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The glucocorticoid response in a free-living bird predicts whether long-lasting memories fade or strengthen with time

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For decades, scientists have used threat conditioning (traditionally termed 'fear conditioning') to study the link between glucocorticoids and the consolidation of long-term memories (i.e. memories that last hours to weeks) in model organisms in the laboratory. We assessed this relationship in a free-living species, and examined a possible relationship between glucocorticoids and the retention of long-lasting memories (i.e. memories that last months to a lifetime). We developed a novel threat-conditioning protocol by which free-living Florida scrub-jays, *Aphelocoma coerulescens*, were either chased by a novel predator or exposed to a control. We measured flight initiation distance (FID) 48 h, 11 months and 2 years after conditioning or control exposures, and compared these measures to levels of stress-induced glucocorticoids. Conditioned subjects maintained significantly longer FIDs for at least 2 years. Furthermore, the long-term memory consolidation of conditioned subjects positively correlated with their stress-induced glucocorticoid response, similar to results from laboratory studies. Surprisingly, individuals with a moderate stress response exhibited an exaggerated defence response (i.e. FIDs increased) at 11 months and 2 years post-conditioning, whereas low and high stress responders exhibited memory decay or extinction (i.e. FIDs decreased). We speculate that the recently discovered processes of memory reconsolidation and system consolidation may help explain why some Florida scrub-jays exhibit more fearful-like behaviour with time.

Memories of emotionally arousing experiences tend to be better remembered than non-emotive memories (McGaugh & Roozendaal, 2002) in part due to the effects of stress-induced glucocorticoids (reviewed by Cahill & McGaugh, 1998; Rodrigues, LeDoux, & Sapolsky, 2009; Roozendaal, 2002). Glucocorticoids are adrenal cortex-derived steroid hormones that usually circulate at relatively low baseline concentrations but increase following activation of the hypothalamic–pituitary–adrenal (HPA) axis in response to a stressor, such as exposure to a live predator or a predator cue (e.g. Canoine, Hayden, Rowe, & Goymann, 2002; Cockrem & Silverin, 2002a; Jones, Smith, Bebus, & Schoech, 2016; Park, Zoladz, Conrad, Fleshner, & Diamond, 2008).

Memory consolidation is the process by which short-term memories become long-term memories (McGaugh, 2000), and it occurs within hours of initial learning (Clopath, 2012). The retention of consolidated memories can be diminished or prevented by memory decay (i.e. forgetting) or memory extinction (i.e. the masking of a previously learned association by a different, newly learned association; Myers & Davis, 2007; Pavlov, 1927; Quirk, 2002). So-called 'fear' conditioning is a form of Pavlovian conditioning that has been used extensively to evaluate the effects of acute stress on the consolidation of long-term memories in laboratory animals (e.g. McGaugh, 2015; Ng & Gibbs, 1991; Sandi & Pinelo-Nava, 2007; Sandi & Rose, 1994). Fear conditioning is a type of associative learning that is thought to have evolved because it allows animals to learn from past events to avoid future threats (Maren, 2001). Fear conditioning occurs when a stimulus that is innately threatening, known as an unconditioned stimulus (e.g. foot shock), is paired with a relatively benign stimulus (e.g. an audible tone). The benign stimulus then becomes what is known as a conditioned stimulus and is able to elicit a conditioned response (e.g. freezing in laboratory rats) on its own. Although conditioned stimuli are often described as nonthreatening or neutral, rats that are not conditioned often exhibit some degree of freezing when presented with only a conditioned stimulus (e.g. Corley, Caruso, &

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Takahashi, 2012; Lee et al., 2011; Rau, DeCola, & Fanselow, 2005). 'Fear', as it is used in fear-conditioning research, is caused by exposure to a stimulus that is associated with a threat, which in turn triggers defensive behaviours and activates associated neuroendocrine circuits (Perusini & Fanselow, 2015). However, LeDoux (2014) advocated that the use of 'fear' should be reserved for the subjective state of feeling afraid and not the nonconscious neural systems that mediate defence behaviours. We agree, and hereafter use 'threat conditioning' in place of 'fear conditioning' and 'defence response' or 'antipredator response' instead of 'fear response'.

The physiological stress response following a threatening stimulus is mediated by the amygdala, an area of the limbic system also known for its role in mediating emotional responses. In addition to activating an initial sympathetic nervous system response, the amygdala triggers the HPA axis, ultimately leading to a surge of glucocorticoids secreted from the adrenal cortex (Rodrigues et al., 2009). The physiological stress response is self-limiting, as negative feedback within the HPA axis, hippocampus and basolateral amygdala inhibit further release of glucocorticoids (Boyle, Kolber, Vogt, Wozniak, & Muglia, 2006; Herman & Cullinan, 1997; Herman, Dolgas, & Carlson, 1998). Additionally, the basolateral amygdala and hippocampus are key regulatory centres in the formation and retention of threat-conditioning memories (reviewed by Rodrigues et al., 2009) and have been implicated in the regulation of the overall magnitude of the glucocorticoid response following HPA axis activation (Herman, Ostrander, Mueller, & Figueiredo, 2005). Although our understanding of the neuroendocrine pathways of the physiological stress response and learning and memory have been derived from rodent models, the relevant cerebral homologies between the avian and mammalian brain are similar in function and nomenclature (Jarvis et al., 2005).

