

Behavioral Toxicology:† Do Toxicants Alter Behavior?

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The toxicology section of the Iowa Veterinary Diagnostic Laboratory was started in 1965. The Veterinary Toxicology group has a staff of 6 veterinarians and 4 chemists. Three of the veterinary toxicologists are Diplomats of the American Board of Veterinary Toxicology. The section is responsible for the teaching, research, and service functions of veterinary toxicology. Both undergraduate and graduate level training is offered. The Behavioral Toxicology Laboratory, started in 1967, is one area of the present research program in toxicology.

Behavior is a broad term used to describe the things that man and animals do to maintain life; for example, learning, remembering, detecting sensory stimuli, reacting, decision making, seeking food or shelter, and performing motor tasks. It is the role of the behavioral toxicologists to determine if exposure to toxicants results in changes in normal behavioral patterns or behavioral capabilities. Behavioral alterations can range any-

where from total disruption of a learned skill to more subtle changes such as altered perception or detection of stimuli, lapses in memory, slowed reaction times, and difficulty in learning new skills. It is changes of this nature that are of special interest to the behavioral toxicologist.⁵

One of the objectives of behavioral toxicologic research is to provide information which is applicable to man. Industrial and agricultural workers are exposed in their jobs to chemicals which may have an effect on the nervous system. Examples include insecticides such as DDT, dieldrin, parathion, and carbaryl; gases such as ozone, carbon monoxide, and carbon dioxide; and metals such as lead, mercury, thallium, and cadmium. The general population is exposed to therapeutic agents capable of altering behavior such as tranquilizers, sedatives, and stimulants. The present drug abuse problem offers a number of chemical agents that are of interest to behavioral toxicologists. A closely related area is behavioral pharmacology or psychopharmacology, which deals with psychotropic drugs.¹²

Based on reports of clinical observations and epidemiology investigations, there are reasons to suspect that some environmentally occurring toxicants are causing behavioral changes in selected populations. Significant pesticide exposure may interfere with the flying abilities of aerial applicators.² Recalling that a spray plane

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may be flying 10 feet off the ground at 80 mph, it is easy to understand why the pilot needs to be 100% functional. Of concern here are subtle changes in behavior that may not be easily detected by co-workers or by the pilot. Pesticide exposure of people employed as pesticide applicators has also been suggested as a causative factor in changes in sleep patterns and changes in attitude.

Another industrial poison suspected of causing behavioral changes is lead. It has been suggested by an industrial physician that workers with elevated lead exposure suffer both an increased number and more severe accidents.³

The role of the behavioral toxicologist is to determine whether these suggested changes occur as a result of exposure to the toxicant, and if so, what is the exposure threshold and how long the changes in behavior persist? One pending commercial application of a behavioral toxicologic technique is the electronic device that prevents a driver with less than normal mental functioning from starting his car if he can not correctly remember a sequence of 5 numbers.

Behavioral toxicology is an interdisciplinary effort crossing several historically academic boundaries. Areas represented in our group include toxicology, neurophysiology, biomedical engineering, analytical chemistry, computer science, and experimental psychology. In the behavioral toxicology laboratory, a number of behavioral tasks have been used in an attempt to find tests which are sensitive for detecting drug induced behavioral changes. The experimental animals used have been the domestic sheep and the squirrel monkey (*Saimiri Sciureus*).

BEHAVIORAL TESTS USED WITH SHEEP

Conditioned Avoidance Task

In the conditioned avoidance task, the sheep were trained to make a jumping response following the turning on of a light (stimulus) to avoid an electric shock. Each animal was placed in a 4 feet by 8 feet rectangular chamber which was 7 feet

high. The chamber was divided into two equal areas by a 12 inch high center barrier. A cable supported by an overhead trolley was connected to skin electrodes and was used to deliver an electric shock. At the start of the testing session, each trial began with illumination of the side of the chamber in which the animal was standing. The animal was required to jump across the barrier within 5 seconds after the light was turned on to avoid the electric shock. As soon as the animal jumped across the barrier the light was turned off. After a short intertrial interval, the light above the sheep was turned on again and the animal was required to jump to the other side of the chamber. Since sheep normally go toward light, they were trained to jump from the light side to the dark side of the test chamber. After the animals were trained to criterion, the electric shock generator was deactivated. The sheep were then exposed to dieldrin, a chlorinated hydrocarbon insecticide, and tested daily on the conditioned avoidance task. After a period of days, the animals learned that they no longer needed to respond to the onset of the light to avoid the non-existent electric shock. This phenomena of unlearning the old response or in essence learning a new response is called extinction. The exposed and control animals extinguished the response at the same rate; therefore, it was concluded that this task was insensitive to disruption by dieldrin exposure.⁹

