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Glucocorticoid negative feedback as a potential mediator of trade-offs between reproduction and survival

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ABSTRACT

A large increase in glucocorticoid hormones can inhibit or completely shut down breeding in wild animals. Because of its critical role in reducing glucocorticoids after exposure to stressors, hypothalamic–pituitary–adrenal (HPA) negative feedback could be an important mediator of the ecological trade-off between investing limited resources into survival/self vs. reproduction/offspring. Although assessing negative feedback in a standardized way using injections of the synthetic glucocorticoid dexamethasone is a straightforward procedure, we show that several different approaches are used to report negative feedback in the literature, and then demonstrate that this can in turn affect the statistical results and conclusions of a study. We then review six specific predictions about adaptive within- and across-species patterns in glucocorticoids based on the relative costs and benefits of maintaining or abandoning breeding attempts when animals are faced with prolonged strong stressors, and examine evidence for these predictions in the context of HPA negative feedback. Thus far, evidence supporting these predictions for negative feedback is mixed, with the strongest evidence supporting a link between poor body condition and weak negative feedback in breeding animals. However, more research is necessary to assess the importance of changes in HPA negative feedback, especially in reptile, fish, and amphibian species. Furthermore, future research would benefit from reporting negative feedback ability in a standardized way, or at least making raw data available for the computation of alternate measures, to more easily compare studies in this growing area of research.

1. Introduction: What is glucocorticoid negative feedback, and why does it matter?

Wild animals must respond appropriately to all the different stressors they encounter in their day-to-day lives, including everything from predator attacks to severe winter storms. The physiological response to stressors includes two different integrated systems. First, a rapid arm (the "fight-or-flight" response) causes secretion of catecholamines mere seconds after a stressor that result in increased blood flow to brain, heart, lungs, and skeletal muscles and the mobilization of energy stores (Goldstein, 1987). Secondly, a slower arm (the glucocorticoid response) takes place over minutes to hours and helps animals continue to mobilize energy, suppresses non-essential functions, and prepares them for future stressors they may encounter (Sapolsky et al., 2000).

The glucocorticoid response involves a hormonal cascade (the hypothalamic–pituitary–adrenal or HPA axis; Fig. 1) that begins with higher brain areas signaling the hypothalamus to secrete corticosterone releasing factor, which causes the release of adrenocorticotropic hormone from the pituitary gland, which causes the rapid synthesis and release of corticosterone or cortisol (Cort) from the adrenal or interrenal glands. Cort binds to receptors throughout the body and causes major changes in gene expression that are critical for long-term adaptive responses to stressors (Sapolsky et al., 2000). Although helpful in the short-term, when activated for longer periods of time, the Cort response is also associated with a number of deleterious effects, including metabolic dysregulation, cognitive impairment, and suppression of reproductive and parental behaviors (Dallman et al., 2003; de Kloet et al., 2005; Wingfield and Sapolsky, 2003). Thus, the ideal Cort response to environmental challenges may represent a delicate balance: a rapid, robust hormonal response that can then be quickly shut down (i.e., hormones reduced back to baseline concentrations).

Cort has often been proposed as a key mediator of one of the most

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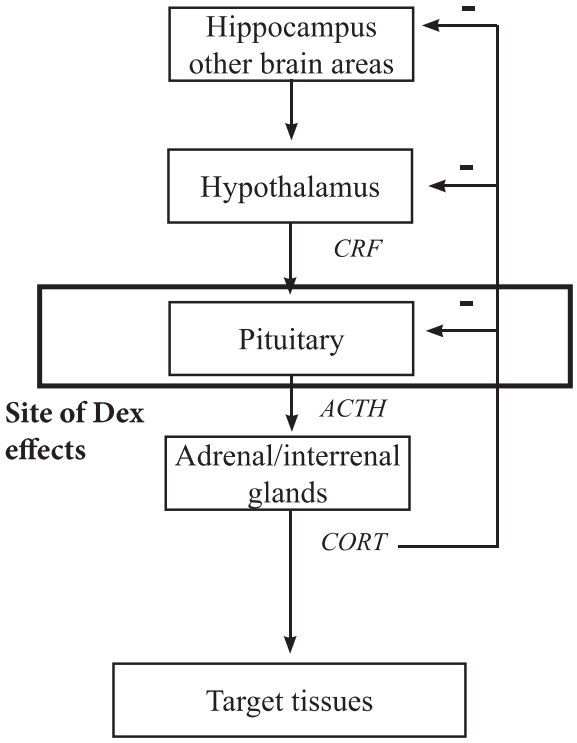


Fig. 1. The hypothalamic-pituitary-adrenal axis (hypothalamus-pituitary-interrenal axis in fishes and amphibians) and negative feedback. Higher brain areas including the hippocampus signal the hypothalamus to secrete corticosterone releasing factor (CRF), which causes the release of adrenocorticotropic hormone (ACTH) from the pituitary gland, which causes the rapid synthesis and release of corticosterone or cortisol (CORT) from the adrenals. Cort binds to receptors in different target tissues and has a number of effects on the body, including shutting down reproduction. Cort also binds to receptors in the brain and pituitary to shut down its own release. The synthetic glucocorticoid dexamethasone (Dex) can also stimulate negative feedback, but only at the level of the pituitary.

important ecological trade-offs all animals face – between investing limited resources into survival (self) vs reproduction (offspring) (Bokony et al., 2009; MacDougall-Shackleton et al., 2013; Wingfield et al., 1998). This can also be thought of as a trade-off between current and future reproductive attempts. Indeed, there is evidence that a large influx of endogenous or exogeneous Cort can cause wild animals to delay or abandon breeding (Boonstra et al., 1998; Silverin, 1986; Spee et al., 2011). Because exposure to high levels of Cort for prolonged periods of time causes reproductive failure, the HPA negative feedback system appears critical in mediating this potential trade-off. In negative feedback, Cort, the end product of the HPA axis, binds to receptors in the brain and pituitary to inhibit its own release (Herman et al., 1992; Jacobson and Sapolsky, 1991; Keller-Wood and Dallman, 1984). (Note that in this review, we will use the terms "glucocorticoid negative feedback" and "HPA negative feedback" interchangeably.) There is often wide variation in negative feedback ability among individuals, and this variation has been related to reproductive responses: for example, female tree swallows (*Tachycineta bicolor*) with weaker negative feedback were more likely to abandon their nests after stressors (Zimmer et al., 2019). Similarly, laboratory rat pups that experienced low rates of maternal care developed weaker negative feedback, stronger behavioral responses to stress, and low rates of maternal care with their own offspring (Liu et al., 1997; Meaney, 2001).

Because it determines an animal's overall Cort exposure in response to stressors, examining individual variation in HPA negative feedback ability could be an especially fruitful metric to understand adaptive ecological trade-offs between survival and reproduction in wildlife. However, at this point, data is limited, and wider assessment of negative feedback ability will be necessary across a wider range of ecological contexts and species. One major barrier to comparing results across studies is a lack of standardization in how negative feedback is quantified. In this review, we (1) discuss and compare the different approaches used to quantify HPA negative feedback in the literature; (2) provide recommendations for reporting negative feedback in future studies; (3) examine specific predictions about what we might expect to see if negative feedback helps adaptively mediate trade-offs between survival and reproduction; (4) summarize current evidence related to these predictions.

