents, or tranquilizers may serve as models on which the screening procedures are to be based, although, the ideal compound may not fit into these categories.

The pharmacology and various techniques of study of psychopharmacological agents (hallucinogens, psychomimetic agents, etc.) have been reported and reviewed by numerous authors (1-12). From the array of techniques and methods available, the screening procedure which follows, we believe, constitutes sufficient depth and versatility to be most effective.

METHODS

A. TOXICITY SCREENING: In order to establish the potency of a material, mice have been chosen as the first experimental animal. In addition, by careful observation, especially in regard to characteristic actions in mice of various accepted psychopharmacological agents, one may be alerted to new possibilities.

Mice, purchased from
will be held in temperature-controlled quarters for one week prior to compound administration in "shoe box" type cages with free access to water and Purina Laboratory Chow. The compound dissolved in water or other suitable vehicles will be administered orally at graded doses to four mice per dose level (after range finding) to determine the oral LD_{50} and minimum effective dose. The signs which will be noted range from the most subtle, such as social interaction, to death. The degree of effect will be noted as slight, moderate, or severe. The signs to be recorded are listed below:

- Social Interaction
- Alert
- Respiration
  - Rapid
  - Slow
  - Labored
  - Arrhythmic
- Ocular
  - Lacrimation
  - Swollen Lids
  - Ptosis
  - Miosis
  - Mydriasis
- Reflexes
  - Corneal
  - Pinnal
  - Righting
  - Tail
  - Myotactic
  - Hypersensitive
- Tremors
  - Localized
  - General
  - Intermittent
- Convulsions
  - Clonic
  - Tonic
- Depression
- Analgesia
- Ataxia
- Narcosis
- Prostration
- Hypothermia
- Hyperthermia
- Salivation
- Mouth Dry
- Defecation
- Urination
- Piloerection
- Vasoconstriction
- Vasodilation
- Cyanosis
- Behavior Unusual
  - Straub Tail
  - Vocalization
  - Circling
  - Hyperactive
  - Stretching
- Death
- Other

The mice will be observed frequently following administration of the test material so that the onset and duration of activity will be known.

B. SPONTANEOUS MOTOR ACTIVITY (Mice): The use of photocell devices is a widely accepted procedure and many reports concerning the effects of psychopharmacological agents are available in the literature (12).

Mice purchased, acclimated, and housed as previously described will be used as experimental subjects. A maximum of three doses (maximum tolerated to no effect) will be selected on the basis of previous results and the compound will be administered to four treated mice per dose level. The mice will be placed in an | for 12 hours and the spontaneous activity | at 15-minute intervals will be obtained. The spontaneous activity of six control (vehicle) mice will be recorded at the same time.

C. SPONTANEOUS ELECTRICAL CEREBRAL ACTIVITY: The effects of psychopharmacological agents on the electrical activity of the brain have been reviewed by Evarts (13,14) in respect to psychomimetic agents, and by Brazier (15) in respect to various drugs acting on the central nervous system. The preparation we propose to use has been reported by Renaldi and Himwich (16,17) to be sensitive to 1 to 5 micrograms/kg of lysergic acid.

For each compound tested two rabbits will be curarized, artifically respirated, and fitted with surface electrodes. Increasing intravenous doses will be given at 15 minute intervals. The spontaneous cerebral electrical activity (EEG) will be recorded. The minimum (cumulative) effective dose, the type of response, and the duration of effect will be reported.

A minimum of two dogs per compound will be used. The animals will be fasted overnight, anesthetized with phenobarbital sodium and suitably prepared for right vagal stimulation and to record blood pressure, EKG, and intestinal activity. After stabilization, control recordings of the above parameters will be obtained.

After parameters return to normal, the test compound will be administered intravenously at doses selected on the basis of previous data. Changes in baseline values will be noted. The animal will

be treated with

as before and changes in responses in terms of the parameters listed above will be noted.

This procedure will be repeated in every animal with increasing doses of the test compound.

