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An IACUC Perspective on Songbirds and Their Use in Neurobiological Research

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Abstract

Laboratory research using songbirds as a model system for investigating basic questions of neurobiological function has expanded rapidly and recently, with approximately 120 laboratories working with songbirds worldwide. In the United States alone, of the approximately 80 such laboratories nearly a third have been established in the past 10 years. Yet many animal facilities are not outfitted to manage these animals, and as a consequence laboratories often use alternative housing arrangements established by institutional animal care and use committees (IACUCs). These committees invariably differ in their expertise level with birds and thus guidelines also vary considerably from one institution to another. In this article I address a number of factors to consider for effective oversight of research involving songbirds.

Overview: Songbird Research, Species Diversity, and Implications for Oversight

Research use of songbirds falls into three broadly defined areas: vocal learning and production (the most popular areas in terms of number of labs; Aronov et al. 2008; Mooney 2009; Schmidt 2009; Suthers and Margoliash 2002), hormonal mechanisms of adult (seasonal) plasticity and neurogenesis (Nottebohm 2004; Remage-Healey et al. 2010), and sexual dimorphism of brain and body (Wade and Arnold 2004). Because much of this work seeks to link brain mechanisms with behavior, songbird laboratories often cover a wide range of experimental procedures, such as neural recordings in brain slices, pharmacological manipulations in awake behaving animals, and genomewide screens of signaling cascades associated with aspects of vocal behavior. The need to link brain function to behavior in songbirds, and in particular in the zebra finch (*Taeniopygia guttata*), a favorite model in songbird research, has pushed the development of technological methods to record and manipulate brain function in these small (~14 g) birds to extreme levels. For example, new technologies enable the recording of airflow in the trachea of singing birds (Goller and Cooper 2004), action potentials from identified neurons in vocalizing juvenile zebra finches (Aronov et al. 2008), and, using miniaturized wired or wireless devices, neural activity in birds interacting in a natural social setting (Schregardus et al. 2006).

One of the great attractions of studying songbirds is their enormous diversity—among all species of birds approximately half (~5,000) are songbirds (order Passeriformes) (Sibley and Monroe 1990). Identifying the different strategies with which different species of songbirds learn and produce their song can be tremendously informative. The field is still relatively young—most work at the neural level originated from a series of seminal papers in the

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mid-1970s (Kelley and Nottebohm 1979; Nottebohm and Nottebohm 1976; Nottebohm et al. 1976). Many of the early studies used canaries (*Serinus canaria*), but over the past 20 years most labs have switched to zebra finches and recently labs have begun to complement this work with studies in different species to address scientific questions that are not possible in zebra finches or are better addressed in other species.

The introduction of diverse species of songbirds in the laboratory means that a one-size-fits-all model for songbird care will not work. In addition to the obvious differences in dietary needs, different songbird species have different husbandry requirements (Bateson and Feenders 2010, in this issue). Some birds, for example, perform well in relative solitude while others require housing conditions that at least partly mimic their normal social structure.

The changing landscape of songbirds in research from use of a single experimental species to a greater number of species calls for flexibility in IACUC oversight of songbird research. Institutional oversight should also be adaptive to appropriately monitor different species. In addition, with an increasing number of laboratories recording and manipulating (both genetically and pharmacologically) brain function in awake behaving birds, it is important to understand the strengths and weaknesses of different species in their tolerance of the various manipulations.

In this essay I highlight some of the issues related to the care and maintenance of songbirds in the laboratory. I focus mostly on zebra finches but try, when possible, to include concepts that would apply to other species as well. I do not address issues concerning parrots, which are also used as animal models for vocal production and learning, but refer the reader to an excellent article on parrot husbandry in this issue (Kalmar et al. 2010). I conclude with a recommendation for the development of standardized care and maintenance procedures that will preserve the flexibility and accommodation of different needs across multiple species of songbirds.