2. Different approaches to measuring and reporting negative feedback

2.1. Natural negative feedback and the dexamethasone suppression test

The capture stress protocol is one of the most widely-used methods to assess HPA function in wild animals (> 200 species to date) (Romero and Wingfield, 2016; Sapolsky, 1982; Wingfield et al., 1982). With this procedure, researchers use the effects of capture, handling, and restraint stress on animals as a standardized way to assess the stress response across different species. A blood sample taken within 2–3 min of capture reflects baseline Cort concentrations before they start to rise from the stress of capture and handling (Romero and Reed, 2005). Animals are then restrained using a standardized method (e.g., songbirds are usually put into clean, breathable cloth bags) and additional blood samples taken at various time points post-capture to assess animals' ability to raise Cort in response to a strong psychological stressor. In birds and mammals, this "stress-induced" sample is typically taken after 30 min of restraint, although some protocols use shorter or longer time periods.

Negative feedback can be measured as an extension of this protocol. One way to assess negative feedback is to simply take blood samples at 60 min post-capture or later and compare these to earlier samples (e.g., 15 min or 30 min post-capture). However, animals may still be mounting a stress response to capture and handling at this point, and negative feedback may not initiate until much later. For example, Dayger and Lutterschmidt (2017) found that after 4 h of restraint, female garter snakes (*Thamnophis sirtalis parietalis*) caught at den sites in the spring were able to bring Cort levels back down to baseline, whereas females caught on roads were still increasing Cort concentrations. Therefore, to more quickly assess negative feedback in a standardized way, many researchers have adapted a clinical procedure for use with the wild species they study – using the synthetic glucocorticoid dexamethasone (Dex) as part of a Dex suppression test (Carroll et al., 1981). Dex is a glucocorticoid agonist that binds to and activates glucocorticoid receptors in the periphery. When it binds to receptors in the pituitary gland, this has the effect of shutting down the synthesis of endogenous Cort (Fig. 1), resulting in decreased Cort titers (Cole et al., 2000). The stronger an animal's negative feedback, the larger the decrease in Cort titers after a Dex injection. Theoretically, other

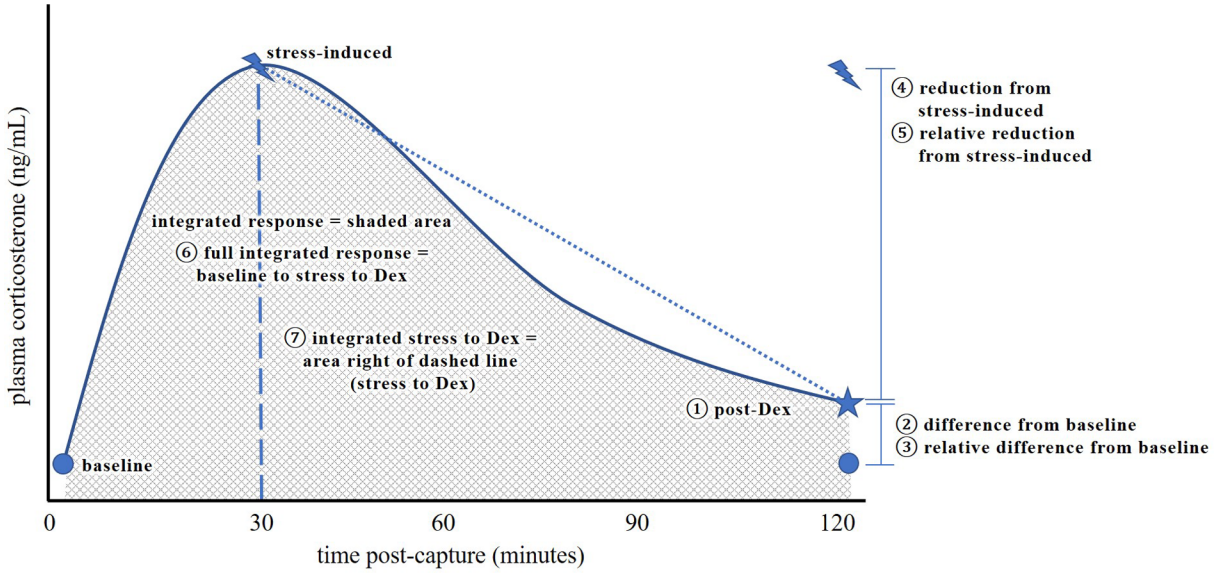


Fig. 2. A graphical representation of typical fluctuations in a vertebrate's corticosterone titers following an acute stress response and a dexamethasone (Dex) injection to measure negative feedback. Seven possible approaches to quantifying negative feedback found in the literature are labeled with encircled numbers. See Table 1 for more detailed descriptions. The dotted line represents the presumed straight line when calculating an integrated response, but note that if researchers collect multiple blood samples during this time period, more accurate integrated values can be determined. The timing of post-Dex sampling shown here is based on validation experiments by Lattin et al. (2012) in house sparrows (*Passer domesticus*) and must validated for other species.

glucocorticoids could be used in a similar way, but to our knowledge, Dex is the only glucocorticoid agonist that has been extensively used and validated in a variety of species to test HPA negative feedback.

There are three important considerations with this method. First, it is necessary to use a Cort assay (typically an enzyme-linked immunoassay or radioimmunoassay) with antibodies that do not cross-react with Dex, so only endogenous Cort in response to Dex injection is measured. Second, the appropriate Dex dose and time course post-injection must be determined with validation experiments for each new species. Third, because Dex does not cross the blood–brain barrier, it does not trigger negative feedback at the level of the hypothalamus, hippocampus, or other higher brain areas (Fig. 1), so it is not completely analogous to natural negative feedback, which operates at both the brain and pituitary (de Kloet et al., 1998).

2.2. Approaches for evaluating negative feedback: literature search and analysis

Once researchers have completed a stress series using Dex in their species of interest and determined endogenous Cort concentrations in pre- and post-Dex blood samples, there are still several different approaches that can be used to quantify negative feedback. We performed a literature search to determine the most commonly used approaches. The following criteria were applied to select relevant articles:

1. Because our primary interest was comparative endocrinology (particularly in wildlife), we excluded human studies and studies on laboratory rats and mice. We did include studies on other types of domesticated animals (cows, sheep, etc.) and primate studies. Animals could be housed in captivity or studied in the field.
2. The study had to quantify negative feedback efficacy using Dex.

To find relevant articles, the following search query was entered in Web of Science on 12 April 2019: *TOPIC: "negative feedback" AND cort* NOT human NOT patient* NOT child NOT rat NOT mice*. Filtering for primary literature refined our initial database of 629 articles down to 532. Thirty-five more articles were added to this list by screening the references of relevant articles. Therefore, a total of 567 articles were manually screened for relevance. Of these, 70 articles were deemed relevant (see Supplementary Material for a complete list). Because some studies used more than one approach, this search yielded a total of 75 reports of quantifying negative feedback. For each report, we categorized the approach used to report negative feedback as well as: 1) type of animal (domesticated, primate, other wildlife); 2) vertebrate class (mammal, bird, reptile, amphibian, fish); 3) species.

There were seven different approaches used among the initial 567 articles (Fig. 2, Table 1): (1) raw post-Dex Cort concentrations, (2) the raw difference between baseline and post-Dex Cort (difference from baseline), (3) the relative (%) difference between baseline and post-Dex Cort, (4) the raw difference between stress-induced and post-Dex Cort (reduction from stress-induced), (5) the relative (%) decrease in Cort from stress-induced concentrations, (6) the full integrated stress response (baseline to stress-induced to post-Dex Cort), (7) the latter half of the integrated response (stress-induced to post-Dex Cort).

