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Glucocorticoids and the Development of Agonistic Behaviour during Puberty in Male Golden Hamsters

J. C. Wommack, A. Salinas and Y. Delville
Psychology Department and Institute for Neuroscience, The University of Texas at Austin, Austin, TX, USA.

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Abstract

During puberty, the agonistic behaviour of male golden hamsters undergoes a transition from play fighting to adult aggression. Repeated exposure to social stress early in puberty accelerates this transition. The present study investigated the possible role of glucocorticoids on the maturation of agonistic behaviour. First, we compared serum cortisol levels following a 20-min restraint stress during early puberty, mid-puberty or adulthood. Across puberty, animals exhibited a two-fold increase in post-restraint cortisol levels. We also compared corticotrophin-releasing hormone (CRH) immunoreactive fibres projecting to the median eminence between animals in early puberty and adulthood. The CRH fibre density was two-fold greater in adults compared to juveniles. Furthermore, we investigated the effects of stress hormones on the maturation of agonistic behaviour. Male hamsters were injected daily with dexamethasone, a corticosteroid receptor type II agonist (0, 10 or 40 μg/100 g), early in puberty from postnatal day 31 (P-31) to P-36. When paired with a smaller and younger intruder on P-37, attack frequency did not differ between groups. However, dexamethasone-treated animals showed a dose-dependent decrease in the percentage of play-fighting attacks and an increase in the percentage of adult attacks. In summary, puberty can be described as a period of increasing hypothalamic-pituitary-adrenal activity in male golden hamsters. Moreover, increasing glucocorticoid levels influence the maturation of agonistic behaviour. These data shed new light on the neuroendocrine mechanisms that regulate the maturation of social behaviours during puberty.

Puberty is marked by significant behavioural and neuroendocrine transitions. This developmental period is commonly characterised by increased activity of the hypothalamic-pituitary-gonadal (HPG) axis and the maturation of reproductive behaviours. However, other social behaviours, such as aggression, also undergo substantial pubertal transitions. In most mammalian species, the transition from play fighting to serious aggression occurs during puberty (1). For example, play fighting attacks gradually decline during puberty in male golden hamsters whereas adult attacks increase during this time (2, 3).

The HPG axis is not the only neuroendocrine system that undergoes significant development during puberty. There is mounting evidence that hypothalamic-pituitary-adrenal (HPA) axis changes considerably during puberty, although the nature of these changes varies between species. For example, prepubertal male rats show prolonged adrenocorticotrophin-releasing hormone (ACTH) release and delayed corticosterone responses (4–6). In this species, baseline HPA activity does not change during puberty (4–6). By contrast, baseline glucocorticoid levels increase gradually throughout puberty in other species, including tree shrews (7), golden hamsters (8) and humans (9–11). Male golden hamsters also show a pubertal increase in postdefeat cortisol levels (8).

Could changes in stress hormone levels influence the development of agonistic behaviour? Studies on social stress in male golden hamsters suggest that this is the case. Social subjugation is a potent stressor that produces numerous behavioural and neuroendocrine effects (12). In male golden hamsters, repeated social stress produces unique and context-specific enhancement in the development of agonistic behaviour (2, 13, 14). Male hamsters socially subjugated during early puberty show an accelerated transition of offensive responses from play fighting to adult aggression when animals are paired with a smaller and younger intruder (2, 14). It is unlikely that the behavioural effects of social stress are related to the HPG axis as subjugated animals have lower testosterone levels than controls (8). Moreover, manipulations of testosterone levels during puberty do not affect the development of agonistic behaviour (15). Social subjugation causes a daily increase in plasma cortisol levels and is a stressor to which juveniles are incapable of habituating (8,

Correspondence to: Joel C. Wommack, Psychology Department, 1 University Station, A8000, The University of Texas at Austin, Austin, TX 78712, USA (e-mail: joelwommack@aol.com).

14). Because puberty is a time of increasing stress responsiveness in hamsters, it is possible that the daily activation of corticosteroid receptors is a mechanism that drives the development of offensive aggression.

The aims of the current study were to investigate peripubertal changes in glucocorticoid levels and its influence on the maturation of agonistic behaviour.

Materials and methods

Animals and treatment

Animals (male golden hamsters) were obtained from a breeding colony housed within the laboratory derived from Harlan Sprague-Dawley (Indianapolis, IN, USA). Approximately 1 week after birth, all litters were culled to six pups, including males and females. The males were weaned on postnatal day 25 (P-25) and individually housed in Plexiglas cages ($20 \times 33 \times 13$ cm). Within 2 days, each animal was briefly (for a few seconds) observed in the presence of an adult intruder. Individuals that immediately fled from the adult were considered to be inherently fearful (approximately one in 12) and were removed from the experiment. All animals received food and water *ad lib* and were housed under a reversed light/dark cycle (14 : 10 h, lights off 09.00 h).

