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## Behavioral Studies of Bats in Captivity

### *Methodology, Training, and Experimental Design*

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**S**TUDYING BAT BEHAVIOR IN CAPTIVITY is a valuable complement to field studies. Many classical studies, especially of sensory biology (e.g., Griffin et al., 1965; Schnitzler, 1968; Simmons, 1973) and social behavior (e.g., Porter, 1979), have been conducted in the laboratory. Despite the development of new, sophisticated methods that enable us to eavesdrop on bats in the wild in considerable detail (see Kerth and Dechmann, this volume; Parsons and Szewczak, this volume), in the future we will continue to depend on captive studies to unravel many of the unsolved secrets of bat biology.

Captive studies have proven useful in addressing details of all kinds of natural behavior that are difficult to observe in the wild. For example, many gleaning bats are difficult to observe in the field; vegetation obscures direct visual observation, and their echolocation calls are often too low in amplitude for acoustic monitoring. Much of our knowledge on many aspects of foraging behavior, echolocation, and prey capture in gleaning bats therefore comes from captive studies (e.g., Marimuthu and Neuweiler, 1987; Ryan and Tuttle, 1987; Faure and Barclay, 1992; Arlettaz et al., 2001; Swift and Racey, 2002; Siemers and Ivanova, 2004). Likewise, work on social interactions and communication in bats relies heavily on captive studies (e.g., Esser and Schmidt, 1989; Balcombe and McCracken, 1992; Boughman, 1998), because it is often impossible to identify individual signals, senders, and receivers in wild colonies that are densely packed with hundreds of individuals.

In general, controlled laboratory conditions allow more precise measurement of many aspects of bat biology. Bats can be tested individually, and individuals can be clearly identified and linked to their responses. Captive studies enable explicit tests of hypotheses. The experimenter can manipulate testing conditions in ways that are difficult if not impossible in the field. Examples include studies of echolocation behavior (see Parsons and Szewczak, this volume), sensory ecology (see

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chapter on olfaction by Kalko and Ayasse, this volume), psychophysics (e.g., Schmidt et al., 1984; Masters and Jacobs, 1989; Mogdans and Schnitzler, 1990; Moss and Simmons, 1993; Wiegrebe and Schmidt, 1996; Koay et al., 1997; Winter et al., 2003; Grunwald et al., 2004), locomotion (e.g., Riskin and Hermanson, 2005), and learning (e.g., Gaudet and Fenton, 1984; Bates and Fenton, 1990; Boughman, 1998; Siemers, 2001; Ratcliffe et al., 2003; Ratcliffe and ter Hofstede, 2005; Page and Ryan, 2006). In addition, standardized conditions are needed for the comparison of the sensory, cognitive, or motor abilities of different species (e.g., Ratcliffe and Dawson, 2003; Siemers and Schnitzler, 2004). Neuroethological studies involving invasive techniques such as electrophysiology also require captive conditions, but they will not be addressed in this chapter.

While captive studies are extremely powerful tools for the investigation of bat behavior, caution should always be taken in the interpretation of laboratory results. Bats may respond differently in laboratory settings than they do in the wild. For example, the echolocation calls of bats in a captive environments have been shown to differ in structure from the calls of the same species recorded in the wild (Surlykke and Moss, 2000; Siemers, 2004); differences in call intensity are also possible. While captive studies provide an invaluable window into the intricacies of bat behavior, experimental results should always be examined critically.

#### PERMANENT HUSBANDRY OR SHORT-TERM CAPTIVITY?

Bats for behavioral studies may be kept in captivity for a short period of time or permanently. The capture of any wild bats requires the approval of the responsible wildlife regulatory authorities (see Simmons and Voss, this volume). Depending on the applicable animal welfare laws and the nature of the planned experiments, an additional license from the animal welfare and veterinary authorities may be required. When possible, males of the species should be used preferentially to females. In many bat species, the contribution of males to reproduction is restricted to courtship and mating in a limited time of the year. Furthermore, male reproductive success is often highly skewed (Rossiter et al., 2000). Presumably, males taken out of the population can readily be replaced by others, whereas a loss of females would mean the loss of potential offspring. Thus, it is advisable to capture only males for behavioral studies whenever possible to minimize the impact on the population. In species where males establish harems, capture of harem males may be inappropriate; however, studies aimed at understanding social behavior or mother-infant interactions will, of course, require capturing females as well. Captive breeding or cap-

turing pregnant females and rearing the young in captivity will likewise be necessary for studies that investigate innate versus learned components of bat behavior. While these questions are scientifically interesting, they should be restricted when possible to studies of abundant, non-threatened species.

Short-term captivity is the method of choice for research questions on natural behavior of experimentally naïve bats. For conservation reasons, it may be the only feasible approach to study endangered species, especially when larger sample sizes are required. We have had good experience keeping bats for periods of several days up to approximately eight weeks. The longest temporary captivity period we know of was for greater and lesser mouse-eared bats (*Myotis myotis* and *M. blythii*) that were kept for behavioral studies for 14 months (Arlettaz et al., 2001) and successfully released to the wild afterwards. One bat was radio-tracked before and after this captive period and found to return immediately to its habitual foraging areas after release to the wild (R. Arlettaz, pers. comm.).

The bats should always be released at the site of capture at the end of experiments to enable them to easily relocate roosts and colony members. Special care should be taken to release animals in good physical condition. For this reason, bats should have daily flight exercises while they are in captivity. For both the sake of the individual and the sake of wild populations, bats should only be released when in good physical health. For a detailed discussion of bat health and captive care, see the chapter by Barnard in this volume. In temperate regions, bats should be released early enough in autumn so that they have ample time to resettle in their natural environment before the onset of hibernation.

Permanent husbandry is preferable for studies that require long-term training of bats but smaller sample sizes. One example is psychophysical studies aimed at establishing quantitative relationships between physical stimuli and their perception by bats. It is not known whether the development of a fully functional echolocation system might require learning and experience in a natural environment. Because a systematic study of this question is still lacking, as a measure of caution, research into the psychophysics of echolocation should be performed with wild-caught bats. Other psychophysical or cognitive studies (e.g., Winter et al., 2003; von Helversen 2004; Winter et al., 2005), as well as studies on social behavior (e.g., Porter, 1979; Ratcliffe and ter Hofstede, 2005), have been conducted with captive-born bats from successful captive breeding colonies, especially with species of nectar-feeding or fruit-eating species.

It is important to individually mark captive bats so that they can be kept in a group and unambiguously identified. Marking methods such as banding, PIT tagging, bleaching, or fur clipping for identification of bats are discussed

by Kunz and Weise (this volume). To obtain natural behavior under a natural arousal level, it is important that experiments be conducted during the natural activity period of the bats (i.e., at night). However, it may be desirable to time-shift the bats. Maintaining bats under an inverted light regime will switch their night to day, which is more the convenient for the human experimenter. Time shifting is recommended only if the bats are expected to be held in the lab for several weeks. Bats need to be back-shifted before release to the wild. Time shifts in both directions should be executed stepwise over several days with a maximum shift of two hours per day (H. Erkert, pers. comm.).

### HOW MANY BATS ARE NEEDED FOR A MEANINGFUL EXPERIMENT?

Before beginning an experiment, one must first determine the appropriate sample size for the study. One must choose a sample size that is large enough to obtain reliable results but not so large that the study wastes effort and resources, tests animals needlessly, or becomes infeasible. The optimal sample size for a given study will depend on a number of factors, among them, the magnitude of the effect the researcher wishes to be able to detect. If the minimum difference one wishes to detect is large, a small sample size will suffice. Alternatively, if one wishes to detect fine-scale differences, a larger sample size will be required. Likewise, a large sample size will be required to argue that an experimental effect in fact results in no significant difference. The nature of the study itself will dictate sample size. In studies measuring psychometric functions of absolute ability, where little variation among individuals is expected, experiments can be conducted with only a few subjects. Conversely, larger sample sizes are needed to investigate more qualitative questions aimed at assessing the natural variation in a population. For example, experiments with only three individuals were sufficient to demonstrate that glossophagine bats have ultraviolet vision (Winter et al., 2003). In contrast, the echolocation calls of 386 individual greater horseshoe bats (*Rhinolophus ferrumequinum*) were analyzed to investigate seasonal and lifetime changes in vocalizations (Jones and Ransome, 1993).

