Target Organ Toxicology Series

Nervous System Toxicology

Editor

Clifford L. Mitchell

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Preface

This volume deals with one of the most important yet least understood fields of modern toxicology. Interest in nervous system toxicology has been growing in recent years, largely the result of increased public concern over the impact of toxicants on human health. Of particular interest are implications of permanent damage to the nervous system when exposed during development to low concentrations of a variety of agents (e.g., alcohol, narcotics, and other drugs).

Unfortunately, real or potential risks to the nervous system are difficult to assess because of the complexity of the system. Some of the problems in assessment are associated with the wide variations in function that can occur, yet still lie within the classification of normal. Others are associated with the plasticity of the nervous system, and still others with our incomplete understanding of precisely what is being measured by certain tests. Clearly, no single test or approach will suffice to examine the functional capacity of the nervous system.

This volume deals, therefore, with an interdisciplinary approach to nervous system toxicology. The focus is on animal test procedures for the assessment of toxic effects. Conceptual and methodological problems are discussed in detail in an effort to show both their usefulness and limitations.

Several chapters in the volume focus on behavioral assessment of toxic effects. The first chapter provides a background on behavioral principles for the reader unfamiliar with behavioral research and its terminology. Other chapters deal with advantages and disadvantages of examining so-called naturally occurring behaviors versus conditioned behaviors, the assessment of toxic effects on specific sensory modalities, learning and memory, and approaches to screening for behavioral toxicity.

In addition, the volume covers reviews of neuropathological, electrophysiological, and neurochemical approaches to the detection of nervous system toxicity. The possible uses of tissue cultures are examined as well as the contribution of changes in vascular permeability to neurotoxic effects. Variables confounding the interpretation of data obtained using the various approaches are presented throughout the text.

This volume, then, brings together information previously available only from scattered sources. It will be useful to researchers in nervous system toxicology, teachers in toxicology and the neurosciences, and those scientists in government and industry who must make decisions about the future of a chemical on the basis of data generated using methods described in this book.

The Editor

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Behavioral Principles for Use in Behavioral Toxicology and Pharmacology

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Behavioral and neurological toxicology is an interdisciplinary area of research closely related to psychopharmacology or behavioral pharmacology. In either case, methods derived from experimental psychology, physiological psychology, neurology, and neuropharmacology are used to study the effects of chemical agents and other factors on behavior. For the traditional pharmacologist or toxicologist trained primarily in biochemistry, physiology, and/or pathology, behavioral experiments are often difficult to interpret because they are described in behavioral or psychological terms. The purpose of this chapter is to provide a basis for the understanding of behavioral principles and to give examples of how behavior can be affected by pharmacological and toxicological agents.

Before delving into the fundamentals of behavior, it should be pointed out that behavioral methods can be used in at least two different ways by behavioral pharmacologists and toxicologists. It is important to make this distinction since the behavior selected for analysis depends on the intent of the study. First, behavioral methodology can serve as a "tool" for the evaluation of chemical or environmental factors. In this case, a well-established procedure or battery of tests might be chosen for comparison of unknown agents to an agent with known effects or having a known mechanism of action. Similarities in effects or profiles of effects between a known and unknown agent might suggest similarities in mechanism of action or provide the basis for more mechanistic experiments. An extension of this "behavioral tool" approach, as it might apply to neurotoxicology, is the administration of pharmacological agents having known mechanisms of action to toxicant-exposed animals to discover subtle alterations in responsiveness. Shifts in the behavioral response to a pharmacological agent in toxicant-exposed animals might provide some insights as to how and where in the nervous system the toxicant is acting.

Another type of experimental strategy is the "pharmacological tool" strategy, in which chemical agents or environmental factors are used to study neural processes that underlie or mediate behavior. For example, if it is of interest to determine how certain areas of the brain might control a behavior such as spontaneous locomotor activity, this might be studied directly by selectively depleting catecholaminergic neurons with specific neurotoxicants, such as 6-hydroxydopamine (6-OHDA), and measuring changes in motor activity. In this case, the purpose of the experiment is to determine what controls motor activity rather than how 6-OHDA affects catecholaminergic neurotransmission.

Regardless of the general approach, the problems of experimental design and data interpretation are formidable. A systematic evaluation of these problems is well beyond the scope of this chapter, but the interested reader is directed to an excellent guide for research design by Sidman (42). Certain limited aspects of problems in experimental design will be dealt with in succeeding chapters.

