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A single exposure to an acute stressor has lasting consequences for the hypothalamo–pituitary–adrenal response to stress in free-living birds

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ABSTRACT

In vertebrates, activation of the hypothalamo–pituitary–adrenal (HPA) axis in response to unpredictable events results in elevated glucocorticoid secretion. Repeated exposure to stressors alters subsequent glucocorticoid secretion, either by inducing chronic stress or as a result of habituation. However, most studies of repeated stress focus on the impacts of multiple and frequent exposures to acute stressors, and few have been carried out in free-living animals. We investigated whether a single exposure to a novel stressor was sufficient to produce long-lasting alterations in HPA function in free-living eastern bluebirds (*Sialia sialis*). We subjected adult females to a capture/restraint protocol in which we collected serial blood samples over an hour of restraint to be analyzed for corticosterone. We administered this protocol to three groups of females during the nestling phase of their first and/or second brood of the season: Repeaters (sampled during brood 1 and brood 2), Naïve-Brood 1 (sampled only during brood 1), and Naïve-Brood 2 (sampled only during brood 2). Repeaters had attenuated corticosterone responses to the second restraint bout compared to the first, and in brood 2, Repeaters had lower responses than Naïve-Brood 2 females. However, Naïve-Brood 1 and Naïve-Brood 2 birds did not differ in their responses to restraint. Thus, as little as one prior experience with an acute stressor was sufficient to alter subsequent HPA responsiveness, and this effect was not due to a natural change in HPA responsiveness as the breeding season progressed. These data may have important implications for understanding how acute stressors can alter a free-living animal's ability to cope in the face of subsequent stressors, and for longitudinal field studies in which individuals are repeatedly sampled for glucocorticoid responsiveness.

1. Introduction

Perception of environmental stimuli as noxious or threatening results in activation of the hypothalamo–pituitary–adrenal (HPA) axis in vertebrates. A consequence of this perception is a rapid and substantial increase in secretion of glucocorticoids (Sapolsky et al., 2000; Norris, 2006) that can alter behavior and physiology consistent with an emergency response (Wingfield et al., 1998). Acute elevations in plasma glucocorticoids, for example, orchestrate changes in reproductive and territorial behavior, immune function, foraging efficiency, glucose metabolism, and locomotion, all of which may help an individual to cope in an unpredictable situation (partially reviewed in Wingfield et al., 1998).

Across vertebrate classes, HPA function, and consequently glucocorticoid secretion, can be modulated in response to a variety of factors. Such factors may include body condition (Tilbrook et al., 2008; Tilbrook and Clarke, 2006; Perfito et al., 2002), age (Heidinger et al., 2006), sex (Astheimer et al., 1994; Wingfield et al., 1995; Perfito et al., 2002; Romero et al., 2006; Tilbrook and Clarke, 2006), and season or reproductive state (Wingfield et al., 1992, 1994, 1995; Astheimer et al., 1994, 1995; Romero et al., 1998a,b, 2006; Tyrell and Cree, 1998; Holberton, 1999; Romero and Remage-Healy, 2000; Perfito et al., 2002; Tilbrook and Clarke, 2006; Schradin, 2008). In addition, glucocorticoid secretion in response to stress may differ depending on the type of stressor to which an individual is exposed (e.g., Canoine et al., 2002; Bowers et al., 2008). Mounting evidence also indicates that alterations in HPA function within individuals may arise over time as a result of repeated exposure to short-term stressors both during development (e.g., Bhatnagar et al., 2005; Walker et al., 2005; Richardson et al., 2006) and in adulthood (see below).

Studies spanning several vertebrate taxa indicate that adult animals exposed to repeated stressful events exhibit reduced glucocorticoid secretion in response to subsequent stressful events. For example, repeated exposure to short-term stressors such as brief, repeated periods of handling, restraint, social crowding, or exogenous glucocorticoid treatment result in attenuation of the hormonal stress response in rats (e.g., Gadek-Michalska and Bugajski, 2003). Often, these effects are profound and relatively quick to manifest. For example, in mature rats, 1 min bouts of handling repeated for only three days were sufficient to reduce the

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subsequent glucocorticoid response to restraint by more than 40% over controls (Gadek-Michalska and Bugajski, 2003). Reduced glucocorticoid secretion in response to repeated handling has also been documented in a variety of laboratory and domesticated animals, including mice (Chen and Herbert, 1995), cattle (Andrade et al., 2001), sheep (Hargreaves and Hutson, 1990), and chickens (Freeman and Manning, 1979; Litten and Cockrem, 2001). Similar reductions in HPA activity following repeated exposure to handling stress have been demonstrated in non-domesticated species in captivity. For example, in wild birds either held in or raised in captivity, repeated capture/restraint resulted in decreased glucocorticoid secretion to the same stimulus (e.g., European starlings, *Sturnus vulgaris*, Romero and Remage-Healy, 2000; great tits, *Parus major*, Cockrem and Silverin, 2002; American kestrels, *Falco sparverius*, Love et al., 2003). These results have largely been attributed to habituation or acclimation to the stress of handling with repeated exposure (i.e., the animal no longer perceives the event as being as threatening; Cockrem and Silverin, 2002; Love et al., 2003; Romero, 2004).