Each approach has its advantages and disadvantages. Certainly, the most straightforward way to describe negative feedback is approach 1: simply report the post-Dex Cort concentrations and run analyses on these values. This was also the most commonly used approach in the comparative endocrinology literature (63% of articles; Table 2). However, negative feedback is an integrative ability - the ability to decrease plasma Cort back to baseline following elevation due to a stressor - and post-Dex Cort alone is not an integrative measure (Box 1).

Box 1

: Examples demonstrating the limitations of some measures of glucocorticoid negative feedback compared to approach 5.

① Post-Dex Cort.

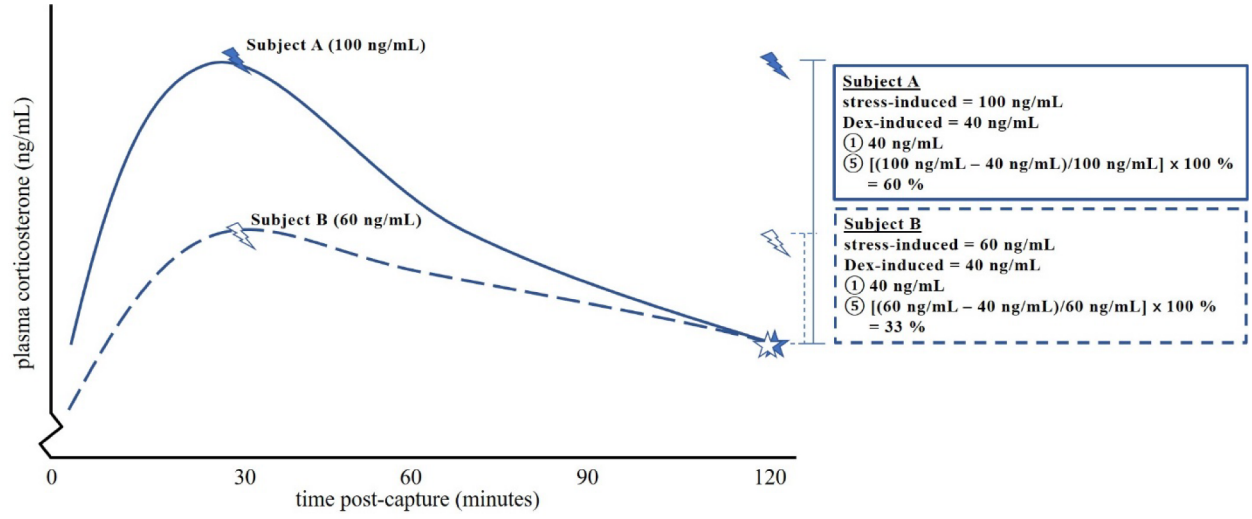
This approach assumes that the lower the post-Dex Cort concentration is, the better a subject's negative feedback efficacy. However, the stress response is not included in this measure. The purpose of measuring negative feedback efficacy is to know how well a subject can reduce Cort concentrations after exposure to an acute stressor. Without including the stress response, this measure may be less useful.

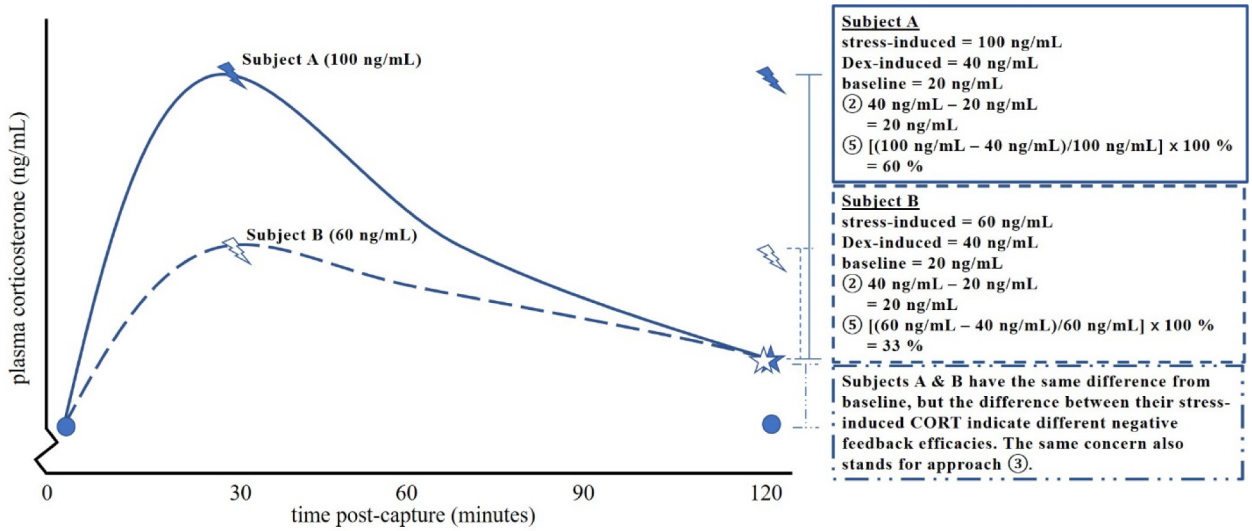
Example: Subject A and B both have post-Dex Cort values of 40 ng/ml. Reporting these values would indicate they have the same negative feedback efficacy. However, Subject A had stress-induced values of 100 ng/ml, whereas Subject B had stress-induced values of 60 ng/ml. While it seems obvious that Subject A has better negative feedback efficacy than Subject B, this is not apparent from raw post-Dex Cort alone.

② (& ③) Difference from baseline.

This approach assumes that the closer the post-Dex Cort concentration is to baseline levels, the better a subject's negative feedback efficacy. However, the stress response of the subject is not included in this measure. Similar to the first example, this measure may be less useful because the stress response is not considered in this approach.

Example: Subjects A and B have similar differences between baseline and post-Dex Cort (20 ng/mL). Reporting the difference between post-Dex Cort and baseline Cort would indicate that they have similar negative feedback efficacies. However, subject B had a much higher stress response (100 ng/mL) than A (60 ng/mL), indicating a greater capability of subject B to shut down the stress response because it reduced circulating Cort concentrations by a much greater proportion.

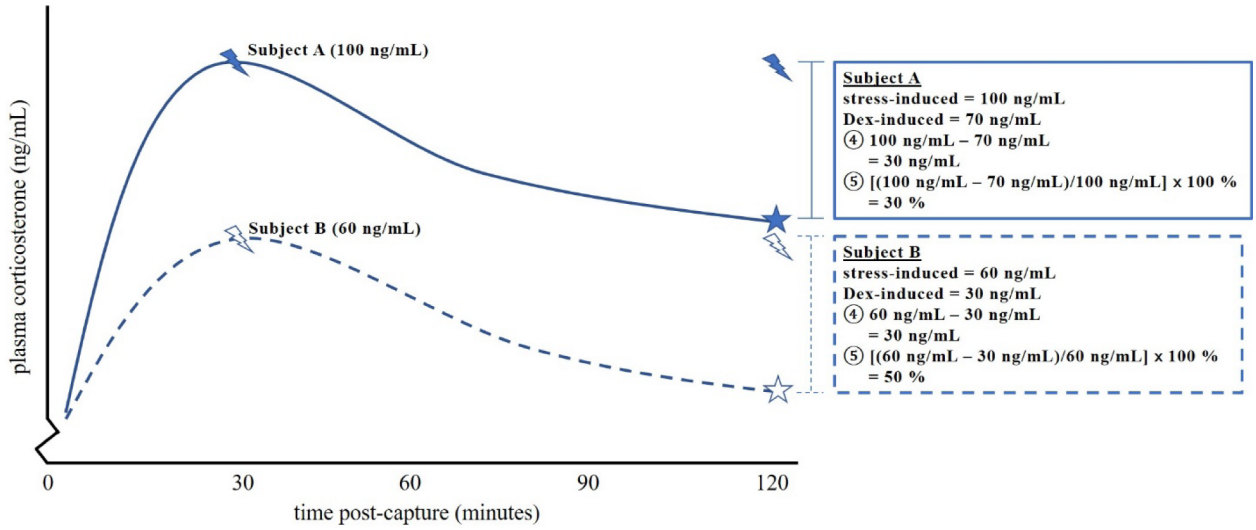




④ Reduction from stress-induced.

