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Maternal stress and plural breeding with communal care affect development of the endocrine stress response in a wild rodent

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ABSTRACT

Maternal stress can significantly affect offspring fitness. In laboratory rodents, chronically stressed mothers provide poor maternal care, resulting in pups with hyperactive stress responses. These hyperactive stress responses are characterized by high glucocorticoid levels in response to stressors plus poor negative feedback, which can ultimately lead to decreased fitness. In degus (*Octodon degus*) and other plural breeding rodents that exhibit communal care, however, maternal care from multiple females may buffer the negative impact on pups born to less parental mothers. We used wild, free-living degus to test this hypothesis. After parturition, we manipulated maternal stress by implanting cortisol pellets in 0%, 50–75%, or 100% of adult females within each social group. We then sampled pups for baseline and stress-induced cortisol, negative feedback efficacy, and adrenal sensitivity. From groups where all mothers were implanted with cortisol, pups had lower baseline cortisol levels and male pups additionally had weaker negative feedback compared to 0% or 50–75% implanted groups. Contrary to expectations, stress-induced cortisol did not differ between treatment groups. These data suggest that maternal stress impacts some aspects of the pup stress response, potentially through decreased maternal care, but that presence of unstressed mothers may mitigate some of these effects. Therefore, one benefit of plural breeding with communal care may be to buffer post-natal stress.

Introduction

Maternal effects are important contributors to phenotypic variation among individuals (Mousseau and Fox, 1998). Independent of genetic contributions, mothers can significantly influence the phenotypes of their offspring through habitat selection and resource allocation during development. After birth, mothers and social group members can continue to affect offspring phenotypes by altering the quality and quantity of parental care. These parental care behaviors can significantly influence offspring fitness by affecting future reproductive output (Lindstrom, 1999), growth rate (Metcalfe and Monaghan, 2001), immune function (Saino et al., 1997), and stress responsiveness (Francis et al., 1999; Liu et al., 1997). Low rates of parental care have generally been associated with negative or maladaptive offspring development (Cottrell and Seckl, 2009; Meaney et al., 2007) although there has been increasing recognition of maternal effects as potential adaptive responses to environmental heterogeneity, as what may be considered a "maladaptive phenotype" in a benign environment can be beneficial in a stressful or challenging environment (Dantzer et al., 2013; Mousseau and Fox, 1998; Sheriff and Love, 2013).

For mammals, parental care encompasses many different behaviors including thermoregulatory huddling, nursing, predator protection, and licking and grooming. In rodents, licking and grooming, which are almost exclusively performed by the mother, are important for development of the hypothalamic–pituitary–adrenal (HPA) axis (Francis et al., 1999; Liu et al., 1997). Chronically stressed rodent mothers have lower rates of licking and grooming (Brummelte and Galea, 2010), and pups receiving relatively low amounts of licking and grooming increase glucocorticoid (GC) secretion (Liu et al., 1997). Pups exposed to higher, sustained levels of GCs are more likely to develop hyperreactive stress responses (Francis et al., 1999; Liu et al., 1997), which are characterized by increased GC output in response to stressors, plus poor negative feedback. Together, these factors increase long-term GC exposure, which has been linked to lower adult survival and reproductive success (Cottrell and Seckl, 2009; Matthews and Phillips, 2010; Monaghan et al., 2012).

Because the stress response is a key component in maintaining physiological homeostasis (McEwen and Wingfield, 2003; Romero et al., 2009), maternal-induced alterations to the offspring HPA-axis can have widespread effects on numerous physiological and behavioral

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processes. When an animal encounters a stressor, corticotropin-releasing factor (CRF) is secreted from the hypothalamus, which stimulates release of adrenocorticotropic hormone (ACTH) from the anterior pituitary, which then increases secretion of GCs from the adrenal glands. When circulating GC levels are elevated, a variety of physiological effects occur that essentially shift energy away from non-necessary processes to those crucial for survival. In the short-term, these effects include increased energy mobilization, inhibition of the reproductive system, and priming of the fight-or-flight response (Sapolsky et al., 2000). However, these "adaptive" physiological effects can shift to being pathological if GC levels remain elevated for long periods of time, resulting in diabetes, immunosuppression, reproductive shut-down, and cardiovascular disease (McEwen, 1998; Sapolsky et al., 2000). Animals with hyper-reactive stress responses may be more susceptible to developing these pathologies (Romero and Wikelski, 2010), as amplified GC responses to stressors and poor negative feedback cause greater, integrated GC exposure.