To determine the appropriate sample size for a given experiment, one must first estimate the variation present in the population. Large sample sizes will be needed to assess populations with considerable variation, whereas more homogeneous populations can be assessed with reasonable precision using smaller sample sizes. Ideally, one would have an estimate of population variance as a basis for computing the appropriate sample size for the study using an a priori power analysis test (Kraemer and Thiemann, 1987; Cohen, 1988; Lipsey, 1995). Many statistical

software packages include power analysis tests. They are specific to the type of statistical test and can be used to determine the appropriate sample size given the desired detection difference and power. Free power analysis software is also available over the web (Erdfelder et al., 1996). To date, power analysis has rarely been used in studies on bat behavior because it is often difficult to know how much variation to expect before the study is conducted. When available, comparisons with previous studies may help. For example, Ratcliffe and Dawson (2003) used data from Faure et al. (1993) to estimate the variance in the echolocation calls of the northern long-eared bat (*Myotis septentrionalis*) and the little brown myotis (*M. lucifugus*), and then used an a priori power test to determine an appropriate sample size for their study. In many cases, comparative data will not be available. Thus, exploratory pilot studies may help to assess population variation and determine the appropriate number of subjects needed for a study. In many behavioral studies on bats, logistical constraints make it impossible to accurately assess population variation before the experiment begins, and in these cases, appropriate sample size must be estimated rather than calculated by power analysis.

Another factor that affects the sample size is the type of statistical test used; for example, smaller sample sizes are acceptable if each animal acts as its own control. This is the case for matched or paired designs and for repeated measures tests. Because these tests compare the differences in performance within an individual rather than comparing means across groups, these tests are intrinsically very powerful, and thus it is possible to detect the same real differences with a smaller number of individuals.

In some experiments, learning by the test subject will bias the results of the tests, and thus only naïve individuals should be tested. If only the first response of a naïve individual is of interest, then the more powerful statistical tests described above (repeated measures, paired designs, etc.) cannot be used. In these cases, larger sample sizes will be essential.

In determining appropriate sample size, there are often factors outside of statistical design that must also be considered. Is the species of interest threatened or endangered? The conservation status of many species limits the number of experimental animals that can be collected and used. Are individuals of this species difficult to capture? For practical reasons, the optimal sample size for a particular experiment may be impractical for the species in question. Depending on the species, bats also may be difficult to maintain in captivity, or they may require extensive training before behavioral tests can begin. In more complex training paradigms, some bats will not cooperate at all and thus will not produce any data. These practical constraints ultimately will limit the number of individuals that can be tested in behavioral studies.

## THE ROLE OF LEARNING IN BEHAVIORAL STUDIES

Bats are often able to learn quickly. Thus, in behavioral studies it is important to consider the impact of possible learning effects on the observed results. Of course, learning effects are not always undesirable. If one is training a bat for a specific behavioral task, learning is the desired outcome. Because the performance of bats may improve through learning during the course of an experiment, one usually starts testing only after a certain performance level is reached (see below).

If one is interested in natural responses in experimentally naïve bats, learning may pose problems. Bats can quickly be conditioned to many cues for a food reward. Thus, it is important to conduct tests before the bats have “caught on” to an experimental design. The experimenter will be able to conduct only a few or perhaps only one test trial per individual if truly naïve individuals are required (see Ratcliffe et al., 2003, for an example of single-trial learning of taste aversion). However, learning should not be viewed merely as a laboratory artifact; it is likely that learning also plays an important role in natural foraging behavior. This seems to be especially true for bat species that search for food that is difficult to find (e.g., if it is masked by echo-cluttering vegetation). There is indeed behavioral evidence for rapid learning abilities of gleaning bats in a foraging context (Siemers, 2001; Page and Ryan, 2005; Page and Ryan, 2006). Flower-visiting glossophagine bats that live in a complex environment, with food sources that replenish over time and are distributed in three dimensional space, have an excellent spatial memory and high capacity for learning spatial patterns (Thiele and Winter, 2005; Winter and Stich, 2005). There is also recent evidence for rapid learning in an aerial hawking context (Hristov and Conner, 2005) and for learning habitat-specific echolocation calls (Wund, 2005). Studies that focus on learning per se aim at quantifying and comparing learning rates (Gaudet and Fenton, 1984; Bates and Fenton, 1990; Wund, 2005). We suggest that it will be a promising goal to compare the learning capacities of different bat species, exploring the relation of these abilities with the specific demands of their respective ecological niches.

## TESTING ARENAS

Testing arenas vary from small, confined enclosures to large, outdoor flight cages. They can be transient structures that are lightweight and portable, such as flight tents erected in the field (Siemers, 2004). Large mesh tents are relatively inexpensive; they can be easily purchased from outdoor equipment stores, and they are suitable for many types of experiments. Alternatively, testing arenas can be permanent structures, such as specially modified

rooms in buildings or stand-alone, screened flight rooms. The appropriate experimental setting will depend on both the bat species to be tested and the nature of the questions being asked.

In preparing a test arena, one must ensure that the space is adequate to accommodate the type of experimental test and the equipment necessary to record the signals and the bats' responses. For experiments in which sound is critical, such as experiments in which acoustic stimuli are broadcast from speakers or the bats' echolocation calls are recorded, it is important to cover all reflective surfaces with sound absorbing material. Concrete floors and metal supports produce strong echoes and thus should be covered with sound-absorbing acoustic foam. Sound-absorbing foam is widely available online. This material is generally used in music recording studios or for general noise control and is tailored for attenuating sound frequencies in the human hearing range, although it also is useful for absorbing sounds in the ultrasound range. In the case of experiments that involve the use of video filming or photography, background materials should be visually nonreflecting. Some surfaces that are dark to the human eye strongly reflect infrared (IR) light and are thus unsuitable background surfaces for video filming under IR illumination. For example, most cotton cloths are bright and shiny under IR illumination, irrespective of their color in the visible spectrum. Leather, some velvets, and acoustic foam are more suitable backgrounds. Testing the equipment, the background materials, and the experimenter's clothing in the experimental setting should reveal potential problems with reflective surfaces that can be addressed before an experiment begins.

Just as it is essential to maintain the thermoneutral temperature and appropriate humidity in a bat's home cage, climatic conditions must be similarly controlled in the test arena. This task is simple if experiments are conducted in outdoor flight rooms or flight tents in the bat's natural habitat. These conditions may be more difficult to control in situations where the bat is removed to an indoor setting, or taken to a different habitat entirely (e.g., when bats are translocated from tropical to temperate regions). Methods used to control climatic conditions are discussed by Barnard (this volume).

Installing a double door at the entrance to the testing arena can be invaluable for preventing bats from escaping. The experimenter should enter and close the outer door before opening and entering the inner door. Double doors can be as simple as mesh vestibules constructed out of mosquito netting.

Studies aimed at eliciting natural behavior usually require experimental conditions that mimic the conditions found in nature. For example, studies of foraging ecology can be conducted with artificial ponds, trees, leaf litter, vegetation, fruit, or flowers (e.g., Kalko and Condon, 1998; Thies et al., 1998; Siemers and Schnitzler, 2000; Arlettaz

et al., 2001; Jones et al., 2003; Siemers and Ivanova, 2004; Korine and Kalko, 2005; Ratcliffe et al., 2005). Ruczyński et al. (2007) used natural logs with artificially caved entrance holes and cavities to study the sensory basis of tree roost detection. Such tests can be conducted with flying bats exhibiting natural responses that are part of their behavioral repertoire in the wild. Psychophysical experiments that require very controlled conditions, on the other hand, are often conducted most efficiently in a confined test chamber (see below).

## PREPARING BATS FOR EXPERIMENTS IN CAPTIVITY

Before training or testing can begin, it is important to accustom the bats to their new environment. This should be done for both for wild-caught bats introduced into captivity for the first time, and for permanently captive bats entering a novel test arena. Upon releasing bats into the test arena, they need time to explore the area, to find appropriate perches, and to settle into their new surroundings. Rushing this process can be counterproductive, as the bats will be less likely to exhibit natural behavior and less likely to cooperate during experiments.

For both ethical reasons and for the integrity of the experimental results, steps should be taken to ensure that captive bats are calm, healthy, and appropriately cared for. If bats are held in captivity for long periods, routine health checks are advised so that disease or injury can be treated promptly (for assessing and treating injury and diseases, see Demma et al., this volume). For shorter-term stays, close observation is usually sufficient to monitor health. Healthy bats will seem alert, they will show active ear motions, and they will respond readily to species-appropriate stimuli.

It is always important to handle bats gently. Different species of bats, and even different individuals within a species, may vary tremendously in their temperament. While rough handling may have little effect on one bat, it could frighten another to the degree that it will cease to exhibit normal behavior in captivity. Overly fearful bats may show undesired responses (e.g., repeated escape attempts) or no response at all in planned behavioral experiments. Likewise, it is always important to act calmly around bats. One should move slowly, talk softly, and avoid loud or sudden noises in their presence. Bats rely extensively on auditory signals, so it is important to be sensitive to their “acoustic world.” The rustling of clothes (especially synthetic material such as raingear) as well as the sound of rubbing fingers over clothes, skin, or hair can produce broadband noise or clicks that extend far into ultrasound. It is also noteworthy that whispering contains considerably more high frequency components than normal human speech. These actions may seem harmless to us, but can be quite stressful to unhabituated bats. Addi-

tionally, these actions may provide unintended cues that may confound the interpretation of the experimental results.

