Ethological and Experimental Approaches to Behavior Analysis: Implications for Ecotoxicology

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Laboratory research in toxicology has progressed far beyond reliance on measures of mortality to make use of sophisticated behavioral preparations that can evaluate the consequences of sublethal toxicant exposure. In contrast, field studies have not evolved as rapidly. Approaches developed by experimental psychologists and ethologists provide powerful and complementary methodologies to the study of environmental pollutants and behavior. Observational data collection techniques can easily be used to broaden the number of questions addressed regarding sublethal exposure to toxic agents in both field and laboratory environments. This paper provides a background in such techniques, including construction of ethograms and observational methodologies, and the use of laboratory analogues to naturally occurring activities such as social behavior, predation, and foraging. Combining ethological and experimental approaches in behavior analysis can result in a more comprehensive evaluation of the effects of environmental contaminants on behavior. — Environ Health Perspect 104(Suppl 2):299–305 (1996)

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Introduction

The study of animal behavior is an ancient vocation with its origin in the dim reaches of prehistory. Early members of the family *Hominidae* were hunter–gatherers. An accurate knowledge of the behavior of animals that shared their habitat was a vital necessity for both obtaining prey and avoiding predation. Cave paintings and other archaeological evidence demonstrate that people of the time actively engaged in natural observations of animal behavior.

According to Tinbergen (*1*), to truly comprehend behavior one must be able to answer four questions. These questions are of immediate causation, ontogeny, evolution, and function. Causation in this context refers to the internal and external stimuli, processes, and contingencies that precede the behavior of interest. Ontogeny refers to the development of behavior over the lifetime of an individual, which is mediated by complex interactions between genetic and environmental factors. Evolution refers to changes in behavioral processes across generations that may contribute to the process of speciation. Function refers to questions of adaptation, i.e., how behavior contributes to maintaining the relationship between an organism and its environment. Wilson (*2*) classified the first two questions as those of proximal causation. How do endogenous and exogenous variables interact to produce the behavior of interest at a given point in time? The latter two questions were classified as those of ultimate causation. Why do these behaviors occur and how do they contribute to the perpetuation of a species?

Questions of proximal and ultimate causation reflect the early approaches of two different schools in the study of animal behavior. American comparative psychologists addressed primarily questions of proximal causation through experimental manipulation under controlled laboratory conditions. They hoped to uncover universal principles of learning, such as the law of effect, which they believed would be applicable across species and conditions. European ethologists, through unobtrusive observation of animals in their natural habitats, were interested predominantly in describing the evolution and adaptive significance of behavior. These two groups disagreed sharply at times over the relative merits of their respective methodologies. In the past 25 years, however, a reconciliation between these two schools has emerged, leading to an integration of laboratory and field techniques, as well as the types of questions addressed (*3*). Skinner (*4*–*6*), for example, stressed the common operation of environmental contingencies in the evolution of species and the shaping of the behavior of organisms.

Ethological approaches typically use observational techniques to describe the occurrence of behavior in natural environmental settings. The types of behavior are generally species specific, but they ordinarily deal with basic activities related to survival, such as reproduction, parental behavior, defense, and food gathering (foraging). Ethological approaches also typically focus on the dynamics of group behavior. Several response classes that are often defined topographically may be measured. The data are generally in the form of incidences from which analyses of the probability of occurrence and the sequencing of different response classes can be made. In contrast, the experimental approach to behavior analysis is primarily a manipulative approach. Its focus is on the behavior of individual subjects and the conditions under which behavior is acquired and maintained. Studies are carried out in relatively restricted but well-controlled environments. A relatively small number of response classes is studied; these response classes are defined functionally by their common effects on the environment. Since the test environment is often automated, a continuous measurement of discrete responses is achieved from which analyses of the frequency and the temporal pattern of behavior can be made.