Maintenance and Care of Songbirds

The housing and care of songbirds vary considerably depending on the species involved. In this section I describe considerations for the care and maintenance of songbirds, in particular zebra finches, used for experimental studies in the neural and behavioral sciences. Bateson and Feenders (2010) provide a full discussion of general husbandry for songbirds; in addition to common issues such as diet, light cycle, and housing, they stress the importance of an adequate social setting for captive birds because it can directly affect health and behavior and consequently the neural circuits that drive the animals' behaviors.

Captive bird species most commonly used for neurobiological research include, in order of popularity, the zebra finch, canary, and other small finches such as the Bengalese finch (*Lonchura striata domestica*; also known as society finches). The popularity of zebra finches as a model animal is due in part to the ease of breeding and maintaining them in captivity. They also sing a single, stable, and highly stereotyped song all year long and are very resilient to experimental manipulation (Immelmann 1969; Sossinka and Boehner 1980; Yu and Margoliash 1996). Reasons for choosing other birds vary but include the fact that some birds sing a single song that has variable song syntax (e.g., the Bengalese finch) while others have a large repertoire of different songs (Sober et al. 2008). Each of these behavioral variants offers advantages depending on the scientific question(s) under study.

Many investigators have also started studying birds not raised in captivity; the following commonly used birds are caught in the wild and then brought to the laboratory: European starlings (*Sturnus vulgaris*), song sparrows (*Melospiza melodia*), swamp sparrows

(*Melospiza georgiana)*, white-crowned sparrows (*Zonotrichia leucophrys*), brown-headed cowbirds (*Molothrus ater*), and brown thrashers (*Toxostoma rufum)*. Use for experimentation can range from the day of capture to extended periods (up to years). The maintenance and care of songbirds is therefore a function of both species and time (e.g., housing for a single day may differ greatly from long-term housing).

Breeding versus Purchasing Songbirds

Researchers may purchase their birds from large local bird farms or breed them in their laboratories. Breeding is in many ways preferable because the experimenter can control housing density, diet, and light cycle. Even more important for many researchers is the fact that they have a relatively good understanding of each bird's acoustic environment and may even know the exact history of a bird's exposure to song. Because all of these factors (from diet to song exposure) have profound effects on a bird's ability to copy a tutor song and produce a “normal” song as an adult (Nowicki et al. 2002; Zann and Cash 2008), having a good knowledge of these facts and the ability to control them can have significant consequences for experimental interpretation.

Unfortunately, in most cases in-house breeding can be prohibitively expensive as the experimenter must pay husbandry costs until sexual maturity, which in zebra finches occurs between 120 and 150 days posthatch. Costs are usually about $2/cage/day and maximal housing per conventional cage is typically limited to 5 birds, so a single 150-day-old bird will cost a laboratory approximately $60. For many experiments, this cost is well justified if the details of song tutoring are critical to the investigator.

Laboratories often cannot justify this high cost and choose to purchase adult birds from large bird farms, reducing the cost to $8 or $10 per bird. This approach raises two problems, one pragmatic and the other experimental. The first problem is that with the increase in laboratories using zebra finches over the past decade, availability from the relatively few bird farms can unexpectedly become limited, resulting in delivery delays of up to several weeks and slowing research. This problem could be exacerbated by further growth in the number of laboratories using zebra finches or by more aggressive targeting of bird suppliers by animal welfare organizations, a phenomenon that began in 2008.

The second, perhaps more serious concern is that many bird farms receive their supply of zebra finches from local breeders whose identity and thus breeding conditions are unknown. The result may be uneven quality of birds, a factor that is less obvious in the birds' health or plumage than in their songs. The uneven quality suggests that the birds probably experienced a suboptimal social environment, a potentially serious general concern because it may directly affect experimental outcomes. Specifically, evidence that early experience shapes adult plasticity and learning (Peña and DeBello 2010) suggests that the results of many recent studies of adult vocal plasticity (e.g., Fee and Scharff 2010) could be confounded by heterogeneity in animal background. Use of “low-quality” birds may also lead to an increase in the number of animals used in order to achieve a statistically relevant cohort—an outcome that conflicts with the “Three Rs” (replacement, reduction, and refinement; Russell and Burch 1959).