Handling of bats should be kept to a minimum. With the use of automatic perch scales, body mass can be regularly monitored without handling (see below). To minimize stressful handling, it may be helpful to train bats to enter a small transport cage that can be moved from the home cage to an experimental room. This will minimize chasing bats around the cage and thus will facilitate the transfer process. Bats can be trained to enter a transport cage by placing it near their roost perch and initially offering a food reward inside. Training bats to enter a transport cage is an especially valuable technique for bats that will be kept and tested in captivity for long periods. For bats that are held for only a few days and then released back into the wild, this additional training effort may not be worth the effort.

It is important to maintain a consistent daily routine. Training should always begin at the same time each day for each individual. If bats are not trained daily, the long-term training schedules should still maintain a constant pattern; (e.g., five days training, two days break, etc.). It is important to work only during the activity period of the bats. While bats may show some behavioral responses during their sleeping hours, natural behavior is best measured when the bat activity levels are highest.

## OBSERVING NATURAL BEHAVIOR IN UNTRAINED BATS

Behavioral studies in captivity can be divided into two broad categories: those that require extensive training before testing, and those that require minimal training. Studies in the later category are aimed primarily at the characterization of natural behavior and are often conducted to complement field-based studies that focus on the behavior of bats in the wild (see Kerth and Dechmann, this volume). In studies that aim to elicit natural behavior, it is often preferable to test bats quickly following capture to avoid confounding factors associated with learning. In cases in which learning will alter the experimental results and learning is not the focus of the study, it may be necessary to limit the number of tests per bat (e.g., Ratcliffe et al., 2005), and in some cases use only the first response per bat.

The most common types of experiments conducted with untrained bats are those that elicit natural foraging behavior. By triggering prey-capture behavior, experiments can investigate sensory basis of prey detection, prey assessment and identification, and prey preference and selection. Actual prey items, or isolated cues associated with prey items, are presented to the bat and its response is quantified. There are many methods of prey presentation. Some examples include moths tethered to a thread or

pinned to a substrate (Ratcliffe and Dawson, 2003; Siemers and Ivanova, 2004), crickets thrown onto a platform (Fuzessery et al., 1993), mealworms hidden in leaf litter (Jones et al., 2003), and frogs dragged across a substrate (Marimuthu and Neuweiler, 1987; Ryan and Tuttle, 1987; Ratcliffe et al., 2005). Offering a bat live versus dead prey, or presenting prey under different levels of illumination, can provide cues to stimulate foraging behavior (Schmidt et al., 2000; Arlettaz et al., 2001; Eklöf and Jones 2003). The manipulation of artificial food dummies can also elucidate the role of specific food cues. For example, Thies et al. (1998) altered the surface structure, form, and olfactory cues of artificial piper fruits to investigate foraging behavior in two species of neotropical fruit-eating bats (*Carollia perspicillata* and *C. castanea*). To isolate the role of distinct sensory modalities, an isolated food-specific cue can be presented to the bat. Acoustic cues can be broadcast from loudspeakers, such as the frog mating calls that attract the frog-eating bat, *Trachops cirrhosus* (Tuttle and Ryan, 1981; Page and Ryan, 2005).

Studies that elicit natural behavior are highly species-specific. While it may seem obvious that an aerial insectivore will not approach an artificial flower in search of food, and likewise, a nectar-feeding bat may not take a tethered moth from a wire, it is important to keep these species differences in mind when designing and conducting experiments. If the test species is not responding to the experimental design, it may be worthwhile to collect additional natural history data and more closely match the experimental conditions with natural ones.

Escape is another type of behavior that can be elicited without training. Studies that investigate the sensory cues used during escape from a maze investigate questions concerning the hierarchy of cue use, that is, which sensory modality bats weigh most heavily when information from different sensory modalities are in conflict (Chase, 1981, 1983; Mistry, 1990).

Response to communication calls can also be tested with untrained bats. Discrimination between stimuli can be assessed with the use of a habituation test. Boughman and Wilkinson (1998) used this technique with greater spear-nosed bats (*Phyllostomus hastatus*) to investigate their perception of the screech calls produced by roost mates versus screech calls produced by non-roost mates. In this testing paradigm, a single call type is presented repeatedly until the test subject habituates (ceases to show a response). Subsequently, a novel call type is presented. If the subject perceives the new stimulus as different from the previous stimulus, it will dishabituate and exhibit a response. If the subject perceives the two stimuli as the same, it will continue to show no response. In this testing paradigm, it is important to use probe trials in addition to test stimuli (i.e., present the subject with a control stimulus that should easily elicit a response) to ensure that the test subject is still responsive in general. Habituation tests are

a powerful technique that can be used to assess perceptual differences with naïve individuals (for examples with other taxa, see Wyttenbach et al., 1996; Eimas et al., 1971; Cheney and Seyfarth, 1988; Nelson and Marler, 1989; Hauser, 1998). Similar results can be obtained with psychophysical tests, but these require training (see further discussion below).

Other studies of social communication quantify the recognition of vocalizations. These tests have been used with Brazilian free-tailed bats (*Tadarida brasiliensis*) to test recognition of a pup's vocalizations by its mother (Balcombe, 1990) and recognition of the mother's vocalizations by its pup (Balcombe and McCracken, 1992). Response to chemical cues is another test that can be conducted with naïve animals. Bloss et al. (2002) used a Y-maze paradigm to show that big brown bats (*Eptesicus fuscus*) can discriminate between colony-mates and non-colony-mates on the basis of chemical cues alone.

## EXPERIMENTS THAT REQUIRE TRAINING

### The Reward

Food rewards are a critical component to many behavioral experiments. When training bats, it is important that the bats know from the beginning that an experimental task will be rewarded. Usually, bats receive food only during the experiments, but have ad libitum access to water in their home cage. While in most cases one large meal a day is sufficient for insectivorous or carnivorous bats, nectar-feeding bats have high energy turnovers and need access to food for at least several hours (see Voigt et al., this volume). In any case, it is advisable to regularly weigh bats to make sure they are eating enough to maintain their body mass.

One should choose a reward that can be administered quickly and conveniently during the experiments. Each single reward should be small enough that the bats will not become quickly satiated and unresponsive, but large enough to ensure the bats will be motivated to perform. For example, *Myotis* species of about 10 g will eat 10 to 15 mealworms per daily session, *Eptesicus fuscus* will eat 15 to 20, and *Rhinolophus ferrumequinum* will eat up to 30. The type of reward depends on the bat species under study. Food items that are part of a bat's natural diet are a logical choice and can be used in experiments that mimic natural foraging conditions. For example, piper fruits were used in experiments with *Carollia castanea* and *C. perspicillata* (Thies et al., 1998); frogs were used in experiments with *Cardioderma cor* (Ryan and Tuttle, 1987); and moths were used in experiments with *Eptesicus fuscus* (Hristov and Conner, 2005).

Alternatively, bats can be given food rewards that are substitutes for food items they eat in the wild. Nectar-drinking glossophagine bats can very effectively be rewarded with sugar solution that they drink from artificial

flowers (Winter et al., 1998; von Helversen and von Helversen, 2003; Simon et al., 2006). Winter and Stich (2005) provided a detailed description of computer-controlled feeders that deliver tiny drops of sugar water (e.g., 10  $\mu$ l) when a bat hovers in front of them and breaks a light barrier with its snout. In a fully automated flight room, glossophagine bats can make up to several hundred behavioral decisions for food rewards from these artificial flowers in a single night (Winter et al., 2003). Blood-filled syringes that release a delimited quantity of blood upon being compressed by a computer-controlled lever have been used to reward vampire bats in a psychophysical training paradigm (L. Wiegrebe, pers. comm.). A similar automated apparatus can be used to reward insectivorous bats with a liquid or pastelike blend of preferred foods (Thompson et al., 1990). Such mixtures can be made from blended mealworms, cottage cheese and water (Koay et al., 1997); blended mealworms, baby food, and vitamin supplements (Lollar and Schmidt-French, 2002); or high-quality pet food (see Barnard, this volume, for details on balanced diets for captive bats). Mealworms are the standard reward for insectivorous bats. Usually mealworms are offered alive or, depending on the experimental condition, freshly killed by briefly boiling, freezing, or severing with a sharp blade. Halved mealworms can be used for small bat species and can also be used to increase the number of trials an experimenter can conduct per day with larger bat species.