Experimental design

The following experiments were conducted to test the development of the HPA axis and its influence on the pubertal transition from play fighting to adult aggression. First, we sought to investigate stress responsiveness following restraint stress on developmental days corresponding to early puberty (P-28), mid-puberty (P-45) and adulthood (P-70, 8, 16). Second, we compared the density of corticotrophin-releasing hormone (CRH) immunoreactive (-ir) fibres projecting to the median eminence between male hamsters during early puberty and adulthood. Finally, we tested the hypothesis that the glucocorticoids influence the development of agonistic behaviour by injecting animals with dexamethasone (Dex) and later testing these animals for offensive responses. Immunocytochemistry was performed on animals sacrificed 4 h after an injection of Dex, cortisol or vehicle to confirm that Dex activated corticosteroid type II receptors within the brain.

Cortisol assays

Plasma cortisol levels were assayed in samples collected immediately following restraint on P-28, P-45 or P-70. Animals were sacrificed by rapid decapitation immediately following a 20-min period of restraint stress ($n = 7$–8/day). Trunk blood samples were collected and centrifuged at 5000 r.p.m. for 5 min. Sera were saved at −20 °C until assayed using Cortisol Correlate-EIA^{TM} kits (Assay Designs, Inc., Ann Arbor, MI, USA). Samples were assayed in duplicate from 10-μl aliquots. Intra-assay variability was 5.2%. Inter-assay variability was not calculated as all samples were used in a single assay. Plasma levels of cortisol were expressed as ng/ml. The cross-reactivity of the antibody supplied with the assay kit was 4.0% for 11-deoxycortisol, 3.6% for progesterone and less than 1% for all other endogenous steroids. The antibody also had a 27.8% cross-reactivity with corticosterone; however, corticosterone levels were below 1 ng/ml.

CRH immunocytochemistry

Male golden hamsters were sacrificed by rapid decapitation on either P-35 ($n = 8$) or P-70 ($n = 7$). Following sacrifice, brains were collected and fixed by overnight immersion in 10% acrolein in 0.1 M potassium phosphate-buffered saline (KPBS) buffer (pH 7.2) at 4 °C and later saved in 20% sucrose/KPBS. The brains were then sectioned into 40-μm thick coronal sections with a freezing rotatory microtome and were stored in a cryoprotectant (17) at −20 °C until labelled by immunocytochemistry using a previously described protocol (18). Briefly, free-floating sections were pretreated in 1% sodium borohydrite (to remove residual aldehydes) followed by a preincubation in a solution containing 20% normal goat serum, 1% hydrogen peroxide and 0.3% Triton X-100 (respectively, to block nonspecific labelling, eliminate endogenous peroxidase activity, and permeabilise the tissue). Sections were then incubated in a rabbit polyclonal antibody to Human/Rat CRH (dilution 1 : 6000; Peninsula Laboratories, Inc., San Carlos, CA, USA), containing 2% normal goat serum and 0.3% Triton X-100 for 48 h at 4 °C. After washing, the sections were incubated in the secondary antibody (biotinylated goat anti-rabbit IgG; 7.5 μg/ml; Jackson Immunoresearch Laboratories, West Grove, PA, USA) followed by a tertiary incubation (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA, USA). Between incubations, sections were washed in 0.05 M Tris-buffered saline (pH 7.6). Finally, the sections were labelled with diaminobenzidine (DAB, 0.5 mg/ml) and 0.05% hydrogen peroxide. Labelled sections were mounted on gel-coated slides, dehydrated in a series of alcohols, and coverslipped with permount.

CRH fibre density was quantified by using NIH Image Software (version 1.62; NIH, Bethesda, MD, USA) by an observer blind to age groups. Brain sections containing the posterior hypothalamus were observed through a ×20 objective as digitised images recorded by a CCD camera mounted on a microscope and imported to a computer using a frame grabber (LG3, Scion Corporation, Frederick, MD, USA). The density of CRH-ir fibres was expressed as size of the area covered by immunoreactive fibres within a standard sample placed over matching zones of the posterior hypothalamus near the arcuate nucleus en route to the median eminence (19). This brain area has previous been described in studies on vasopressin fibre density in golden hamsters (20). The sample (60-μm diameter circle) was placed over the digitised area. Measurements were taken after normalising for background and foreground differences (20). Several measurements ($n = 7$) were taken from each sides of the brain in consecutive sections and averaged for each individual.

