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# Adult males buffer the cortisol response of young guinea pigs: Changes with age, mediation by behavior, and comparison with prefrontal activity

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ABSTRACT

In the guinea pig, the presence of the mother buffers hypothalamic-pituitary-adrenal (HPA) responses of her young during exposure to a novel environment, and can do so even if she is anesthetized. In contrast, under comparable conditions other conspecifics (siblings, other adult females) are less effective or ineffective in doing so. However, we recently observed that an unfamiliar adult male reduced plasma cortisol elevations and increased Fos in the prefrontal cortex of preweaning pups exposed to a novel enclosure for 120 min. Here we found adult males buffered the adrenocortical response of preweaning pups at 60 as well as 120 min and of periadolescent guinea pigs if exposure was of 120 min. Further, because males vigorously engaged in social interactions with the young during exposure, we examined the effect of behavior by comparing the impact of conscious and unconscious (anesthetized) adult males. When tested with a conscious but not unconscious male, pups exhibited reduced plasma cortisol elevations. Pups, particularly females, had greater Fos induction in the prefrontal cortex when with a conscious versus unconscious adult male. Overall, we found that an unfamiliar adult male can buffer the cortisol response of guinea pigs both before and after weaning, though more-prolonged exposure appears necessary in the older animals. Further, unlike buffering by the biological mother, the effect of the male is mediated by behavioral interactions. Thus, the buffering of the infant guinea pig's cortisol response by the mother and an unfamiliar adult male involve different underlying mechanisms.

## 1. Introduction

The physiological impact of a stressful event often depends on the social conditions under which it occurs. Activation of the hypothalamic-pituitary-adrenal (HPA) axis can be greatly reduced or eliminated in the presence of particular social partners. Rodent and nonhuman primate studies have found the maternal figure can often buffer HPA responses of their infants in challenging situations (Hill et al., 1973; Smotherman et al., 1979; Shionoya et al., 2007; Wiener et al., 1987). Similarly, the presence of the infant has been observed to reduce the response of the mother (Ritchey and Hennessy, 1987; Wiener et al., 1987). These effects extend to adult partners that exhibit attachment-like adult social bonds, particularly pair-mates in monogamous species (Hennessy et al., 1995b; Rukstalis and French, 2005; Smith and Wang, 2014; Remage-Healey et al., 2003; Sachser et al., 1998). Although the existence of an attachment-like bond appears to be the best predictor of whether a partner can buffer HPA activity, other variables, such as genetic relatedness or species-typical social grouping patterns, have been associated with buffering ability as well. For instance, in adult male rats, an unfamiliar male of the same or closely related genetic strain reduced plasma corticosterone elevations to a tone conditioned to foot shock, whereas a male of more-distantly related strains did not (Nakamura et al., 2016). Further, in juvenile sheep, which tend to flock in threatening situations, lack of access to pen-mates elevated plasma corticosterone levels in the presence of a human intruder, whereas in juvenile goats, which are more-likely to disperse under threat, pen-mates were without effect (Lyons et al., 1993).

Guinea pigs offer a clear example of buffering by the maternal attachment figure. The pups are born physically mature, begin following the mother almost immediately, and display evidence of filial attachment (Hennessy and Ritchey, 1987; Jäckel and Trillmich, 2003; Pettijohn, 1979). When housed with mother and littermates in standard laboratory caging, pups exposed to a novel environment either alone or with littermates show robust HPA activation that is blunted or abolished if tested with the mother (Hennessy and Moorman, 1989; Hennessy et al., 2015; Ritchey and Hennessy, 1987). Other adult females buffer the pup's cortisol response, but not as consistently or potently as does the mother (Graves and Hennessy, 2000; Hennessy and

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Ritchey, 1987). However, after the pups are weaned on about Day 25, selectivity wanes in that any adult female appears to be as effective as the mother in reducing HPA responsiveness of the young (Graves and Hennessy, 2000; Hennessy et al., 2006; Maken and Hennessy, 2009). In an initial study examining the buffering ability of adult males, offspring that were ~40–50 days old and just approaching sexual maturity (referred to here as periadolescents) exhibited elevations of plasma cortisol 20 and 60 min following exposure to a novel cage that were as great when in the presence of a conscious adult male as when alone (Hennessy et al., 2002). However in a recent study, we found quite different results in younger animals. Preweaning (Day 16) pups exposed to a novel cage for 120 min showed reductions in plasma cortisol to baseline levels when tested with either the adult male or the biological mother (Hennessy et al., 2015). Moreover, only the presence of the adult male induced increased levels of the neural activation marker, Fos, in the pups' prelimbic cortex, a region of prefrontal cortex known to inhibit HPA activation in rats (Jones et al., 2011; Radley et al., 2006). Thus, increased Fos activity might reflect a neural mechanism through which buffering by the male was achieved. These findings were particularly unexpected since there is no evidence of affiliation between pups and adult males or of paternal care in the guinea pig, and because the pups had never been directly exposed to an adult male prior to testing.