A possible solution to the problem of uneven quality in birds from breeders might be the development of bird farms dedicated to raising birds for research purposes only, where the quality of song exposure, diet, and social context are well controlled. Similar repositories and production facilities exist for rodents and might therefore be sustainable for songbird research as this field of research expands. Such an approach would likely raise purchase costs substantially, but would provide important benefits, including better-quality animals for researchers and therefore the need to use fewer animals.

Need for Common Procedural Guidelines for Songbirds

There is a need for some degree of commonality—for example, standard operating procedures—for the maintenance and care of zebra finches and other songbirds. Federal regulations and policies are routinely updated but often relatively stable, whereas the same cannot be said for their interpretation at the institutional level, where procedures and practices can vary significantly and seemingly arbitrarily from one institution to the next. Procedures and practices are often determined by the attending veterinarian and the IACUC, neither of which may have expertise in songbirds. For a variety of very good reasons, many research institutions are "rodent-centric," which is good for rodent users but creates difficulties for scientists using nonrodent research models.

The need to develop songbird-specific standards is well illustrated by the way temperature and humidity are regulated in many animal facilities. These two environmental conditions are typically held constant according to their use for rodents; indeed, conditions for birds often seem to be modeled after those for rodents. Temperatures are typically allowed to fluctuate by only a few degrees before alarms are set off. However, although a temperature of 75°F is not harmful for a zebra finch it is not ideal either, especially for breeding birds that usually like humid and hot conditions. Given that zebra finches are desert birds accustomed to large fluctuations in both humidity and temperature—temperatures in the desert can range from −1°C (30°F) to 45°C (113°F), with typical daily fluctuations, especially in the winter, reaching 25°C (~40°F) (Zann 1996)—strict requirements for constant environmental conditions are certainly not necessary and are possibly even suboptimal for maintaining healthy zebra finches. The *Guide for the Care and Use of Laboratory Animals* (NRC 2010) offers general guidance on the negative impacts of inappropriate ranges of temperatures and humidity, but offers little commentary on the great flexibility of many species (e.g., songbirds) to remain healthy in a great variety of micro- and macroenvironments.

This is but one illustration of the inapplicability of rodent standards for birds. This example also highlights the importance of understanding the behavioral ecology of different bird species housed in aviaries—large temperature fluctuations might be beneficial for zebra finches but not for other birds.

Veterinary Oversight and Training

A significant and increasing number of laboratories are using birds caught in the wild. IACUCs may have little familiarity not only with such species but also with field capture techniques. Here again, the establishment of guidelines for the four or five avian species most commonly used in the laboratory would be beneficial both for the laboratories that use these birds and for the institutions that oversee research involving them. Guidelines with clear requirements for conditions would also greatly benefit laboratories that might wish to start working with wild-caught birds.

One of the concerns about research with wild-caught birds is that campus veterinarians usually have little or no experience in avian medicine and even less experience with wild birds. Many investigators with years of experience working with wild-caught birds have remarked that it is hugely beneficial to help veterinarians that lack experience with these animals learn how to manage individual bird illness or disease outbreaks. In many cases the first signs of illness in wild birds may be subtle (e.g., sustained sitting on the perch or bottom of the cage, fluffed feathers, difficulty breathing) and recognizing them in time to successfully treat the birds requires husbandry personnel who are well trained and experienced with birds.

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The Public Health Service policy has language about facilities being managed by a "scientist trained and experienced in the proper care, handling, and use of the species," so it is advisable that scientists whose knowledge of "their" species exceeds that of the veterinarian participate both in the maintenance of the species and in the training of laboratory animal care staff involved in bird handling.

