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Psychophysical Assessment of Visual Dysfunction

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Selected psychophysical techniques for nonhuman animals are described. These include operant learning methods and a new reflexive technique that may prove especially efficient. Problems of particular interest for toxicological research include control of the physical stimulus, choice of species, separation of stimulus from attentional effects, response bias, and pre- and post-training efficiency.

Animal psychophysics offers a variety of sophisticated tools to the behavioral toxicologist. Available methods permit us to assess sensory functions in nonhuman subjects with remarkable precision; it follows that we can also measure toxin-induced effects on sensory systems. Recent reviews (*1, 2*) survey the techniques of animal psychophysics and consider their problems. In the present paper we shall consider only a few of those methods, selected for their applicability to toxicological research.

The association between stimuli and responses is the basis of most animal psychophysics. In most cases this association is established through training. By using the techniques of operant or classical conditioning, animals can be taught to discriminate among stimuli by associating different responses with different stimuli. The animal psychophysicist starts with an easy discrimination and then varies the stimulus along the desired dimension in order to find a "threshold" or point at which the discrimination breaks down. Figure 1 describes a simple example. Hungry pigeons were rewarded with food for pecking a disk illuminated with light of a particular wavelength. When illumination was provided by any other wavelength, pecks were not reinforced. The function in Figure 1 describes the pigeon's ability to discriminate among wavelengths in each of two spectral regions by showing the probability of incorrect responses to wavelengths near the correct value. The slope of the curve is an index of discriminability. These functions indicate that the birds discriminate better in the 600 nm region than in the 540 nm region of the spectrum. This example illustrates a relatively unsophisticated example of Skinner's operant conditioning methodology. As in most animal psychophysics, reward or reinforcement substitutes for the verbal instructions available in human experiments.

While operant learning methods are most widely used, classical and reflexive techniques may be more appropriate in some instances. Later in this paper we shall illustrate the special applicability of a reflexive technique, and subsequent papers will describe a variety of other psychophysical methods.

In choosing and applying the techniques of animal psychophysics, the particular sensory modality is only one factor. Other considerations include the clear establishment of the stimulus-response relationship and the separation of sensory and nonsensory effects. For toxicological research, efficiency is also an important consideration.

Establishment of the Psychophysical Relationship

A relationship basic to all psychophysics is known as the psychometric function. It describes the variation in a response measure with the variation of a a stimulus along a clearly-defined continuum. Figure 2 illustrates a psychometric function obtained

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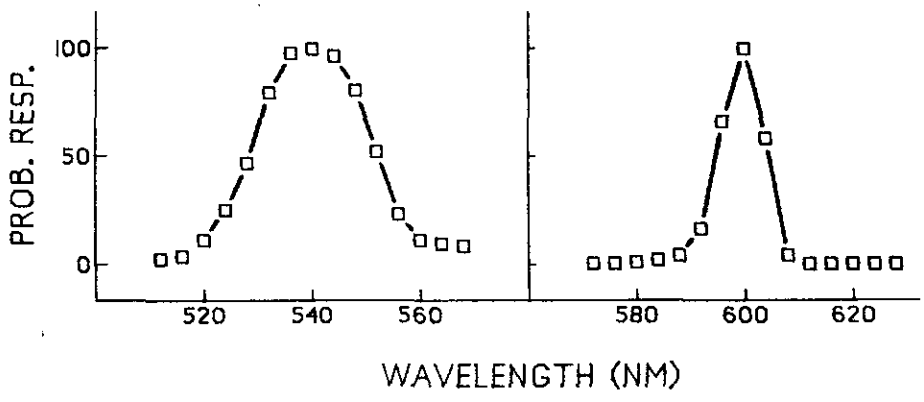

FIGURE 1. Probability of response to a continuum of wavelengths: (left) data from pigeons rewarded only for responding to 540 nm; (right) data from birds rewarded only for responding to 600 nm. Responses to other wavelengths result from the birds' inability to discriminate them from the rewarded value. Thus, the slopes of the two functions reflect wavelength discriminability for two regions of the spectrum.

for pigeons. The response measure was simply the probability of the key peck; the stimulus varied along the dimension of luminance. Reward was available only when the response key was illuminated. On half the trials the key was dark and no responses were reinforced. Thus, this function indicates the bird's ability to detect the presence of lights of varying luminance.