This approach assumes that the greater the reduction from stress-induced Cort, the better a subject's negative feedback efficacy. While this may generally be true, it does not consider how different one individual's stress response might have been from another individual's response.

Example: Subjects A and B have similar reductions in Cort concentrations following exposure to Dex. Reporting the difference between stress-induced Cort and post-Dex Cort would indicate that these subjects had the same negative feedback efficacy. However, subject A had a much higher stress response (100 ng/mL) than B (60 ng/mL), so if we think of negative feedback as the ability to shut down "most of" the stress response, approach 5 indicates subject B has stronger negative feedback because it reduced circulating Cort concentrations by a greater proportion.



Approaches 2 (raw difference from baseline) and 3 (relative difference from baseline) are certainly integrative; negative feedback is sometimes referred to as a subject's ability to bring hormone titers back to baseline and this measure reflects that. However, most wildlife researchers are interested in negative feedback as an animal's ability to suppress the stress response, and these approaches do not incorporate the stress response in any way (Box 1). Both were infrequently used in the literature, although they did appear in some primate studies (raw difference: 3%; relative difference: 6%; Table 2). The relative difference from baseline numbers can also be difficult to interpret: if an animal is not able to bring Cort back down close to baseline after Dex injections, or if baseline values are very low (e.g., ~0.01 ng/ml), this value can be several thousand percentages and result in heavily right-skewed data. Furthermore, most researchers have no way of knowing if a wild animal they capture has just escaped from a predator or experienced another kind of stressor, which could cause "baseline" Cort to be much higher in some individuals than others. This could be a source of additional variation in these measures.

Approaches 4 (raw difference from stress-induced) and 5 (relative difference from stress-induced) are both integrative measures that include the stress response. They were the second and third most common approach used to quantify negative feedback (raw difference: 12%; relative difference: 10%), particularly prevalent in wild species (Table 2). We believe approach 5 has advantages over approach 4 that should favor its usage. Specifically, if two individuals have the same raw decrease in Cort concentrations, but one individual has a much higher stress-induced response than the other, approach 4 will treat them as equal while 5 will not (Box 1). Also, because approach 5 is a relative decrease, this makes it much easier to compare negative feedback ability across studies and species. This is especially important considering that different methods used by different labs (e.g., assay kits and antibodies) can have significant effects on raw Cort concentrations (Bokony et al., 2009).

Approaches 6 (full integrated response) and 7 (integrated stress-induced to post-Dex Cort) are the most integrative, and occurred in the literature infrequently (the full integrative response was found in a few studies using natural negative feedback but no studies using Dex; stress-induced to post-Dex: 5%). We maintain that approach 6 is too integrative, and that including the full baseline to stress-induced Cort response is not appropriate for a "negative feedback" measure. Approaches 6 and 7 both have other flaws that we believe make them worse than approaches 1 and 5. Specifically, the slope of the relationship between sampled data points is assumed to be linear, and this assumption may not be accurate. However, if researchers collect multiple blood samples during this time period, more accurate integrated values could be determined. Approaches 6 and 7 are also not as comparable across studies because the amount of time necessary to wait to take a blood sample after a Dex injection may vary across species, and increasing the time between sampling points has a dramatic effect on the numbers obtained for integrative measures.

Overall, mammals (57%) and birds (37%) were the primary focus of studies investigating negative feedback efficacy, leaving fish and reptiles understudied (2 studies each). Surprisingly, we found no studies investigating glucocorticoid negative feedback in amphibians, suggesting a major gap in the literature, especially in light of global amphibian declines (Cohen et al., 2019). An important consideration in reporting raw rather than relative Cort concentrations in response to Dex (all approaches except 3 and 5) is that it may limit the ability to compare responses across taxa. For example, female lemmings can have Cort concentrations as high as 8000 ng/ml during the breeding season, compared to ~80 ng/ml for breeding songbirds (Romero et al., 2008). Additionally, poikilotherms typically have much lower Cort concentrations than homeotherms (Nevarez et al., 2011). Thus, reporting the relative change between sampling points and expressing the efficacy of negative feedback as a percentage more easily allows studies to be compared across taxa, and the interpretation of this value may be more intuitive to readers as it does not require taxonomic knowledge of typical Cort concentrations.

Table 1
Summary of possible approaches to assess negative feedback using an injection of the synthetic glucocorticoid dexamethasone (Dex). Baseline corticosterone (Cort) refers to cortisol or corticosterone concentrations in blood before the subject is stressed by capture and handling (~2–3 min post-disturbance). "Stress-induced Cort" refers to hormone levels after an acute stressor, such as 30 min of restraint in a cloth bag (a standardized stress protocol used in wild birds). See Fig. 2 for a graphical representation of these different methods. Note: Dex dose and the time course of maximum response post-Dex injection should be determined for each new species using validation studies.

Approach	Calculation	Considerations
① Post-Dex Cort	None; this is the amount of raw Cort circulating in the subject's bloodstream following an injection of Dex	PRO: Easy to evaluate; most commonly used in the literature CONS: Difficult to compare raw values across taxa; not an integrative measure RECOMMENDATION: OK but not as good as ⑤
② Difference from baseline Cort	The absolute change between baseline and post-DEX Cort concentrations: $= baseline - postDex$	PROS: More integrative CONS: Difficult to compare raw values across taxa; does not incorporate the stress response of the individual (see Box 1) RECOMMENDATION: Discouraged
③ Relative difference from baseline Cort	The relative change (percent) between baseline and post-Dex Cort concentrations: $= \left(\frac{postDex - baseline}{baseline}\right) \times 100\%$ Note: Negative values indicate post-DEX Cort *below baseline*; 0% indicates the same Cort concentration for baseline and post-DEX	PROS: Similar to ② but more easily enables comparisons across taxa CONS: Does not incorporate the stress response of the individual; values are susceptible to skewed data if baseline values are close to 0; expressed as a percentage, so must use an appropriate statistical analysis; variable baseline Cort among subjects makes for difficult interpretation RECOMMENDATION: Discouraged
④ Reduction from stress-induced Cort	The absolute change between stress-induced and post-Dex Cort concentrations: $= stressinducedCORT - postDex$	PROS: More integrative; includes the stress-induced response CONS: Difficult to compare raw values across taxa; does not incorporate variation in the stress response among individuals RECOMMENDATION: Discouraged
⑤ Relative reduction from stress-induced Cort	The relative change (percent) between stress-induced and post-Dex Cort concentrations: $= \left[\frac{stressinduced - postDex}{stressinduced}\right] \times 100\%$ Note: Negative values indicate Cort *increases* after a Dex injection; 100% indicates complete inhibition of Cort	PROS: More integrative; incorporates the stress-induced response; easily comparable across species and studies CONS: Expressed as a percentage, so must use an appropriate statistical analysis RECOMMENDATION: Top choice
⑥ Full integrated response	Add the area under the curve calculated above to the area under the curve, assuming a straight line, from baseline to post-Dex sampling points: $= integrated\ stress\text{-}induced\ to\ post\text{-}Dex + [area\ of\ baseline\ to\ stress\text{-}induced\ triangle] + area\ to\ X\text{-}axis$ $= integratedstressinducedtopostDex + \left[\frac{minutes \times (stressinducedCort - baselineCort)}{2}\right] + (minutes \times baselineCort)$ *minutes = time from baseline – post-Dex samples*	PROS: Very integrative CONS: Too integrative, reflects more than just "negative feedback"; difficult to compare across taxa; assumes relationships between data points RECOMMENDATION: Discouraged
⑦ Integrated stress-induced to post-Dex Cort	Calculate the area under the curve, assuming a straight line, from the stress-induced to post-Dex sampling points: $= [area\ of\ stress\text{-}induced\ to\ Dex\ triangle] + area\ to\ X\text{-}axis$ $= \left[\frac{minutes \times (stressinducedCort - postDexCort)}{2}\right] + (minutes \times postDexCort)$ *minutes = time from stress-induced – post-Dex samples*	PROS: Very integrative CONS: Difficult to compare across taxa; assumes relationships between data points^ RECOMMENDATION: Discouraged