A simplified conceptual framework for relating the different approaches to behavior analysis is presented in Figure 1. Ethological

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	Field (Monitoring)	Laboratory (Modeling)
Animal		
Human		

Figure 1. Conceptual framework for relating field and laboratory investigations of behavior in animals and in humans. Field research is primarily descriptive in nature whereas laboratory research is primarily manipulative.

approaches monitor (describe the occurrence of) behavior in the field or natural environment, which is characteristically specified but uncontrolled. Epidemiological studies may be considered an analogous effort to monitor behavior and disease in humans in the field. On the other hand, experimental approaches to behavior analysis model basic features of behavioral processes (e.g., learning, sensation, motivation, and performance) under well-controlled, manipulable test environments in both laboratory animals and humans (i.e., clinical studies). Health research has to a large extent focused on the behavior of laboratory animals, before and after toxicant exposure, in the hope of predicting effects that may occur in the human population under similar conditions of exposure. In some instances, behavioral processes studied in animals can also be studied in humans under controlled laboratory conditions (*7*). While these types of studies can increase our confidence in the utility of animal models, assumptions must be made regarding the significance of any clinical findings for the population at large. An alternative strategy involves monitoring the behavior of animals in field studies to evaluate environmental quality. Such a strategy uses the behavior of animals as sentinels to warn of changing environmental conditions that may adversely affect human health. Burrell (*8*), for example, was the first to show the utility of canaries for detecting excessive levels of carbon monoxide in mines at levels below those that would adversely affect the miners.

It must be emphasized, however, that the distinction between field and laboratory research is not rigid and that a number of hybrid approaches are possible. For example, animals (either toxicant-exposed or not) can be retrieved from the field and studied in the laboratory. Peakall (*9*) has reviewed a number of studies of this sort. For example, a considerable amount of research on the mechanism of action of formamidine insecticides (*10,11*) has shown that stimulation of octopamine receptors is involved in light produced by fireflies (*12*) and the abnormal flight patterns of exposed moths (*13*). Likewise, a variety of observational techniques are widely used in laboratory research on the behavioral effects of drugs and environmental contaminants (*14,15*). On the other hand, natural environments can be more precisely structured to determine the effects of controlled contaminant exposures on wildlife. By the same token, laboratory tests derived from experimental analyses of behavior can be adapted for use in natural environments (*16*).

While it has been generally easy to identify differences in the approaches to behavior analysis taken by ethologists and experimental psychologists, under close scrutiny these differences may not be so great. Consider, for instance, the ultimate goals of behavior analysis of the two approaches. According to Silverman (*17*), an ethologist will "attempt...to recognize elements of the animal's own behavior and to identify situations where they occur reliably enough for experimental use." Similarly, the experimental approach to behavior analysis formulated by Skinner (*18*) has focused on the identification of functional units of behavior, the conditions under which they reliably occur, and the variables that modify their occurrence. Our basic position is that much has been learned about the determinants of behavior from these two approaches, that a fusion of the disciplines is warranted (*19*), and that one major beneficiary of such a fusion will be ecotoxicology.

The following sections provide details on current research efforts in ecotoxicology and on some laboratory analogs of real-world behavior.

Potential Causes of Population Decline

The most basic measure of toxicity in both field and laboratory research has traditionally been mortality. Laboratory research in toxicology has, however, progressed rapidly in its investigation of toxicant effects on subtle aspects of behavioral function that occur at exposure levels considerably below those that are life threatening (*20*). In contrast, field studies have generally not evolved as rapidly. Research into the causes of population decline provides a good example of the types of phenomena that can be addressed within ecotoxicology research. A decline in population (individuals of a single species) or in species richness (the number of different species in an ecosystem) may be due to any number of variables besides mortality. A population decline will, of course, result from widespread mortality if toxicant levels are high enough. Consider, however, how more subtle effects on various aspects of behavior could produce the same outcome (Table 1).

Parental behavior, for example, is a complex group of disparate activities (nest building, retrieval of young, defense from predators, feeding, etc.). Failure to perform optimally in any of these activities will result in decreased survivability of the young. Grue et al. (*21*), for example, found that starlings exposed to an organophosphate insecticide exhibited reduced parental attentiveness. Exposure to lithium (*22*) and lead (*23,24*) have also produced changes in parental behavior with resultant delays in offspring maturation.