BEHAVIOR DEFINED

Behavior can be conceptualized as the end-product of a variety of sensory, motor, and integrative processes occurring in the nervous system. As such, an alteration in behavior following exposure to chemical or physical factors might be a relatively sensitive indicator of toxicant-induced changes in nervous system function (5,53-55). Thus, it is of importance to have a clear idea of what behavior means.

Behavior can be defined as the movement of an organism or its parts within a temporal and spatial context. Behavior can be thought of as being comprised of units termed *responses*, which can be defined operationally as whatever covary with effective controlling variables. By definition, an aspect of the environment that controls behavior in a functional or lawful manner is a *stimulus*. Behaviorists focus on what is termed the functional analysis of behavior, i.e., the relationship between stimuli, behavior, and the consequences of this behavior in the environment. The behavior of an organism at any one moment is determined not only by the currently acting environment, but also by the organism's previous experience with these or similar environmental conditions. Typically, two types of responses are characterized, those that are *respondent*, or *elicited*, and those that are *operant*, or *emitted*. In addition, responses can also be *unconditioned* (unlearned) or *conditioned* (learned). Responses demonstrate certain physical characteristics, such as topography (form) and effect (i.e., rate, force, duration, latency), which are the dependent variables studied in behavioral experiments. A summary of some of the more salient features of the behavioral classification that follows can be found in Table 1.

Unconditioned (Unlearned) Behavior

Respondent Behavior

As a response class, respondents are characterized by the fact that they are elicited by a known environmental stimulus, usually one with specific temporal relationships to the occurrence of the response. The frequency of the occurrence of a respondent depends primarily on the frequency of the occurrence of its eliciting stimulus. This frequency is not usually affected by the consequences of the response, which are the environmental events that follow. There are at least four types of behaviors that constitute the respondent class. *Kinesis* is a response by an organism that is environment directed in a global sense. For example, paramecia in solution may spread

TABLE 1. *Classification of behavior*

A. Unconditioned (unlearned) behavior
  1. Respondent
    a. Elicited by known, observable stimulus
    b. Responses typically include those of smooth muscles, glandular secretions, autonomic responses, environment-elicited effector responses
    c. Data are measures of response magnitude, probability, latency, or related to intensity of eliciting stimulus
    d. Taxonomy of respondents include the following:
      (1) Kinesis, environment-directed, and movement is random
      (2) Taxis, stimulus-directed, and movement is specific response of whole organism
      (3) Reflex, object-directed, and movement involves specific effect or system
      (4) Species-specific, stimulus-specific, and movements are sequences of behaviors (fixed-action patterns)
  2. Operant
    a. Emitted, with no known, observable eliciting stimulus
    b. Responses typically include those mediated by CNS, such as skeletal muscular movements that operate on and change the environment
    c. Data are measures of response probability or frequency

B. Conditioned behavior
  1. Classically conditioned (respondent or type S learning): Response (CR) is elicited by a new stimulus (CS) as the result of close temporal pairing of that stimulus (CS) with another stimulus (US), which originally elicited the response (UR)

$$US \rightarrow UR$$
$$CS \rightarrow CR$$

  2. Instrumentally conditioned (operant or type R learning): Response (R) changes in frequency of occurrence as a function of the response consequence (S^R)

$$s \ldots R \rightarrow S^R$$

themselves outwardly in a random fashion in the presence of a carbon dioxide bubble. A second type of respondent behavior is *taxis*, a form of behavior in which the whole organism orients itself in a specific fashion toward or away from an identifiable environmental stimulus. A special example of this behavior is the trophistic movement of plants toward the sun. *Reflexes* are similar to taxes in that behavior is guided by a specific stimulus. However, in the case of a reflex, there is a specific effector system involved in the response. That is, there is no change in the orientation of the organism's whole body, as in the case of taxis. Examples of reflexes include withdrawal of limbs following painful stimuli, the eye blink reflex, and the constriction of the pupil to light. Finally, there are *species-specific behaviors*, or, as some prefer, instincts. These are actually a fixed sequence of several responses (fixed action patterns) that are released by a specific environmental sign, or releaser. It is generally recognized that before a releaser is totally effective in activating behavior, there must be some optimal internal state, such as appropriate blood levels of a hormonal substance. A frequently used example of species-specific behavior is courtship sequences in fowl.