^See Fig. 2 for a visual depiction of this straight-line assumption (dotted line). However, if researchers collect multiple blood samples during this time period, more accurate integrated values can be determined.

3. Reporting negative feedback: does approach matter?

Given the wide range of different methods used to report negative feedback, we questioned how much it determines the results of a particular study. If most of these various approaches give the same answer, it may not matter which a researcher chooses. To compare and contrast these different approaches, we reanalyzed data from Lattin et al. (2012) on seasonal variation in negative feedback efficacy. In this study, adult house sparrows (*Passer domesticus*; n = 58, a mix of males and females) were caught during six different life-history stages: molt, early and late

Table 2
Summary of the approaches used to report negative feedback in wildlife. Articles were obtained from a literature search in Web of Science (see text for details) as well as screening the references of returned relevant articles for a total of 70 articles. Six of these articles reported negative feedback efficacy using two methods, for a total of 75 reports of negative feedback efficacy. Articles were then classified based on their study species being wildlife (64% of studies), domesticated species (10%), or a primate (wild species that could be in the lab or in captivity; 26%). Classes of study species represented within each classification are designated with symbols. In total, Mammals were used in 41 of the studies (57%); 27 (37%) in Birds; 2 in both Fish and Reptiles (3% each).

Approach of reporting negative feedback	Number of occurrences; proportion	Within classifications		
		Wildlife *Birds†, Reptiles‡, Fish^, Mammals**	**Domesticated** *Mammals, Birds*	**Primates** *Mammals*
① raw post-Dex corticosterone	47; 63%	29; 62% †, ‡, ^, *	4; 100%	14; 59%
② difference from baseline	2; 3%			2; 8%
③ relative difference from baseline	5; 6%			5; 21%
④ reduction from stress-induced	9; 12%	7; 15% †, *		2; 8%
⑤ relative reduction from stress-induced	8; 10%	7; 15% †, *		1; 4%
⑥ full integrated response				
⑦ integrated stress-induced to post-Dex	4; 5%	4; 8% †		
	Total	47	4	24

winter (non-breeding), pre-laying, breeding, and late breeding. In each stage, HPA function was evaluated by measuring baseline and stress-induced Cort, negative feedback in response to Dex, and maximum adrenal response through an injection of adrenocorticotropic hormone. Birds were then brought into captivity and the HPA function tests repeated five days later. For this reanalysis, we considered only the first series of samples and did not include data on the maximum adrenal response (adrenocorticotropic hormone was given after the post-Dex sample was collected).

We used R Statistical Software version 3.4.1 (R Core Team, 2017) for all analyses. We calculated negative feedback in the seven different ways described above (Fig. 2, Table 1). We first evaluated each approach for normality (*skewness* and *kurtosis* commands in the *moments* package; Komsta and Novomestky, 2015) and transformed

Table 3
Correlation of different approaches for assessing negative feedback efficacy in response to an injection of the synthetic glucocorticoid dexamethasone (Dex). Data are from Lattin et al. (2012) (house sparrows, n = 58). Bolded cells highlight correlations with the relative reduction of corticosterone (Cort) after Dex treatment (approach 5, originally used in Lattin et al. 2012). Statistically significant contrasts ($p < 0.05$) are bolded and italicized. Baseline Cort was not taken in < 3 min for four subjects resulting in a reduced sample size for approaches that require baseline Cort in their calculation (approaches ②, ③, and ⑥).

	① raw Cort post-Dex	② difference from baseline	③ relative difference from baseline	④ reduction from stress-induced	⑤ relative reduction from stress-induced	⑥ full integrated	⑦ integrated stress-induced to post-Dex
① raw Cort post-Dex	r	-0.96	0.27	0.60	-0.73	0.60	0.73
	n	54	54	58	58	54	58
	p	***<0.001***	***0.047***	***<0.001***	***<0.001***	***<0.001***	***<0.001***
② difference from baseline			-0.47	-0.65	0.74	-0.49	-0.60
			54	54	54	54	54
			<0.001	***<0.001***	***<0.001***	***<0.001***	***<0.001***
③ relative difference from baseline				0.37	-0.37	0.10	-0.02
				54	54	54	54
				0.005	***0.01***	0.36	0.16
④ reduction from stress-induced					-0.89	-0.24	-0.09
					58	54	58
					<0.001	0.08	0.52
⑤ relative reduction from stress-induced						-0.03	-0.19
						54	58
						0.83	0.14
⑥ full integrated							0.99
							54
							<0.001

Table 4
Comparison of one-way ANOVA (or ^ = Welch's non-parametric) results for different approaches of assessing negative feedback efficacy after an injection of dexamethasone (Dex). Data are from Lattin et al. (2012), investigating differences in negative feedback efficacy among six different times of year in wild house sparrows (*Passer domesticus*), which used approach 5 to quantify negative feedback. Statistically significant results ($p < 0.05$) are bolded and italicized. Because not all baseline Cort samples could be collected in < 3 min, samples sizes and degrees of freedom for measures incorporating baseline Cort differ. See text for more details of analysis.

Approach	F	df	p
① raw post-Dex corticosterone	4.503	5, 52	***0.002***
② difference from baseline	3.438	5, 48	***0.010***
③ relative difference from baseline	5.596	5, 48	< ***0.001***
④ reduction from stress-induced	2.145	5, 52	0.074
⑤ relative reduction from stress-induced	2.780	5, 52	***0.027***
⑥ ^full integrated response	8.530	5, 21	< ***0.001***
⑦ ^integrated stress-induced to post-Dex	7.790	5, 21.8	< ***0.001***

appropriately: the relative change (percent) between baseline Cort and post-Dex Cort and the difference between stress-induced and post-Dex Cort concentrations as well as their relative (percent) change were squared. All other variables were normally distributed.

3.1. Correlations among different approaches

We first evaluated whether the multiple approaches to measuring negative feedback were correlated by creating a correlation matrix using the *rcorr* command in the *Hmisc* package (Harrell, 2019). A Levene's test assessed homogeneity of variances (*leveneTest* command in the *car* package; (Fox and Weisberg, 2011) and found both integrated approaches to require non-parametric statistical approaches. As we have advocated for reporting the relative decrease from stress-induced Cort (method 5), and this was what was originally reported in the Lattin et al. (2012) study, we interpreted our findings with reference to this approach.