One strategy that has been effective in the care of songbirds, and in particular wild-caught birds, is to encourage facility veterinarians to consult with outside veterinarians who have training and experience with exotic species. It is important to bear in mind, though, that many institutions need to have an approved Animal Welfare Assurance filed with the Public Health Service and that all clinical management and care of university-owned animals must therefore be overseen by the institution's attending veterinarian, even if performed by an outside veterinarian.

Experimental Procedures and Behavioral Testing in Songbirds

Experimental procedures in the behavioral and neural sciences cover a wide range of techniques. Due to the nature of the types of questions addressed, many procedures (survival and nonsurvival) in songbirds involve direct or indirect manipulation of brain tissue. Nonsurvival procedures include removal of the brain for in vitro slice physiology or for mapping of gene expression patterns, or the recording of neural activity in anesthetized birds that are then sacrificed for histological reconstruction. For much of this research, existing guidelines are satisfactory. Survival procedures often involve surgery and the implantation of electrodes or other probes in the brain or airway for long-term recording or drug delivery. Some research may involve the creation of small lesions targeted to specific brain areas to assess effects on behavior for weeks to months. Survival procedures in nonbrain tissue might include removing the cochlea or cutting the nerve that innervates the vocal organ musculature.

In this section, I discuss two concerns for IACUCs regarding experimental procedures in songbirds. The first relates to anesthetic use, in which a "one-size-fits-all" approach is not a viable solution for many of the experiments performed in songbirds. The second issue concerns the recording of birds over long time periods.

Use and Choice of Anesthetics and Analgesics in Songbirds

Early experiments that required birds to be anesthetized for stereotaxically targeted brain lesions or injections did not concern themselves too much with how the anesthetics might alter brain function. Researchers typically used equithesin, a cocktail of chloral hydrate, magnesium sulfate, and sodium pentobarbital (Doupe and Konishi 1991) that is effective at providing anesthesia, is well tolerated by songbirds, and lasts for a duration that is useful for most experimental procedures. But as researchers began to probe auditory responses in areas of the brain that serve a sensorimotor function, it became clear that this drug is not viable because it suppresses neural activity in response to auditory stimulation (Doupe and Konishi 1991).

Use of Anesthetics When Recording Neural Response Properties—Research in the 1980s and 1990s revealed the importance of anesthetic choice when recording from the brain: many neurons in selected sensorimotor areas of the songbird's forebrain responded selectively to playback of a prerecorded version of the bird's own song (BOS) (Margoliash 1986)— and the selectivity of these responses was highly dependent on the type of anesthetic used (Vicario and Yohay 1993). Recent work has also shown that subtle changes in the bird's behavioral state can have a profound effect on the strength and selectivity of neural responses (Cardin and Schmidt 2003; Rauske et al. 2003): neurons in selected forebrain areas in sleeping birds or birds treated with certain anesthetics respond strongly to the BOS and very little, if at all, to auditory stimuli such as conspecific songs or artificial stimuli such as white noise or tones. In contrast, when the bird is awake, neurons tend to lose their selectivity to the BOS and become responsive to other auditory stimuli (Cardin and Schmidt 2003).

Because a bird's neural selectivity is strongly influenced by the mechanism of anesthesia or sedation, researchers have used a number of different drugs over the past decade: urethane (Lewicki 1996; Mooney 2000), mixtures of ketamine and xylazine (Schmidt and Konishi 1998), sedation with diazepam alone (Cardin and Schmidt 2004), melatonin to induce a sleep-like state (Hahnloser et al. 2002), and even, for the nonsurgical portion of a recording experiment, unanesthetized restrained animals (Vicario and Yohay 1993). Each of these approaches has advantages and disadvantages. Urethane, for example, probably provides the most robust neural selectivity to the BOS and has been used by many laboratories with great success. Because it is not metabolized, however, experiments using this drug must be nonsurvival surgeries. Additionally, urethane, a carcinogen, poses a risk to personnel working with it and requires oversight by the institution's health and safety group. Isoflurane, which works very well for other sorts of surgery in songbirds, does not support auditory responsiveness. Drugs such as diazepam and other benzodiazepines do not have analgesic properties and thus are not appropriate for surgical procedures but are critical for studies of the mechanism(s) by which behavioral state controls sensory responsiveness. In our own laboratory, we have found that brief arousing stimuli (such as a toe pinch or air puff) to a diazepam-treated bird temporarily but completely suppress auditory responsiveness. Interestingly, this effect is observed only in the forebrain sensorimotor areas, not in the auditory forebrain where responses remain stable throughout the transition from a sedated to an aroused state.