Although most studies using respondent behavior employ a conditioned, or learned, response, there are numerous examples in the literature in which unconditioned, or unlearned, respondents are utilized. For example, a battery of observational tasks has been described by Marshall and his colleagues (20,21) in which a stimulus is presented to an animal and the presence or absence of a localization or orientation response is recorded. Effects of brain lesions on auditory, visual, somatosensory, and olfactory function have been studied using this technique. Simple orientation responses and sensorimotor reflexes are used extensively in the Soviet Union for evaluation of neurotoxicants (29) and in the United States to screen CNS active drugs (17).

As a class of behavior, unconditioned respondents may not be entirely suitable for many toxicological or pharmacological studies. Although these behaviors are highly reproducible and easily quantified, they may not be sensitive to the subtle effects produced by long-term exposure to relatively low amounts of neurotoxicants.

Operant Behavior

Operant, or emitted, responses are not elicited by a single, identifiable, temporally cued stimulus in the environment (Table 1). These responses occur within the context of many environmental stimuli, but there is no single eliciting stimulus, as in the case of respondent behavior. Operant behavior is most frequently discussed in the context of learned, or conditioned, responding. Horizontally directed motor activity and exploratory behavior are examples of unconditoned operant responses; the frequency of these responses may vary according to known variables, such as diurnal cycles, deprivation schedules, hormonal cycles, and experimental or environmental factors, but no single stimulus elicits them. Experimentally, motor activity may be studied by various means (36), such as in openfield and residental mazes, rotating wheels, jiggle cages, photocell cages, and cages placed on platforms surrounded by capacitance or magnetic fields. Activity measures are frequently used in behavioral toxicological studies, as discussed in another chapter in this book.

Behavior Controlled by Learning

Most psychopharmacological and behavioral toxicological studies utilize some type of behavior modified by learning. Behaviorists have identified two types of conditioning, *respondent, or classical,* and *operant, or instrumental*. The description of conditioning as being either operant or respondent might be confusing, in that behavior *per se* is also identified by similar terms. The issue becomes clearer if it is realized that operant and respondent conditioning are operationally defined procedures of behavioral modification, whereas operant and respondent responses are descriptions of two classes of behaviors, which are modifiable through conditioning. Obviously, respondent, or reflexive, behaviors can be conditioned using classical procedures. That reflexive or respondent behaviors can be modified through operant techniques has been demonstrated by Miller and his colleagues (26,27). This discovery has been viewed with considerable interest by experimental psychologists and is the principle underlying the currently popular "biofeedback" phenomena. Emitted responses theoretically cannot be classically conditioned, since, by definition, no discrete stimulus precedes them. As discussed later, stimuli preceding an emitted response, by being paired with reinforcement, can come to control responding.

Laws of Respondent Conditioning and Extinction

Respondent (classical or type S) conditioning refers to a set of operational procedures elucidated by Ivan Pavlov (30), the Russian physiologist. In this type of learning, the approximately simultaneous presentation of two stimuli, one of which belongs to a genetically determined stimulus-response relationship (i.e., a reflex) existing at the moment in some strength, can create an increase in the strength of another reflex composed of the response resembling the one in the original reflex and the other stimulus. An example of this *Law of Respondent Conditioning* is the leg flexion response. In this procedure, an animal is restrained so that only withdrawal movements of the limbs are permitted. If a brief electric shock is applied through electrodes to one of the limbs, a reflexive withdrawal response is elicited. In classical conditioning terms, the shock is an unconditioned stimulus (US) and the leg flexion is an unconditioned response (UR). If the onset of a light repeatedly precedes the onset of the US, it can become a conditioned stimulus (CS). That is, the light can eventually elicit a conditioned response (CR) in the absence of the US. The CS-CR reflex represents a new entity, and, although the CR (leg flexion) resembles the UR, they may be differentiated qualitatively and quantitatively. During acquisition, the strength of the CR increases, up to a point, as measured by increased probability of the CR, decreased response latency, and stimulus intensity required to elicit the response. If a CR is repeatedly elicited by the CS alone, the strength of the CR declines. This is called *extinction* and effects on response parameters opposite to those obtained during conditioning are observed. Figure 1 illustrates the processes of acquisition and extinction.