E. BEHAVIOR PROFILE (Cats)

Irwin (1) along with Norton and DeBeer (23) have suggested the use of standardized multidimensional observations that are especially useful in the evaluation of drugs acting on the central nervous system. Using the procedures outlined below in cats, which were selected because of unusual signs elicited after treatment with certain type of CNS agents, we propose to establish a behavior profile for each compound.

A group of four cats acclimated to the procedure will be tested once weekly in a quiet temperature-controlled room. The animals will be restrained in overlapping areas by leashes. Observations will be made one-hour before administration of the drug and hourly thereafter, using as criteria: wakefulness, upright position, total locomotion, spatial activity, contact seeking, grooming, play, fearfulness, restlessness, vocalization, auditory response, aggressiveness, body tone, limb weakness, ataxia, nictitans relaxation, pupil size, heart rate, and respiration rate. The drug will be administered orally to a minimum of two sets of four cats in graded doses (based on previous results) with one cat receiving no active ingredient.

F. BEHAVIORAL EFFECTS (Rhesus Monkeys):

(1) Effect on Social Behavior: First the order of dominance in group-housed monkeys will be definitively ascertained. Following this, compound will be administered orally to one monkey at a

time from various levels of the social structure. The effect on the behavior of the treated monkey toward other members of its society will be observed. Other behavioral effects that will be observed include the order in which the monkeys eat and drink and the effect on the small cliques which are normally present.

(2) Effect on Aggressiveness: The "taming" effect of these compounds will be measured in rhesus monkeys by the method of Heise and Boff (24). This method consists of measuring aggression (forward lunging at the experimenter, baring teeth, and attacking experimenter's glove) and activity (general activity level or response to visual or auditory stimuli or ataxia). After drug administration a ratio, aggression score (per cent of own control)/activity score (per cent of own control), is determined. Taming is defined as a ratio of 0.5 or less; a ratio of about 1.0 indicates that drug action or aggression is not specific.

G. OPERANT BEHAVIOR (Squirrel Monkeys)

The use of changes in operant behavior induced by drugs has in recent years increased markedly (25-30). Two broad types of operant behavior are differentiated on the basis of the type of reinforcement, reward versus punishment (30,31). Sidman (11) has suggested that behavior controlled by different modes of reinforcement (food reward versus electrical shock negative reinforcement) may be controlled by different levels of drive. We propose to train animals to a multiple Fixed Ratio (FR) - Fixed Interval (FI) schedule (31). Half of the animals will be reinforced with food and the other half reinforced by avoiding electrical shock (8). During the Fixed Ratio portion of the schedule, the animal is reinforced after responding (lever pressing) a given number of times. In the Fixed Interval portion (usually taken to

indicate temporal discrimination, 31, 32) the animal is reinforced by the first response after the lapse of a given time interval. The two components are cued by different visual stimulus and a third visual stimulus is presented in the reinforcement period or when responses elicit no effect on the reinforcement of the animal.

Four squirrel monkeys (2 for each mode of reinforcement) trained to relative responding stability will be administered the test material orally, 30 minutes before testing begins (additional times will be allowed if a delayed onset of action has been noted previously). The animals will be tested the day of administration and daily thereafter until the behavior returns to normal. Three dose levels per compound will be tested. The analyses of data will consist of FR responding rate, FI responding rate, a mathematical index of FI performance (33), and responses in reward or time out portions of the schedule.

DISCUSSION

The first year would be devoted to obtaining equipment, training animals and running a series of positive controls. It is estimated that 40 to 60 compounds could be screened per year. This rate of screening in the second and third year of the contract would allow additional time for research directed toward improving the techniques of the screen, additional basic work on compounds of interest, or further work (LD_{50} and subacute toxicological studies) so as to permit limited use in man.

The procedures as described herein will provide basic pharmacological background in respect to acute toxicity, site of action, duration of action, species difference, and side effects. Behavioral or psychopharmacological parameters related to mood, drive, visual and temporal discrimination, memory, social interaction, aggressiveness, and motor coordination will have been evaluated.

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COST ESTIMATE

SUMMARY

First Year

Second Year

Third Year

Total