Population decline can also result from changes in rates of predation. As predators will generally select potential prey based upon perceived vulnerability (*25*), sublethal exposure to toxicants can have a large impact on predator–prey interactions. Hedtke and Norris (*26*) found that low concentrations of ammonium chloride reduced the number of juvenile chinook salmon (*Oncorhynchus tshawytscha*) consumed by brook trout (*Salvelinus fontinalis*) while higher levels significantly increased the number consumed. These biphasic results were attributed to stimulant properties of the compound at low doses and to depressant properties at higher concentrations. Ionizing radiation produces greater susceptibility to predation in mosquitofish (*27*), as does sublethal mercury exposure (*28*). Galindo et al. (*29*) found that bobwhite quail (*Colinus virginianus*) treated with methyl parathion were more likely to be caught and killed by a domestic cat introduced into an observation field.

The complex social caste system of honey bees and other members of the order Hymenoptera provides a further example of the many avenues through which adverse

Table 1. Population decline may result from a number of factors in addition to overt mortality.

An adverse effect on	Results in population decline due to
Parental behavior	Fewer offspring reaching reproductive maturity
Reproductive behavior	Fewer matings occurring, fewer offspring born
Avoidance of predators	Increased mortality by predation
Predation	Increased mortality by starvation
Migration	All of the above

effects may be expressed and measured in natural populations. Because bees and other valuable pollinators often come in close proximity to pesticides, there has been a large amount of research on sublethal effects (*30,31*). Individual hive populations are divided into different age-based castes, each with a specific task (foraging, brood care, hive maintenance, etc.). This division of labor can be adversely affected by sublethal exposure to pesticides such as parathion (*32*). The phenomenon of bee dancing, in which the distance and direction to food sources communicated to other members of the hive, is disrupted by exposure to methyl parathion (*33*), and foraging for new food supplies is disrupted by exposure to permethrin (*34*). Changes such as these can drastically reduce a bee colony's chances of survival and produce a substantial economic impact for those that depend on bees as crop pollinators.

Methodology

Given the importance of understanding the sublethal effects of chemicals on natural populations, what are the best means for their assessment? The many advantages of controlled laboratory studies may be offset by uncertainties in the generalizability of results to complex field environments. Observational field studies raise equally difficult questions regarding the relative lack of control and inability to manipulate key variables, e.g., the amount of exposure between control and experimental groups. We address these issues by first describing the methodology used in field studies to define and quantify behavior and then by demonstrating the application of these observational methods in laboratory research to produce sensitive and informative hypothesis testing.

The methodology of natural observation can be as detailed as that of any laboratory endeavor. What follows is an overview of the major principles and the techniques commonly used. Thorough reviews of observational research methods have been prepared by Hinde (*35*) and Altmann (*36*).

The Ethogram

The ethogram is a means by which several behaviors of interest are categorized and operationally defined. Figure 2 provides an example of an ethogram in which various components of reproductive behavior in the golden orb-weaving spider, *Nephila clavipes*, are presented. Hinde (*35*) distinguished two ways in which behavior can be defined. A molecular description is based on the topography or the physical characteristics of behavior. For example, the male *N. clavipes* might rapidly drum its palps (modified appendages used as copulatory organs) on the ventral surface of a female before attempting to copulate (defined as palp pounding). Behavior can also be defined in terms of its consequences (*35*). For example, in Figure 2, any behavior by the female spider that results in a male's ceasing copulatory behavior and moving away is termed a fend. In the ethogram, codes for each behavior are noted in the order in which they occur, providing a serial record of behavior for a specific time period.

Reproductive behavior in the golden orb-weaving spider, *Nephila clavipes*.