There were significant correlations between the relative reduction in stress-induced Cort (approach 5) with all other approaches except the two integrated approaches (Table 3). The correlations between these different approaches are not too surprising, given that they all use common variables in their calculations: baseline Cort, stress-induced Cort and raw post-Dex Cort. The fact that they are correlated suggests that they reflect similar variation in the data and may provide similar interpretations of negative feedback efficacy. However, because integrated responses were not significantly correlated with as many of the more straightforward measures of negative feedback efficacy, this suggests that using an integrated response is quite a different approach and may not be as appropriate for assessing negative feedback. This is one more reason to discourage researchers from these integrated approaches, although they may still be useful when investigating questions such as how the total amount of Cort secreted may affect physiological parameters, such as body condition.

3.2. Contrasting statistical findings for different approaches

In the original Lattin et al. (2012) study, life history stage was shown to significantly impact negative feedback ability (defined as the relative decrease in Cort from stress-induced concentrations). Specifically, negative feedback was weaker in the pre-breeding period compared to the late winter and breeding periods. Here, we assessed whether the method of reporting negative feedback can affect significant differences among life history stages, and thus, the conclusions of a study. Because the raw post-Dex Cort, difference from baseline, relative difference from baseline, and reduction from stress-induced approaches were correlated with the relative reduction in stress-induced Cort (Table 3), we predicted these approaches would produce similar significant differences among seasons. Because integrated measures were not correlated with the relative reduction from stress-induced Cort, we predicted these measures would show different results.

We used ANOVAs to examine how the different approaches used to report negative feedback may affect statistical results and interpretation. However, more powerful statistical approaches exist (e.g., linear mixed models, "character state" approaches) that may allow for the incorporation of multiple measures in a single model (Baugh et al., 2014). We used Welch's ANOVA using the *oneway.test* command in the *stats* package (R Core Team, 2017) in cases where the assumption of homogeneity of variances was not met (both integrated approaches as per *leveneTest* command in the *car* package (Fox and Weisberg, 2011)). Otherwise, we used one-way ANOVAs using the *aov* command in the *stats* package (R Core Team, 2017) to test for differences in negative feedback efficacy among six different times of year. In keeping with the original analysis, we did not control for possible covariates like sex or body mass. When we found significant differences among groups, we ran Tukey's HSD test as a multiple comparison procedure using the *TukeyHSD* command in the *stats* package (R Core Team, 2017). In cases where the assumption of homogeneity of variances was not met (both integrated approaches), we ran Games-Howell Tests using the *gamesHowellTest* command in the *userfriendlyscience* package (Peters, 2017).

All approaches except for the raw reduction from stress-induced Cort (approach 4) showed significant seasonal variation in negative feedback ability (Table 4). However, significant post-hoc differences varied by the approach used (Fig. 3; Supplementary Material, Table S1), and, unexpectedly, no two methods gave the same results. What was especially surprising was that methods that seemed roughly similar (e.g., the raw vs. relative reduction in Cort from stress-induced to post-Dex) gave such different results. This raises concerns because the interpretation of results relies upon what significant differences emerge from the data. Although a study examining six different times of year may be especially prone to finding differences depending on analysis method, we also saw this problem crop up in other studies from our literature search. For example, Desantis et al. (2018) and Liebl et al. (2012) both examined post-Dex Cort (approach 1) as well as the raw reduction from stress-induced to post-Dex Cort (approach 3) in their species of interest for a smaller number of life history stages (Desantis et al., 2018: southern flying squirrels (*Glaucomys volans*) during breeding and non-breeding seasons; Liebl et al., 2012: house sparrows during breeding and molt). Both studies found a significant seasonal difference in negative feedback using approach 1 but not 3, consistent with our results in Fig. 3 (Table S1). Thus, the approach used to investigate negative feedback efficacy will affect the conclusions of the study and our ultimate understanding of HPA function. Furthermore, such variable results may make it difficult to compare results across studies.

Moving forward, we have several specific suggestions. Ideally, all researchers would use a common approach to describe negative feedback efficacy, and we believe the relative decrease in Cort from stress-induced concentrations (approach 5 in Table 1, Fig. 2) is the most informative and integrative measure of negative feedback for the reasons detailed above (see Section 2.2). Another option would be to report multiple measures of negative feedback, but this is time consuming and inconsistent results may be confusing to synthesize. At the very least, researchers should make their raw baseline Cort, stress-induced Cort, and post-Dex Cort data available so alternative measures can be calculated. We also advise caution in comparing results among studies using different methods to assess negative feedback.

4. HPA negative feedback as a potential mediator of trade-offs between survival and reproduction: predictions and evidence

Cort can inhibit or completely shut down breeding and cause the reallocation of an organism's resources away from reproduction

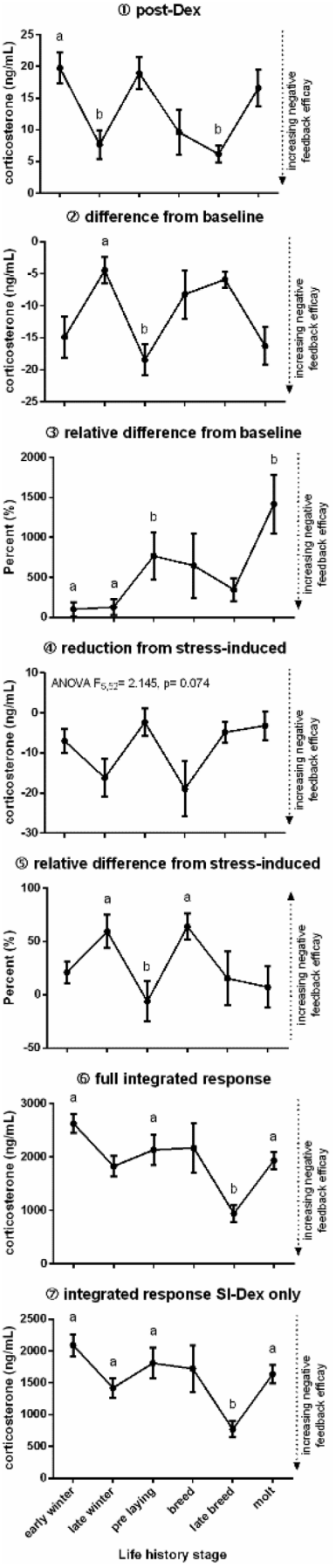


Fig. 3. Different approaches for calculating negative feedback efficacy yield different statistical results. With a dataset of wild house sparrows caught at six different times of year (n = 58), we first quantified negative feedback using the seven approaches described in Fig. 1, then did an ANOVA to examine seasonal variation among life history stages. When the overall model effect was significant, we compared different stages using Tukey's HSD post-hoc tests (Games-Howell for non-parametric). Significant differences ($p < 0.05$) are indicated by letters. Differences of $p < 0.10$ are reported in Table S1. Arrows on the right y-axis indicate the direction of the strength of negative feedback. SI = stress-induced; Dex = dexamethasone.

towards immediate survival. HPA negative feedback partly controls an organism's overall Cort exposure after it encounters an environmental stressor. If animals are responding adaptively, what patterns might we expect to see across and within vertebrate species related to the potential role of HPA negative feedback in mediating trade-offs between survival and reproduction?