Bats on a perch or platform can be offered mealworms with blunt forceps. It is important to remember that the bats cannot resolve the experimenter's hand, the forceps, and the mealworm by echolocation or sight in as much detail as we can in our visual world. Thus, the bat will not know exactly where the mealworm is and may need to move its head to find it. One should touch the bat's mouth gently with the mealworm. The mealworm should protrude sufficiently from the forceps to keep the bat from biting the forceps and damaging its teeth. Plastic forceps are gentler on teeth than metal ones.

Mealworms can also be used as rewards for flying bats. A convenient method to attach a mealworm to a thin nylon thread is to stick the mealworm onto the needle of a syringe, insert the thread into the hole at the tip of the needle, and then push the mealworm from the needle onto the thread. The hemolymph of the mealworm provides enough adhesion to attach the mealworm to the tether, but the bats have no difficulty removing it from the thread in flight with their uropatagia or wings. Other methods to offer mealworms are to use feeding trays, dishes, or experimental feeding boards (Wiegrebe and Schmidt, 1996; Schmidt et al., 2000; Swift and Racey, 2002; Weissenbacher et al., 2002; Hübner and Wiegrebe, 2003).

Generally, a reward is offered after each trial that an experimental subject successfully completes. Intermittent reinforcement, in which not every trial is rewarded, is

well established in experimental studies as an alternative to continuous reinforcement (Ferster and Skinner, 1957) and is known to produce high response rates in common laboratory subjects such as rats, pigeons, and humans. When rewards are not offered in every trial, experimental subjects do not become satiated as quickly, and once the experimental task is acquired, variable reinforcement schedules can result in higher levels of response than continuous ones (Ferster and Skinner, 1957; Baum, 1993; Cole, 1994; Lattal and Neef, 1996; Bizo and Killeen, 1997; Bizo et al., 2001). We are not aware of any published behavioral studies that compare response rates with intermittent reinforcement versus continuous reinforcement with bats. However, given that intermittent reinforcement elicits high levels of responsiveness in other animals, it may be worthwhile to adopt this method for simple rewarded tasks with bats. While intermittent rewarding is likely to work well for simple, directed tasks such as localizing a sound source or navigating an obstacle course, it will be less appropriate for choice experiments in which the intermittent presence or absence of a reward would dissociate the desired stimulus (S+) from the reward and possibly confound experimental results (see below). In a study on localization abilities of frog-eating bats, intermittent reinforcement has proven effective (Page and Ryan, 2008).

### The Perch

Experiments with flying bats often require that the bats begin and end experiments from a set position in a test cage. This perch should be a comfortable substrate from which the bats can hang (e.g., a piece of cork, a rough wooden bar, or a cloth-covered frame). The (final) position of the perch should be in a sheltered place such as a corner, and high enough that the bats have a good overview of the flight room, as they rest undisturbed.

The perch can either be a starting point or an end point for the experimental task. With the perch as a starting point, the bats are required to make a behavioral decision from the perch (e.g., choose between stimuli presented from two equidistant loudspeakers). In this case, the bats receive a reward for the correct response at the stimulus site, not at the perch. Thus, it is more straightforward for the experimenter to associate the reward with the task and not with the perch from the beginning of the training.

Conversely, with a perch as an end point of the experimental task, a bat should land at the perch after completing a task to receive its reward, for example, after flying through an obstacle course. In this case, one can train the bat to the perch using the reward. One possibility is to bring a bat into a flight room in a small transport cage. The experimenter then opens the cage and offers a reward. With the reward close to the bat so that it can smell, see, or possibly echolocate it, the experimenter tries to attract the bat to leave the cage and climb onto the perch

that is held directly in front of the cage. In the next step, the perch is presented at some distance, so that the bat has to make a short flight to reach it. It then is moved farther and farther away until reaching its final location in the flight room. Depending on the bat's cooperation, this procedure can be accomplished in a few days or, at most, several weeks. This method has proven effective in training horseshoe bats to follow a desired flight trajectory or go to a given perch (H.-U. Schnitzler, pers. comm.; Klemen Koselj, pers. comm.). If the experiment requires that a bat fly between two perches, the transport cage can finally be replaced by a second perch. If not, the cage can remain in place so the bat can retreat to it at the end of the session to return to the home cage.

Training a bat to perch can be considerably easier if the desired perch is the only place in the flight room from which it can comfortably hang. Studies involving sound recording or playback may require lining the flight room walls with sound-absorbing foam. In such a room, a bat will be able to perch anywhere. In this case, special care must be taken to make the perch attractive. If bats are tame enough, they can be brought to perches by hand. Bats should be allowed to rest on the perch and discouraged from hanging elsewhere by slowly approaching them and giving them a gentle tap. Many bats learn to fly or crawl into the transport cage from the perch at the end of an experimental session, which saves them and the experimenter stressful "hunts" and contributes to making

the perch a "sacred" place where bats are not disturbed. When the walls of the flight room are coated with substrate on which the bats can easily perch, a useful technique for training bats to a perch is to initially cover the entire flight room except the desired perch with slippery plastic sheets. The bat will be unable to perch on the plastic and should automatically choose the desired perch. Once the bat is consistently landing only on the perch, the plastic can be gradually removed. A few gentle taps may be necessary to complete the training. Another helpful technique is to only allow the bats to eat food rewards once they have returned to the perch. Bats should be discouraged from eating anywhere else in the flight room by approaching and gently tapping them if they land anywhere but on the desired perch.

The perch can be an important tool for automatically monitoring body mass. A piece of cork suspended from a computer-controlled precision balance will allow automated monitoring (Fig. 17.1A). This can be instrumental for studying metabolic costs of certain behaviors (Winter et al., 1998; Winter and von Helversen, 1998; Winter, 1998) as well as for experiments in which the bat is tested only when motivated to feed.

### The Training Platform

Many psychophysical experiments require bats to sit on a platform to perceive the test stimuli and then to crawl toward a rewarding location to indicate their behav-

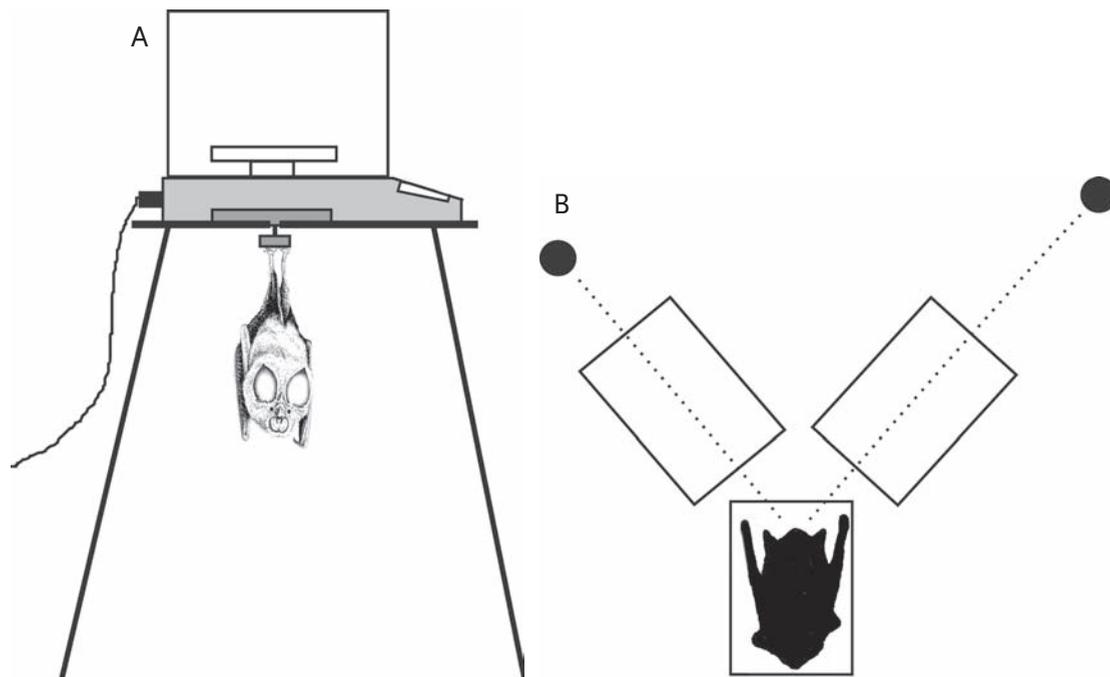


Figure 17.1. Experimental setup. A, perch suspended from a computer-controlled precision balance; B, Y-platform used in a 2-AFC paradigm. Bats crawl from the starting position of the Y-platform to one of the two arms in front of it to indicate a behavioral decision. In this illustration, the bat must decide which of the two echo targets (black circles) is closest. *Illustration by Ingrid Kaipf.*