Term	**Definition**
Cop	Pair *in copula*
PP	Palp pounding, male rapidly drums his palps (modified appendages used as copulatory devices) on epigynum of the female, 1 sec separating individual occurrences
Bout	Observed palpal insertions of at least 5 sec duration
BC	Hemaetodochal bulb contraction rate (per min)
FF	Female fends, any female behavior that either terminates a copulatory bout or causes a male to move off of or away from her ventrum
Alt Pl	Alternate plucking, male plucks on individual strand of web with alternating front legs
Sh	Shake, rapid shuddering behavior by male
B	Bounce, male rocks up and down in place on web
↓ or ↑	Male moves down to or up and away from female
Other	Other, discrete male behavior not defined

Time, min	**Behavior**	**Notes**
1:00		
2:00		
3:00		
4:00		
5:00		
6:00		
7:00		
8:00		
9:00		
10:00		

Figure 2. Sample ethogram and data sheet for recording a 10-min sequence of *Nephila* reproductive behavior. Codes for each behavior are noted in the order in which they occur, providing a serial record of behavior.

There are a number of factors that an ethogram should embrace.

- The behavioral categories included in an ethogram should be exhaustive. All relevant behavior during the allotted observation period should be accounted for, even if the experimenter's view of the animal is obstructed. This can usually be addressed by including a generic category of other.
- Behavioral categories should also be mutually exclusive, i.e., a subject is not recorded as doing more than one thing at a time. For example, an observation of a rhesus monkey eating a piece of fruit while moving around an enclosure would not be recorded as feeding and moving at the same time. In such situations, one behavior should be defined as taking precedence over the other. An alternative would be to have separate categories for combinations of behavior, e.g., feeding while moving versus feeding in place.
- Behavioral categories should be labeled objectively, thereby minimizing the need for judgments by the experimenter. One reason for this is that the same behavior may occur in a variety of contexts. For example, if you have ever observed two large dogs engaged in rough-and-tumble play, you probably saw behaviors such as growling, biting, lunging, and chasing. Variations of these same behaviors can occur during predatory attacks, territorial conflicts, and reproduction. Due to the nature of these types of interactions, a growl occurring in the context of play could quickly recur as an aggressive cue. It is better, therefore, to define discrete, objective terms such as growl, bite, or chase rather than more subjective labels such as displays fear or hunts prey. These discrete behaviors can be organized into more functional groupings during data analysis when the entire range of behaviors can be used to make better informed judgments as to the functional significance of the behavior.

Sampling Methods

As it is usually impractical to conduct natural observations on a 24-hr basis, a variety of techniques have been developed to obtain representative samples of behavior. Which technique is most appropriate depends upon the nature of the experimental question; the characteristics of the individual, group, or groups to be observed (such as number of individuals, baseline activity levels, etc.); and the time and resources available. For example, the techniques used to observe group dynamics in a large flock of starlings would certainly differ from those used in a pack of wolves. What follows are some of the most common observational methods (*36*).

Ad Libitum ***Sampling.*** *Ad libitum* sampling is used most frequently for recording unusual, infrequent events and requires the investigator to simply record his or her observations informally in a field notebook. Often, investigators making use of more formal, organized systems of data collection will include a place for comments and casual observations (*35*).

Serial Recording. Serial recording is collecting data on all behaviors in the order they occur during a given block of time. In the absence of detailed video or sound recording, a more practical approach is to select those behaviors specifically of interest and to record each occurrence. Because around-the-clock surveillance is usually not practical, serial recording is often used in combination with a time sampling schedule. The focus may be on an individual subject, in which case observation of behaviors initiated by or directed toward that individual are recorded at regular intervals, e.g., every 10 min or 1 hr. Alternative approaches make use of a sampling focus in other contexts without necessarily concentrating on an individual. These include recording all occurrences of some behaviors across individuals and recording all behavior at a specific location (e.g., a watering hole, lek, etc.). Serial recording permits calculation of rates of occurrence, sequences of behavior, and interactions between individuals. It is a more time-consuming approach than some of the alternatives, and the large amount of collected data often requires complex statistical analyses.

