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Hormones and Behavior xx (2005) xxx – xxx

Hormones
and Behavior

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Difficulties and special issues associated with field research in behavioral neuroendocrinology

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Received 2 March 2005; revised 26 April 2005; accepted 3 May 2005

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 samples 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.

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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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56 Not only can the context of behavior in captivity differ
 57 from that in the wild, captivity itself can also have
 58 tremendous effects on behavior and physiology. For
 59 example, deficits in social experience during ontogeny can
 60 cause abnormal behavior in the black-headed gull (*Larus*
 61 *ridibundus* Groothuis and Vanmulekom, 1991). Further-
 62 more, in both mammals and birds, behavioral deprivation
 63 may alter brain structures (see Barnea and Nottebohm,
 64 1994; Healy et al., 1996; Rosenzweig and Bennett, 1996).
 65 For instance, hippocampal volume is reduced in captive as
 66 compared to free-living dark-eyed juncos (*Junco hyemalis*
 67 Smulders et al., 2000). Furthermore, comparative studies
 68 indicate that testosterone levels are generally higher in free-
 69 living than in captive birds (Wingfield et al., 1990), and the
 70 identical pharmacological treatment results in different
 71 behavioral responses in free-ranging as compared to captive
 72 male European stonechats (*Saxicola torquata rubicola*)
 73 (Canoine and Gwinner, 2002a,b). Hence, deprivation of
 74 important environmental and social cues, restraint in space,
 75 a limited nutritional spectrum, absence of predation and its
 76 perceived risks (Bednekoff and Lima, 1998), and many
 77 other factors can dramatically influence the neuroendocri-
 78 nological and behavioral output of an organism in the
 79 laboratory (Künzl and Sachser, 1999). These findings
 80 emphasize the need for field experiments in behavioral
 81 neuroendocrinology. Or, as Fernando Nottebohm put it:
 82 “Unless you understand the needs, the habits, the problems
 83 of an animal in nature, you will not understand it at all. Take
 84 nature away and all your insight is in a biological vacuum.”
 85 (in Specter, 2001). However, field experiments come with
 86 their own set of drawbacks and challenges. The purpose of
 87 this review is to highlight some of the issues that this group
 88 of researchers has come across while conducting field
 89 studies in behavioral neuroendocrinology on vertebrates
 90 (mostly birds) in various locations around the world. We
 91 also offer specific recommendations to overcome logistical
 92 problems frequently encountered in field neuroendocrine
 93 research.

94 Experimental design

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

104 *Designing field studies*

105 Small samples sizes (6–10 or lower) are common in field
 106 behavioral neuroendocrinology due to the need to limit the

107 impact on wild populations. Final sample sizes can be
 108 further reduced due to the difficulty of observing and
 109 catching wild animals. Small sample sizes and high
 110 variability in the data make careful experimental design a
 111 crucial part of successful neuroendocrine field studies. A
 112 simple design with only few treatment groups is therefore
 113 advantageous because it will maximize the power of
 114 statistical tests. Examples include comparisons between
 115 reproductive states (Canoine and Gwinner, 2002a; Foidart
 116 et al., 1998), sexes (Schultz and Schlinger, 1999), phenotypes
 117 (Miranda et al., 2003; Schlinger et al., 1999; Wikelski et al.,
 118 2005), or endocrinologically manipulated versus control
 119 animals (Romero et al., 1998; Semsar et al., 2001; Soma
 120 et al., 2002). In some experiments, a repeated-measures design
 121 is feasible where the same individual is measured repeatedly
 122 under different conditions or before and after a treatment.
 123 This can also be a mean to improve the power of detecting
 124 differences in a data set with a low sample size. However,
 125 one problem often encountered in field studies using a
 126 repeated-measures design is that not all individuals can be
 127 recaptured each time. At the same time, additional data
 128 points may be available from individuals that originally
 129 were not included in the study. Attempts at solving this issue
 130 statistically include estimating missing data from repeated
 131 measurements, combining data from different time points,
 132 taking the mean of repeated measures for each individual, or
 133 randomly choosing just one measurement for each individ-
 134 ual for statistics on independent data (Hau et al., 2002,
 135 2004b; Sands and Creel, 2004).

