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March 4, 1968

Dear Doctor

Enclosed are six copies of our final report on "Studies of Screening of Chemical Compounds for Detection of Behavioral Effects."

Please note that only the title page bears the letterhead of our laboratory and the appropriate signature. This page can be removed from any or all copies of the report at your discretion.

If there are any questions or comments, please do not hesitate to let us know.

Sincerely yours,

C00021828

DR.

TASK ORDER NO.

CONTRACT NO.

STUDIES OF SCREENING OF CHEMICAL COMPOUNDS FOR DETECTION OF BEHAVIORAL EFFECTS

Date: March 4, 1968

FINAL REPORT

STUDIES ON SCREENING OF CHEMICAL COMPOUNDS FOR DETECTION OF BEHAVIORAL EFFECTS

INTRODUCTION: This report encompasses three phases of work. Initially, the task was to select from numerous published procedures the best techniques for defining the behavioral and neurological profile of a chemical. In addition, a literature review on muscle receptors and drugs affecting these sensory organs was prepared.

Potentially useful screening methods were tested utilizing four model compounds

Test procedures deemed worthy of further study were chosen on the basis of results obtained with these four compounds. These procedures were applied to a further series of compounds for classification purposes and to gain further information about the tests.

The second phase of work undertook a study of the behavioral profile of a series of well known clinically and experimentally used compounds, as well as of 10 relatively unknown experimental materials submitted for screening. The screening tests selected in the initial phase of this work were used for this purpose.

A third aspect concerned in-depth studies of interesting materials selected on the basis of screening results. A particular compound, which showed unusual and sustained behavioral effects was subjected to detailed investigation using pharmacologic inhibitors and potentiators to elucidate the mechanism of action. A method was developed for studying drug effects on motivation and then put into practice using the 10 experimental compounds submitted for screening.

PHASE I

A. LITERATURE REVIEW-PHYSIOLOGY AND PHARMACOLOGY OF THE REGULATION OF POSTURE AND MOVEMENT: An extensive review of the literature was made in the physiology and pharmacology of proprioceptors, and on the transmission of impulses from these receptors and the integration of sensory signals in the central nervous system. This review was submitted with the first interim report.

B. SYSTEMATIC STUDY OF THE EFFECTS OF FOUR COMPOUNDS IN VARIOUS TEST PROCEDURES: Compounds tested were the

1. In Cats: On Induced Rage and on Aggressiveness to Mice: Cats of each sex received and in later tests to induce a rage reaction. The ability of each of the four compounds to subdue this behavior was tested. Additionally, the behavior of the subject cats to mice was tested. The details were submitted in the first interim report. Results are summarized in a later section of this report.

2. On the Maze Performance of the Rat: Long-Evans strain hooded male rats were trained to run a Lashly III alley maze for a food reward. The procedure and maze were as described by Carlini, E. A., and Kramer, D., Effects of on Maze Performance

of the Rat,
The effect of each of the four compounds on the performance of a trained rat was determined by the time and the number of errors required to complete the maze.

3. On Shock-Induced Fighting Response (Mice): Fighting in mice was examined. Pairs of male mice selected because they were previously shown to fight were intravenously injected with graded doses of the test compound 30 minutes before testing. Five pairs of mice were used per dose level. The results are summarized below.

4. Spontaneous Activity (Mice): Spontaneous activity of mice was studied. The test compounds were administered intravenously at graded dose levels (three mice per level). The mice from each level were immediately placed in a
The activity (number of times a beam of light was interrupted) was recorded for 60 minutes after injection. The results are summarized below.

5. Sequential Response Performance (Rat): Male Long-Evans hooded rats were trained to a criterion of performance on a B-D schedule of reinforcement (water) in a Polidora Apparatus (J. exper. anal. Behav. 6, 271-277, 1963). Graded doses of test compounds were administered intravenously and the effects on rewards and total responses were recorded for an interval of 10 minutes immediately afterward and 2, 4, and 6 hours post-treatment. The results were reported in the first interim report.

COMPARISON OF COMPOUNDS IN BEHAVIORAL TESTS

DISCUSSION: This battery of behavioral tests was used to determine minimal (MED) and median (ED_{50}) effective doses of the test drugs and differences in patterns of response. From the results summarized above, it may be seen that yield similar effects, as do but that differences in dosage and spectrum of activity can be discerned between these pairs.

The most sensitive tests are those for Grouped Activity of Mice and the Polidora Sequential Behavior for rats. Both were effective in increasing activity of mice and in inhibiting responses of rats in these tests at a level of However, increased responses in the sequential response (Polidora) apparatus at and occasionally at lower levels. was much more potent in inhibition of mouse fighting behavior than was . Similarly more effectively inhibited learned goal-oriented behavior of rats in the Lashley III maze at lower dosage levels . Both effectively inhibited the induced rage response of cats , whereas even at the largest doses given, did not fully inhibit the "snarling" reaction.

