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Behavioral Assessment of Visual Toxicity

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A wide variety of behavioral methods has been employed with animals to assess visual changes induced by drugs or toxicants. The methods range from simple to complex, from broad screening devices to narrowly focused techniques. Their relative advantages for the environmental toxicologist are discussed. Manipulation of stimulus values is an essential ingredient in the identification of specific sensory functions. The percentage of correct choices from a discrete-trial, multiple-choice discrimination procedure is to be preferred to measures of response rate, speed or reaction time when experiments require answers about specific visual functions.

Introduction

Visual impairment is a consequence of exposure to any of a large variety of substances (*1, 2*). Given the obvious importance of visual function in health and most aspects of human activity, it is surprising to find such a meager literature on visual changes in experimental toxicology with animals. As elsewhere in toxicologic research, morphologic change has been the most frequently reported endpoint. The white rabbit is probably the most common preparation for assessing occular toxicity (*3*). Substances are presented directly to the eye and consequences are evaluated by examining tissues. The pupiliary reflexes represent another frequent toxicologic index, but a central nervous system poison such as methylmercury can cause profound visual impairment in the absence of observable changes in the eye (*4*).

This paper surveys behavioral methods for assessing visual function; it is for the toxicologist who asks "does this substance actually change the animal's ability to see?" Although visual science currently is a lively and sophisticated field, the toxicologist will find neither a benchmark test for vision nor any substantial body of research on visual toxicology. Therefore, this paper takes a wide-angle view of the various behavioral methods that have been employed in animal experiments and summarizes their advantages and disadvantages for the assessment of visual toxicity.

The techniques seem to fall into one of two categories. Some are complex and obviously designed for the precise and detailed determination of specific aspects of vision. Other methods are clearly useful for detecting a broad range of behavioral effects in addition to visual impairment and thus could be useful at the early stages of toxicity testing when one is compelled to screen for any of a wide variety of toxic effects. This latter group will be discussed first.

Mazes

Mazes and shuttle-boxes often require animals to attend to a visual discriminative stimulus. Toxicants such as lead have reported to alter such performances by rodents (*5–7*). These procedures require little investment in time or equipment. Their advantages for screening purposes become disadvantages if one desires to determine whether vision, rather than learning and memory, motor coordination, motor activity level, etc. are responsible for the change in performance. All of the above behavioral factors have been described as influencing performance of mazes and shuttle-boxes (*8*). Thus, altered performance in these situations provides weak evidence of visual changes. Another disadvantage, at least of simple mazes, is that they are not very sensitive to central nervous system insult (*9*).

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Operant Reinforcement Schedules

A quite different approach, employing operant reinforcement schedules in assessing learned behavior (*10*), has examined drug effects upon discriminative behavior. The multiple fixed-interval/fixed-ratio schedule is the most frequently used (*11*). The rate and pattern of responding upon a single response device are correlated with changes in visual discriminative stimuli. Discriminative stimuli influence the control performance and also modulate the drug response (*12, 13*).

An example is provided by a study of drug-induced changes in color vision (*14, 15*). Before drug exposure, pigeons had been trained to obtain food by pecking on a lighted disk. Food could be obtained according to a variable-ratio schedule whenever the disk was illuminated by either of one or two different wavelengths, but not when several other wavelengths were present. Before exposure, pigeons responded at a consistently high rate in the presence of the two reinforced wavelengths but responded very little in the presence of the other wavelengths. During drug exposure the animals responded uniformly at all wavelengths or shifted their preference to nonreinforced wavelengths. As with the maze studies described above, visual changes are but one of several possible explanations of the results. These results might also reflect nonvisual effects such as drug-induced amnesia or the rate-dependent manner in which many agents decrease high baseline response rates while at the same time causing an increase in low rates (*16, 17*).

A key advantage of operant behavior with previously trained animals is that each animal can serve as its own control, thereby reducing the between-animal variability and providing results from a small number of animals. But the disadvantage, aside from requiring more time and instrumentation than mazes, is the limitation in inferring sensory changes from response rates. Response rate often has been found unrelated to stimulus discrimination (*18–20*).