While the relationships between post-parturition maternal stress, maternal care, and the offspring stress response have been well studied in laboratory rodents (Hennessy, 2003; Liu et al., 1997; Own and Patel, 2013), this has remained relatively unexplored in wild, free-living animals. Additionally, there are few studies examining whether care from other members within a social group can significantly contribute to the development of the offspring stress response (Birnie et al., 2013; Branchi et al., 2013; Harris et al., 2013). We assessed whether post-parturition maternal stress affected the development of the offspring stress response in wild, free-living degus (*Octodon degus*). Degus are social, caviomorph rodents endemic to central Chile. Cortisol is the predominant glucocorticoid hormone in degus (Kenagy et al., 1999), and while degus are precocial rodents, their post-natal basal glucocorticoid profiles are more similar to altricial rodents such as rats and mice than to other precocial caviomorph rodents such as domestic guinea pigs (*Cavia porcellus*) (Gruss et al., 2006). Degus are plural breeders which means that several females reproduce and raise their offspring within a group (Hayes, 2000). Additionally, degus exhibit communal care, where mothers within the group will lick, groom, and even nurse other group member's offspring (Hayes et al., 2009). There are obvious fitness consequences to plural breeding with communal care, but a recent study using a long-term dataset suggests that degus benefit from plural breeding with communal care only during years of low food availability (Ebensperger et al., 2014). The mechanisms behind this relationship are unknown, however, as several laboratory and field studies have failed to find any direct benefits degus could be deriving from this unique reproductive strategy (Ebensperger et al., 2007, 2011a, 2011b, 2015; Hayes et al., 2009). One possible benefit degus may receive from plural breeding with communal care is a "buffering effect" against post-natal stress; if a chronically stressed mother is not providing sufficient care for her offspring, other mothers within the group may be able to partially compensate.

Wild, free-living adult female degus were radio-collared to determine social group membership (Hayes et al., 2009). Directly after parturition, each social group was assigned to one of three treatments: CORT, Mixed, or Control. In CORT groups, all females were implanted with 21-day release cortisol pellets, while 50–75% and 0% of females were implanted in Mixed and Control groups, respectively. When pups emerged from burrows at 4 weeks of age, we sampled them to determine baseline and stress-induced cortisol levels. Additionally, we assessed their negative feedback efficacy via a dexamethasone suppression test and their adrenal sensitivity via an ACTH challenge.

The purpose of this study was to address two hierarchical questions and their associated hypotheses: 1) how does post-parturition maternal stress affect the development of the offspring stress response in wild, free-living degus and 2) how does plural breeding with communal care influence these effects of maternal stress on offspring stress response development? If maternal stress affects the development of the offspring stress response (Hypothesis 1), then we predicted that pups from CORT groups would have hyper-reactive stress responses (high stress-induced GCs and weak negative feedback) compared to pups from Control groups (Fig. 1a–b). If plural breeding with communal care helps buffer post-natal stress (Hypothesis 2), then we predicted that only pups from CORT groups would have hyper-reactive stress responses compared to pups from Control and Mixed groups (Fig. 1c).

Methods

Study animals

This study was conducted from August 3rd–October 29th, 2011 and August 9th–November 9th, 2012 near Santiago, Chile at Estación Experimental Rinconada de Maipú (33°23′S, 70°31′W, altitude 495 m), a field site owned and maintained by the Universidad de Chile. Although degus are precocial, pups spend the first 4 weeks of life in underground burrows (Bauer et al., 2014). When pups emerge aboveground, they forage near the maternal burrow but will continue nursing for another