Conducting field studies and coping with stochastic events 136

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

161 is known, care can be taken to capture the subject before the
162 critical time (Wikelski et al., 2003a). Else, one needs to resort
163 to another method of capture or at the very least keep detailed
164 records to later account for these issues with statistical tools
165 (for example, see Dittami and Gwinner, 1990). An efficient
166 passive capture method for many vertebrates consists of
167 walk-in traps, which may contain a food bait as an attractant.
168 However, even such apparently benign traps have their
169 drawbacks as they can impose stress on the animals once
170 they realize they are trapped (Place and Kenagy, 2000;
171 Romero and Romero, 2002). Additionally, the bait may alter
172 the endocrine responses of the trapped animal since it can
173 provide additional nutritional resources (e.g., Romero and
174 Romero, 2002). Even swift immobilization procedures such
175 as darting, often used in studies on wild mammals, can
176 influence the stress axis (Devilliers et al., 1995; Sapolsky,
177 1985).

178 After a subject is caught in the field, the time it takes
179 until the biological sample is obtained can be longer than in
180 laboratory studies. This could be because standard methods
181 take longer in difficult field conditions or because the
182 animal needs to be transported to the laboratory for further
183 procedures. However, the stress resulting from capture and
184 transport and the resulting increase in glucocorticoids,
185 which typically occurs within minutes after capture, can
186 alter the physiology of the individual (Martin et al., 2004;
187 Sapolsky et al., 2000; Wingfield et al., 1997). For example,
188 corticosterone can suppress the secretion of gonadal steroids
189 in a wide variety of vertebrates (Moore et al., 1991;
190 Wingfield, 1988). In some cases, testosterone may increase
191 after acute stress (Heiblum et al., 2000; Place and Kenagy,
192 2000; Sapolsky, 1986). Again, careful advance planning and
193 meticulous record taking are important. If an unstressed
194 condition is important for the experiment, taking the sample
195 within 2 min is of the essence (Romero and Reed, 2005;
196 Romero and Romero, 2002). Alternatively, if the time
197 elapsed from capture to obtaining the sample is recorded,
198 one might be able to control for effects of stress on the
199 biological sample using regression statistics.

200 Despite careful experiment planning, there are many
201 ways in which natural events can impinge on a project. The
202 most constructive way of dealing with stochasticity is to
203 take advantage of unpredictable events as 'natural
204 experiments'. Examples in field endocrinology where
205 researchers have made excellent use of such natural experi-
206 ments include studies on the endocrine effects of inclement
207 weather (Smith et al., 1994; Wingfield et al., 1983), social
208 stimulation (Goymann et al., 2001; Wikelski et al., 1999),
209 environmental contamination (Wikelski et al., 2002), or
210 drought (Romero and Wikelski, 2001).