One reason for the establishment of a relationship like this is that drugs and toxins seem to have their clearest effects when external control of behavior is weak (*3*). For example, Evans (*4*) has shown that scopolamine interacts with stimulus discriminability in its effect on performance. To describe the early or subtle effects of toxins, it seems most useful to consider stimuli in the range where discrimination is poor; these would be values in the intermediate portion of the psychometric function. Establishment of the complete function is important, however, because a clear relationship indicates good stimulus control of the response. (Animals can be quite expert at detecting extraneous variables that the experimenter has failed to control.)

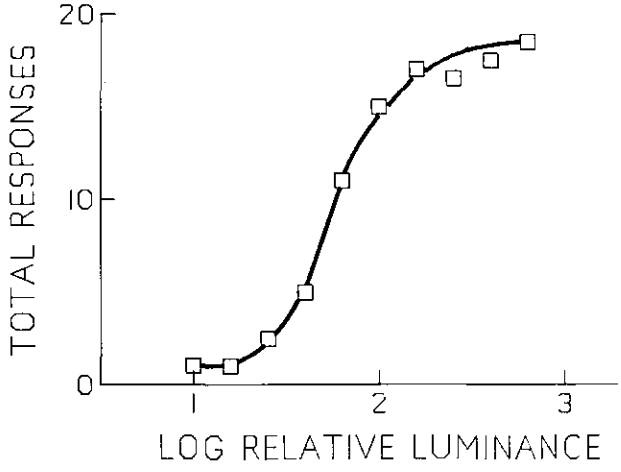

FIGURE 2. Number of responses to lights of varying luminance. There were 20 opportunities to respond to each of the 10 values. The pigeons were rewarded for responding to the lighted keys, but not to dark ones. Thus, failures to respond reflect the birds' inability to detect the light and the function provides an index of the absolute threshold.

Special Problems in Vision Research

The control of the stimulus side of the psychometric function requires special sophistication with regard to the sensory modality under investigation. For example, the stimuli described in Figure 2 varied in luminance alone, because neutral density filters were used to control the energy of the light. Had changes in electrical resistance been used to vary the output of the stimulus lamp, the stimulus continuum would have represented a mixture of wavelength and luminance changes. Analogously, the wavelength continuum shown earlier did not vary in luminance, because filters corrected the lights so that they would have equivalent luminances for the pigeon's eye. These are just two examples of the complex problem of controlling the visual stimulus; technical details are discussed by Boynton (*5*), among others.

A second consideration that specifically pertains to the study of vision concerns the duplexity of the visual system. Subjects working in well-lighted conditions probably use photopic or daylight vision, mediated by the retina's cones and good for tasks

requiring acuity or color vision. Subjects who are adapted to darkness use the scotopic visual system, mediated by rods and especially sensitive to dim light. There are other procedures for separating the two systems; for example, the photopic system is relatively more sensitive to long wavelengths and to lights flickering at high speeds. Isolation of photopic and scotopic systems can be helpful in understanding the nature of treatment effects. For example, recent research of Merigan (*6*) has elucidated the effects of methylmercury by varying both adaptation conditions and frequency of a temporally modulated light. His findings, suggesting that the photopic visual system is involved, help to clarify the constriction of the visual field seen after mercury poisoning.