It is well established that glucocorticoids enhance memory consolidation (reviewed by McGaugh, 2000, 2015; Roozendaal, 2000, 2002; Sandi & Pinelo-Nava, 2007). However, studies have typically examined 'long-term' memories only hours to days beyond learning (e.g. Akirav et al., 2004; Roozendaal, Carmi, & McGaugh, 1996; Roozendaal, Okuda, Van der Zee, & McGaugh, 2006; Salehi, Cordero, & Sandi, 2010), or at most 1 week after learning (e.g. Cordero & Sandi, 1998; Flood et al., 1978; Frost, Castellucci, Hawkins, & Kandel, 1985; Shors, Weiss, & Thompson, 1992). This is surprising given that many animals can retain memories for years (Balda & Kamil, 1992; Bednekoff, Balda, Kamil, & Hile, 1997; Vaughan & Greene, 1984) or even decades (Beran, Pate, Richardson, & Rumbaugh, 2000). Studies of long-term memory have often used 'long-lasting' to describe memories that persist for days to weeks (e.g. Marin, Hupbach, Maheu, Nader, & Lupien, 2011; Quirk, 2002; Zoladz, Fleshner, & Diamond, 2012). However, we use the term 'long-lasting memory' to refer to memory that is retained for months to throughout the lifetime of an individual (see McGaugh, 2000). To the best of our knowledge, the influence of stress physiology on long-lasting memory is unexplored.

The mediation of memory by stress physiology has been well studied in model organisms in the laboratory, but few have studied this relationship in captive wild animals (Mateo, 2008; Pravosudov, 2003; Thaker, Vanak, Lima, & Hews, 2010), or free-living animals (Croston et al., 2016; Thaker et al., 2010). Investigating the effects of stress on memory in free-living animals is valuable for at least two reasons. First, the behaviour and physiology of free-living animals frequently differ markedly from that of their domesticated and laboratory counterparts (e.g. Calisi & Bentley, 2009; Fleming, Agustsson, Finstad, Johnsson, & Bjornsson, 2002; Künzl, Kaiser, Meier, & Sachser, 2003; Stryjek, Modlinska, Turlejski, & Pisula, 2013). Second, wild-caught animals kept in captivity can suffer from chronic stress, which can alter HPA axis function (Dickens, Delehanty, & Romero, 2010; Dickens, Earle, & Romero, 2009; Morgan & Tromborg, 2007; Terio, Marker, & Munson, 2004), and thus confound experiments designed to evaluate the effects of acute stress on memory.

We developed a protocol, based on the threat conditioning (traditionally termed 'fear conditioning') paradigm developed in the laboratory (reviewed by Maren, 2001), to assess the effects of glucocorticoids on the consolidation and retention of long-lasting memory in a free-living species, the Florida scrub-jay, *Aphelocoma coerulescens*. We hypothesized that exposure to glucocorticoids during a threatening event would mediate the consolidation and long-lasting memory of that event. Specifically, we predicted that the amount of endogenous glucocorticoids an individual produced in response to a standardized stressor, which is repeatable within an individual for years (Small & Schoech, 2015), would positively correlate with the consolidation and retention of long-lasting memory of a novel predator attack.

METHODS

Study System

The Florida scrub-jay is a nonmigratory, socially and genetically monogamous, cooperatively breeding avian species endemic to the fire-dependent Florida scrub ecosystem (Quinn, Woolfenden, Fitzpatrick, & White, 1999; Townsend, Bowman, Fitzpatrick, Dent, & Lovette, 2011; Woolfenden & Fitzpatrick, 1984). We studied a population of ca. 200 individuals at Archbold Biological Station, Highlands County, Florida (27°19′N, 81°2l′W, elevation 38–68 m). We used the Florida scrub-jay as a model to study long-lasting memory and stress physiology in a free-living animal because individuals are easily located within year-round territories and are readily identified by unique combinations of colour bands. Also, the population at Archbold Biological Station readily eats and caches peanuts, which we use to bait traps and motivate individuals to participate in behavioural tests (Schoech, Mumme, & Moore, 1991; Schoech et al., 2007). As caching species, *Aphelocoma* jays have well-developed cognitive and memory capabilities (e.g. Bebus, Small, Jones, Elderbrock, & Schoech, 2016; Bednekoff et al., 1997; Brown, 1997; Clayton, Dally, & Emery, 2007), making them an ideal model to study long-lasting memory in a free-living animal. Additionally, Florida scrub-jays have baseline and stress-induced levels of corticosterone (CORT; the primary glucocorticoid in a number of vertebrate taxa, including birds) that are repeatable within individuals across life history stages (Rensel & Schoech, 2011) and multiple years of adulthood (Small & Schoech, 2015).

Threat Conditioning

We developed a novel threat-conditioning protocol to be used in free-living birds to study memory because threatening experiences tend to be better remembered (McGaugh & Roozendaal, 2002) and can stimulate glucocorticoid secretion (Canoine et al., 2002; Cockrem & Silverin, 2002a; Jones et al., 2016). We conditioned randomly selected jays to avoid novel 'predators' (an 'umbrella' in late March through April 2012, and a 'puppet' in April 2013; Fig. 1) by simulating an attack; we chased subjects with the predators for less than 5 s from within 4 m. Many animals learn the dangers of predators via threat conditioning, which is the mechanism by which innately threatening stimuli (unconditioned stimuli; e.g. an attack, an alarm call) become associated with the predators themselves (conditioned stimuli; Griffin, Blumstein, & Evans, 2000; Griffin, 2004).