Detour Maze Task

The detour maze task consisted of an alley leading to a small open field. The goal object, food, was placed in the open field. The animals were trained to walk down the alley and to find the goal object. Following initial training, barriers were placed in the open field which prevented direct access to the goal object. Barriers used were of 2 types. One barrier was a wire screen which allowed the sheep to view the goal object, and the second barrier was plywood which prevented the sheep from directly viewing the goal object. Animals exposed to dieldrin were as successful in learning to solve the maze

and reach the goal object as were control animals. It was concluded that this task was not sensitive to disruption by dieldrin exposure.⁶

Y-Maze Visual Discrimination Task

The Y-Maze visual discrimination task consisted of a two-choice visual discrimination between geometric symbols presented simultaneously. The sheep were allowed 5 seconds in which to walk down a short alley and press a pedal in front of the geometric symbol. If the animal selected the correct symbol, an electric shock was avoided. If an incorrect choice was made or if the animal took longer than 5 seconds to make a choice, then a mild electric shock was delivered and the trial was scored incorrect. The sheep were trained on two problems to a criterion of 70% correct responses for 3 consecutive days. As soon as criterion was reached on one problem, the animals were trained on the second problem. Beginning with training on the third problem, the animals were exposed to dieldrin. Following 30 days of training on the third problem, the animals were tested for recall of the first problem. The control animals required 67% fewer trials to relearn while the exposed animals required 61% more trials to relearn the previously learned problem. From this it was concluded that dieldrin could disrupt behavior, but it could not be determined whether dieldrin was interfering with learning, memory, or visual processes.¹⁰ A more recent experiment also using a visual discrimination task has produced data which indicates that dieldrin interferes with the ability of sheep to learn a visual discrimination.

Auditory Detection Task or Vigilance Task

Exposure to dieldrin in both man and animals of various species results in high voltage, slow waves in surface electroencephalograms.^{10,11} Similar changes in brain electrical activity have been associated in man with decreased ability to maintain attention and to maintain a high level of performance on a continuous per-

formance task. The auditory detection task used in sheep consisted of placing the animal in an 8 foot square chamber equipped with a food delivery device and a response pedal.⁷ A white noise source was used to mask background noise and to provide a continuous level of auditory stimulation. A brief (0.1 second) tone was presented randomly to the subject with the tone superimposed upon the white noise. The animal had 5 seconds in which to press the pedal in order to receive food reinforcement. If the animals failed to respond or waited longer than 5 seconds, no food was delivered. Sheep exposed to dieldrin or elemental lead performed poorly on this task. In the case of dieldrin, the severity of behavioral decrement was linearly related with log dosage. Dose-response experiments have not been done with elemental lead as the neurotoxicant. In work completed to date the auditory detection task has been the most sensitive indicator of dieldrin induced behavioral changes.^{8,9,10}

BEHAVIORAL WORK WITH SQUIRREL MONKEYS

Behavioral work with squirrel monkeys was initiated in order to study the effect of neurotoxicants in an animal phylogenetically closer to man. The squirrel monkey was selected over other primates because of their small size, availability, ease of maintenance, and relatively minor disease problems.⁴ A picture of one of our squirrel monkeys was recently published in this journal.¹ In retrospect, the squirrel monkey has been a temperamental animal easily disturbed by changes in environment, procedures, and handling. The squirrel monkey has also proven to be a fastidious animal. Considerable time was spent in selecting a suitable food that the animals would consistently work to obtain. Initially pellets of sucrose, monkey chow, dried milk, banana flavor, or fructose were tried. Frequently, the animals would eat the pellets for a few days and then refuse to work for pellets or would perform erratically. After additional experimentation it was found that diluted sweetened condensed milk was uniformly rewarding to

squirrel monkeys. An interesting problem developed with the milk reinforcement. Several animals refused to eat the sweetened condensed milk part way into the daily session. Consultation with dairy food specialists revealed that brass produces an off-flavor in milk that was apparently detected by some of the monkeys. Replacing brass fittings in the milk feeding system with stainless steel solved the problem.