Finally, in using nonhuman subjects for vision research, one must consider the special qualifications of each particular species. This problem is complex. Not only do different species appear to respond differently to toxic agents; there are also differences in certain features of their visual systems. Most mammals, for example, are more dependent on scotopic vision than are humans. The macaque monkey, an exception, has been an excellent subject for visual studies (*4*, *6*). A less expensive species is the pigeon, which has excellent photopic vision. However, we do not yet know how toxic agents affect its visual system and whether there is useful comparability between birds and humans in this respect. It is worth noting here that species differences, when understood, can often serve as tools to help clarify factors affecting vision.

Isolation of Sensory Effects

It is well known that toxic agents affect many aspects of behavior. The assessment of strictly sensory effects requires special precautions to avoid confounding behavioral deficits. Recent research of Hayes (*7*) illustrates this problem. These experiments attempted to separate the sensory effects of certain drugs from their effects on the subject's attention. A drug that leads to an attentional deficit should affect performance in all phases of a discrimination task, regardless of its difficulty. If the effect is strictly sensory, however, we would expect to see the deterioration limited to difficult discriminations.

Hayes' research used a more sophisticated application of the operant method than those seen in our earlier examples. Again, pigeons were trained to discriminate among wavelengths of light. In this case, however, there were two response keys available. The birds were rewarded for pecking the one on the left if the wavelength was shorter than 572 nm; for longer wavelengths they were rewarded for pecking the right-hand key. The functions in Figure 3 illustrate an attentional deficit induced by pentobarbital. As in the earlier examples, the slope of the curve is an index of sensory function; steeper slopes indicate better discriminability or smaller differential thresholds. In contrast, the asymptotes of these curves provide a measure of attention (*8*). If attention were perfect, the left asymptote would be at zero and the right, at 100% correct; that is, the pigeon would never peck the right key when the wavelength was very short, and it would always do so when the wavelength was very long. Hayes' findings indicate that pentobarbital affects both the slopes and the asymptotes of these psychometric functions. Thus, the drug effect on a sensory system was confounded by its effect on an attentional system.

A related problem concerns the separation of response bias from stimulus sensitivity. Suppose, for example, that a certain toxin led to an increased activity level that, for a pigeon, was reflected in an

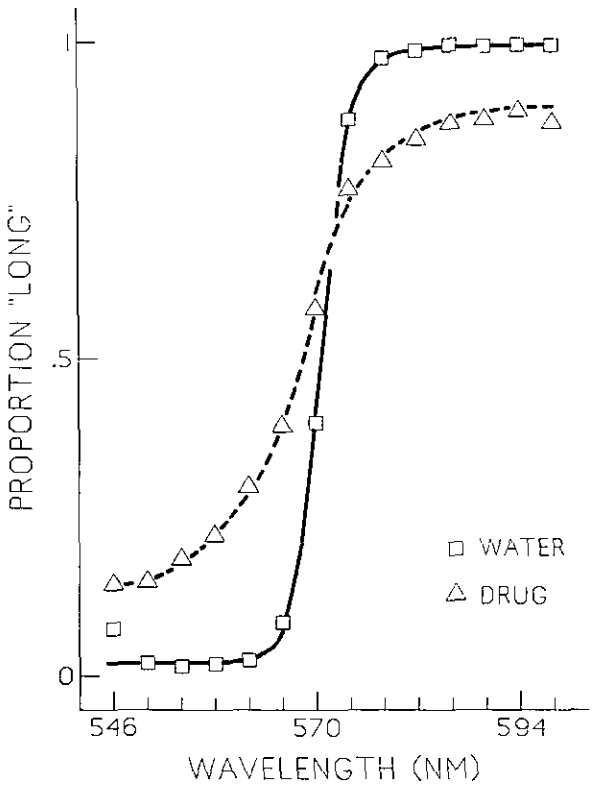

FIGURE 3. Psychometric functions obtained (□) before and (Δ) after pentobarbital treatment with pigeons. Two responses were available. The one shown was correct only when the stimulus wavelength was greater than 572 nm. The drug appeared to reduce attention since treatment led to more errors on easy as well as hard discriminations. Data are from the research of Hayes (*7*).

increased likelihood of the pecking response. Had the procedure been the "go/no-go" method illustrated in Figure 1, the effect would have been an increase in response probability to all wavelengths and an apparent, but misleading reduction in sensitivity. In fact, there are data (*9*) that do show a selective effect of a drug on bias. The theory of signal detection (*10*) provides us with techniques for coping with this problem. Blough and Blough (*1*) discuss their application to animal psychophysics. When the two-key method used by Hayes is employed, it is possible to evaluate effects on bias as well as those on attention.