Time-based Sampling. Time-based sampling techniques are used to obtain a "snapshot" of behavior at the conclusion of a regularly spaced interval. Sampling intervals typically are on the order of a few minutes at most and depend upon factors such as group size and the frequency with which the behaviors of interest occur. Scan sampling (*36*) is typically used in situations where the experimenter is interested in monitoring a large group of individuals, e.g., determining the percentage of time in which specific behaviors occur or during which a percentage of individuals is in a specific location. Typically, the experimenter scans a field of view at a constant rate, recording what each individual is doing the instant it is observed. Consistency of observation is particularly important to minimize bias. Timed intervals must be rigidly followed, and the scan must always occur in the same manner, e.g., left to right, preferably observing each animal in the same order each time. One zero sampling (*36*) is a variation of scan sampling in which a behavioral category is assigned a 1 if the behavior occurs at least once during a predetermined interval and a 0 if it does not. The actual number of times the behavior occurs within the interval is irrelevant. Information on behavioral sequences, rates, and duration, etc., is lost using this method. Its primary advantages are ease of data collection and analysis.

Observational Methodologies in the Laboratory

While controlled manipulations in a field setting often require special considerations, the observational techniques outlined above can be easily adapted for laboratory use. Observational assessments (cage-side observations) have, of course, been long used in assessing the effects of a wide variety of environmental and pharmaceutical compounds (*37*), and refinements of these techniques have been incorporated into screening batteries for assessing the neurotoxic potential of compounds (*38*). There are many examples of individual and social behavior that can be studied in the laboratory as well as in field settings. Use of these techniques may produce two related benefits. First, one may identify changes that can serve as behavioral indicators of incipient toxicity in a field environment. Second, it may be easier to generalize effects of a compound on an observed naturally occurring activity, such as maternal behavior, to its equivalent field setting than an effect on behavior produced in a highly controlled artificial environment.

Social Behavior

Social interactions among traditional laboratory animals are often overlooked by behavioral toxicologists. Nonetheless, such data can often be informative and can be gathered quite easily in the laboratory by, for example, studying the effects of toxicants on parent–young interactions. Holloway and Thor (*39*), for example, looked at the effect of neonatal lead (Pb) exposure via lactation on play behavior in rat pups. The authors developed an ethogram containing three categories of play: *a*) social investigation, which was defined as sniffing, grooming, or following a newly introduced conspecific; *b*) crossover, which occurred when the subject completely transversed the dorsal or ventral surface of the conspecific; and *c*) pin, which occurred when one rat held another on its dorsal surface while standing above it. Other measurements of maternal behavior (pup retrieval) and pup open-field activity were included. Pb exposure failed to produce any measurable changes in general activity, pup growth rates, or maternal behavior. It did, however, produce significant increases in measures of the three play categories.

Behavioral abnormalities have been identified using similar observational techniques among female rodents and their young exposed to lithium (*22*), and lead (*23,24*), as well as in analogous field studies involving parental behavior in birds (*9*). Beck and Cooper (*40*) used a similar methodology to determine that a partial inverse benzodiazepine agonist, FG 7142, specifically reduced aggression in pair-housed rats. While measures of overall social interaction remained unchanged, aggressive behaviors decreased significantly. Compensatory social behaviors, such as approach and avoidance, increased as aggression decreased.

Often, the use of naturalistic observation methodologies in a laboratory setting requires that native habitats be simulated. Depending upon the complexity of the subject's environment, this can be achieved with some creativity. To examine the effects of ammonium chloride on predation by brook trout (*Salvelinus fontinalis*) toward juvenile chinook salmon (*Oncorhynchus tshawytscha*), Hedtke and Norris (*26*) constructed an artificial laboratory stream. The stream, complete with currents, stones to hide among, and two species of fish, served as a convenient arena in which to record the behavioral effects of the compound. Such a system could easily provide a rich data set by defining specific predatory and defensive behaviors in an ethogram and noting what effects treatment may have on more narrowly defined dimensions of behavior.

An alternative to attempting to reproduce a habitat within the confines of the laboratory is to duplicate the relevant stimuli produced by the particular habitat or conspecifics therein. Dutta et al. (*41*) devised a means to assess the optomotor response (important to maintain spacing within one's habitat and in fish schooling) by reproducing the stimuli that elicit the behavior. They placed bluegills (*Lepomis macrochirus*) in a 1-gal jar suspended within a bucket. Black electrical tape was placed within the bucket so that the fish were presented with a series of equally spaced diagonal bands. When placed upon a turntable, the bucket would rotate around the fish and cause the illusion of a continuously moving downward slope. Fish in such an apparatus will typically turn in the direction of the rotation, just as an individual fish will turn in the direction of conspecifics in its school. The data were collected using a serial-recording procedure in which 90° changes in position were termed quarter turns, movement with the rotation was termed following, and movement in the opposite direction was termed reversal. The organophosphate pesticide diazinon (30 µg/l) produced significant alterations in optomotor responding. These results may have important implications for assessing the effects of pesticide runoff on aquatic species as well as providing intriguing hypotheses regarding population changes in contaminated streams.