211 Special techniques required in field neuroendocrinology

212 Fieldwork has requirements that are very different from
213 those for laboratory studies and vary depending on the

geographic location of the study area, accessibility, available 214
facilities, species studied, etc. In this review, therefore, we 215
will focus on special technical requirements of field 216
behavioral neuroendocrinology studies. Typically, experi- 217
ments of this kind involve hormonal manipulations that may 218
need to be continued for a certain period. In the laboratory, 219
this can be achieved by repeated (i.e. daily) administration 220
of the substance. In the field, it is often difficult or 221
impossible to capture the same individual more than once 222
or twice. Thus, it is necessary to implant devices that deliver 223
drugs continuously. One of the most commonly used 224
devices consists of a piece of silicon tubing (Silastic 225
Medical Grade silicon tubing, Dow Corning Inc.) that is 226
filled with the substance to be administered after which the 227
two ends are closed with silicon glue. The tube is then 228
inserted subcutaneously and releases the substance for a 229
period that depends on the length of the tubing, the 230
thickness of the walls, and the amount of drug. The rate 231
of passage from the inside of the tubing to the outside, 232
however, depends mainly on intrinsic properties of the 233
substance itself, in particular on its lipophilic nature. Thus, 234
while silastic tubing is useful for lipophilic hormones like 235
steroid hormones, it cannot be used for hydrophilic 236
substances. If one or both ends of the tubing are left open, 237
silastic can be used also for hydrophilic substances, 238
however, in this case, the release is relatively fast. 239
Alternative drug delivery devices are osmotic pumps and 240
time-release pellets. The release rate of an osmotic pump is 241
more or less independent of the solubility properties of the 242
drug. However, the drug must form a stable solution for the 243
whole duration of the experiment. Osmotic mini-pumps 244
(Alzet, Charles River) which can release drugs for up to 4 245
weeks have been now used successfully in several studies 246
(Fusani et al., 2001a; Soma et al., 2000). Time-release 247
pellets are also a good way to deliver drugs for longer 248
periods (Fusani et al., 2003). Innovative Research of 249
America (Sarasota, Florida, USA) produces pellets for the 250
most common hormones and anti-hormones at several 251
dosages and release period length. The big advantage of 252
these pellets is that the drug is included in an organic matrix 253
that is slowly reabsorbed and thus it is not necessary to 254
recapture the animals to remove the implant like for silastic 255
tubing or osmotic mini-pumps. 256

257 Another limitation of field studies is the difficulty of
258 using invasive techniques in animals that need to be released
259 in their home area immediately after the treatment.
260 Procedures that involve long recovery times must be
261 excluded to avoid the risk of hypothermia, increased
262 predation, and social/territorial challenges to the exper-
263 imental subject. Sometimes it is not possible to observe the
264 animal in the hours following the treatment, therefore any
265 procedure that may potentially put at risk the health of the
266 animals should be first tested on few individuals when used
267 in a new species.

268 Experimental animals could be fitted with radio trans-
269 mitters to ascertain their location (Cochran et al., 2004; 269

270 Goymann and Wingfield, 2004) or even physiological
271 parameters such as heart rate and respiration, and these
272 techniques have just become available for the use on small
273 animals (Bowlin et al., in press).

274 Certain methods such as blood sampling of birds from the
275 wing vein have now become standard field procedures and
276 are relatively harmless provided that the experimenter has
277 been properly trained. In a few cases, researchers have
278 developed methods that transfer complex laboratory techni-
279 ques to the field. One such example is the method developed
280 by L. Michael Romero for intracranial drug administration in
281 wild songbirds (Romero et al., 1998). Many neuroactive
282 hormones and drugs do not pass the blood–brain barrier and
283 therefore cannot be administered peripherally. In the
284 laboratory, this can be bypassed by intracranial administra-
285 tion using a stereotaxic apparatus. Romero developed a
286 small, portable stereotaxic device that he used successfully to
287 inject neuropeptides into the third ventricle of small passer-
288 ine birds in harsh field conditions (Romero et al., 1998).

289 Technical issues in field neuroendocrinology

290 In the following sections, we will briefly review some
291 technical challenges commonly encountered in field neuro-
292 endocrinology. This is not meant to be an exhaustive review
293 but rather an overview of available techniques and solutions.

294 Field stations

295 A good field station or field base can greatly simplify
296 fieldwork. The best field stations can provide safe storage of
297 samples and equipment, bench space for sample processing,
298 electricity, water, telephone and Internet connection, and
299 above all the knowledge and experience of station managers
300 and other researchers. In addition, the station manager can
301 provide researcher with updated information about research
302 permits, safety of study areas, and contacts with local
303 authorities. Thus, the availability of a field station should be
304 highly valued when planning a field study.