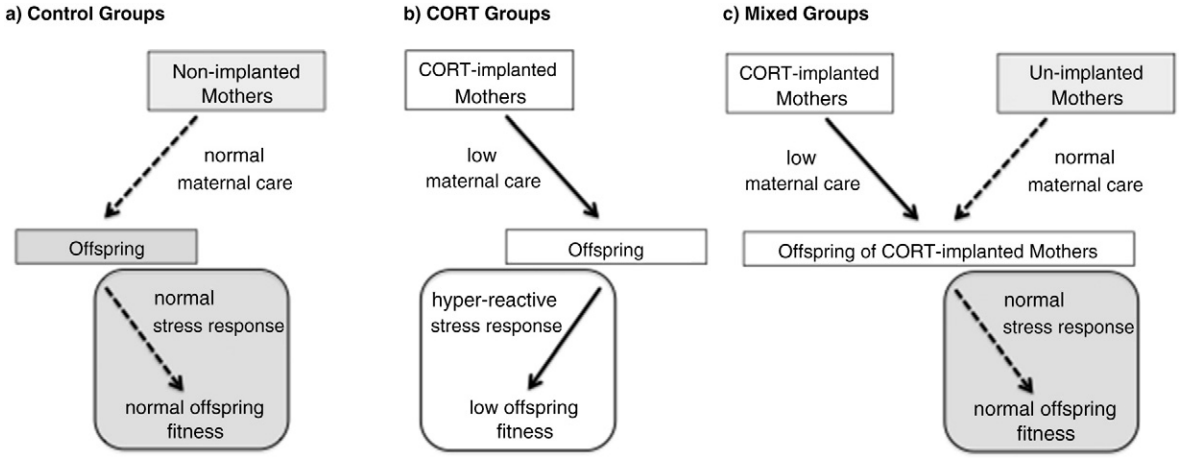

Fig. 1. Relationship between maternal stress, maternal care, the offspring stress response, and offspring fitness. Hypothesis 1 posits that maternal stress can affect the development of the offspring stress response. If stressed mothers provide low rates of maternal care to offspring, this may cause offspring to develop hyper-reactive stress responses, which may then lead to decreased fitness. Under this hypothesis, we predicted that compared to pups from a) Control groups (all mothers unimplanted), pups from b) CORT groups (all mothers implanted with CORT) would develop hyper-reactive stress responses. Hypothesis 2 posits that because all mothers within the group provide care for all pups, then the potential consequences of maternal stress are buffered. Under this hypothesis, we predicted within the c) Mixed groups (~50% of mothers implanted with CORT), *all* pups should develop normal stress responses and avoid negative fitness consequences. If plural breeding with communal does not help buffer the stress response, then we predicted that ~50% of pups from Mixed groups would have normal stress responses, as those with CORT-implanted mothers would have hyper-reactive stress responses.

1–3 weeks (Becker et al., 2007). Laboratory studies have confirmed that mothers will nurse both their pups and other group member pups (Ebensperger et al., 2002, 2006), as well as provide other forms of maternal care such as licking, grooming, and huddling. While male degus may also provide some of the later forms of care (Braun et al., 2013), their contribution to parental care does not impact offspring quality or survival (Ebensperger et al., 2010).

Social group determination

To capture degus, we used Tomahawk live traps (Tomahawk Live Trap Company, Hazelhurst, WI, USA) baited with plain, rolled oats. Newly captured degus were sexed, ear-tagged for identification purposes, and weighed to the nearest 0.1 g. For female degus, pregnancy status was determined via abdominal palpation. All female degus captured in this study were pregnant and showed signs of lactation after parturition, typical of this field site (Ebensperger et al., 2011a, 2011b).

Degus were then fitted with either 8 g (BR radiocollars, AVM Instrument Co., Colfax, CA, USA) or 7–9 g radiotransmitters (RI-2D, Holohil Systems Limited, Carp, ON, Canada, and SOM-2190A, Wildlife Materials Incorporated, Murphysboro, IL, USA). All radiocollars had unique pulse frequencies that could be tracked with hand-held three-element Yagi antennas (AVM Instrument Co., or Advanced Telemetry Systems, Isanti, MN, USA) fitted with either LA 12-Q (for 150.000-151.999 MHz frequency transmitters; AVM Instrument Co.) or FM-100 receivers (for 164.000-164.999 MHz frequency transmitters; Advanced Telemetry Systems). We used the same radiotelemetry techniques, trapping schemes, and data analysis as described in Hayes et al. (2009) to determine social group membership. Briefly, individuals that spent 50% or more of their nights together in the same burrow system were assigned to the same social group. We removed radiocollars immediately prior to parturition as changes in group membership typically occur between mating and early/mid pregnancy.