More specific evidence of visual impairment can be found in accuracy of choice (see below). For example, an operant schedule can be combined with a discriminative procedure to provide evidence on both sensory and motor changes. In one such instance, reinforcement was contingent upon a forced-choice color discrimination following completion of a conventional fixed-interval schedule (*21*). If one also wished to determine whether the visual or motor performance were preferentially affected, more elaborate experiments would be required in order to equate the response cost and other aspects of the "difficulty" of the two performances. The concept of task difficulty as a determinant of the effects of drugs and toxicants was recently reviewed by Evans and Weiss (*9*).

Signal Detection

The signal detection approach most commonly employs a simple behavioral task, with a stimulus either "on" or "off" and two responses equivalent to "yes I see it" or "no I don't see it." This approach was used to measure drug-induced changes in dark adaptation (*15*). Here, too, there is risk of the results being influenced by non-visual effects, particularly if the two responses have different topographies. For instance, if an active response indicated detection and the absence of a response were interpreted as indicating "I don't see it," then any toxicant that reduces the animal's willingness to respond might lead to the erroneous conclusion of a reduced visual sensitivity.

The formal theory of signal detection evolved to help separate non-sensory influences from estimates of sensory capability (*22*). A pure measure of sensory capability (d'), can be calculated with non-sensory factors lumped together in a second measure, "bias."

A more complex detection task is visual perimetry, the mapping of the visual fields. Although a useful clinical technique with humans, objective visual perimetry is very difficult with animals (*23*). Confrontation with small bits of food could easily demonstrate constriction of the visual field following chronic methylmercury exposure (*4*), but a more formal procedure could not reveal constriction or other impairment prior to the occurrence of overt intoxication (*24*). Applications of perimetry in experimental toxicology probably have been limited because the techniques were devised for use with humans, where one can give verbal instructions and expect cooperation from the experimental subject.

Temporal Discrimination

A flickering light will appear to be steady as the flicker frequency is increased and as the luminance of the light is decreased. Exposure to methylmercury increased the critical fusion intensity, i.e., the luminance required for a flickering light to be discriminated from a steady light (*25*). The frequency of flicker was held constant while luminance was varied. The complementary procedure is referred to as the critical flicker frequency test (CFF); luminance is held constant while frequency is manipulated until the flicker disappears. Lead-exposed workers did not differ from controls in a test of

two-flash fusion threshold, nor on two other, nonvisual, tests (*26*). Toxicants have not been studied with CFF, but the majority of psychoactive drugs tested in humans cause a decrease in the CFF (*27*). The CFF may not be the most sensitive index of visual impairment, since extensive ablations of the cerebral cortex have little effect on the test results (*28*).

Spatial Discrimination

We now consider techniques proven at the higher tiers of toxicity testing in the determination of specific visual mechanisms. These methods focus more narrowly upon vision, even at the expense of ignoring nonvisual effects. As would be expected, they all share the disadvantage of requiring more time and equipment than the simpler screening techniques, but they offer the least ambiguous results and are more likely to detect subtle effects. At this level, nearly all research employs nonhuman primates because their rich visual capabilities closely parallel ours. The pigeon offers an economical alternative species having well-documented visual capabilities. However, substantial differences between species are more frequently encountered in vision than in other types of behavioral research.