305 Blood sampling and processing

306 Blood sampling is probably the most common procedure
307 in field neuroendocrinology studies. Sampling methods
308 differ according to species, however, processing almost
309 invariably involves centrifugation and separation of plasma
310 for hormone measurements. There are portable centrifuges
311 on the market which can be operated with batteries (e.g.,
312 ZipSpin, LWScientific, Inc., Lawrenceville, GA) or even
313 manually (Armin Baack, Schwerin, Germany).

314 Tissue preparation

315 To maintain the structure of the tissue as intact as
316 possible, it is necessary to fix the samples. The fixative

rapidly penetrates the cell membranes and immobilizes the
macromolecular material. This can be done in several
ways:

Perfusion

The most commonly used method is intracardiac
perfusion with paraformaldehyde or neutrally buffered
formalin (e.g., Goodson et al., 2004; Leitner et al., 2001).
In the laboratory, a peristaltic pump is generally used, but at
many field locations, electricity may not be available.
Alternatives to peristaltic pumps are large syringes or
gravity. Generally, a good perfusion can be achieved by
hanging the bottles containing the formalin 2 m above the
ground. For some histological techniques, such as in situ
hybridization, it is essential to use sterile solutions and
materials (Leitner et al., 2001). In such cases, it is more
difficult to transfer laboratory methods to a field setting.
Hence, depending on the technique that will be later used to
process the tissue, the following alternative fixation
methods can be considered.

Acrolein

Recently, acrolein has been used to fix tissue in field
studies. Acrolein fixation is obtained by simple immersion
of the tissue in the fixative. We still know little about
potential interferences of acrolein with staining techniques,
i.e. immunohistochemistry. Hence, artifacts caused by
acrolein cannot be excluded (Butler et al., 1999). However,
the few studies that have used this fixative have provided
satisfactory results (Butler et al., 1999; Iturriza and Thibault,
1994; Maney and Ball, 2003). Acrolein is highly toxic but
could be a valid alternative to formalin in field work
situations when perfusion is unpractical.

Freezing

Rapid freezing is not only an alternative to fixation in
histological studies but also the choice method for proce-
dures such as enzyme assays or receptor binding. The best
results will be obtained using liquid nitrogen (Fusani et al.,
2001b; Soma et al., 1999). The block of tissue to be frozen
is placed on a small aluminum weighing dish that is floating
on the liquid nitrogen. Liquid nitrogen can be kept in special
containers for long periods of time, but transportation can be
a logistic challenge. Alternatively, tissues can be frozen on
dry ice (Canoine et al., submitted for publication; Ritters et
al., 2001; Schlinger et al., 1989). It is important that the
tissue freezes as quickly as possible to avoid formation of
water crystals that might alter the histological structure.
Thus, the dry ice should be kept in a deep styrofoam box so
that cold air will surround the tissue. This is critical when
working in hot climates. The best results can be obtained by
filling a metal container with ethanol and putting it in a
styrofoam box surrounded by dry ice. Evaporation of the
alcohol will decrease the temperature even further. The
tissue is frozen on a small aluminum weighing dish that is
floating on the ethanol.

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370 *Storage and transportation*

371 Tissue or plasma are best stored at very low temper-
 372 atures, i.e., -80°C or lower. Only few field biologists will
 373 be lucky enough to conduct their fieldwork at a place
 374 where they can store their samples in a -80°C freezer.
 375 Even if there is a freezer available at the field station,
 376 eventually the samples will have to be transported from
 377 there to the laboratory. After the hard work of properly
 378 collecting the samples, the transportation is a very critical
 379 step for the samples (and the investigator!). Transportation
 380 on an airplane can be particularly challenging because
 381 security regulations may require that the container is
 382 opened to inspect the content.