ioral decision. Often, Y-shaped platforms with one starting and two rewarding arms are used (for a review see Moss and Schnitzler, 1995; see Fig. 17.1B for an illustration). Depending on paradigm and experimental setup, elongated platforms can be employed, where bats move back and forth (e.g., Koay et al., 1997) or left and right from a central starting location (Masters et al., 1997). In preparation for such experiments, one must accustom bats to the experimental platform and train them to sit and to crawl, but not fly. In some experiments, this task will be easy because it is possible to use confined test chambers that are too small for flight (e.g., Esser, 1998). Many acoustic psychophysical studies, however, require one to several meters of clutter-free space between the bat and the echo targets or the loudspeakers (i.e., enough space for the bats to fly). The willingness to take flight from the platform depends on how maneuverable the species is in small spaces; it will also vary among individuals within a species. One possibility to train bats to remain positioned is to abort the experimental session (i.e., the food supply) when they begin to fly. In this way, bats can learn to signal when they have reached a level of satiation at which they are no longer motivated to work for a food reward. Big brown bats (*Eptesicus fuscus*) have been used for many psychophysical tests on platforms. After time spent in the laboratory, some individuals are reluctant to fly even in the home cage and prefer to walk and climb. Most individuals become accustomed to sitting and crawling on the platform within days. For *Rhinolophus*, an analogous U-shaped hanging stand was used by Emde and Menne (1989), under which the bats hang and walk instead of sitting and crawling.

In general, bats should be sufficiently accustomed to the experimenter before they are introduced to the platform so they do not attempt to escape in response to the stress of being handled. Highly active individuals may become accustomed to the platform more readily if they are allowed to inspect the test chamber in flight during the first few days after being introduced. From the onset of training, bats should be fed only on the platform and no longer in the home cage. It is important to start the actual training for the experimental task as soon as they accept the platform as a perch.

### GENERAL TECHNIQUES FOR TRAINING BATS

Before an experiment can begin, the test subject must first be able to perform the experimental task. In many cases, this is straightforward. As above, experiments that elicit natural behavior require little or no training. For tasks that require training, shaping is a powerful training technique. Shaping involves two components: the reinforcement of successive approximations of the desired response, and nonreinforcement of earlier, more general

responses (Shettleworth, 1998; Domjan, 2003). The experimenter begins by rewarding the subject for any response that approximates the desired response. In gradual steps, the experiment hones the subject's response by rewarding responses that more and more closely match the desired response, and by ceasing to reward other responses. The technique has been shown to work with a wide variety of taxa (e.g., Platt, 1973; Pear and Legris, 1987), and is an effective technique in experiments with bats. For example, Barber et al. (2003) used shaping techniques to train pallid bats (*Antrozous pallidus*) to associate dead prey items with a noise burst from a speaker. Initially, the bats would not take dead mealworms from a speaker. To train this task, Barber et al. broke the task into a series of smaller steps; the bats learned to associate a sound burst from a speaker first with live crickets, then with anesthetized crickets, and finally with dead mealworms.

Another successful method for training a behavioral response is stimulus fading (Terrace, 1963). Whereas shaping involves the gradual honing of a response while the stimulus remains constant, fading involves the gradual alteration of the stimulus while the response remains constant. Fading involves the gradual introduction or removal of a stimulus. For example, in a discrimination task, the experimenter can first present the unrewarded stimulus (S-) infrequently and at low intensity, while presenting the rewarded, desired stimulus (S+) frequently at full intensity. The intensity and duration of the S- presentation is increased gradually so that the subject never responds to this stimulus. Eventually, the experimenter is able to present the S- with the same intensity and frequency as the S+ without discrimination error from the subject. This technique has been used widely in experimental psychology studies (Fields, 1978; Doran and Holland, 1979; Ploog and Williams, 1995). In a recent study, Page and Ryan (2005) gradually faded túngara frog mating calls (signaling a palatable prey item) into cane toad mating calls (signaling a poisonous prey item) to demonstrate that the frog-eating bat (*Trachops cirrhosus*) can flexibly reverse its associations for the calls that signal palatable and poisonous prey. The bats rapidly tracked this change. It should be noted that bats can sometimes erroneously key in on the high intensity of S+ as the desired experimental cue and thus have difficulties discriminating S- from S+ when they are presented at equal intensity (S. Walther, pers. comm.).

Training should be conducted in a rigid, stereotyped manner. Standardized training trials will facilitate training and allow comparisons between animals. It is possible to compare learning curves (the number of training trials required to reach a specified criterion) among individuals only if training trials are standardized.

To accustom bats to the test arena and to train them to the experimental task, it may be helpful to house several bats together. Social learning is widespread among

mammals, and has been demonstrated in several species of bats (Gaudet and Fenton, 1984; Wilkinson and Boughman, 1999; Ratcliffe and ter Hofstede, 2005; Page and Ryan, 2006). In training for a specific task, such as the navigation of an obstacle course for a food reward, the fastest and most effective training technique may be to allow inexperienced bats to observe an experienced one. Unless the experiment is specifically designed to test more than one bat simultaneously, the bats should be separated once they have reached testing criterion (see below) and are ready to begin the experiment. Bats should be tested individually to avoid the confounding effects of interactions among animals.

## TEST PARADIGMS

If one wishes to ask bats about how they perceive the world, we must ask in a way that allows the bats to “understand” the question and us to understand the answer. There are two basic testing paradigms that are frequently used in bat behavioral and psychophysical studies: (1) the “go/no-go” or “yes/no” paradigm and (2) choice experiments (Moss and Schnitzler, 1995). For both approaches, it is important to clearly identify the behavior that is defined as the experimental animal’s “reaction” or “decision.” Further, one should ensure that the animals are motivated to participate in the experiment. If bats are expected to work for a food reward, but are not hungry, the resulting behavioral data will be suspect. Thus, it is recommended to start and end each experimental session with a very easy trial, in which it is clear that the bat will show the correct reaction or decision if it is motivated to feed.

### Go/No-go Paradigm

In this testing paradigm, if the bat perceives the test stimulus, it responds and receives a reward. If the bat does not perceive the stimulus, it does not respond. In a very simple form, this paradigm can be used to measure how bats perform in a naturalistic prey-detection task. Siemers and Schnitzler (2000, 2004) presented tethered mealworms to wild-caught, edge-space aerial foraging or trawling bats at some distance to vegetation or to a “clutter-screen” in a flight cage. Tethered mealworms mimic airborne insects or spiders and caterpillars on threads, and most bats started to capture the mealworms spontaneously. After the bats had learned to reliably search for tethered prey in the flight tent, the mealworms were presented at systematically varied distances from the clutter background. The bats were allowed to search for a given time period before the trial was stopped. If they captured the mealworm within this time window (“go”), the trial was scored as successful detection. If they flew past the prey in search flight but did not capture it (“no-go”), it was assumed that they had not detected the mealworm. The percentage of

successful detections was then used to quantify prey detectability as a function of the distance between prey and clutter background. Experiments of this kind require little training and can be accomplished with wild bats in short-term captivity because the experimental task mimics foraging tasks the bats confront in nature.

Classically, the go/no-go paradigm is used to assess perception thresholds (e.g., hearing thresholds; Koay et al., 1997). In this paradigm, the bat must first be trained to associate a specific stimulus with a food reward. The most common training method involves a conditioning procedure. In the case of hearing threshold tests, a clearly audible tone is presented and immediately afterwards the bat receives a reward. Next, the bat must learn to indicate that it hears the tone and expects a reward. Usually, the bat sits at one end of an elongated platform to listen to the stimulus and then crawls to the other end of the platform to receive the reward. The experimenter can teach the bat to do this by luring it forward with the reward. For an automated test protocol, test boxes with two compartments are used: one for perceiving the stimulus and one for receiving the reward. If the bat perceives the “go” stimulus, it crawls from the first compartment to the reward compartment. It is also possible to implement this paradigm with bats that have to fly from a test perch to a feeder. The goal of training is that the bat moves from the starting position to the reward position upon perceiving the test stimulus (“go”) and stays at the starting position if it does not perceive anything (“no-go”). Koay et al. (1997) used a conditioned avoidance procedure, in which a hungry bat remained at a feeder as long as no stimulus was present and left when it perceived a stimulus. Instead of positive reinforcement (reward) for a correct response, this and some other psychophysical studies use punishment (e.g., a mild electric shock) for a missed detection.