Each animal is maintained in an individual cage in the colony room. Daily each animal is transferred from its home cage to an identical test cage. The test cage is enclosed by a soundproofed environmental chamber so that the animal is not distracted by outside auditory stimuli (Figure 1). A closed circuit television camera, mounted inside the chamber, is used to monitor the animal. Geometric patterns are presented simultaneously on two small screens. A transparent press plate is mounted over each of the screens. The monkey makes its choice by pressing one of the plates following presentation of the stimuli. If the monkey makes a correct response, then a few drops of sweetened condensed milk are delivered in a miniature cup. If an incorrect response is made, no food reinforcement is given and the animal must wait an additional few seconds for the next trial. Each animal is tested 30 minutes/day during which a maximum of 500 trials can be obtained. When the animal makes 15 consecutive correct responses without making an incorrect response, the sense of the task is reversed. That is, the previously correct geometric symbol becomes incorrect, and the previously incorrect symbol becomes correct. It is the task of the animal to develop a win-stay, lose-shift strategy. That is, if the animal receives reinforcement then the optimum strategy is to respond to that symbol (win-stay). If the response is not rewarded, then the strategy is to shift responding to the other symbol (lose-shift). The right-left position of the correct symbol is determined randomly in each trial. After several months of experience in this test situation, some of our squirrel monkeys have developed a win-stay, lose-shift strategy.

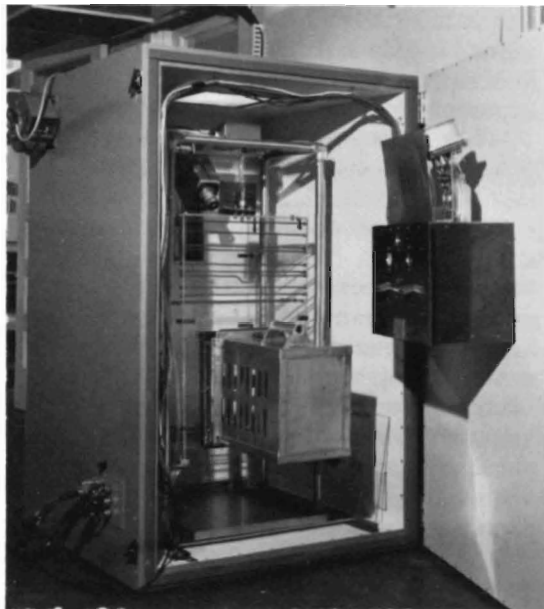


Figure 1. Test chamber used in Squirrel Monkey Behavioral Tests. The intelligence panel is the metal box mounted on the door. The intelligence panel houses the food delivery, stimuli presentation and response devices. The monkey is taken from the home cage to the test chamber in a transfer cage (perforated metal cage with handle) shown attached to the test chamber in the lower center. The test chamber has its own light source and ventilating fan. The closed circuit television camera is mounted above and behind the clear plastic animal cage. When the door is closed, the front of the intelligence panel approximates the front of the animal cage. A water font is mounted at the top of the animal cage.

On each trial the following data is recorded: stimuli used, position of correct symbol, sense of task (normal or reversed), correct or incorrect response, and response latency to the nearest 0.01 second. During one 30 minute experiment 3000 data points are generated by each animal. Because of the large amount of data collected and also to facilitate a detailed analysis of the data, a laboratory computer is used for data acquisition and analysis (Figure 2). At the end of each daily session a summary of the results for each animal is listed by the computer. The summary includes: total trials, correct trials on the left and the right, total correct trials, total percent correct, and percent correct on the