383 *Liquid nitrogen*

384 Liquid nitrogen is the best and easiest solution to store
 385 samples. Liquid nitrogen can be kept in special containers
 386 for long periods of time—from a couple of weeks up to 6
 387 months or longer, depending on the size and model of
 388 container—and should be seriously considered for storing
 389 tissue and plasma when conducting field studies in remote
 390 places. The initial expenses are high, and both the pressure-
 391 sealed container and the liquid nitrogen itself are not easy to
 392 handle. In many countries, liquid nitrogen can only be
 393 transported in special safety-containers. These are indis-
 394 pensable for transportation on airplanes. The disadvantage
 395 of these containers is that they do not allow storing liquid
 396 nitrogen for long periods of time. It is not allowed to
 397 transport liquid nitrogen in the passenger cabin of airplanes.
 398 Some airlines do not allow liquid nitrogen on board at all.

399 *Dry ice*

400 Dry ice is a good alternative for short-term storage and
 401 transportation, and handling is less problematic than with
 402 liquid nitrogen. The disadvantage is that dry ice evaporates
 403 rapidly and cannot be used to store samples for longer than a
 404 few days. Dry ice should be kept in a styrofoam container
 405 that is well closed but not sealed to avoid the building-up of
 406 pressure as the dry ice evaporates. Dry ice will last longest if
 407 stored at -80°C or at the lowest possible temperature.
 408 Transportation of samples on dry ice is simple, but not all
 409 airlines allow passengers to carry dry ice and if they do, they
 410 usually restrict it the amount to 2.5 kg. It is recommendable
 411 to enquire about dry ice restrictions before booking the
 412 flight.

413 *Transportation of animals*

414 A general problem of field studies is the difficulty to
 415 control the experimental conditions. If possible, it is
 416 desirable to set up an experimental room at the field site
 417 or nearby (e.g., [Hau et al., 2000b](#)). For studies involving
 418 specific recording equipment that cannot be taken to the
 419 field, it is necessary to transport the wild caught animals to
 420 the laboratory. Such complex studies were conducted, for

example, by E. Gwinner and his co-workers at the Max 421
 Planck Institute for Ornithology in Andechs (Germany) 422
 ([Fusani and Gwinner, 2004](#); [Gwinner and Scheuerlein,](#) 423
[1999](#); [Partecke et al., 2004](#); [Wikelski et al., 2003b](#)). 424
 Different bird species were caught in several parts of the 425
 world and transported back to the home laboratory. This is a 426
 very difficult task and requires long-term advance prepara- 427
 tions. Permits for catching, export, transportation, and 428
 import are essential. Quarantine regulations have to be 429
 fulfilled, and certificates from veterinarians are required. 430
 Furthermore, in some situations, special animal care is 431
 indispensable, for example, when working with bird 432
 hatchlings that need to be fed at frequent intervals. In such 433
 cases, the animals need to be with the passengers during the 434
 flight. In the past, the airlines usually gave permissions to do 435
 so, but recent changes in security policies render such 436
 undertakings increasingly difficult. Apart from the formal- 437
 ities that have to be dealt with in advance, the actual 438
 transportation of the animals has to be planned in detail. 439
 Special cages are required to reduce the space needs and to 440
 minimize the possibility of injury for the animals. The 441
 International Air Transport Association (IATA) provides 442
 guidelines for transportation of animals on airplanes which 443
 vary according to the species, and most airlines will not 444
 allow the animals to be boarded unless these guidelines are 445
 respected. Researchers should consider that transportation 446
 represents a stressor, especially for wild-caught animals, and 447
 this could have tremendous effect on their behavior and 448
 physiology during and after the trip. Thus, an appropriate 449
 period of acclimatization should elapse between the animals 450
 have reached the laboratory and the beginning of the 451
 experiments. 452