Laboratory Analog of Real-world Behavior

Many of the procedures used in traditional behavioral toxicology studies are laboratory analog of real-world events. For example, avoidance paradigms can be related to antipredatory behavior, and spatial learning tasks and operant schedules of reinforcement can be compared to foraging behaviors.

Predation

Galindo et al. (*29*) studied the effect of parathion on cat–quail interactions in an enclosed arena. Following dosing, quail were released into the arena and behavioral observations of motor activity (walking, running, flying) were recorded. After 5 min, a cat previously trained to prey on quail was released into the arena. Behavioral observations continued, and the latency to capture quail was recorded up to a maximum of 15 min. Quail in the higher dose groups were less active in the presence of the predator than were controls or the lower dose group(s) and were less successful in avoiding predation.

Spiders can provide a useful model for studies on the sublethal behavioral effects of toxicants (*42*) because both the orb-weaving and ground-dwelling species can usually be maintained in the laboratory with little difficulty. As obligate predators, they will accumulate toxicants to which their prey were exposed. Spider behavior, moreover, is easily quantifiable into discrete units, and the web produced by orb-weavers can provide a "snapshot" of the current pharmacological (or toxicological) state of the animal (*43*). Interestingly, one of the few papers examining the sublethal effects of pesticides on spider behavior used spiders as prey rather than as predators. Everts et al. (*44*) exposed spiders of the Linyphiidae and Erigonidae families to deltamethrin, a synthetic pyrethroid insecticide. Three behavioral tests included walking speed, avoidance of unfavorable environmental conditions (excessive dryness), and avoidance of predators (Caribid beetles). Spiders exposed to 0.6 ng deltamethrin were slower and less successful in avoiding both the arid environment and the predatory beetles.

Foraging and Bait Shyness

While observational techniques are the raison d'etre of ethology, field researchers have certainly been able to conduct careful, manipulative studies in purely field settings (*45*). However, as natural environments cannot be structured and manipulated to the extent that laboratory environments can, experimental analyses will be indispensable in identifying the variables that control behavior. Laboratory studies allow systematic manipulation of one variable at a time, often over a range of values, so that its influence on behavior can be firmly and unequivocally established. Such a detailed isolation and dissection of a corresponding variable is likely to be impossible in a natural environment.

The study of foraging and bait shyness may be a good example of the collaboration that is possible between ethologists and experimental analysts of behavior. Foraging for food is an activity common to all species. Foraging involves an extended series of response sequences including searching for and identifying food items and procuring and consuming the food. In addition, should an animal come into contact with a contaminated food source, toxic effects may interfere with avoidance of predators, further food gathering, and a host of other activities. Two questions then arise. First, what is the nature of foraging and the sublethal effects of contaminated food consumption? Second, to what extent can animals discriminate food that is potentially harmful? Both these questions have important implications for natural populations and can be addressed effectively in a laboratory setting.

Three variables that can influence foraging are the types of food available, its relative abundance, and its distribution. Traditional approaches to the experimental analysis of behavior have not been well-suited for laboratory studies on foraging: the amount of a standard food is fixed, and testing takes place in an open economy with supplementary food available in the home cage after the test session. Relatively recently, however, innovative laboratory approaches have been undertaken in the study of foraging (*46–48*). Extended sequences of foraging behavior are created in time rather than space, with response requirements manipulated to simulate the effortfulness of response and the probability of finding food. The types of food available have also been varied systematically (*49*). Many of these manipulations would be difficult to arrange in a natural environment. It should be noted, however, that while these developments offer promise, to date there has been no systematic laboratory investigation of the effects of environmental contaminants on foraging behavior.