Other data, mainly from behavioral pharmacology, should also lead us to be wary in our selection of psychophysical tools. For example, rate measures (number of responses over an extended trial) are prevalent in the operant discrimination literature. Yet a considerable body of data suggests that drugs have complicated effects on response rate (*11*). In some cases, a particular agent may raise an initially low rate, but lower one that is initially high. Thus, rate measures are unsuitable for the assessment of sensory effects. The experiments illustrated in Figures 1, 2 and 3 used trialwise procedures. Each trial began with the presentation of the stimulus and lasted only briefly. The resulting measure, the probability of a particular response during a brief trial, is a more reliable index than the traditional rate measure.

Improving Efficiency

Collection of data such as those illustrated above is notoriously time-consuming. For example, it took 11 weeks to gather the data shown in Figure 3. The necessity for lengthy training is one source of difficulty; animals typically improve over time, and stable pretreatment performance may not be reached for several weeks. A second problem concerns limitations on the amount of data that may be obtained in a single session. In typical operant studies, this limitation is imposed by the amount of the food or water reward that can be consumed without satiating the subject. For certain toxic agents it may be desirable to administer the toxin only once and to maximize the amount of data that can be collected immediately afterward. One way to improve the efficiency of single sessions is to reduce the probability or the amount of reward. When these values are low, satiation occurs less quickly, and a session can consist of more trials. We have found that pigeons will maintain good stimulus control when correct responses are reinforced with a probability as low as one out of 32 occurrences. Thus, the lengthy training required for operant psychophysical methods can pay off with quickly obtained, reliable post-treatment data.

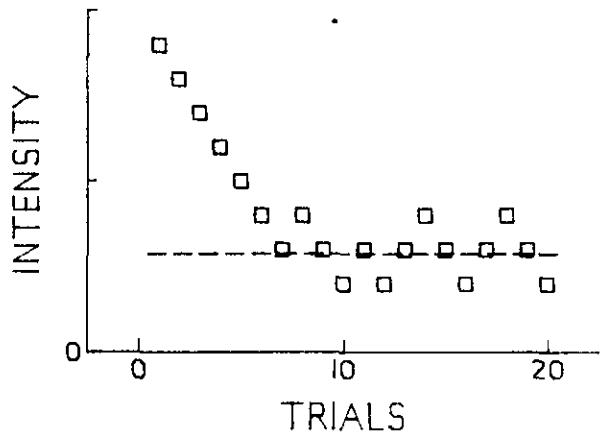

FIGURE 4. Schematic representation of a modified tracking procedure. The subject's behavior on each trial determines stimulus intensity for the following trial. A correct detection decreases intensity; when the subject fails to detect the stimulus, its intensity is increased. The dashed line indicates an intensity that might be taken as absolute threshold.

Another method of improving efficiency in the post-treatment stage is to maximize presentations of test stimuli in the region of the threshold. A useful set of methods, known as "tracking" (*12*), uses feedback from the subject to determine stimulus values. The stimulus initially diminishes in strength until the response measure indicates that the subject no longer detects it. Strength is then increased until a criterion for detection is reached; again strength is decreased and so forth. Figure 4 illustrates this procedure. Stebbins (*13*) has modified the tracking method and applied it in his extensive research in studies of ototoxicity. It has also proven effective in assessing the effects of neural deficit on visual function in cats (*14*).