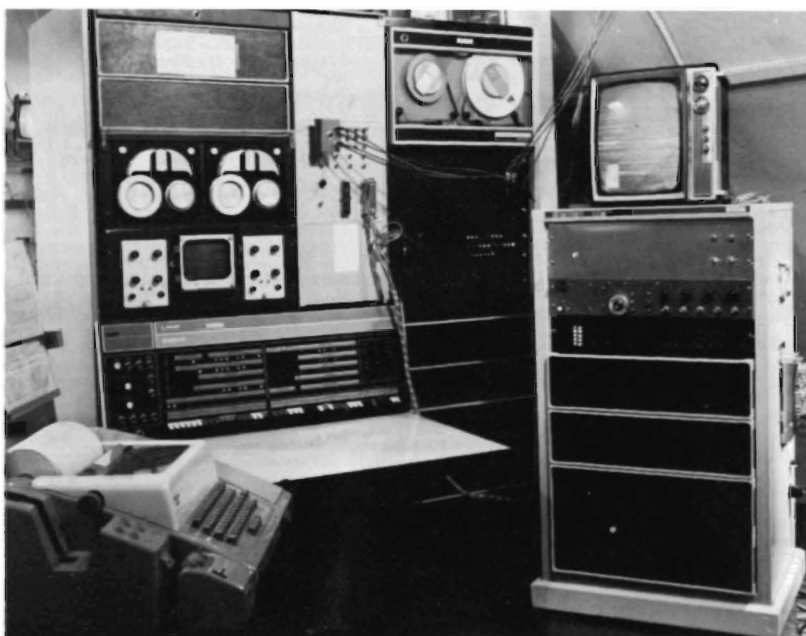


Figure 2. Laboratory computer (LINC-8) used for data acquisition and analysis. On the right is the solid state logic controller which has been interfaced to the computer. The closed circuit television monitor is shown on top of the controller. The computer has a 12 bit, 4K core memory and 32K disk, dual mini-tape and incremental tape bulk storage devices. Two real-time, buffered clocks and a general purpose data interface were designed by the laboratory staff for use in behavioral studies. The computer is also used by other investigators for data reduction and analyses.

right and left. This information is used to evaluate the overall progress of each animal. In addition, the trials for each reversal are analyzed and the following data printed: number of correct and incorrect trials and mean latency for correct and incorrect responses. This information is used to evaluate the progress of each animal toward a win-stay, lose-shift strategy. The data tape for each testing session is also transferred to an incremental magnetic tape in a format compatible with an IBM-360/65 data processing computer. When all the data is collected, it will be analyzed using available multifactor, statistical programs. Some of the animals in this experiment have been exposed to dieldrin, but collection of data has not been completed.

DISCUSSION

Behavioral toxicology is a relatively new area of research.¹² It will probably be a number of years before data is generated in enough laboratories to fully assess the usefulness of this method of evaluating the functional integrity of an organism in a complex situation. Based on our own findings and those of other laboratories, it appears that tasks which are more complex are also more easily disrupted by toxic chemicals. Just as a sulfobromophthalein clearance test is an indication of the functional integrity of the liver, it is possible that the determination of experimental behavior will prove to be a reliable indicator of the functional integrity of the central nervous system.

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Field Testing of Milking Machines by the Veterinarian

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The question of veterinary involvement in milking machine (M M) analysis is controversial. It is the opinion of some that M M matters should be left to the M M serviceman's judgement.³ The veterinarian should have an advisory role in M M management. With adequate knowledge and proper testing equipment a veterinarian can give an accurate and unbiased evaluation of M M function. The veterinarian should involve himself more in evaluating M M function and less in making recommendations on specific equipment designs.

The requirements¹ for analyzing M M systems are:

1. A knowledge of basic principles of the M M.
2. Testing equipment.
3. Evaluation forms.
4. Interpretation of data.

A fast, reliable and inexpensive analysis is necessary. The procedures explained

here are adequate for this purpose.

The objectives of these procedures are to determine the vacuum system's capacity, its activity at the teat end and to appraise the equipment.

Equipment needed for this analysis is a vacuum recording device, (preferably a dual channel recorder) and an air flow meter. A mercury manometer would be helpful to periodically check the accuracy of the vacuum gauges of the recorder and air flow meter.

Figure 1 shows a form which when completed would contain the necessary data for the analysis. If possible the M M serviceman should be present at the evaluation. Step I through IV of the analysis are completed prior to milking. Step V and VI are completed during the milking operation.

Step I. Vacuum Pump Performance

Determine the air flow at the vacuum pump by breaking into the system as close

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† The American Society of Mechanical Engineers Method measures volume of air at sea-level pressure (Free Air).

‡ The New Zealand Method measures volume of air at 15 inches Hg vacuum.