Conservation considerations 453

Neuroendocrinology studies on wild animals are some- 454
 times designed to improve our knowledge of the species 455
 reproductive physiology. In many cases, conservation itself 456
 can be the goal of the research (cf. [Cochrem](#), this issue). 457
 Manipulating wild animals in their natural habitat can have 458
 widespread consequences not only for the experimental 459
 individual itself, but also for its neighbors and potentially 460
 for its offspring. For example, testosterone-manipulated 461
 individuals increase their interactions with neighbors, who 462
 in turn increase their endogenous testosterone, possible to 463
 live up to the challenge ([Wingfield et al., 1990](#)). Similar 464
 effects can occur in females that live in dense and thus 465
 interaction-rich colonies ([Eising et al., 2001](#)). As maternal 466
 hormones deposited in the egg can significantly affect 467
 offspring phenotype in birds ([Schwabl, 1996](#)), it is clear that 468
 endocrine manipulations in the field can have long-term and 469
 even cross-generational consequences at least on local 470
 populations. Experimental manipulations will also have 471
 direct implications for individuals. Repeated capture can 472
 induce avoidance strategies in animals which again could 473

474 have negative effects on populations but also make
 475 recaptures more difficult reducing sample sizes. Manipu-
 476 lated individuals may also be preferentially selected by
 477 predators. Capture itself may affect the characteristics of the
 478 stress response because it can be perceived as a predation
 479 attempt (cf. Canoine et al., 2002; cf. Scheuerlein et al.,
 480 2001). Some neuroendocrinological experiments require
 481 terminal sampling of individuals. Obviously, such invasive
 482 studies cannot be conducted on populations that are
 483 endangered or small. Overall, ethical considerations should
 484 rank highly in the decision making process of whether and
 485 how a neuroendocrinological study should be conducted in
 486 the wild. Ethical concerns will also affect the decision of
 487 how many individuals will be used for the experiments.
 488 Sample sizes should take into account that field experiments
 489 have intrinsically more variation compared to laboratory
 490 experiments due to imponderabilities of environmental and
 491 social conditions. Furthermore, some animals will vanish for
 492 unknown causes (emigration, predation). Thus, to conduct
 493 solid experiments, field neuroendocrinologists need to plan
 494 for considerably larger sample sizes than would normally be
 495 necessary to achieve sufficient statistical power. Pilot
 496 experiments are therefore a strong requisite of any field
 497 study involving invasive techniques (in the broadest mean-
 498 ing). Fortunately, the ecological community has already put
 499 forward guidelines for the use of wild animals in research
 500 that should be adhered to (Oring et al., 1988). To date, the
 501 authors are not aware of neuroendocrinological studies that
 502 endangered or harmed local populations of wild animals, a
 503 tribute to the ethics of researchers in this field.

504 Permits and health considerations

505 An often overlooked complication for field experiments
 506 is the fact the multiple agencies need to be contacted for
 507 acquiring the necessary permits for catching animals, for
 508 experimental manipulations, and for terminal sampling. In
 509 the case the field study is conducted in a country different
 510 from that in which the samples will be analyzed, export and
 511 import permits for animal samples need to be applied for
 512 well in advance. Regulations not only differ between
 513 countries but also between regions, like among US states.
 514 Obviously, the primary and main concern of the regulatory
 515 agencies is the health and well-being of humans, domestic
 516 animals, and livestock that could be affected by animal-
 517 borne diseases introduced in the country via research
 518 samples. For example, mammalian and avian samples could
 519 potentially contain viruses that are detrimental to humans or
 520 livestock, such as West Nile or New Castle disease germs.
 521 Agencies are less concerned about fish and reptilian samples
 522 because fewer possibilities for cross-infections of livestock
 523 exist. Even if the possibility of cross-infections and spread
 524 of diseases is generally extremely rare, serious precautions
 525 should be taken, in particular in the packaging of samples
 526 for transportation.

Conclusions

In this brief review, we have addressed the most common
 issues associated with studying behavioral neuroendocrinol-
 ogy in the field. We have also provided solutions to some
 typical technical and logistic problems that we have
 encountered while conducting our studies. Despite the
 challenges presented by field studies, we believe that they
 are necessary for understanding the complex interactions
 between brain, hormones, and behavior in an evolutionary
 perspective.

Acknowledgment

We dedicate this paper to the late Ebo Gwinner, who
 impressed upon us the importance of studying behavioral
 endocrinology in the field. Ebo was a most inspiring and
 enthusiastic mentor for all of us.

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