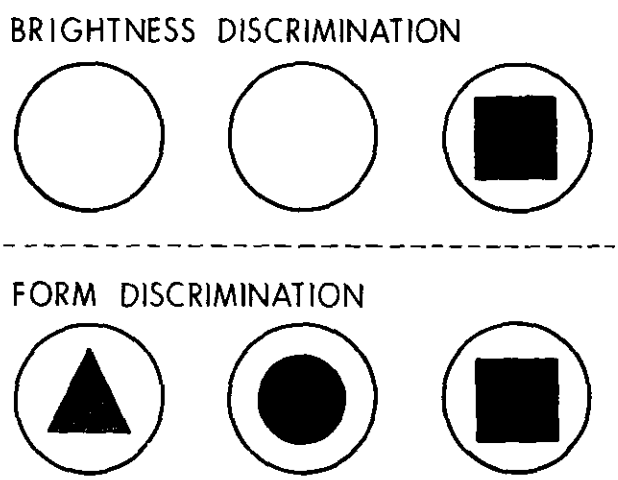

FIGURE 1. The three-choice visual discrimination tests. Prior to exposure, each monkey was trained to sit inside a light-and-sound proof enclosure. The monkey faced three pressure-sensitive disks which could be illuminated under computer control. The monkey could obtain a sip of fruit juice by pressing the disk upon which a square was illuminated. On half of the trials (brightness discrimination) only the square was illuminated, with the other two disks remaining dark. This task did not require the ability to discriminate forms, and thus would be less affected by impairment of acuity than by blindness or reduced sensitivity to light. The remaining half of the trials required a form discrimination. A two-dimensional form, a circle, triangle, or square, was projected on each of the three disks, with the position randomized from trial to trial. Once again the monkey was required to select the square, but now the task required the discrimination of three different shapes of equal brightness. Selection of the circle or triangle, or a failure to respond within ten seconds, terminated the trial without juice and postponed the start of the next trial.

Probably the best method of evaluating visual capabilities is the discrete-trial, forced-choice discrimination. The animal is confronted with two or more stimuli stimultaneously, each differing in some visual dimension such as color, brightness, shape, etc. This technique's advantage is the elimination of a default response; every choice is indicated by a simple positive response such as touching a pressure-sensitive disk upon which is displayed the selected stimulus. The motor response of pointing to the precise location of the stimulus is less ambiguous, and easier to verify, than is a verbal response such as "yes I see it."

Figure 1 illustrates the visual form and brightness discrimination used to study chronic methylmercury intoxication (*4*). By confronting the animal with three choices instead of the customary two choices, the accuracy of discrimination at chance levels ("guessing") is reduced to 33% correct instead of 50% correct with two choices. The expanded range between perfect (100%) and chance should increase the likelihood of detecting a small change in accuracy of discrimination. Greater numbers of stimuli and responses are practical now that laboratory minicomputers are widely available.

Preliminary results suggest that the earliest indices of chronic methylmercury intoxication can be revealed by manipulation of two characteristics of the visual stimuli: luminance and complexity (*4*). The curve in the upper portion of Figure 2 portrays the accuracy of form discrimination throughout a 21-week exposure to methylmercury. With stimuli of high luminance ("bright") accuracy is consistent at 100% through the first 20 weeks of exposure. With low luminance ("dim"), in comparison, accuracy began to decline after 10 weeks, at a time when high luminance discrimination was unaffected. The decline in accuracy with the dim stimuli was the earliest sign. Overt neurological signs appeared after the 20th week at about the same time as the marked decline in accuracy with bright stimuli. These signs are compatible with the distribution of Hg and of pathologic changes in the brain (*30, 31*).

Psychophysical techniques, which involve the manipulation of stimulus parameters, help identify the specific visual aspects of the results. The book edited by Stebbins, *Animal Psychophysics*, is required reading (*32*). In the present example, manipulation of luminance (Fig. 2) and of task complexity (Fig. 1) (*33*) provide the requisite evidence. Thus, this animal model provides a simple, yet