These examples illustrate the importance of anesthetic choice when undertaking procedures where the type of anesthetic might directly affect the neural properties being recorded. The fact that the effect on neural tuning is so dramatic in sensorimotor areas of the songbird forebrain and not in primary or secondary sensory areas suggests that behavioral state can have very profound effects on some types of neural processing and only limited effects on others. It also highlights the need to consider the experimental question being investigated when choosing the method of anesthesia.

Anesthetics for Nonneural Procedures—For many procedures the effect of brain state on neural responsiveness is not an immediate concern and a greater variety of anesthesia methods can be considered. A recent development implemented in many laboratories is the use of gas anesthesia with isoflurane to perform peripheral surgeries such as cochlear removal, electromyographic implants in syringeal muscles, and targeted brain lesions. In some cases, where selectivity of neural responses is not an issue, gas anesthesia has also been used for neural recording. One of the advantages of this method is that birds can be anesthetized relatively rapidly and returned to a nonanesthetized state with equal rapidity. Several laboratories have adapted isoflurane delivery masks to stereotaxic head holders for adult birds (Cooper and Goller 2006), and one laboratory has pioneered the delivery of isoflurane via cannulation of the air sac for cases in which a mask or intubation is not appropriate (Nilson et al. 2005).

For the above reasons and because isoflurane has consistent analgesic properties, IACUCs may tend to focus on it as a universal anesthetic for most potentially painful procedures (e.g., surgery, stereotaxis). It may indeed be appropriate for surgery when neural recording begins well after recovery, but alternative anesthetics must be considered because standard methods may interfere with the science, a concern that the IACUC must consider.

Determining the right anesthetic for the songbird and for the procedures performed must involve the close collaboration of the scientist, veterinarian, and IACUC to ensure both the highest level of welfare for the anesthetized animal and the scientific validity of the model.

Longitudinal Studies in Songbirds

Much research in songbirds attempts to link singing behavior to its neural underpinnings by investigating the effects on song output of hormone manipulation (Meitzen et al. 2007), targeted gene knockdown (Haesler et al. 2007), intracerebral drug infusion (Olveczky et al. 2005), or brain lesion in select brain areas over many consecutive days of singing (Kao and Brainard 2006; Meitzen et al. 2007). Such research typically requires longitudinal studies that can last days or months, especially when studying vocal learning, to cover the period of vocal ontogeny during which juvenile songbirds learn their song, typically from 25 to about 120 days posthatch (Derégnaucourt et al. 2004; Sossinka and Boehner 1980). Important factors in effective longitudinal studies are appropriate housing, husbandry, and monitoring as well as the use of technological resources that can enhance both animal welfare and scientific results.

Housing, Husbandry, and Monitoring—A key component of successful song recording over long time periods is obviously the creation of conditions that are favorable for singing behavior. For zebra finches, if the research requirement is simply to collect song, relatively little effort is necessary as these birds sing abundantly in cages even in the soundproof chambers that are necessary to ensure high-quality acoustic recordings. In many cases these birds sing hundreds of songs a day even when alone in the cage. For other species, whose abundance of song in a small cage setting, especially in a soundproof chamber, is diminished, a number of strategies (e.g., placement of females in the cage, possibly along with other males) are available to enhance song production. Researchers have also boosted song production levels in male birds by implanting small subcutaneous testosterone pellets (Meitzen et al. 2007).