Three compounds tested blocked cats' aggression to mice at different minimally effective dosage levels was not tested in this fashion).

were active in inhibiting rats tested in the Polidora apparatus and the Lashley III maze, but the effective doses were higher than those for the other two compounds.

With grouped mouse spontaneous activity showed similar biphasic responses of at similar dose levels. However, effectively inhibited

cats' aggression to a mouse at [redacted] whereas [redacted] was necessary in this test. In mice, induced fighting was effectively inhibited by a smaller dose of [redacted] than of [redacted]

[redacted] induced effects at much lower doses than did [redacted] could be distinguished from [redacted] on the basis of a stimulant effect in which low doses effectively increased responses in the Polidora apparatus. Other tests were necessary to distinguish [redacted]

C. ELECTROENCEPHALOGRAPHIC RESPONSE TO AROUSAL (RATS AND RABBITS): A technique for monitoring EEG response to stimuli (sound or light) was investigated in rabbits and rats. The use of the rat with this procedure appeared to be of possible value for incorporation into a primary screen. Samples of recording were included in the first interim report.

PHASE 2

On the basis of the preliminary work described in the preceding section, a series of screening procedures were established for the study of candidate compounds.

The screening procedures included:

A. ACUTE TOXICITY AND PRELIMINARY SCREENING: Mice (2 per dosage level) received test compounds orally or intravenously at 1/2-log dosage intervals. The pharmacological signs which were sought were listed on a sample work sheet which was appended to the first and second interim reports. For this test, signs which were observed at each dose, the number of mice reacting, the

time of onset of these signs, the degree of their severity, and the time for recovery were recorded. The median lethal dose (LD_{50}) and the median effective dose (ED_{50}) were estimated. This information was tabulated by electronic data processing equipment, and was presented in tabular form. An explanatory sheet, which is a guide to the tables, was also appended in the second interim report.

B. LOCOMOTOR ACTIVITY: Mice (male, 3 per dose level) received graded doses of test compounds intravenously. The mice of each dosage group were then placed together in the | The activity (number of times a beam of light was interrupted) was recorded by digital accumulation and printing equipment at five minute intervals for 60 minutes after injection. The tabulated data (number of counts for each five-minute interval) were also presented graphically in the second interim report.

C. PHYSICAL AND NEUROLOGICAL EXAMINATION OF CATS: The purpose of this screen was to determine effects of test compounds on physical status, proprioceptive function, and other behavioral and neurological parameters. Cats (2 per dosage level) received test compounds orally or intravenously, and were examined periodically after treatment. Doses were determined usually by levels which yielded significant effects in the mouse toxicity screen. Because of limited quantities of test compounds, only a few animals could be used for each.

Observations included determination of heart rate, respiration rate, body temperature, pupillary size and reaction to light, and neurological signs and reflexes elicited as described by McGrath (Neurological Examination of the Dog,

Lea & Febiger, Phila., 2nd Ed. 1960), modified as appropriate for the cat. A list of the test reflexes and signs was attached to the second interim report. An interval of at least two weeks was allowed for recovery from drug effects before using any one cat again. The data were included in the first and second interim reports.

One monkey was also tested with one compound in a preliminary experiment and reported in the second interim report.

D. EFFECTS ON THE AUTONOMIC NERVOUS SYSTEM (DOGS): Mongrel dogs, anesthetized with [redacted] were used in a test system in which baseline recordings of the electrocardiogram, arterial blood pressure, respiratory movements, gastrointestinal activity, and surface electroencephalogram were obtained. The responses to [redacted] in each preparation were noted. Selected test compounds were injected into the femoral vein until an effect was noted. The responses to previously fixed doses of [redacted] after the administration of the test compound were noted. The data were submitted in the first interim report.

E. POLIDORA SEQUENTIAL RESPONSE APPARATUS: The technique was utilized as described in the preceding section. The data were submitted with the first and second interim reports.

TEST COMPOUNDS

Compounds tested were assigned code numbers in order of receipt as follows:

Code No.	Compound

PHASE III

SPECIAL STUDIES

A. The compound showed interesting pharmacological effects and was a subject of special study. The studies included prolonged investigation of its effect on locomotor activity of mice, on behavior of rats in the Sequential Response Behavior apparatus, and interaction with possible potentiators and inhibitors. The details were presented in the second interim report.

It was concluded that the behavioral effects of compound were mediated

B. DRUG EFFECTS ON MOTIVATION: To supplement the various testing procedures employed in this program, a method for the study of drug effects on motivation was tested.

c. To further screen candidate compounds,