An Alternative Psychophysical Approach

Although operant methods can be modified to provide efficient posttreatment data, the need for extensive pretraining can be a serious drawback. For example, when equipment and manpower are limited, operant methods are more appropriate for research requiring a small number of subjects. In toxicological research, where irreversible treatments are combined with a desire to describe a complete dose-response relationship, it is often desirable to use a large number of subjects and thus more efficient pretreatment procedures.

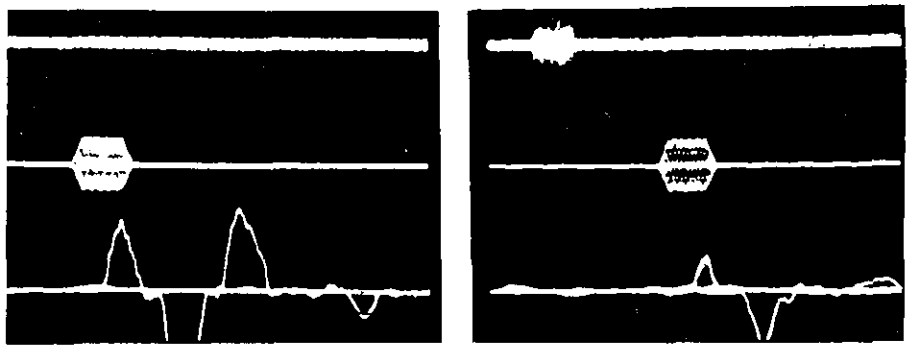

FIGURE 5. Oscillographic records of startle trials (left) without and (right) with a noise burst stimulus preceding a startle tone; (upper trace) background noice and noise prestimulus; (middle trace) 10 kHz startle tone; (bottom trace) startle response.

Researchers at several institutions, including Johns Hopkins University, have been working on the development of a psychophysical procedure that promises to be simple and quick to implement and which may prove nearly as sensitive as procedures based on extended operant training. This method is based on the inhibition of the acoustically elicited startle reflex by a prior stimulus.

Several authors have described the phenomenon known as prestimulus inhibition (*15*). In essence, low intensity stimuli, when presented 20 to 200 msec before the startle-eliciting stimulus, reduce the amplitude of the startle response. Figure 5 illustrates the phenomenon. The prestimulus may be auditory (*16*, *17*), visual (*18–20*) or cutaneous (*21*, *22*). The prestimulus need not be the onset of a stimulus; termination of an ongoing stimulus (*23*) or changes in the frequency distribution of an ongoing noise (*24*) will also inhibit startle. Startle amplitude reduction appears to be maximal when the prestimulus and startle stimulus are separated by 40–80 msec; the particular maximum seems to vary slightly depending on the nature of the prestimulus Similar inhibitory effects of preceding stimuli are also seen with other reflexes, such as the airpuff- or tap-elicited eyeblink.

From the perspective of the animal psychophysicist, the reflex inhibition finding is of interest because the amount of inhibition varies with the strength of the prestimulus. Further, at least in audition, the intensity of the prestimulus just required for inhibition is quite close to the absolute threshold of detection (*25*, *26*). This observation suggests that the reflex inhibition technique could be useful as an animal psychophysical procedure. Since it is based on a reflex rather than a learned response, the training procedure could be eliminated and the pretreatment period considerably shortened. Thus, large numbers of subjects could be tested relatively quickly. Virtually all mammals seem to show the startle reflex and may therefore be used as subjects. While birds do not exhibit auditory startle, they have a visual startle response which is similarly modulated by prestimulus presentations. Indeed, the stimulus parameters effective for modulation of the two reflexes seem nearly identical (*27*).