Cortisol implants

Social groups were divided into three different treatments: Control, Mixed, and CORT. Control groups ($n = 13$) received no cortisol implants, whereas 50–75% of females within each Mixed group ($n = 5$) and 100% of females within each CORT group ($n = 9$) were implanted with cortisol pellets. Because social groups from a separate study were opportunistically used as Control groups, we had more Control groups than Mixed or CORT groups. Because surgery is a major stressor, we did not implant Control females and certain Mixed females with placebo implants because the aim of our experiment was to manipulate stress levels, not solely cortisol levels. Consequently, surgical implantation of a placebo is the proper control for cortisol increases, but lack of surgery is the proper control for stress per se. Female degus were implanted 1–2 days after parturition. Females were anesthetized via inhaled Isoflurane and pellets were implanted on the back of the neck, under the skin. In 2011, we used 60-day release, 35 mg hydrocortisone pellets (Innovative Research of America, Sarasota, FL, USA). In 2012, we switched to using smaller pellets that gave the same daily dose (21-day release, 10 mg hydrocortisone pellets, Innovative Research of America). We switched to these pellets because we found in 2011 that pups emerged aboveground at 3–4 weeks of age, and therefore maternal care after 3 weeks of age was unlikely to have significant effects on HPA-axis development in pups. We verified that cortisol implants elevated circulating cortisol levels in female degus 2–4 days after implantation by determining baseline cortisol levels in a sub-sample of un-implanted and implanted female degus (166 ± 42 vs. 357 ± 93 ng mL^{-1}, respectively, mean ± sem; $t_{(13)} = 2.2$, $P = 0.02$). Inducing and/or mimicking chronic stress in a free-living animal is difficult because chronic stress is more than just GC exposure and also includes activation of the fight-or-flight response (Cyr and Romero, 2006) and exposure to CRF and other hormones higher up in the HPA-axis. However, by manipulating cortisol levels, we were able to mimic at least one arm of the chronic stress response.

Blood sampling

Degu pups were trapped and sampled between 0700 and 1300, but time of day does not correlate with baseline cortisol levels in this species (Bauer et al., 2013). One to two observers with binoculars continuously monitored nearby burrow systems so exact time of capture could be determined. Pups were then bled from the saphenous vein into heparinized, microhematocrit capillary tubes for a baseline blood sample (~30 μL) within 3 min of capture (Romero and Reed, 2005). Pups were then ear-tagged, sexed, and weighed to the nearest 0.1 g. Stress-induced blood samples (~15 μL) were taken 30 min after capture, immediately after which pups were injected intra-peritoneally with a 1 mg kg^{-1} of body weight dose of dexamethasone (Bauer et al., 2013). Dexamethasone (DEX) is a synthetic glucocorticoid that inhibits endogenous glucocorticoid release and is commonly used to test negative feedback efficacy (Boonstra and Singleton, 1993; Carroll et al., 1981; Romero and Wikelski, 2010; Sapolsky and Altmann, 1991). A blood sample (~30 μL) was taken 90 min after DEX injection, after which pups were given an intra-peritoneal injection of ACTH (adrenocorticotropic hormone from porcine pituitary, Sigma Aldrich, St. Louis, Mo., USA) at a dose of 50 IU kg^{-1} of body weight. This dose should induce maximal secretion of cortisol, as it is much higher than that used in most small mammal studies (Boonstra et al., 1998, 2008, 2011). A final blood sample (~15 μL) was taken 15 min after ACTH injection. The total amount of blood taken (~90 μL) was below 1% of each individual's body weight, as per the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes et al., 2011). Between blood samples, pups were kept in shaded traps and supplied with oats. Pups were then released at their home burrow system.

Radioimmunoassays

Blood samples were kept in a cooler with ice packs for no more than 9 h and were then centrifuged for 2–3 min at ~230 G. Plasma was drawn off and stored in sealed Eppendorf tubes at −20 °C until further analysis. Samples were measured for cortisol with commercial I^{125} radioimmunoassay kits (Corti-Cote Solid Phase Component System, MP Biomedicals LLC, Irvine, CA, USA) that we previously validated for measuring cortisol in degus (Bauer et al., 2014). Total sample volume was brought up to 50 μL with distilled water and samples were then assayed in duplicate. Assay sensitivity was 0.7 ng mL^{-1} and intra- and inter-assay variation were 3.8% and 9.6%, respectively.