Dexamethasone treatment

We tested the effects of daily treatment with Dex during early puberty on the development of agonistic behaviour. On P-31, male golden hamsters were weighed and screened for offensive responses using a resident–intruder paradigm (21). Smaller (10–20%) younger animals were placed in the home cage of experimental animals for 10 min. The size difference between the resident and intruder favours offensive responses (22). Offensive responses such as attacks and pins (see below for a detailed description) were recorded during this time. After confirmation that each resident readily performed offensive aggression, animals were separated into experimental groups balanced for litter and body weight. Animals received daily injections of Dex (0, 10 or 40 μg/100 g; $n = 9$–10 per dose) from P-31 to P-36. These doses of Dex are within the range used in rats in learned helplessness studies (23).

Dex was chosen as a corticosteroid receptor agonist due to its long half-life and its ability to selectively activate the low affinity, type II corticosteroid receptors in hamsters (24).

Behavioural responses

On P-37, 1 day following the last injection, animals were tested for offensive aggression using a resident intruder paradigm (21). Smaller (10–20%) and younger intruders were placed into the home cages of experimental animals for 10 min. Behaviours were recorded with a Sony video camera and later analysed using iMovie software (Apple Computer Inc., Cupertino, CA, USA) by an observer blind to treatment. Several behavioural responses were recorded as previously described (2). Attacks were recorded when the resident approached and attempted to bite the intruder. Attack latency was recorded as the amount of time that transpired between the initial pairing of the resident and the intruder and the first attack. Attacks were separated into four distinct categories based on the region of the intruder initially targeted by the resident during an attack. In play fighting attacks (PF Att), the resident first targeted the face and/or cheeks of the intruder. Side attacks were recorded when the resident target the flanks and/or the dorsal midsection of the intruder, and adult attacks (Ad Att) were recorded when the resident target the belly and/or rear of the intruder. A fourth attack type, classified as a walk-in attack, was recorded when the resident attacked the intruder when it was lying on its back or righting itself from a previous attack. The percentage of attacks that were PF Att or Ad Att was calculated for each individual by dividing the each subcategory by the total number of attacks. Due to the ambiguous nature of the region of the intruder targeted during walk-ins, this type of attack was excluded from calculations of attack percentages. Pins were recorded when the resident forced the intruder into the supine position during an attack. Contact time was recorded as the number of seconds the resident initiated and maintained contact with the intruder.

Dex treatment and GR immunocytochemistry

To test whether Dex activated corticosteroid receptors within the brain, female hamsters were injected in their home cage (40 μg/100 g), cortisol (40 μg/100 g) or vehicle (propylene glycol) on P-26 or P-27. At this age, female hamsters have very low circulating cortisol levels, approximately 1 ng/ml (3), and therefore should have low basal levels of corticosteroid type II receptor activation. Four hours after injection, animals were anaesthetised with nembutal (35 mg/kg) and then perfused with 4% paraformaldehyde/KPBS. Following extraction, brains were sectioned as described earlier and later labelled for corticosteroid type II receptor immunocytochemistry. The sections were labelled using a procedure slightly modified from the CRH protocol, using a rabbit polyclonal antibody against the carboxy terminus of human corticosteroid type II-α receptor (1.5 μg/ml catalogue no. SC-1002; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The secondary antibody was biotinlylated goat antirabbit IgG (7.5 μg/ml; Jackson Immunoresearch Laboratories). All solutions were prepared with 0.1 M PBS (pH 7.4).

Neurones containing corticosteroid type II receptor immunoreactivity were counted within the paraventricular hypothalamic nucleus (PVN), specifically the anterior parvicellular subdivision of the PVN (19). In the same brains, receptor immunoreactivity was also quantified within the rostral portion of the dentate gyrus. Four brains were used for the cortisol and vehicle groups, while three brains were used for the Dex group. The analysis of immumocytochemical labelling was performed by an observer blind to treatment groups. Cells were counted using a 200 μm × 200 μm grid using a camera lucida attachment under ×40 magnification. Once counted, neurones were subdivided into two groups: (i) cells with clear nuclear and cytoplasmic immunoreactivity and (ii) cells with only cytoplasmic immunoreactivity. A cell was defined as nuclear labelled if there was a visibly dense and round compartment within the centre of the cell. Cells without nuclear labelling often had clearly visible processes cytoplasmic labelling with an absence of an identifiable nuclear compartment. Similar immunocytochemical approaches have been used to determine the intracellular location of corticosteroid (25) and other steroid receptors (26, 27). For each brain, the total number of cells, total number of nuclei and the percentage of cells without nuclear labelling were calculated.