These results raised two questions addressed here: (1) why the cortisol findings of the two earlier studies differed; and, (2) the process by which males produced the buffering effect in pups. Regarding the first question, not only the age of the subjects, but the time point at which blood was obtained for cortisol analysis differed between the two studies, i.e., periadolescents sampled at 20 and 60 min and preweaning pups sampled at 120 min. Thus the discrepancy in findings may have been due to either the different ages/developmental states of the offspring or to the time points at which blood samples were obtained for cortisol analysis. The first purpose of the current study was to distinguish between these possibilities. As for the second question, the one clue as to how the adult male may have suppressed the pup's cortisol response was the behavior occurring between the animals. Pups and adult males showed much more social interaction than did pups tested with either the mother or littermates. These interactions consisted of a "greeting" response in which animals touch noses, anogenital and other investigation of the young by the male, and mild agonistic interactions directed at the pup, particularly the male pushing or lifting the pup with its snout. Therefore, the second purpose of the present study was to determine the effect of the adult male's behavior on plasma cortisol and prelimbic Fos levels by comparing pups tested with fully conscious and anesthetized adult males. In all experiments, we recorded social behaviors occurring between subjects and adult males. Additionally, for comparison with cortisol results, we scored behavioral responses typical of young guinea pigs during isolation in a novel environment.

## 2. Method

### 2.1. Animals

Albino guinea pigs (*Cavia porcellus*) of the Hartley strain were bred in our laboratory. Following birth (Day 0), each mother was housed with her litter in an opaque plastic cage (73 cm × 5 cm × 24 cm) with a wire front and sawdust bedding. Water and guinea pig chow were available ad libitum. Animals were maintained on a 12:12 light:dark cycle with lights on at 07:00 h. All procedures were approved by the Wright State University Institutional Animal Care and Use Committee. Adult males from our breeding colony served as stimulus animals. The stimulus male was never the father of, and always unfamiliar to, the test subject. Mothers and litters were housed in the general colony room until several days before testing, and then transferred to the laboratory colony room that housed only mothers and litters. Thus, while no subject had direct experience with adult males, all had previously been exposed to odors of all male breeders. At least six different stimulus males were used in each condition.

### 2.2. Experimental conditions

Experiment 1 addressed the discrepancy in results of our earlier studies by asking how timing of blood sample collection affected buffering of the cortisol response of pups by adult males. Day 16 ( ± 1) pups were either removed from the home cage prior to any disturbance (Undisturbed) or were exposed to the test cage for 60 or 120 min while either alone (Alone 60, Alone 120) or in the presence of a fully conscious adult male (ConMale 60, ConMale 120) prior to blood sample collection. Six males and six females from 12 different litters were tested in each of the five conditions. Experiment 2 assessed the effect of behavioral interactions on the ability of the male to reduce the plasma cortisol response and increase prelimbic Fos levels of pups. Day 16 ( ± 1) pups were tested in four conditions: Undisturbed, Alone 120, ConMale 120, and UnconMale 120. In the latter condition, the male was anesthetized with an IP injection of ketamine (35 mg/kg) and xylazine (5 mg/kg) cocktail just prior to placement in the test cage and introduction of the pup. Six or seven males and six females from 10 to 12 different litters were tested in each of the four conditions. Four of these males and four of these females were randomly selected for measurement of Fos. Finally, Experiment 3 examined whether adult males could buffer the cortisol response of periadolescents if the animals had a full 120 min to interact prior to blood sample collection. Day 46 ( ± 1) periadolescents were tested in the Undisturbed, Alone 120 and ConMale 120 conditions. Seven or eight male and eight female periadolescents from 14 or 15 different litters were tested in each of the three conditions.

### 2.3. Social buffering tests

For testing, the subject was removed from its home cage and taken quietly in a carrying cage to the nearby testing room where it was placed into a clear, empty plastic cage (55 cm × 32 cm × 18 cm) under full room lighting either alone or together with an adult male for either 60 or 120 min as designated. A trained observer behind one-way glass scored behavior during the first and last 30-min segments of the 2-h tests. These intervals were chosen to best capture behaviors of the active and passive stages, respectively, that are characteristic of young isolated guinea pigs (Hennessy et al., 1995a). We tallied the number of times subjects emitted the whistle vocalization (Berryman, 1976), the primary behavior of the active phase, as well as the number of 1-min intervals in which pups exhibited the crouched stance, eye-closure, and extensive piloerection typical of the passive stage. Our dependent measure ("full passive" behavior) was a composite of the number of 1-min intervals in which pups exhibited all three passive behaviors. On occasion, crouching transitioned into lying down, which could be substituted for crouching, i.e., the full passive response was scored if eye-closure, piloerection, and either crouch or lying down were observed in the same 1-min interval. As in our previous study, we scored the frequency of both non-agonistic (nose-nose, anogenital investigation, fur sniff) and mildly agonistic behaviors (kick, nip, thrust/lunge/lift) between the subject and adult male. See Table 1 for full definitions of behavioral categories. A microphone transmitted vocalizations to the head phones of the observer who tallied them with a hand counter. Other behaviors were scored on check sheets. Test cages were cleaned with detergent after each test.

### 2.4. Blood sample collection and cortisol determination

Blood was collected either following no disturbance or immediately following exposure to the novel test cage, between 1330 and 1600 h. All samples were collected on heparin under CO2 anesthesia either by decapitation or by cardiac withdrawal with a tuberculin syringe. In