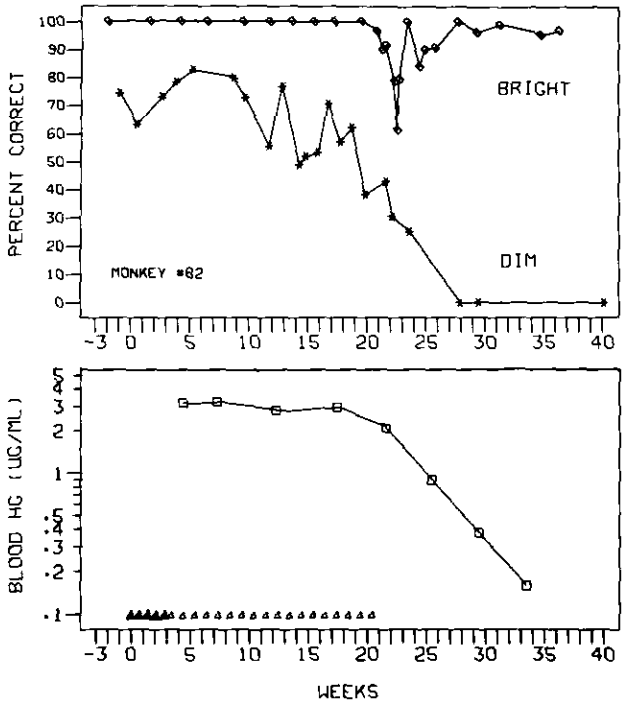

FIGURE 2. Plots of (top) accuracy of form discrimination and (bottom) whole blood Hg concentration of macaque No. 82 during exposure to methylmercuric chloride. Two luminance values (bright and dim) were selected for testing on the basis of normative results. Tests were conducted in the dark, following a 10-min adaptation. In the lower graph, ▲ = 1.0 mg Hg/kg priming doses at 5-day intervals; △ = weekly maintenance doses of 0.5 mg Hg/kg. Dosing stopped when overt signs appeared. From Evans (*29*).

quantal, index of specific visual deficits.

Other types of stimuli, resembling the Landolt rings used in acuity testing, have been employed to document visual impairment following irradiation (*34*) or ingestion of lead (*35*). The latter experiment employed a common procedure for forced-choice discriminations with monkeys, the WGTA (Wisconsin General Test Apparatus) (*36*). The main advantage of this specific technique is the large literature concerning its use with discriminative behavior. Because the technique is only partly automated, it is time-consuming and may be more vulnerable to errors and variability. The precise manipulation of stimuli, required for psychophysical studies, is more difficult with the WGTA. Since performance on the WGTA, like maze performance, usually requires a more complex sequence of motor responses than is required by the operant discrimination procedures, performance of WGTA or of mazes is more likely to be disrupted by toxicants which cause motor impairment, and these motor changes might overshadow the visual impairment that is being investigated. An example of this was discussed by Evans et al. (*4*).

General Comments

Studies of animal psychophysics illustrate important principles for all behavioral toxicologists, whether or not they are studying sensory processes. The systematic manipulation of stimulus values or schedule values should be a part of every experiment, just as are dose manipulations. Preliminary experiments which examine a large number of the stimulus values are, indeed, time consuming, but are indispensable if we are to identify the important principles that will permit us to perform simplified experiments in the future.

The toxicologist will encounter a bewildering variety of units of measure in the literature of vision. Some units are peculiar to clinical work, some prevail in foreign laboratories and some are necessary to convey subtle distinctions. However, some appear to overlap needlessly. Vision is worse than most other fields in this respect. Chapters such as those by Kaufman (*37*) or by Boynton (*38*) provide definitions and tables comparing the various units.

It may be surprising to read a chapter about vision without encountering the word "threshold." The concept of threshold has been disappointing to me; I don't know whether the disappointment is peculiar to vision or to some of the methods that I have been using. My thinking about thresholds was conditioned by biological definitions of the lower limits of the capacity of the nervous system.

When the visual threshold is determined behaviorally, should the threshold be defined as any discrimination better than guessing (i.e., better than 33% correct in the example of Fig. 1)? Frequency of reinforcement is low at near-threshold situations. Because of this, performance with threshold stimuli is not very vigorous; it is highly variable and may cease because of inconsistent reinforcement. Suppose threshold is defined as the point at which accuracy of discrimination is substantial and consistent, say, 75% correct. Threshold is also an illusive concept in this situation. Even when animals are highly pretrained, one can detect a constant improvement over many thousands of trials, often spanning periods of months or years (*4, 28*). Thus, in some cases, practice makes perfect.

Finally, further discussion of these issues can be found in reviews of general principles in behavioral toxicology (*9, 39*).

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