One important consideration is that Cort is a complex hormone with distinct physiological and behavioral roles at lower baseline concentrations and higher stress-induced concentrations (Landys et al., 2006). Note that these differential effects are possible because Cort binds to two populations of receptors: mineralocorticoid receptors at baseline concentrations, and both mineralocorticoid and glucocorticoid receptors at stress-induced concentrations. Baseline Cort often increases during energetically demanding stages of the annual cycle (e.g., migration, lactation) in ways that appear to help organisms effectively acquire and mobilize the energy necessary to meet the challenges of those stages (Kenagy and Place, 2000; Piersma et al., 2000). Increases in baseline Cort do not typically shut down reproduction, and may in fact be necessary to successfully breed, which may be why the breeding period is often when many species have a seasonal peak in baseline Cort (Romero, 2002).

Stress-induced Cort is a separate matter altogether. Wingfield et al. (1998) proposed that sustained high concentrations of Cort in response to environmental perturbations have the ability to bring animals into an "emergency life history stage" where normal behaviors for that stage of the annual cycle are abandoned until survival is ensured. However, Cort exposure is partly a function of negative feedback – the stronger the negative feedback, the less Cort the animal is exposed to over the long term, and thus, the less likely that it will abandon normal life history behaviors (Fig. 4). Based on earlier predictions about the modulation of acute Cort levels in breeding animals based on species' ecology (Wingfield et al., 1995), we might thus make the following predictions about negative feedback, if animals are responding adaptively:

Across species predictions:

1) Negative feedback should be different in species with different life history strategies. For example, long-lived species might be expected to behave like "prudent parents" and be less likely to take actions that might compromise their own survival during one of their many lifetime breeding attempts, compared to shorter-lived species (Drent and Daan, 2002). *Thus, we might expect HPA negative feedback to be weaker in long-lived animals compared to shorter-lived animals.*
2) Negative feedback should be different in species with very limited breeding seasons (e.g., migratory species breeding in the Arctic summer) compared to species with lengthy or continuous breeding seasons (e.g., species breeding in tropical regions). For these limited breeding season species, the cost of losing a single breeding attempt is very high. *Thus, we might expect HPA negative feedback to be stronger in animals with short breeding seasons compared to those with longer breeding seasons.*
3) In species where one sex invests substantially more resources into producing and/or raising offspring, the cost of losing a reproductive attempt is higher in one sex than the other. *In these kinds of species, we might expect sexual dimorphism in negative feedback, with the more parental sex showing stronger HPA negative feedback.*

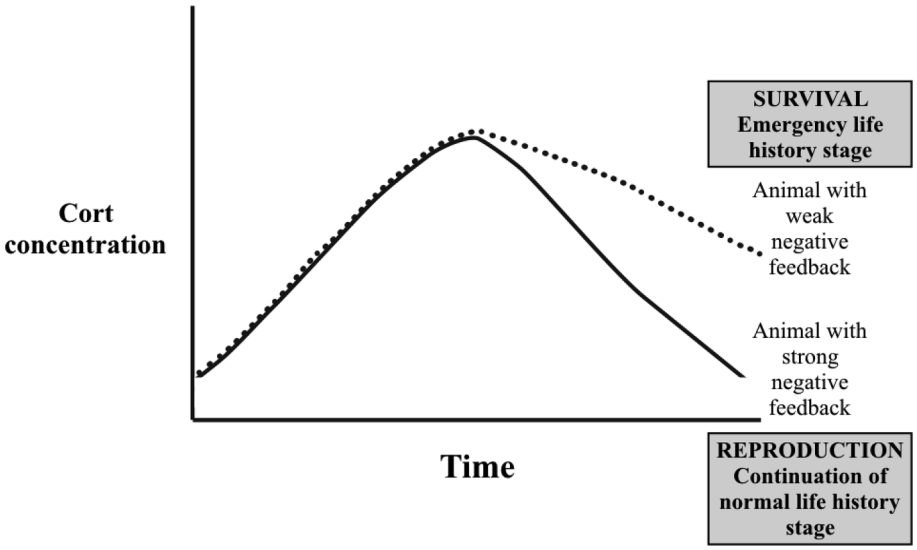


Fig. 4. Theoretical framework for understanding variation in hypothalamic-pituitary-adrenal (HPA) negative feedback ability. All else being equal, after stressor exposure a breeding animal with stronger HPA negative feedback (solid line) will be exposed to lower total concentrations of glucocorticoids (Cort) over time and may thus be less likely to abandon its reproductive attempt compared to an animal with weaker negative feedback (dotted line). Note that because negative feedback is a dynamic trait, these two lines could also represent the same animal in two different body conditions, during two different breeding substages, or at two different ages as per predictions 4–6 above (see text for more details).

Within species predictions:

4) Negative feedback should be condition dependent. An animal in excellent body condition should be able to withstand a stressor (e.g., a severe storm) for longer without compromising survival compared to one in poor body condition. *Thus, in breeding animals, as body condition declines and the ability to withstand a stressor without compromising survival also declines, we might expect to see a concomitant decline in the strength of HPA negative feedback.*
5) For species with parental care, as the breeding season progresses (e.g., from eggs to nestlings to fledglings in birds, or from pregnancy to lactation to weaning in mammals), animals' investment in their broods increase and the cost of abandoning thus also increases. *Thus, we might expect to see stronger HPA negative feedback later during a single breeding attempt relative to earlier during that breeding attempt.*
6) As animals age, the value of current reproductive efforts increases relative to the value of future reproduction and survival. *Thus, we might expect older animals to have stronger HPA negative feedback than younger animals.*

Predictions 4–6 assume that HPA negative feedback ability is dynamic, and predictions 4 and 5 specifically assume that negative feedback ability can be changed relatively quickly. Indeed, negative feedback appears to be a plastic trait, capable of being modulated based on environmental conditions. For example, several studies have demonstrated that wild birds can significantly alter negative feedback efficacy over the course of just a few days (Dickens et al., 2009; Lattin et al., 2012). On an even shorter time scale, marine iguanas (*Amblyrhynchus cristatus*) are capable of modulating negative feedback on a daily basis (Romero and Wikelski, 2006). Seasonal changes in negative feedback have also been reported in several wild vertebrate species (Table S2), so what evidence exists in wild species to support or refute the above-listed predictions?

Prediction 1: Weaker HPA negative feedback in long-lived animals compared to shorter-lived animals. To our knowledge, there are no studies directly comparing negative feedback ability in pairs or sets of species with different lifespans. However, there are certainly studies showing weak HPA negative feedback in response to Dex during the breeding period in several short-lived animals, including brown lemmings (*Lemmus trimucronatus*) (Romero et al., 2008) and a pair of semelparous marsupial species (Bradley, 1990; McDonald et al., 1986). These semelparous marsupials undergo a single round of reproduction and then die - males after breeding, females typically after lactation (Wood, 1970). Prolonged high Cort does not appear to shut down reproduction in these species, and it has been proposed that they may have special adaptations to compensate for negative effects of Cort on the hypothalamic-pituitary-gonadal axis (Wingfield and Sapolsky, 2003), e.g., dramatically reduced Cort receptors in reproductive tissues like brain and gonads. Therefore, more research is necessary to fully test this prediction, ideally a study comparing negative feedback during the first breeding season in a pair of closely related species where one is long-lived and the other short-lived.

Prediction 2: Stronger HPA negative feedback in animals with short breeding seasons compared to those with longer breeding seasons. We do not know of any existing work testing this prediction.