An important variant of go/no-go is a yes/no procedure. For this paradigm, a Y-shaped platform is used. The bat sits on the start position and is trained to move, for example, to the left response platform if it perceives a stimulus and to the right if it does not (e.g., Møhl, 1986; Møhl and Surlykke, 1989; Hartley 1992; for a review, see Moss and Schnitzler, 1995). The procedure resembles a choice test (e.g., the two-alternative forced-choice test described below), in that the bat must move and indicate a decision in each trial, but the possible responses, their interpretation, and the threshold computations are the same as for a go/no-go paradigm. Namely, from the two experimental conditions, “stimulus present” or “stimulus absent” (i.e., above or below threshold), and the two behavioral responses, “go” (“yes”) or “no-go” (“no”), four possible outcomes can result (Table 17.1): correct detection (hit), correct rejection, false alarm, and missed detection (miss).

The start of a trial is indicated to the bat by a light or a different acoustic signal, or simply by the experimenter placing the bat in the starting position. In the test phase,

Table 17.1. The go / no-go paradigm

Stimulus/Bat's Response	Go	No-go
Present	Correct detection (hit)	Missed detection (miss)
Not Present	False alarm	Correct rejection

thresholds are measured by presenting tones at different frequencies and amplitudes and scoring which stimuli the bats can and cannot perceive (for sequence of stimulus presentation and details on threshold computation, see below). Similar to the hearing threshold tests exemplified here, thresholds for other sensory modalities can also be measured (e.g., for a discussion of olfactory detection using go/no-go procedures see Kalko and Ayasse, this volume).

### Choice Experiments

In a choice experiment, we ask the bats which of two or several alternative options is the desired stimulus, and they respond by choosing among the stimuli. The clearest type of choice test is the “two-alternative forced-choice” (2-AFC) paradigm. Bats are trained to discriminate between a wanted, rewarded stimulus (S+) and an unwanted, unrewarded stimulus (S−). They wait for both stimuli at the starting position and then move to one of two alternative rewarding positions to indicate their perception of S+. In contrast to go/no-go procedures, the bats respond in each trial and actively indicate their decision. There are only two outcomes (Table 17.2): either the bat moves to the side where S+ was presented (correct decision), or it moves to where S− was presented (wrong decision).

The bats are trained to make a decision in every trial, hence the paradigm is called a “forced” choice. The rationale behind 2-AFC is that the bats will choose between the two rewarding sites at chance level (50%) if they actually have no idea which stimulus is S+. A performance that deviates significantly from chance is interpreted as evidence that the bats perceive S+ and discriminate it from S−. Accordingly, 2-AFC is often used to measure discrimination thresholds. For example, bats are presented with two metal wires, one on the left and one on the right. They use echolocation to determine the distance between themselves and the wires and then indicate which wire is closer (S+). In this way, an experimenter can determine the smallest difference between two distances that bats can reliably discriminate (range discrimination; Simmons, 1973). Two-alternative forced-choice tests can also be used to measure detection thresholds (e.g., Kick, 1982, using real targets; Kick and Simmons, 1984, using sonar sound playback to simulate so-called phantom targets). In this case, S+ is any perceivable stimulus, e.g., a tone or light, while S− is no stimulus at all (Moss and

Table 17.2. The two-alternative forced-choice (2-AFC) paradigm

Stimulus	Bat's Response
S+	Correct decision
S−	Wrong decision

Schnitzler, 1995; Schmidt, 1995). Winter et al. (2003) rewarded a feeder where no light was presented as S+ to measure visual thresholds. In contrast to go/no-go and yes/no, in this procedure S+ is presented in every trial (either right or left) and correct responses can only be made by moving toward the stimulus. Like the standard 2-AFC test, this procedure results in a two-outcome contingency table (Table 17.2).

The 2-AFC paradigm can be used with sitting or with flying bats. For sitting bats, a Y-shaped platform is the standard tool (Fig. 17.1B). The starting position for the bat is at one end of the platform, and the bat crawls either onto the left or right arm of the platform to indicate its decision. The test stimuli S+ and S− are presented behind and in line with these two arms such that they are clearly associated with either the left or right arm. Many successful 2-AFC experiments have been conducted on the Y-platform with *Eptesicus fuscus* (e.g., Kick, 1982; Masters and Jacobs, 1989; Menne et al., 1989; Simmons et al., 1989; Moss and Simmons, 1993; Denzinger and Schnitzler, 1994; Denzinger and Schnitzler, 1998; Simmons et al., 1998; Masters and Raver, 2000; von Stebut and Schmidt, 2001), as well as with other bat species (e.g., Møhl, 1986; Emde and Menne, 1989; Esser and Lud, 1997; Roverud, 1999; Grunwald et al., 2004).

Another approach to the 2-AFC test requires the bats to fly and was primarily established for *Megaderma lyra*. It includes using a perch as a starting position and two widely separated feeding dishes on a landing platform; the bat must fly from the perch to one of the two feeding dishes to indicate its decision (Schmidt, 1995; Wiegrebe and Schmidt, 1996; Weissenbacher et al., 2002). Additionally, 2-AFC testing paradigms with flying bats can be used for discrimination tasks (Schmidt, 1995; Simmons and Vernon, 1971). In this case, the bat must remain stationary at the perch until it has perceived the test stimuli and only fly after it has made its decision. Finally, 2-AFC has been established for bats that do everything in flight: inspect the test cues, decide, and retrieve the reward (von Helversen, 2004). This fully automated test apparatus for glossophagine bats rotates the test cues in place above a left and a right feeder and releases a droplet of sugar water if the bat sticks its nose into the correct feeder.

Classification tasks have also been addressed using 2-AFC techniques. In this testing paradigm, the bats are trained to classify a single test stimulus that is presented

in the middle of the two rewarding sites. For example, bats are trained to go to the one side if they perceive an acoustic test stimulus as higher as a remembered reference tone, and to the other side if they perceive it as lower (Schmidt, 1995), or else to indicate whether they perceive a computer generated echo as “rough” or “smooth” (Grunwald et al., 2004).

In the training phase for a 2-AFC experiment, bats must learn to associate S+ with the reward. The experimenter can use a conditioning procedure, in which the presentation of S+ is quickly followed by the reward. The bats must be lured to move to the respective rewarding site (left or right) with the reward. Alternatively, bats are rewarded immediately upon spontaneously approaching the correct rewarding site after presentation of a stimulus. In the beginning of the training, the experimenter should use stimuli that accentuate the difference between S+ and S–, so that the two stimuli are highly distinct and easy to discriminate; however, it is important to ensure that bats learn the desired concept of S+ and not key in on extraneous characteristics of the stimuli. For example, in a range discrimination task, bats should learn that S+ is always the closer wire (relative to the other) and not that S+ is the wire at 1.2 m (absolute distance). Thus, one should start training with several different pairs of wires at clearly different distances; the differences should then be gradually decreased. This takes time and patience and can be a difficult distinction to teach to bats. It might help to introduce an additional cue that indicates where S+ is, such as a click or a light. The use of this type of stimulus to distinguish and reinforce S+ is common in experimental psychology studies with mice and pigeons (Lieberman et al., 1979; Thomas and Lieberman, 1990), and has been used successfully with bats (Falk et al., 2005). This additional cue must be removed before the onset of actual testing. In general, overly difficult tasks should be avoided to ensure cooperation by the bats. If the bats do not show good performance in a new step of training within about two weeks, the level of difficulty should be reduced again or the training concept adjusted (for a discussion of fading and shaping techniques for the training phase, see above).

The transition from training to testing can be made once the animals have reached a level of consistently high performance on moderately difficult training stimuli. There are different ways of defining such a stable level of high performance. For example, Denzinger and Schnitzler (1994) required bats to perform above a threshold of 83% correct decisions for three consecutive days. The bats studied by Moss and Simmons (1993) performed above 90% in easy tasks; difficulty was subsequently increased stepwise and performance decreased accordingly down to 50% (chance). Because the criterion for a detection threshold is usually set at 75% correct decisions, it is important that the baseline performance on easy or moderately dif-

icult tasks be clearly above this threshold before the onset of testing.

Several options may be used for rewarding schemes during testing. One can continue to reward bats only for correct decisions. This is the most practical solution for long series of tests, but it is possible that the bats may continue to learn, i.e., they may improve their performance during testing. For this reason, it is very important to begin testing only once stable levels of high performance have been achieved. Alternatively, one can reward all choices during testing. In this case, the bat’s performance will deteriorate with the rewarding of wrong decisions. Finally, one can reward neither of the two choices, which will result in decreased levels of responsiveness. The negative effects of the “reward all” and the “reward none” schemes can be minimized by interspersing test trials singly into a series of training trials. This approach is recommended when testing whether bats have generalized a concept from the training trials, for example, to discriminate a rough from a smooth echo-amplitude structure (Grunwald et al., 2004; “reward all”). In this case, the test stimuli, or at least specific combinations of test stimuli, should be entirely new to the bats.