Most longitudinal avian experiments require long-term specialized housing of experimental animals. Because such an arrangement is not always possible in the standard animal facility, songbirds are likely to be housed in the laboratory, where video and audio recording instrumentation are available. Furthermore, carrying out such experiments in animal facilities away from the laboratory can result in substantially lower-quality experimental conditions, so it is in the interest of the researcher to work closely with the IACUC to get approval to establish satellite facilities in the laboratory.

One of the most important concerns in longitudinal studies is that birds have adequate access to food and water throughout the experiment. This is critical since many of these birds are tethered for periods of hours or days at a stretch and therefore need to be monitored continually, ideally with a camera and video monitor. One potential issue in some of the experiments is that manipulations that target brain areas that control song could adversely affect song output. This might in fact be an expected outcome but how should one distinguish it from discomfort? Because song output and the postural displays associated with singing appear to be controlled by nonoverlapping systems (Feenders 2008), singing can be abolished while postural movements associated with singing are maintained. Under these conditions, video monitoring of song posture is a useful and appropriate way to monitor a bird's comfort level.

Technological Resources—With the increasing ease of collecting and storing data digitally and the availability of sophisticated software to analyze song features, longitudinal studies of song over weeks and months, especially in juvenile birds, have revealed a great deal about the dynamics of circadian changes in behavior (Derégnaucourt et al. 2005) and of vocal learning (Derégnaucourt et al. 2004). Combining physiological recordings with singing is much more challenging because the hardware necessary for the recordings naturally affects the bird's freedom of movement and possibly its well-being. Because of the small size of zebra finches there has been a tremendous amount of innovation to miniaturize devices to record from birds in the least invasive way possible. One impressive development, for example, is the implementation of miniature motorized microdrives that weigh less than a gram and can move electrodes with micrometer precision (Fee and Leonardo 2001). It nonetheless remains critical to ensure that birds in these experiments have the ability to move freely. Fortunately, one of the best measures of a bird's well-being is its ability and willingness to sing. Birds typically do not vocalize if they feel discomfort.

Concluding Remarks

Research on songbirds has dramatically increased over the past decade, yet many universities, veterinarians, and IACUCs have little to no experience with songbirds and training for users is often modeled on procedures for rodent research. As a consequence, protocols are defined locally based on varying degrees of expertise and tend to differ significantly across institutions—what is sometimes acceptable at one location may not be at another. This inconsistency not only is frustrating for individual researchers but may also make it more difficult to compare experimental findings between laboratories.

In this article I have addressed some issues in the husbandry and care of captive and wild-caught birds. I have also discussed the use of anesthetics in songbirds. For both of these issues it is clear that no single solution fits all. As with all experimental animals husbandry procedures must take into account the fact that different species have different needs. With the use of anesthetics, research in songbirds has demonstrated that the choice of anesthetic needs to be carefully considered in the context of the experimental manipulation.

Notwithstanding all the idiosyncratic differences among species and individual animals, there is a real need for a set of basic operating procedures to standardize husbandry practices for the most commonly used songbird species. Such procedures would include guidelines for acquiring, housing, and breeding birds; for using anesthetics, with justifications of when and how to use them; and for ensuring the care of animals during longitudinal studies. The guidelines should also address procedures that routinely raise “red flags” with an IACUC, such as satellite housing and requests for not autoclaving hormone pellets, among others.

The development of standardized procedures should in part be community based with input from individual investigators but should ultimately be the task of a committee of experienced investigators and veterinarians with specialization in laboratory animal medicine and significant experience with birds. Such a committee should also consider the possibility of developing bird production facilities dedicated to raising birds for research purposes only, where the quality of song exposure, diet, and social context are clearly defined and well controlled.

The existence of standard operating procedures would greatly facilitate the job of IACUCs and would also be tremendously helpful for junior faculty trying to set up their laboratories at universities that have little or no experience working with songbirds. Finally, these measures would increase the quality of care for the animals and thus increase the uniformity of research conditions, ultimately leading to reduced and more efficient use of animals.

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