Operant Conditioning

Laws of Operant Conditioning and Extinction

Operant, instrumental, or Type R conditioning is an outgrowth of Pavlovian reflexology and the subsequent research of E. L. Thorndike (51,52). The major work of Thorndike (51,52) was based on experiments in which animals learned to manipulate their environment in order to obtain food or to avoid aversive stimulation. These observations resulted in the *Law of Effect*, which, if put into operational terms, becomes the *Law of Operant Conditioning*, espoused by Skinner (45,46). In essence, when the occurrence of an operant response is followed by the presentation of a reinforcing stimulus, the probability of recurrence of the response increases. A reinforcer, then, is a stimulus that increases the probability (frequency per unit time) of a response. In the classical conditioning paradigm, learning is essentially a result of stimulus substitution; e.g., repeated presentation of the CS with the US eventually results in the ability of the CS to elicit a CR in the absence

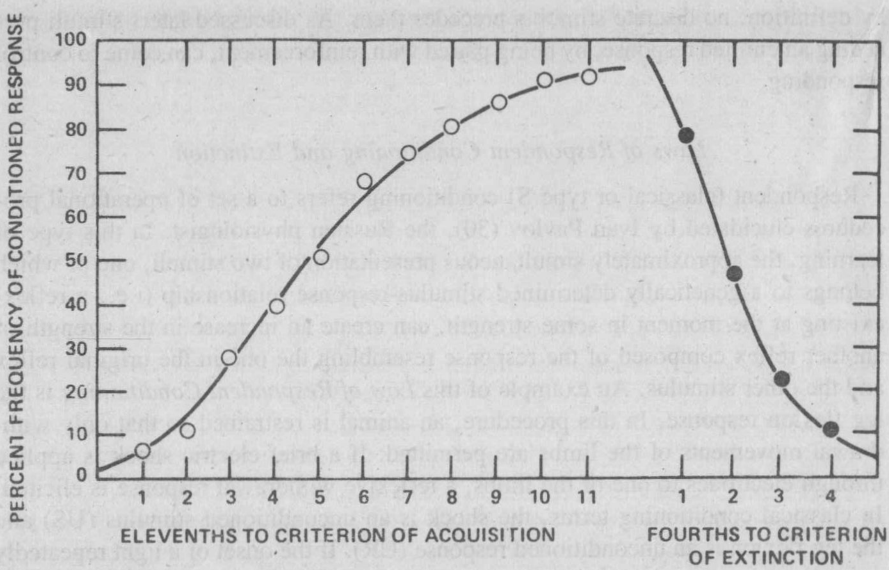

FIG. 1. Acquisition and extinction curves of an instrumental avoidance response in dogs. Data have been combined (Vincentized) to generate representative curves. (From Kimble, ref. 19, with permission.)

of the US. In operant conditioning, a response is paired with a stimulus that follows the response in close temporal order (reinforcing event) rather than a stimulus being paired with another stimulus that elicits a response. Although reference to stimuli preceding the operant behavior is not necessary, this does not mean that such stimuli are irrelevant. Situational cues correlated with reinforcement may come to be an occasion for reinforced responding, as described in a later section of this chapter.

One frequently used example of an operant response studied in the laboratory is the pigeon that has learned to peck at a key as a requirement for obtaining food reinforcement. The experiment takes place in a chamber in which there is a lighted key mounted on the wall (Fig. 2). When a food-deprived pigeon is placed in the chamber, the observant experimenter notices that the pigeon emits pecks spontaneously (unconditioned operant responses). If the pigeon hits the key properly, the experimenter arranges for the pigeon to have access to grain in a nearby hopper for a few seconds. Through a series of training steps called *shaping* and *successive approximations*, key pecking can be established as a highly probable response. If, during the course of the experiment, the reinforcement contingency is terminated, the eventual decline in key-pecking frequency is called extinction.

Concept of reinforcement

Reinforcement is the key concept in the conditioning of operant responses. By definition, a *reinforcer* is a stimulus that follows soon after a response and increases the probability of response recurrence. The presentation of a reinforcer is a description of an operation called *reinforcement*. Reinforcers may be classified as