Apart from 2-AFC, several other types of choice experiments that involve training have been used with bats. When the training and testing protocols do not require that the bats make a decision in every trial, some authors refer to the experiment as “two-alternative choice” (2-AC) instead of two-alternative forced-choice (e.g., Houston and Jones, 2004). This is the case, for example, when bats are offered two prey objects simultaneously to study their preference. They can either choose one or reject both of them. If there are more than two alternative options from which to choose, the chance level must be altered accordingly (e.g., Laska, 1990; Hessel and Schmidt, 1994).

## EXPERIMENTAL CONTROLS

The minimization of confounding variables is key to a well-designed experiment. The more measurement error present in an experiment, the more difficult it will be to detect an experimental effect if there is one. Bats learn quickly and often key in on cues extraneous to the test stimuli. Because many species of bats have acute hearing and olfactory senses, cues that the experimenter cannot detect may directly affect the behavior of the bat and confound the experimental results. Several steps can be taken to minimize the role of confounding variables.

It is important to check and calibrate equipment frequently (see also Parsons and Szewczak, this volume). Equipment can be severely impacted by extremes of temperature and humidity. When working in areas of extreme environmental conditions, such as outdoor flight cages in the tropics, close attention to the state of the equipment is essential. Calibrating the equipment at regu-

lar intervals will ensure that the experimenter is aware of potential problems and able to take appropriate action. Even over the course of a few hours, high levels of humidity can significantly impact speakers and microphones. Speakers and microphones can often be restored to working condition if they are kept in low humidity conditions between experimental sessions. If an air-conditioned storage area for equipment is not available, a small cabinet with two or three light bulbs should keep the equipment at reasonably low levels of humidity.

Bats are quick to learn cues that signal food rewards. This can become problematic if bats respond not to the test stimuli but instead to cues that are unintentionally associated with the test stimuli. Appropriate controls, specific to the experimental design, can allow the experimenter to discriminate between responses to test stimuli and responses to unintentional cues. For example, Balcombe (1990) presented Brazilian free-tailed bats (*Tadarida brasiliensis*) with playbacks of vocalizations from kin and vocalizations from strangers. To ensure that the bats' movement toward one of the two speakers was in response to the test stimulus and not in response to extraneous cues such as olfactory or visual stimuli associated with cloth model bats he had positioned near each speaker, he broadcast a recording of silence as a control, keeping all other conditions constant. Approach to the speaker in test trials, but not in control trials, ensured that the cues to which the bats were attending were indeed the acoustic stimuli Balcombe was testing.

Controlling for motivation is also critical to meaningful experimental results. In addition to careful monitoring of bats' weight and food intake, if it is possible to bracket test trials with motivation trials, one can be sure that a lack of response to a given stimulus is robust and not due to satiation, boredom, or frustration. A trial testing motivation levels should be very simple, with obvious cues. For example, Esser and Lud (1997) used an easy discrimination task as a control for levels of bat motivation in their acoustic discrimination experiment. If the bats did not correctly discriminate between a 20-kHz stimulus and the standard test signal (a discrimination task they had performed easily in the past), the experimenters would discontinue experimental tests for that session.

Experimenters themselves can unwittingly give cues that can affect a bat's performance in an experiment. If it is possible to code experiments with numbers rather than descriptive names and thus conduct "blind" trials, such unintentional cues can be avoided. If it is impossible to run blind experiments, finding a tester who has no investment in the experimental results can serve the same purpose. Fully automated experiments remove the experimenter from the test arena, and thus ensure that the experimenter cannot influence the bat's behavior by providing unintentional cues.

## SEQUENCE AND POSITION OF STIMULUS PRESENTATION

The temporal sequence of experimental tasks can influence behavioral responses of bats. For example, bats may respond strongly to a given stimulus not only because it is attractive or easy to perceive, but also because it is presented early in an experimental session when they are still hungry and motivated to work at the task. Similarly, when several stimuli are presented simultaneously, their spatial positions can influence the response of bats. For example, a bat could for some reason prefer a left position over a right position when presented with two reward sites. If the rewarded stimulus, S+, is presented more often on the left than on the right side, the bat may choose S+ at levels above chance simply as an effect of this preference for position. In this simple example, it is evident that it would be suspect to conclude from the data that the subject learned to discriminate S+ from S-. To control for such biases, experimental designs must be used that control for the effects of temporal sequence or spatial arrangement. In this section, we briefly discuss randomization and pseudo-randomization test protocols that can be used for this purpose. We then sketch the staircase procedure as an alternative to randomized stimulus sequences in psychophysical paradigms.

As an example, we investigate how to determine the consecutive positions (left/right) of S+ for 100 trials in a 2-AFC experiment. The S+ position could be completely randomized for every trial by tossing a coin. However, it would then be possible for an unequal distribution to result (e.g., the coin could come up 48 times indicating left and 52 times indicating right). To avoid the confounding effects of a potential position preference, an equal distribution of 50 left positions and 50 right positions would be preferable. To do this, one could associate a list of 50 left positions and 50 right positions with a list of 100 random numbers (e.g., generated in Excel) and then sort the list by increasing numbers. A random combination of the 100 test trials would result, with S+ presented 50 times from the right and 50 times from the left. However, this random sequence could contain 10 or more left positions in a row, which might favor the establishment of a position preference in the bats (Schmidt, 1995). As a countermeasure, it would make sense to further constrain the test protocol to allow, for example, S+ to be presented from the same side a maximum of four consecutive times. This can be achieved by randomly altering the stimulus sequence within blocks of two left positions and two right positions (i.e., the sequence of 100 trials would be determined by collating 25 independently randomized blocks). Any such constraints that make the protocol deviate from complete randomization result in a so-called pseudorandom test design. Stimulus presentation protocols in psychophysical tests often follow Gellerman (1933).

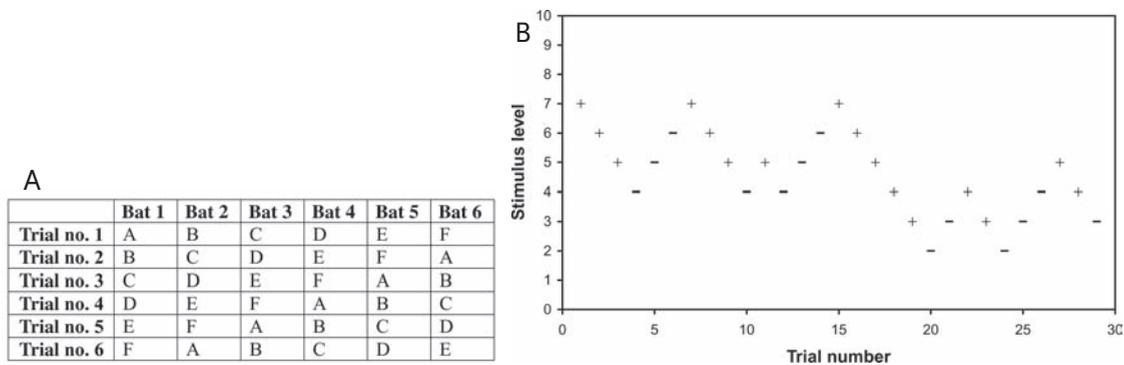


Figure 17.2. Experimental design. A, Latin square design with six experimental subjects and six tasks (designated A–F). A Latin square design controls for a possible effect of task sequence by presenting every task once in each of six possible positions. This and other more sophisticated Latin squares can be found in statistics textbooks, statistics software packages, or on the Internet. B, example of a staircase test design to measure a psychophysical threshold. From one trial to the next, stimulus intensity is decreased if the subject gives a correct response (+), and increased if the subject gives an incorrect response (–).

Pseudorandom designs are generally the method of choice because they balance the effects of chance. A classical pseudorandom design is the Latin square design. As an example, we can imagine that six bat subjects are consecutively presented with six different food types and their reactions are scored to assess food preferences. If we assume that the sequence of presentation can influence the bat's behavior, then a different test sequence should be computed for every experimental subject. Ideally, these sequences should be balanced so that each of the six food types is presented once in each of the six possible positions. This result can be achieved using a Latin square design (Fig. 17.2A). If a Latin square design is used and the bats show a preference for one food type, one can be sure that this result is not influenced by the presentation sequence.

In psychophysical experiments concerned with threshold measurements, there is an alternative to a (pseudo) random sequence of predefined test stimuli (the method of constant stimuli): the so-called staircase method (Fig. 17.2B; Moss and Schnitzler, 1995; Stebbins, 1983). This is a refined method of limits approach, in which the experimenter increases or decreases the intensity of a stimulus until a threshold is reached. Here, one begins with a stimulus that is easily perceptible to the bats and then lowers the intensity of the stimulus in predefined steps from trial to trial, as long as the bat continues to respond correctly. If the bat gives an incorrect response, the intensity is increased by one step. A correct response results in a further decrease. The threshold then is determined by averaging the stimulus intensities of all reversal points.