Statistical analyses

Negative feedback efficacy was calculated as percent decrease from stress-induced cortisol levels after DEX injection, taking into account baseline cortisol levels: (stress-induced cortisol − DEX cortisol) / (stress-induced cortisol − baseline cortisol) * 100. If DEX cortisol levels were higher than stress-induced cortisol levels, then a negative feedback level of zero was assigned since it is not clear what the biological relevance is of having a response worse than no response. Because it was difficult to get multiple blood samples from pups on cold mornings, we do not have all four samples for each individual pup. Therefore, sample size is not the same for all stress response parameters. Because baseline cortisol, stress-induced cortisol, negative feedback efficacy, and adrenal sensitivity are independently regulated (Reul et al., 1987; Romero, 2006), we analyzed each variable separately. Data were analyzed with mixed model ANOVAs (PROC MIXED) in SAS (Version 9.3; SAS Institute, Cary, North Carolina, USA). We controlled for the effect of social group on pup body mass, baseline cortisol, stress-induced cortisol, negative feedback efficacy, and adrenal sensitivity by including

social group as a random variable in the mixed models. We included year, sex, number of adult females within the social group, social group treatment, and sex * treatment as fixed effects. We included year * treatment as a fixed effect because we only had one "Mixed" group in 2011. Effect sizes for significant main fixed factor effects were reported using Cohen's f^2, which is considered an appropriate effect size measure for mixed models (Selya et al., 2012). For significant main effects, we tested for pair-wise differences by using Least Squares Means post-hoc tests. Cohen's *d* was reported as the effect size for pair-wise differences. For significant interactive effects, we examined simple effects separately and controlled for multiple comparisons using Bonferroni's alpha adjustment procedure (Pedhazur and Schmelkin, 1991).

Results

Body mass

There was no significant effect of treatment ($F_{2,61} = 0.03$, $P = 0.97$), year ($F_{1,61} = 1.02$, $P = 0.32$), sex ($F_{1,61} = 0.38$, $P = 0.54$), number of adult females ($F_{2,61} = 0.26$, $P = 0.77$), year * treatment ($F_{1,61} = 2.68$, $P = 0.11$), or sex * treatment ($F_{2,61} = 0.64$, $P = 0.53$) on pup body mass.

Baseline cortisol

CORT pups had significantly lower baseline cortisol compared to Control and Mixed pups (Fig. 2a; $F_{2,59} = 4.63$, $P = 0.01$, Cohen's $f^2 = 0.05$, LSM post-hoc: $P < 0.01$, Cohen's $d = -0.60$ and $P = 0.02$, Cohen's $d = -0.76$, respectively). Year had a significant effect on baseline cortisol ($F_{1,59} = 5.95$, $P = 0.02$, Cohen's $f^2 = 0.08$); and pups from 2011 had lower baseline cortisol levels compared to pups from 2012 (111 ± 17 vs. 230 ± 37 ng mL^{-1}, respectively, mean ± sem). However, there was no significant year * treatment effect ($F_{2,59} = 2.52$, $P = 0.09$). There was also no significant effect of sex ($F_{1,59} = 0.80$, $P = 0.38$), number of adult females ($F_{2,59} = 1.84$, $P = 0.17$), or sex * treatment ($F_{2,59} = 3.12$, $P = 0.06$) on pup baseline cortisol levels.

Stress-induced cortisol

There was no significant effect of treatment ($F_{2,61} = 0.88$, $P = 0.42$), year ($F_{1,61} = 0.71$, $P = 0.40$), sex ($F_{1,61} = 0.27$, $P = 0.61$), number of adult females ($F_{2,61} = 0.55$, $P = 0.58$), year * treatment ($F_{2,61} = 0.81$, $P = 0.45$), or sex * treatment ($F_{2,61} = 0.69$, $P = 0.51$) on pup stress-induced cortisol levels (Fig. 2b).

Negative feedback

While negative feedback efficacy was not significantly affected by treatment ($F_{2,51} = 2.20$, $P = 0.12$), year ($F_{1,51} = 0.12$, $P = 0.73$), number of adult females ($F_{2,51} = 0.23$, $P = 0.80$), or year * treatment ($F_{2,51} = 0.28$, $P = 0.76$), there was a significant effect of sex ($F_{1,51} = 7.70$, $P < 0.01$, Cohen's $f^2 = 0.14$) and sex * treatment ($F_{2,51} = 5.02$, $P = 0.01$). When we examined each sex separately, we found a significant treatment effect in males (Fig. 2c; $F_{2,19} = 4.66$, $P = 0.02$, Cohen's $f^2 = 0.15$) but not females ($F_{2,21} = 0.76$, $P = 0.48$). Within males, Control and Mixed pups had significantly stronger negative feedback efficacy than CORT pups (LSM post-hoc: $P < 0.05$, Cohen's $d = 0.98$ and $P < 0.01$, Cohen's $d = 1.40$, respectively). When we examined each treatment separately, we found that males had stronger negative feedback only within the Mixed treatment (Fig. 2c; Control: $F_{1,17} = 0.24$, $P = 0.63$; Mixed: $F_{1,19} = 12.00$, $P < 0.01$, Cohen's $f^2 = 0.50$; CORT: $F_{1,18} = 1.20$, $P = 0.29$).