Prediction 3: In species with sexual dimorphism in parental care, stronger HPA negative feedback in the more parental sex. Some studies examining negative feedback in breeding or pre-breeding songbirds have found that males have weaker negative feedback compared to females (e.g., Astheimer et al., 1994). Male songbirds typically contribute to parental care but they may spend less time brooding and provisioning than females (Clutton-Brock, 1991), and they certainly invest less in reproduction by not having to lay eggs. Conversely, in degus (*Octodon degus*), where females provide all care to the young, there are no sex differences in negative feedback (Bauer et al., 2014). However, degus do practice plural breeding with communal care, where mothers within the group will lick, groom, and even nurse other group members' offspring (Hayes et al., 2009). It is possible that this could buffer the negative effects of any individual female abandoning her offspring. Interestingly, long-term data suggest plural breeding with communal care only increases offspring survival during years of low food availability (Ebensperger et al., 2014). Again, more studies are necessary that explicitly test this hypothesis.

Prediction 4: Weaker negative feedback as body condition declines in breeding animals. In a study assessing potential stressful aspects of translocation on chukar partridge (*Alectoris chukar*) caught towards the end of the breeding season (mid-July-August), Dickens et al. (2009) found that body mass declined most in a group of birds that had been trapped and translocated, a group that also showed a decline in HPA negative feedback. Similarly, Delehanty and Boonstra (2011) found that male Arctic ground squirrels (*Urocitellus parryii*) caught at the beginning of the breeding season had larger fat stores, fewer visible wounds, and stronger negative feedback than males caught at the end of the breeding season. Reproductive-age female rhesus monkeys with one serotonin transporter gene polymorphism had a phenotype that included lower body weight, lower adiposity, and reduced HPA negative feedback compared to females with a different variant (Hoffman et al., 2007). In breeding male song sparrows (*Melospiza melodia*), there was no

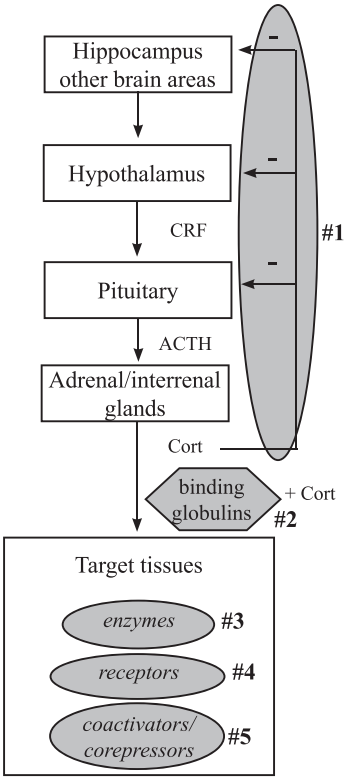


Fig. 5. Changing the strength of negative feedback is not the only way to alter an individual's exposure to glucocorticoid hormones. Although this review has focused on examining evidence that animals adaptively reduce hypothalamic-pituitary-adrenal (HPA) negative feedback (#1) as a way to prioritize immediate survival over reproduction, the HPA axis is an integrated physiological system, and there are several different mechanisms animals can use to change responses to a glucocorticoid (Cort) signal. This includes changing: concentrations of corticosteroid binding globulins in blood (#2), concentrations or activity of enzymes in target tissues that can inactivate Cort or regenerate it from an inactive metabolite (#3), concentrations of Cort receptors in target tissues (#4), and presence of different coactivators and corepressors that can affect the binding of the hormone-receptor complex to DNA (#5). Note that this is not an exhaustive list. Altering HPA function at the tissue level will produce effects that will be more organ or system specific than altering HPA function at the level of hormone secretion.

relationship between the strength of HPA negative feedback and a body condition measure combining mass, tarsus length, wing chord and fat score (Schmidt et al., 2012). However, males with stronger negative feedback did have lower relative heterophil counts and H:L ratios. Although most evidence suggests that breeding animals in better body condition have stronger negative feedback than those in worse body condition, bringing wild house sparrows into captivity was associated both with a ~10% drop in body mass and an *increase* in the strength of negative feedback (Lattin et al., 2012). However, as mentioned previously, this study included more than just breeding birds. Thus, although most evidence to date suggests a link between poor body condition and weak negative feedback in breeding animals, more studies are needed to confirm or reject this prediction.

Prediction 5: Stronger HPA negative feedback later during a breeding attempt relative to earlier during a breeding attempt. In the previously mentioned study of house sparrows during six different stages of the annual cycle, Lattin et al. (2012) found that negative feedback was indeed stronger in breeding birds compared to birds caught during the pre-breeding period. We do not know of any other work testing this prediction.

Prediction 6: Stronger HPA negative feedback in older animals compared to younger animals. Thus far, evidence that negative feedback is stronger in older animals is decidedly mixed. For example, Elliott et al. (2014) found that in two long-lived seabird species, older individuals had lower Cort after a Dex injection compared to younger individuals. Harris and Saltzman (2013) also found stronger negative feedback in older virgin California mice (*Peromyscus californicus*) although this was only true for males, not females. In primates, however, negative feedback is generally weaker in older animals compared to younger animals (Goncharova et al., 2000; Gust et al., 2000; Sapolsky and Altmann, 1991). Donaldson et al. (2005) also found weaker negative feedback in older horses and ponies compared to younger individuals. One additional concern with trying to assess age-related differences in negative feedback is that if individuals with weaker negative feedback have lower survival, this may lead to a population of older animals with stronger negative feedback than younger animals regardless of any potential trade-offs. Thus, longitudinal studies tracking the same animals over time are necessary to fully test this prediction. A final consideration is that in some species, weaker negative feedback in older animals may be a maladaptive, pathological response, caused in part by declines in brain receptor density associated with aging (Sapolsky et al., 1986).

In considering evidence for these predictions to date, we must remember that endocrine systems are made up of many moving parts, and minimizing the effects of Cort on reproductively active animals could happen by altering negative feedback regulation in one species, whereas in another this could occur via changes in adrenal sensitivity to ACTH, changes in the abundance or activity of enzymes that metabolize Cort, a reduction in the number of Cort receptors in reproductive tissues, or any number of other ways (Fig. 5). Furthermore, regulating Cort at these different levels results in very different downstream effects. For example, the semelparous mammals mentioned above that breed once and then die may require high circulating Cort to sustain metabolic demands during breeding, so may be more likely to regulate Cort at a tissue-specific level (by reducing Cort receptors in brain and gonads, or increasing enzymes in brain and gonads that convert Cort into an inactive metabolite) rather than at the level of secretion or negative feedback, which would affect all tissues similarly. To truly understand how ecological constraints affect the evolution of endocrine function, researchers would ideally examine multiple levels of the HPA axis in every study. However, assessing many of the more downstream measures of hormone function such as receptors and enzymes requires euthanizing animals, which is not always possible or desirable. Because HPA negative feedback is an important functional measure, and can be assessed using relatively simple, non-lethal techniques, we hope to see more studies incorporating it, especially in reptiles, fish, and amphibian species, where negative feedback is drastically understudied.

5. Conclusions

Current evidence that HPA negative feedback is an important mediator of adaptive trade-offs between survival and reproduction is mixed, although few studies have directly tested the six predictions stated above. The strongest evidence thus far supports a link between poor body condition and weak negative feedback in breeding animals. It is important to note that published papers relevant to these predictions also use different approaches for assessing post-Dex negative feedback: for example, primate studies are more likely to use negative feedback measures that incorporate baseline Cort, whereas wildlife studies are more likely to use post-Dex Cort or a raw or percent decrease from stress-induced Cort. As shown above, although these approaches are correlated, they can provide different patterns of statistical significance that can influence a study's conclusions. Thus, our ability to synthesize the glucocorticoid negative feedback literature is constrained by the varied approaches of reporting negative feedback efficacy, and this ultimately limits our understanding of HPA function.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.113301.

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