However, changes in HPA function have also been documented in captive and free-living animals in situations in which habituation is not likely. For example, marked changes in HPA function, including lower baseline glucocorticoid levels and lower glucocorticoid secretion in response to handling, were demonstrated in European starlings following repeated short-term exposure to rotating psychological stressors (i.e., a suite of unpredictable stressors; Rich and Romero, 2005; Cyr and Romero, 2007). In addition, repeated acute administration of corticosterone (the primary avian glucocorticoid) to captive white-crowned sparrows (*Zonotrichia leucophrys gambelii*) resulted in higher baseline corticosterone levels, lower corticosterone secretion in response to handling, and changes in body condition consistent with chronic stress (Busch et al., 2008a,b). Thus, in some cases, alterations in HPA activity may not be the result of habituation or acclimation. Rather, an individual's reaction to repeated stressful events may be so severe that the event(s) induce "chronic stress", and mounting an appropriate response to a novel or subsequent stressor is no longer possible (Romero, 2004).

Few studies of repeated stressful events focus on free-living animals. However, recent work has revealed that wild animals that live in areas with high levels of tourism exhibited altered HPA axis function. For example, adult animals living in areas with high levels of tourist visitation had a lower glucocorticoid response to handling/restraint than animals living in more isolated areas (e.g., Galapagos marine iguana, *Amblyrhynchus cristatus*, Romero and Wikelski, 2002; Magellanic penguins, *Spheniscus magellanicus,* Fowler, 1999; Walker et al., 2006), suggesting that repeated disturbance may also alter HPA function in the wild. Studies of the impact of tourism on HPA function in young and juvenile animals reveal mixed results, however (Müllner et al., 2004; Walker et al., 2005), indicating that the effects of repeated exposure to stressors on HPA function may differ according to developmental stage in wild animals. Regardless, whether physiological changes that relate to tourist visitation are the result of habituation or chronic stress remain equivocal. Nevertheless, either scenario (habituation or chronic stress) could have important implications for how free-living animals exposed to repeated disturbances that activate the HPA axis might cope in novel situations. For example, glucocorticoid action helps animals to cope with unpredictable and noxious stimuli (e.g., Wingfield et al., 1998). Thus, an individual whose HPA function is compromised due to chronic stress (i.e., who is unable to mount a sufficient stress response to a novel stressor) may be at a disadvantage in the wild. Alternatively, at least one study suggests that animals that habituate to repeated stressful events may exhibit enhanced stress responses in novel situations. For example, in rats, individuals that had acclimated to a specific "stress" scenario later exhibited different hormonal responses to stress when the stress scenario was altered (Dobrakovova et al., 1993).

Taken together, these studies of both domesticated and non-domesticated vertebrates demonstrate a robust effect of repeated acute stressors on subsequent HPA function. However, most of these studies focus on the impacts of frequent, repeated exposures to acute stressors (i.e., these studies do not provide an indication of how rapidly HPA function may be attenuated), and few studies have been carried out in wild, free-living animals. However, if short-term exposure to a stressor results in persistent alterations in HPA function in free-living animals, this may have important implications for how these experienced individuals cope in the face of subsequent disturbances, and for how field studies investigating glucocorticoid responsiveness to stressors are conducted.

We sought to determine if a *single* exposure to a novel stressor was sufficient to produce lasting alterations in HPA function in free-living, breeding birds during a single breeding season. To this end, we assessed corticosterone secretion in response to capture and restraint in a wild population of eastern bluebirds (*Sialia sialis*) during the first and second broods of the breeding season. We used a capture/restraint protocol modified from that described by Wingfield et al. (1992), a technique that involves capturing an individual and taking serial blood samples to be analyzed for corticosterone (the primary avian glucocorticoid) over a period of 1 h. All birds used in this study were previously naïve to this protocol. To assess effects of prior experience with the protocol on subsequent corticosterone secretion, we administered the capture/restraint protocol to one group of females during the nestling-feeding phase of their first brood of the season (brood 1), and again during the nestling-feeding phase of their second brood of the season (brood 2). To assess whether any changes we might have detected in corticosterone secretion were due to repeated sampling or to the progression of the breeding season (i.e., changes in responsiveness from brood 1 to brood 2), we administered the capture/restraint protocol to two other groups of females: one that was tested only during brood 1, and one that was tested only during brood 2. Because previous studies in captive animals have demonstrated relatively rapid attenuation of glucocorticoid secretion following previous exposure to stressors (Cockrem and Silverin, 2002; Gadek-Michalska and Bugajski, 2003; Love et al., 2003), one plausible hypothesis is that free-living animals should respond in a similar way. From this hypothesis, we predicted that a single exposure to the capture/restraint protocol would alter subsequent corticosterone secretion in free-living female eastern bluebirds, independent of any seasonal changes in HPA axis responsiveness. However, little is known of the time course over which exposure to stressful events can alter HPA responses to subsequent stressors in free-living animals. Therefore, an alternative hypothesis predicts that a single exposure to the capture/restraint protocol would have no effect on subsequent HPA function in free-living eastern bluebirds.

2. Methods

2.1. Birds and study sites

We studied breeding eastern bluebirds in Wayne, Ashland, and Huron Counties, Ohio, during April through August of 2006 and 2007, and April through July of 2008. All birds nested in man-made nest boxes on well-established (>10 years) nest box schemes, and production of two broods per year is common in this study population (Lynn, unpublished data). All individuals were banded with an aluminum US Fish and Wildlife Service band plus a unique combination of colored plastic leg bands for identification. Breeding pairs in this population typically remain mated within a breeding season,