Adrenal sensitivity

Pup cortisol response to ACTH injection was not significantly affected by treatment (Fig. 2d; $F_{2,50} = 0.23$, $P = 0.80$), year ($F_{1,50} = 0.26$, $P = 0.61$), sex ($F_{1,50} = 0.85$, $P = 0.36$), number of adult females

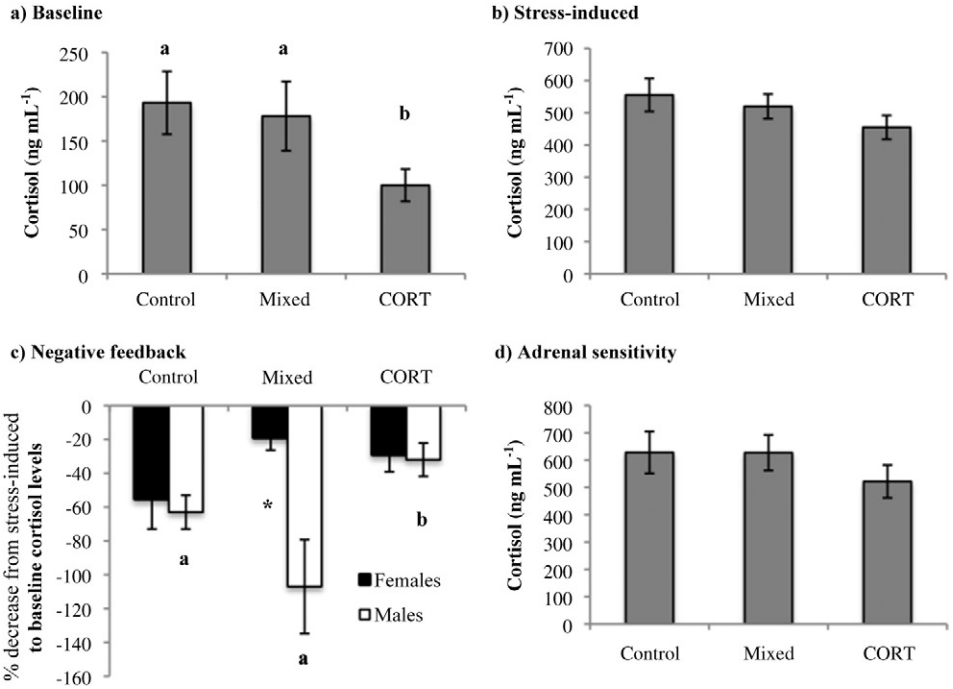

Fig. 2. Mean ± sem a) baseline and b) stress-induced cortisol levels of degu pups from Control ($n = 35$ and 35), Mixed ($n = 26$ and 27), and CORT ($n = 28$ and 29) treatment groups, respectively. Mean ± sem c) negative feedback efficacy of female and male degu pups from Control ($n = 12$ and 15), Mixed ($n = 14$ and 12), and CORT ($n = 16$ and 12) treatment groups, respectively. Note that a larger decrease indicates stronger negative feedback. Individuals with negative feedback values of less than −100% were able to bring cortisol concentrations below baseline levels after DEX injection. Mean ± sem d) cortisol response to adrenocorticotropic hormone (ACTH) injection of degu pups from Control ($n = 32$), Mixed ($n = 22$), and CORT ($n = 25$) treatment groups. Different letters indicate significant differences within treatment (but only within males for negative feedback efficacy). Asterisks indicate significant sex differences within treatments.

($F_{2,50} = 1.41$, $P = 0.25$), year * treatment ($F_{2,50} = 0.66$, $P = 0.52$), or sex * treatment ($F_{2,50} = 0.87$, $P = 0.42$).

Discussion

The purpose of this study was to determine if post-parturition maternal stress affected the development of the offspring stress response in wild, free-living degus, and whether plural breeding with communal care could help buffer this effect (Fig. 1). Our results provide the first evidence that post-parturition maternal stress can significantly influence the development of the offspring stress response in a wild free-living mammal, and that plural-breeding with communal care may help buffer some of these effects.