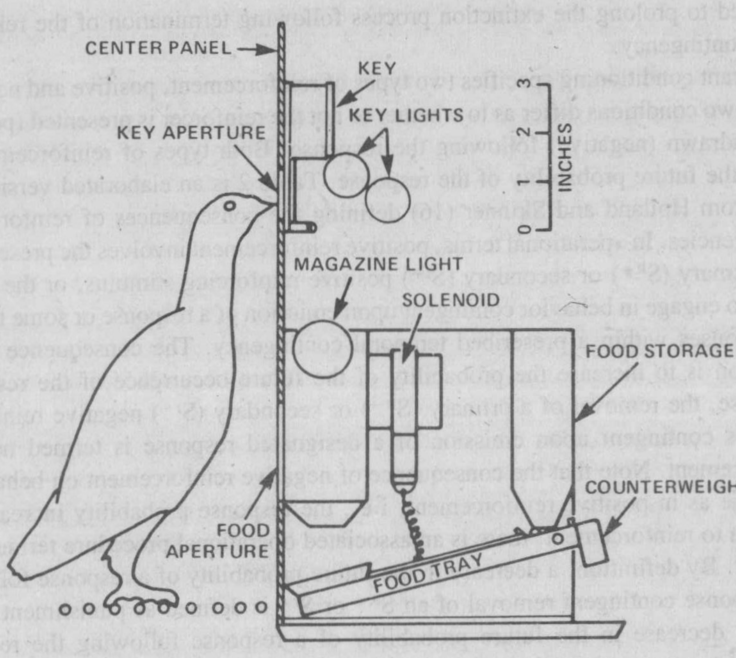

FIG. 2. Schematic of an operant chamber for pigeons. (Redrawn from Ferster and Skinner, ref. 12, with permission.)

being either primary (unconditioned) or secondary (conditioned), as well as either positive or negative.

Unconditioned, or primary, reinforcers increase the probability of behavior without prior conditioning history. They are unlearned, genetic, or innate, and often depend on some degree of momentary deprivation for their reinforcing value. For example, food-deprived organisms learn to operate on their environment to obtain food. However, once the organism is satiated, frequency of food-reinforced behavior declines until deprivation is reinstated. In addition to deprivation-related primary reinforcers, there are species-contrived reinforcers. For example, chimpanzees might learn a complex sequence of responses to obtain the opportunity to play with nuts and bolts or to watch a movie through a slot in their chamber. Rats that are minimally deprived of food might be trained to traverse a maze to obtain access to a novel exploratory area or to press a lever to receive small amounts of current in reinforcing sites of the brain.

Conditioned, or secondary, reinforcers are stimuli that are not originally reinforcing, but can become so by repeated temporal association with a stimulus that is reinforcing. An example of a secondary reinforcer is the presence of a light or sound stimulus that is repeatedly paired with food presentation. If the experimenter then omits the food reinforcement, it can be demonstrated that the light or sound can act as a reinforcer to maintain behavior. Thus, secondary reinforcers would be expected to prolong the extinction process following termination of the reinforcement contingency.

Operant conditioning specifies two types of reinforcement, positive and negative. These two conditions differ as to whether or not the reinforcer is presented (positive) or withdrawn (negative) following the response. Both types of reinforcement increase the future probability of the response. Table 2 is an elaborated version of a table from Holland and Skinner (16) defining the consequences of reinforcement contingencies. In operational terms, positive reinforcement involves the presentation of a primary (S^{R+}) or secondary (S^{r+}) positive reinforcing stimulus, or the opportunity to engage in behavior contingent upon emission of a response or some number of responses within a prescribed temporal contingency. The consequence of this operation is to increase the probability of the future occurrence of the response. Likewise, the removal of a primary (S^{R-}) or secondary (S^{r-}) negative reinforcing stimulus contingent upon emission of a designated response is termed negative reinforcement. Note that the consequence of negative reinforcement on behavior is the same as in positive reinforcement, i.e., the response probability increases. In addition to reinforcement, there is an associated operational procedure termed *punishment*. By definition, a decrease in the future probability of a response following the response contingent removal of an S^{R+} or S^{r+} is defined as punishment. Likewise, a decrease in the future probability of a response following the response contingent presentation of an S^{R-} or S^{r-} is defined as punishment.

The distinction between positive and negative reinforcers has often led to a tendency to accept *a priori* certain stimuli or events as being either one or the other. The definition of positive and negative reinforcers refer to operationally defined effects of a particular stimulus on a given behavior. Whereas certain stimuli, such as electric shock, may be negative reinforcers for some organisms under certain circumstances, it is possible to arrange the past history of an organism so that operant responding is maintained on schedules of electric shock presentation (23,28).

TABLE 2. *Effects of presentation or withdrawal of a positive or negative reinforcer on behavior*

Type of reinforcer	Operation: Presentation	Operation: Withdrawal
Positive	Positive Reinforcement: Presentation of reinforcer follows the response which increases in probability.	Punishment: Removal of reinforcer follows the response which decreases in probability.
Negative	Punishment: Presentation of reinforcer follows the response which decreases in probability.	Negative Reinforcement: Removal of reinforcer follows the response which increases in probability.

