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Difficulties and special issues associated with field research in behavioral neuroendocrinology

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Abstract

Classical behavioral neuroendocrinology has focused on a limited number of domestic mammals and birds. The model systems used in these studies represent a very small proportion of the diversity of hormone–behavior interactions found in nature. In the last three decades, an increasing number of researchers have concentrated their efforts on studying behavioral neuroendocrinology of wild animals. Field behavioral neuroendocrinology presents a series of challenges ranging from the design of the experiments to sample preservation and transportation. The constraints of field conditions limit the number of factors that can be controlled for and the questions that can be addressed. On the other side, many behaviors can be studied only in the field, and only a few species can be kept in captivity. Thus, field studies are necessary to understand the complexity and variety of interactions between hormones, brain, and behavior. In this article, we will review some of the peculiarities and challenges of field behavioral neuroendocrinology, including solutions for some of the most commonly encountered technical issues.

Keywords: Behavioral neuroendocrinology; Wild animals; Field experiment

Introduction

Most studies in behavioral neuroendocrinology are conducted in a laboratory setting. In the laboratory, variables such as climate, nutritional condition, social interactions, dominance, and reproductive status can be controlled. However, in contrast to captive animals, free-ranging populations are exposed to a wide array of ecologically relevant environmental and social stimuli. Thus, free-living animals experience a rich suite of complex interactions with their environment, which results in equally complex neuroendocrine responses. Consequently, laboratory studies face the quandary of producing reliable data under controlled conditions while severely limiting the exposure to natural stimuli and thus the expression of the complete set of behavioral and neuroendocrine traits. For example, wild dusky-footed woodrats (*Neotoma fuscipes*) build 'houses' from twigs that are essential for survival and may be used by generations of woodrats (Monaghan and Glickman, 1992). When the territorial behavior of intact and castrated woodrats was compared in an open field test, both groups of woodrats fought with the same intensity, and the likelihood of becoming dominant was equal, suggesting that territorial behavior was independent of testosterone (Monaghan and Glickman, 1992). However, when the researchers changed the setting and offered the woodrats a 'house', intact woodrats fought more intensively and were more likely to be dominant compared to castrated conspecifics. Hence, in the context of defending a "house", testosterone played a role in the control of territorial behavior, but it appeared to be less important in regulating aggressive behavior displayed in an open field test.

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Not only can the context of behavior in captivity differ from that in the wild, captivity itself can also have tremendous effects on behavior and physiology. For example, deficits in social experience during ontogeny can cause abnormal behavior in the black-headed gull (*Larus ridibundus* Groothuis and Vanmulekom, 1991). Furthermore, in both mammals and birds, behavioral deprivation may alter brain structures (see Barnea and Nottebohm, 1994; Healy et al., 1996; Rosenzweig and Bennett, 1996). For instance, hippocampal volume is reduced in captive as compared to free-living dark-eyed juncos (*Junco hyemalis* Smulders et al., 2000). Furthermore, comparative studies indicate that testosterone levels are generally higher in free-living than in captive birds (Wingfield et al., 1990), and the identical pharmacological treatment results in different behavioral responses in free-ranging as compared to captive male European stonechats (*Saxicola torquata rubicola*) (Canoine and Gwinner, 2002a,b). Hence, deprivation of important environmental and social cues, restraint in space, a limited nutritional spectrum, absence of predation and its perceived risks (Bednekoff and Lima, 1998), and many other factors can dramatically influence the neuroendocrinological and behavioral output of an organism in the laboratory (Künzl and Sachser, 1999). These findings emphasize the need for field experiments in behavioral neuroendocrinology. Or, as Fernando Nottebohm put it: "Unless you understand the needs, the habits, the problems of an animal in nature, you will not understand it at all. Take nature away and all your insight is in a biological vacuum." (in Specter, 2001). However, field experiments come with their own set of drawbacks and challenges. The purpose of this review is to highlight some of the issues that this group of researchers has come across while conducting field studies in behavioral neuroendocrinology on vertebrates (mostly birds) in various locations around the world. We also offer specific recommendations to overcome logistical problems frequently encountered in field neuroendocrine research.

Experimental design

Field studies often generate unique data on the neuroendocrinology of a wild animal in its natural habitat but face the problems discussed above. Almost everything changes all the time in the environment of a wild animal and can introduce considerable variability in the data set, e.g., climate, nutrition, or dominance status, to name but a few. Thus, field experiment needs to be designed carefully to obtain meaningful results in light of the anticipated variability in the data.

Designing field studies

Small samples sizes (6–10 or lower) are common in field behavioral neuroendocrinology due to the need to limit the impact on wild populations. Final sample sizes can be further reduced due to the difficulty of observing and catching wild animals. Small sample sizes and high variability in the data make careful experimental design a crucial part of successful neuroendocrine field studies. A simple design with only few treatment groups is therefore advantageous because it will maximize the power of statistical tests. Examples include comparisons between reproductive states (Canoine and Gwinner, 2002a; Foidart et al., 1998), sexes (Schultz and Schlinger, 1999), phenotypes (Miranda et al., 2003; Schlinger et al., 1999; Wikelski et al., 2005), or endocrinologically manipulated versus control animals (Romero et al., 1998; Semsar et al., 2001; Soma et al., 2002). In some experiments, a repeated-measures design is feasible where the same individual is measured repeatedly under different conditions or before and after a treatment. This can also be a means to improve the power of detecting differences in a data set with a low sample size. However, one problem often encountered in field studies using a repeated-measures design is that not all individuals can be recaptured each time. At the same time, additional data points may be available from individuals that originally were not included in the study. Attempts at solving this issue statistically include estimating missing data from repeated measurements, combining data from different time points, taking the mean of repeated measures for each individual, or randomly choosing just one measurement for each individual for statistics on independent data (Hau et al., 2002, 2004b; Sands and Creel, 2004).

Conducting field studies and coping with stochastic events

For most studies, animals will have to be caught at some time to obtain a biological sample (e.g., blood or tissue). In most laboratory studies, animals are habituated to human disturbance and handling and can swiftly be removed from their cages. However, to catch an individual in the field can take a considerable amount of time and effort and can become the most challenging aspect of the study (e.g., Hau et al., 2004a). The various methods of catching animals all have both benefits and drawbacks. In bird research, it is common to use Japanese mistnets to capture birds (but there are many other methods, see Bub, 1995). In many instances, passive mistnetting—without attracting the bird to the net—is an excellent method. In some situations, however, the bird needs to be attracted using social stimulation either because the sample needs to be obtained at a certain time (e.g., immediately after a behavioral observation Hau et al., 2004a) or because passive capture rate is exceedingly low (Wikelski et al., 2000). A typical social attractant is the playback of conspecific song with or without simultaneous presentation of a decoy (Wingfield, 1985). Social stimulation in itself, however, can stimulate hormone secretion within minutes (Oliveira et al., 2001; Wingfield and Wada, 1989), which in turn might affect a range of physiological parameters. If the time after which hormones increase due to social stimulation