Baseline glucocorticoid levels are commonly used as indicators of an animal's health or condition (Bonier et al., 2009). Degu pups from social groups where all mothers were implanted with cortisol pellets (CORT groups) had lower baseline cortisol levels compared to pups from groups where only some or none of the mothers were implanted (Mixed and Control groups). We did not originally predict that baseline cortisol levels would differ between our treatment groups since rat pups (*Rattus norvegicus*) from low licking/grooming arched-back nursing (LG/ABN) mothers had similar baseline GC levels compared to pups from high LG/ABN mothers (Liu et al., 1997). However, laboratory rat studies that directly manipulated GC levels in mothers or pups found different trends. Juvenile rats whose mothers were exposed to GCs after parturition had decreased baseline GC concentrations (Catalani et al., 1993; McCormick et al., 2001), as did rats whose mothers were implanted with corticosterone pellets 3–18 days after birth (Turner and Taylor, 1976). Our findings may fit better with the latter studies since we directly manipulated adult female degu GC levels in our study whereas Liu et al. (1997) used rat dams with naturally varying LG/ABN rates. Our results also fit well with the single, non-rat mammalian study that also examined the effects of maternal stress and care on offspring baseline GC levels. Weaver et al. (2000) found that male piglets (*Sus domesticus*) separated from their mothers for 10 min/day over the first 2 weeks of life had lower baseline GC concentrations as adults. Therefore, while we had no a priori predictions concerning pup baseline GC levels, our results generally support that pups from burrows where all mothers were implanted with cortisol pellets had hypo-reactive stress responses, as low baseline GC levels decrease an individual's integrated GC exposure.

Negative feedback is an integral part of the HPA-axis, and hyper-reactive stress responses are often typified by weak negative feedback. Male degu pups from CORT groups had weaker negative feedback compared to male pups from Mixed and Control burrows. These findings are in line with our original predictions and are supported by studies demonstrating that rat pups from low LG/ABN mothers also have weak GC negative feedback (Liu et al., 1997; Weaver et al., 2004). However, rat pups given GC-laced drinking water directly after parturition displayed stronger negative feedback (Catalani et al., 2002). Additionally, in studies where neonates were repeatedly separated from their mothers for short periods of time during post-natal development, maternally deprived rats (Ogawa et al., 1994), squirrel monkeys (*Saimiri sciureus*) (Lyons et al., 2000), and male piglets (Weaver et al., 2000) had stronger or similar negative feedback efficacy compared to controls. These maternal deprivation and LG/ABN studies may suggest that the development of the post-natal HPA-axis is affected by multiple maternal behaviors and that different maternal care behaviors can have opposing effects.

Altered levels of maternal behaviors may also affect male and female pups differently. Decreased negative feedback efficacy in only male offspring could be attributed to differential care between male and female offspring. Studies in laboratory rats have shown that licking and grooming may not be evenly distributed among all pups within a litter (Cavigelli et al., 2010) and mothers perform more ano-genital licking on male pups compared to female pups (Moore and Morelli, 1979). If degu mothers show the same sex bias in ano-genital licking and grooming, then it is possible that male degu pups are more sensitive to the effects of post-parturition maternal stress. Additionally, Gruss et al. (2006) found that when repeatedly separating degu pups from their mothers, males had higher stress-induced cortisol concentrations compared to females during the separations. This may suggest that male degu pups are generally more sensitive to stressors during the first few weeks after parturition.

Although increased GC responses to stress are another indicator of a hyper-reactive HPA-axis (Francis et al., 1999), the laboratory rodent literature does not indicate a consistent response. Rat pups that receive low rates of LG/ABN have higher, stress-induced GC levels (Liu et al., 1997; Weaver et al., 2004), but maternal deprivation studies report higher (Banerjee et al., 2012), lower (Ogawa et al., 1994), or unchanged (Schmidt et al., 2014; Weaver et al., 2000) stress-induced GC levels in offspring subjected to repeated, acute maternal deprivation. Studies that directly manipulated maternal stress levels also found mixed results, with some showing increased (Levine and Thoman, 1969; McCormick et al., 2001) or decreased (Catalani et al., 2002; Macri et al., 2007) stress-induced GC concentrations in offspring with stressed mothers. Since maternal stress can affect several different maternal behaviors (Brummelte and Galea, 2010), and because different maternal behaviors may have opposing effects on the development of the offspring stress response, it is perhaps not surprising that the data on stress-induced cortisol did not meet our original predictions. Additionally, we found that our treatment groups did not significantly differ in their cortisol response to ACTH injection. We had no a priori predictions concerning this as there are no studies, to our knowledge, that have examined effects of maternal stress or maternal care on adrenal sensitivity in a mammalian species. However, a recent study on song sparrows (*Melospiza melodia*) found that nestlings fed GC-laced food had increased adrenal sensitivity as adults (Schmidt et al., 2014). Future studies are needed to determine if maternal care and stress may affect the development of offspring adrenal sensitivity in other vertebrate species.