Behavioral Phenomena Studied by Experimental Psychologists

This section will describe behavioral phenomena other than conditioning and extinction that can be affected by toxicants or pharmacological agents. *Spontaneous recovery* is a temporary recovery of response strength that can follow an extinguished CR. The strength of spontaneous recovery depends on variables such as the past history of the organism, the number of times that the organism has experienced the extinction procedure, or, as illustrated in Fig. 3, the time since the end of the extinction series.

In classical conditioning procedures, the CS has empirical physical characteristics, such as the frequency of a tone or the wavelength of a light. Likewise, in the operant situation, stimuli correlated with reinforcement come to control operant behavior as discriminative stimuli (S^D), and, as stimuli, possess the same physical characteristics as the CS in the respondent paradigm. In either case, if one changes slightly the parameters of the stimulus entity, CR will occur because of *stimulus generalization*. The greater the similarity between the test stimulus condition and the one during conditioning, the greater the generalization. Thus, there is a *generalization gradient* in which there is decreased response strength as one deviates from the original conditioning situation (Fig. 4). Moreover, if a response is extinguished in the presence of a specific stimulus entity, a decrement in responding that generalizes from the specific stimulus parameter to adjacent points along a gradient (*generalization of extinction*) occurs. The experimenter can also restrict responding to specific stimulus situations by differential conditioning procedures. For example, in respondent conditioning, a flexion response can be conditioned to

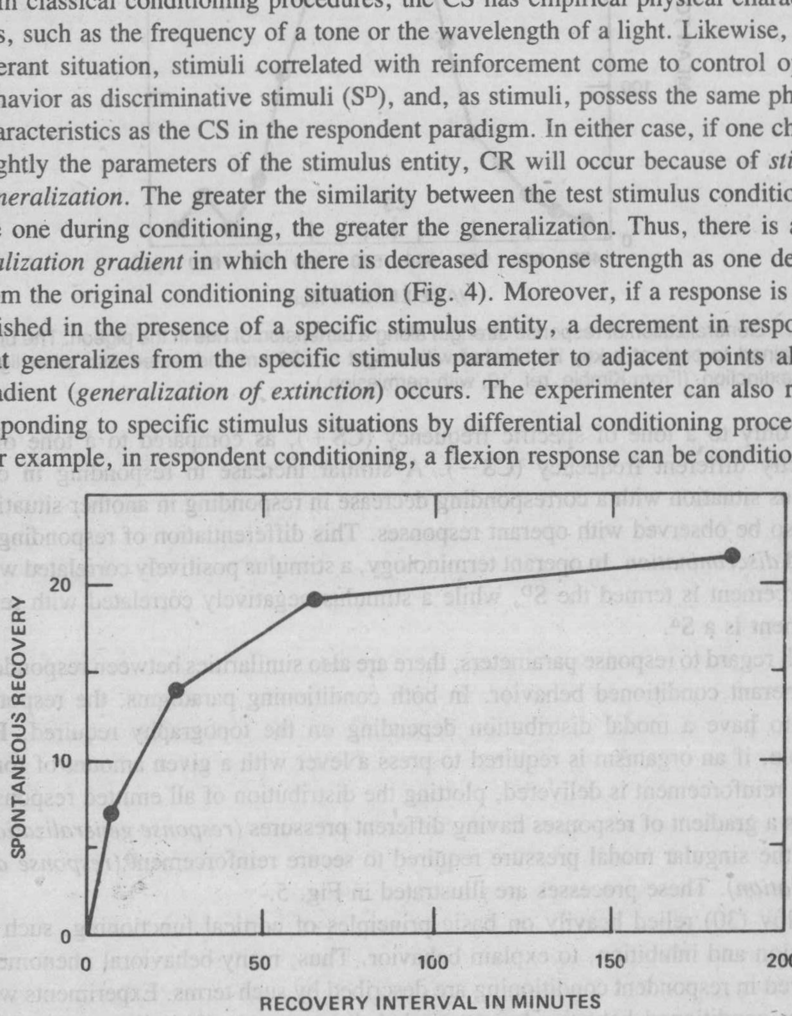

FIG. 3. Spontaneous recovery of the bar-pressing reaction as a function of time since the end of the extinction series. (Data redrawn from Kimble, ref. 19, with permission.)