Although this is a new method, a recent study by Russo (*22*) has applied it to sensory system toxicity. In his research, which examined auditory and cutaneous sensitivity, Russo employed pairs of startle trials. Each pair compared the elicited startle with and without a prestimulus. The criterion of inhibition was that, within each pair of trials, the trial with a prestimulus produced a smaller amplitude response than the trial without the prestimulus. Following each pair of trials, Russo manually adjusted stimulus intensity to track the threshold of startle inhibition. Thresholds for 1/3-octave noisebands and electrocutaneous stimuli were determined in this fashion and were similar to those obtained in operant discrimination studies. Further, the method detected temporary auditory threshold shifts induced by extended exposure to noise and shifts in electrocutaneous thresholds induced by Lidocaine injections.

In preliminary experiments at Johns Hopkins, we are working on a modification of Russo's procedure, which we hope to apply to assessment of visual function. In particular, we are developing a system which relies upon the graded nature of the startle response, rather than an all-or-none criterion of inhibition. This procedure yields psychometric functions that relate the amplitude of the startle response to the parameters of the prestimulus.

Figure 6 shows data from a single rat for a single 72-trial session based on this procedure. For this pilot work the prestimulus was white noise. Vary-

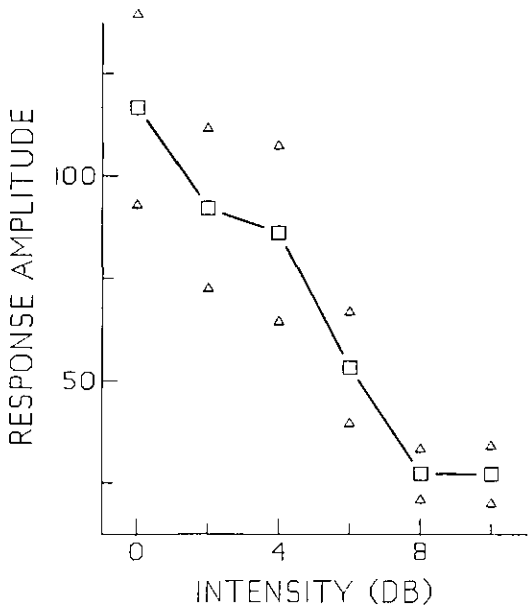

FIGURE 6. Sample data from a reflex-inhibition technique. Amplitude of an elicited startle response was measured as a function of the intensity of an auditory prestimulus. The inverse relation between response amplitude and stimulus intensity reflects the inhibitory effect of the stimulus. Each data point (square) is based on 12 trials during the subject's first exposure to the testing procedure. The small triangles show the standard error of the mean, which decreased by 50% on the following test day.

ing intensities were presented along with control trials in random order (method or constant stimuli). An advantage of the constant stimulus method is that several subjects may be run at the same time, with only a single stimulus-generating apparatus. We think these data are quite acceptable for such a brief run; sessions can be much longer, given appropriate intertrial intervals to preclude the habituation of startle.

Over the past several months, we have been developing an automated test apparatus which will enable us to test up to four subjects at once. We are beginning studies of normal subjects, using a variety of visual tasks as well as pure-tone audiometry and electrocutaneous sensitivity. These data will serve as baselines for a series of positive control tests with toxins known to affect these systems (aminoglycosine antibiotics, MeHg, CS_2).

The reflex inhibition procedure does have several limitations as a psychophysical technique. Its principal constraint is that it has a limited dynamic range, since stimuli of low intensity will produce large decrements of startle amplitude. Further, the method requires extreme care in the control of the testing environment. Extraneous stimuli, even in modalities other than that under study, can inhibit the reflex and make it impossible to partial out the effects of the stimulus under investigation. Lastly, the procedure is necessarily limited to detection studies, as any detected stimulus will alter the reflex. This limitation contrasts with the broader range of discrimination that may be studied with operant or classical conditioning techniques.

Despite its limitations, the reflex inhibition procedure promises to be a psychophysical technique of great utility for the toxicologist, who frequently wishes to test large numbers of subjects. At the present time, it may represent an optimal combination of speed and sensitivity.

Figures 1-4 and 6 were prepared by a computer graphing program (*28*).

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