Although we did not directly test the impact of our manipulations on offspring fitness, evidence suggests that decreased fitness could result from the changes documented here. Weak negative feedback may significantly affect survival, as studies in Galapagos marine iguanas (*Amblyrhynchus cristatus*) found that individuals with poor negative feedback were less likely to survive long-term food shortages caused by El Niño episodes (Romero and Wikelski, 2010) and studies in snowshoes hares (*Lepus americanus*) found that individuals had weaker negative feedback during population declines (Boonstra et al., 1998). Baseline GC levels may also affect survival rates (Goutte et al., 2010; Rivers et al., 2012), although there is no consistent pattern across species (Bonier et al., 2009). Furthermore, there is no correlation between fecal glucocorticoid metabolite levels and adult survival rates in degus (Ebensperger et al., 2013). Further studies are needed to determine which aspects of the degu stress response might be correlated with survival and to determine if correlations between survival and the stress response change with different environmental conditions. If maternal stress is an indicator of current environmental conditions, then maternally-derived stress may adaptively program offspring to either increase offspring or maternal fitness (Dantzer et al., 2013; Sheriff and Love, 2013).

Interestingly, plural-breeding with communal care did seem to help buffer effects of post-natal maternal stress (Fig. 1). Our results are consistent with the hypothesis that un-implanted mothers in Mixed groups partially compensate for the lack of care provided by cortisol-implanted mothers. Another prediction of this hypothesis could be that larger groups are more effective at "buffering." Therefore, while we would have predicted a higher proportion of pups with normal stress responses from larger Mixed groups, the social group size within our Mixed groups (range: 2–4 adult females) did not vary enough to sufficiently test this prediction. Care from other social group members has

also been shown to affect the development of the stress response in young marmosets (*Callithrix geoffroyi*) (Birnie et al., 2013), but not in California mice (*Peromyscus californicus*) (Harris et al., 2013). It is tempting to conclude that buffering of maternal stress might be a benefit of plural breeding with communal care in degus. For this to be true, however, changes to the pup HPA-axis induced by maternal stress must be maladaptive so that reversing the changes through buffering would increase fitness. Future studies will need to show this link in order to support the conclusion that stress buffering is a potential benefit of the degu's unique reproductive strategy.

Because degus live in inaccessible underground burrows, we were never able to verify if our cortisol implants changed the rate or quality of maternal care behaviors. Development of the pup stress response may have been affected by several other mechanisms such as adult inter-female aggression, chemical cues, etc. Additionally, post-parturition maternal stress could affect offspring development via transmission of cortisol during lactation. Laboratory rat studies have shown that maternal GCs can pass into the mother's milk, albeit in very small quantities, and then later absorbed from the pup's stomach into their bloodstream (Zarrow et al., 1970). If cortisol-implanted mothers were significantly affecting pup cortisol levels via lactation, then we would have expected pups from Mixed groups to have stress profiles intermediate to pups from Control and CORT groups. However, we found the opposite, with pups from Mixed groups having stress profiles similar to pups from Control groups. Future studies should test whether maternal stress alters the rate or quality of maternal care in degus, and whether these changes in maternal care are directly responsible for changes in pup HPA-axis development, in addition to exploring other ways maternal stress may impact pup development.

Conclusions

This study demonstrates that maternal stress affects the development of the offspring stress response in wild, free-living degus, and that plural breeding with communal care may help buffer post-natal stress. This is, to our knowledge, the first time post-parturition maternal stress has been shown to affect the development of the offspring HPA-axis in a wild, free-living animal. Future studies should determine if maternal care is the mechanism through which maternal stress affects HPA-axis development in degus. While we doubt plural breeding with communal care evolved solely to help buffer pups from the negative effects of maternal stress, this could be a lasting benefit and potentially a reason this reproductive strategy has been maintained since original evolutionary pressures vanished.

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