## DATA ACQUISITION

Several methods are available to document bats' behavior during experiments. The appropriate method will depend on the research question, the type of experiment,

and the research budget. Regardless of the method of data collection, clear definitions of behavioral categories or responses are critical to transform continuously variable behavior into statistically treatable data. We will briefly outline manual, video-based, and automated data acquisition methods.

The simplest and least expensive method for data acquisition is direct observation and manual annotation on paper or a computer. Direct observation is a robust and reliable technique, and is especially useful in field conditions, such as experiments that are conducted in outdoor flight rooms or in flight tents. A night vision device might be used to augment visual observations.

Alternatively, behavior can be recorded with infrared (IR) sensitive video cameras for online or offline analysis. Several digital video cameras offer a night vision option with increased infrared sensitivity (see Altenbach and Dalton, this volume). We have also had good experiences with surveillance cameras connected to a digital or analog video recorder. Some of these surveillance cameras have built-in infrared filters that can be removed, which increases their IR-sensitivity by several times.

For illumination, IR-lamps (white light with an IR-filter) or infrared LEDs (which consume much less energy than incandescent lights) are commercially available. Digital video cameras often come with a (rather weak) built-in IR-source. These options provide continuous illumination, which is sufficient to film hanging or crawling bats. They are less well suited for a frame-by-frame analysis of the fine details of a flying bat's behavior. A standard European video camera will record 25 frames per second at 40 ms each (i.e., 50 half-frames at 20 ms). Standard U.S. video cameras will record roughly 30 frames (29.7 frames) per second. These speeds are generally too slow to analyze bat flight with adequate precision: bat wings generally move fast enough to blur the image. Stroboscopic illumination that only flashes once per frame or half-frame

for about 1 ms can solve this problem (e.g., Siemers and Ivanova, 2004). Other options for obtaining sharp, finely detailed images of fast moving bats in captivity include high speed video (Riskin and Hermanson, 2005) and multi-flash photography (Siemers and Schnitzler, 2000). Both of these methods require more light than infrared video recording, and may cause more disturbance to the bats. As a result, these methods are less well suited for studying the behavior of freshly captured, unhabituated bats.

Building on stereophotogrammetric techniques established for multiflash photography in the field (Jones and Rayner, 1988; Kalko and Schnitzler, 1989), techniques have been developed to reconstruct 3D positions and flight trajectories of bats from video images taken simultaneously with two or more cameras. In combination with synchronized echolocation recordings, this approach has been used to study prey detection, pursuit, and capture both in the laboratory (Ghose and Moss, 2003, Siemers et al., 2005) and also very successfully in the field (Holderied et al., 2005). Methods of sound recording are discussed in detail in Parsons and Szewczak (this volume).

Under certain circumstances, data acquisition can be fully automated. Automation is possible when the behaviors of interest are sufficiently simple and clear that a human observer is not necessary for their categorization. This prerequisite is met when the experimental subjects are trained to indicate a behavioral decision by going to a predefined location, such as on the left or right arm of a Y-platform or to one of several feeders. In such cases, computer-controlled light barriers (von Helversen, 2004; Winter, 2003; Winter and Stich, 2005) or automated video analysis can unequivocally record the behavior of bats and store it for further analysis.

## DATA ANALYSIS

The final steps of a behavioral experiment are data analysis and representation. Generally, the quantitative data are the most important results of a study; however, proper qualitative descriptions and documentation of the research are also valuable components of the study, especially for little-studied types of behavior or poorly known bat species. Drawings, photos, or video images help to illustrate typical behavior. Detailed descriptions of behavior over time can be used to describe different types of behaviors and to quantify their relative occurrence.

It is generally important to clearly state explicit hypotheses. Predictions should be derived and tested using experimental data to either falsify or corroborate the hypotheses. When possible, the study should include a thorough discussion of null and alternate hypotheses, including those that the study may have been unable to address.

Thresholds for detection (e.g., Simmons et al., 1992; Koay et al., 1997; Winter et al., 2003) or discrimination (e.g., Esser, 1998; von Stebut and Schmidt, 2001; Winter et al., 2003) can be computed from go/no-go, yes/no or choice (2-AFC) experiments using signal detection theory (reviewed in Moss and Schnitzler, 1995). In go/no-go experiments, performance is often standardized to a score from 0 (no hits) to 1 (100% hits with no false alarms) using the following formula (Koay et al., 1997, using a conditioned avoidance procedure):

$$\text{Performance} = \text{Hit Rate} - (\text{False Alarm Rate} \times \text{Hit Rate})$$

Threshold is then defined as the intensity at which the performance measure equals 0.5. For yes/no procedures, a threshold is computed in a similar way (see Hartley,

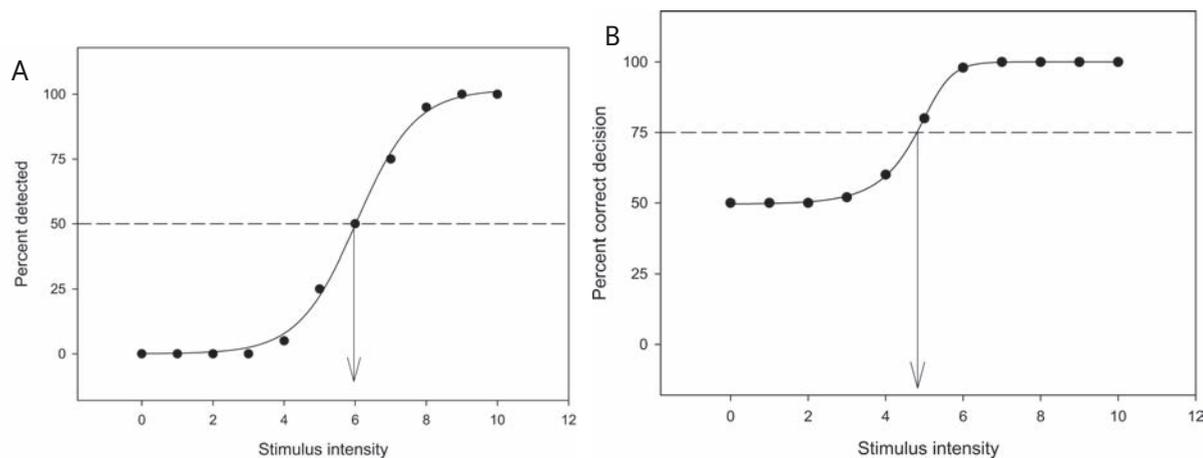


Figure 17.3. Psychometric functions. A, sample illustration of a psychometric function obtained from a go/no go experiment. Data can range from 0% to 100% detection. A sigmoidal curve is fitted to the data (dots). Threshold is usually assumed at 50% detection (dashed line) and read from the stimulus intensity axis (arrow). B, psychometric function obtained from a 2-AFC experiment. Data can range from 50% (chance level) to 100% detection. A sigmoidal curve is fitted to the data (dots). Threshold is usually assumed at 75% correct decisions (dashed line) and read from the stimulus intensity axis (arrow).

—1  
—0  
—+1

1992, for details and discussion). It is a general convention to accept 50% detection as an “arbitrary” absolute threshold for a psychometric function as obtained from this type of psychophysical approach (Fig. 17.3A; Engen, 1972; Levine, 2000; Schiffman, 2001).

For choice experiments with two alternatives, the chance level for correct decisions is 50% (33.3% for three alternatives, etc.). Thus, one should test whether the bat’s performance deviates significantly from this 50% chance level. In simple choice experiments or preference tests, this can be done separately for each experimental subject with a binomial test (Hare et al., 2002) or a chi-square test (use Fishers exact test for small sample sizes). If one has good biological reasons to a priori assume that the bats will perform at or above chance level but not worse than chance, it may be justified to use a one-sided test design. If every subject performed the same number of trials, one could pool the data and test the mean number of correct choices against the number of correct choices expected by chance (Hare et al., 2002).

In contrast to simple choice or preference tests, psychophysical 2-AFC experiments test performance for a series of stimulus intensities. Classically, the threshold is assumed at 75% correct decisions (Fig. 17.3B; for bats, see Moss and Schnitzler, 1995; Esser, 1998; for a general review, see Levine, 2000). A sigmoidal curve is fitted to the data to interpolate the 75% value and to obtain a corresponding error estimate (Schmidt, 1995). For further general reading on classical psychophysical testing methods and data analysis, in-depth discussions can be found in Engen (1972) and Moss and Schnitzler (1995).

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