Bait shyness refers to a feeding aversion, usually seen in rodents and avians, to a food source contaminated with a poison (*50*). Contaminated food sources may have a distinctive flavor that can be detected. Reduced consumption due to a unique flavor or taste of the food will reduce intake of the poison and lessen its toxic effectiveness. As a consequence, animals will avoid consumption of the food source in the future.

Feeding aversions appear to be a basic form of adaptation (learning) that characterizes most mammalian and many other species. Feeding aversions are ordinarily studied experimentally by pairing a distinctively flavored (and preferred) solution with a compound thought to have noxious properties for the organism. For example, rats are ordinarily first adapted to restricted water availability. Once intakes stabilize, a saccharin solution is substituted for water, after which rats receive a dose of lithium or some other toxic compound. The efficacy of the flavor–toxicant pairing is assessed days later when rats are again presented with saccharin either alone (one-bottle test) or simultaneously with water (two-bottle or choice test). The general finding is that vehicle-treated rats consume (prefer) saccharin, while toxicant-treated rats display a dose-dependent reduction in saccharin consumption (i.e., a conditioned flavor aversion). Conditioned flavor aversions have been produced in several species by a wide variety of drugs, metals, pesticides, and solvents (*51*).

Examples of how flavor-aversion conditioning can be applied in assessing environmental contaminants can be found in the work of Peele and colleagues (*52*). In one series of experiments, the efficacy of heavy-metal chelators in counteracting heavy-metal poisoning was assessed. Lead and thallium were first shown to produce dose-dependent conditioned flavor aversions. Two chelators, British anti-Lewisite and dimercaptosuccinic acid, were also shown to produce dose-dependent aversions. Chelator doses that produced either no effect or a moderate effect were then given to rats treated with either lead or thallium. Both chelators were effective in partially blocking the aversion produced by lead but were ineffective in blocking the aversion produced by thallium. Attenuation of lead-induced aversions by the chelators was also shown to be time dependent, i.e., the longer the chelator administration was delayed the less effective it was. These results agreed well with previous clinical reports, but it is not known at this time whether similar interactions can be demonstrated in a field environment.

Feeding (or flavor) aversions have been used by ecotoxicologists to assess the effects of a number of pesticides on food references (*53*). Most of this work has used avians, whereas most experimental laboratory research has used rodents. In addition, ecotoxicologists have studied aversions to foods that have been directly contaminated with the toxicant. Experimental psychologists, on the other hand, have used flavored solutions (e.g., saccharin) independent of the aversion-conditioning agent to more precisely delineate the variables responsible for conditioning. This separation of the flavor from the noxious agent has allowed further exploration of the variables affecting flavor-aversion conditioning. Peele et al. (*54*), for example, also studied the effect of the neurotoxicant trimethyltin on flavor-aversion conditioning. Rats were first treated with either a dose of trimethyltin or a vehicle. Previous work had shown that trimethyltin produced damage to the hippocampus. Flavor-aversion conditioning was arranged using lithium chloride after trimethyltin administration at a time when damage to the hippocampus was maximal (*55*). Unlike many previous studies, Peele et al. (*54*) systematically varied the time between saccharin consumption and lithium chloride administration. Under these conditions lithium produced an aversion in control rats, the magnitude of which was an inverse function of the delay separating saccharin and lithium. Trimethyltin produced deficits in flavor-aversion conditioning after long delays (3 and 6 hr) but not after a short delay (30 min). These results may have important implications for field research in defining the time frame in which aversions may be produced by contaminated food sources.

Conclusions

It should be clear by now that ethological and experimental approaches to behavior analysis are in many ways complementary. While ethological approaches study behavior in natural environments, they suffer in their ability to precisely identify the underlying processes (or variables) responsible for behavior. Experimental approaches distill fundamental features of the environment in a relatively artificial setting to precisely detail the variables responsible for behavior; at the same time, they may raise nagging questions regarding the generality of findings to other environments. A fusion of these two approaches may be required for comprehensive accounts of behavior and the effects of environmental contaminants.

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