Statistical analysis

Post-restraint cortisol levels were compared between animals on P-28, P-45 and P-70 by ANOVA followed by Fisher's *post-hoc* tests. CRH fibre densities on P-35 and P-70 were compared using Student's t-test (two-tailed). In the Dex study, data (PF Att and Ad Att percentages, contact time and body weights) were compared between Dex- and vehicle-treated animals by ANOVA followed by Fisher's *post-hoc* tests. Corticosteroid receptor immunoreactivity was compared within the brains of Dex-, cortisol- and vehicle-treated animals by ANOVA followed by Fisher's *post-hoc* tests.

Results

Cortisol levels

Stress responsiveness increased across puberty. Post-restraint cortisol levels increased by approximately two-fold across puberty [$F_{2,19} = 20.0$, $P < 0.001$, ANOVA] (Fig. 1). On P-45 and P-70 cortisol levels were higher than on P-28 ($P < 0.01$ and $P < 0.001$, respectively, Fisher's PLSD). Cortisol levels collected on P-70 were also higher than P-45 levels ($P < 0.05$).

CRH immunoreactivity

CRH-ir fibres projecting to the median eminence were clearly visible in P-35 and P-70 animals (Fig. 2A). The density of CRH-ir fibres projecting to the median eminence increased by two-fold during puberty (Fig. 3). From P-35 to P-70, the density of CRH-ir fibre density increased from 381.9 ± 67.6 μm^2 to 607.7 ± 35.7 μm^2. This increase was statistically significant [$t(13) = 7.9$, $P < 0.001$, Student's *t*-test].

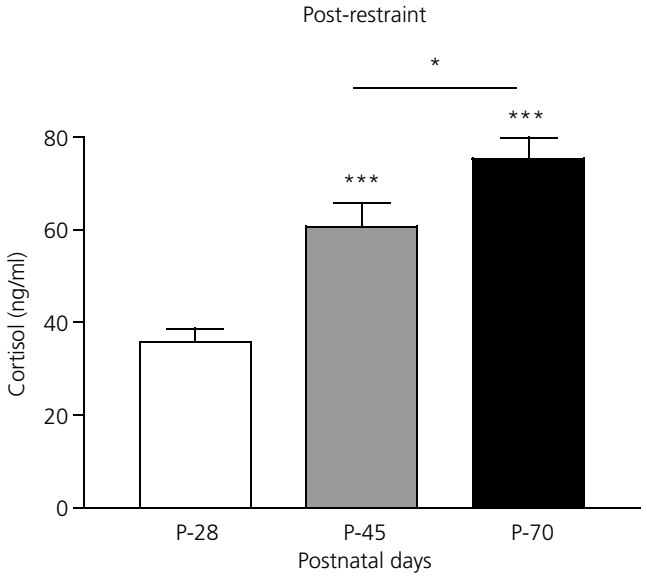

FIG. 1. Plasma cortisol levels assayed immediately following 20 min of restraint stress on postnatal day 28 (P-28), P-45 or P-70. In golden hamsters, these time points correspond to early puberty, mid-puberty and adulthood. (ANOVA, *$P < 0.05$, ***$P < 0.001$).

Dex and behavioural development

Repeated Dex treatment during early puberty accelerated the transition from play fighting to adult aggression (Fig. 4). Dex caused a dose-dependent decrease in the percentage of PF Att [$F_{2,26} = 4.9$, $P < 0.01$]. Compared to vehicle-treated animals, both low-dose and high-dose-treated animals showed a significant decrease in the percentage of PF Att ($P < 0.05$ and $P < 0.01$, respectively). Dex also caused a dose-dependent increase in the percentage of Ad Att [$F_{2,26} = 6.8$, $P < 0.01$]. Animals that received the highest dose of Dex also displayed the highest percentage of Ad Att (compared to vehicle-treated animals, $P < 0.001$). The effects of Dex were specific to the targets of attack because neither attack frequency nor latency differed significantly between groups. No group differences were observed for pins, flank marks and contact time. Importantly, group differences in body weight were not observed, indicating that growth was not affected by repeated Dex treatment.

Dex treatment and GR immunocytochemistry

Dex injections are capable on activating corticosteroid receptors within the brains of golden hamsters (Fig. 2B). Animals treated with Dex or cortisol had a lower percentage of cells without clear nuclear labelling than vehicle-treated animals within the PVN (Table 1). The overall number of cells and nuclei analysed did not differ between treatment groups. It is important to note that no group differences in corticosteroid receptor immunoreactivity were observed between treatment groups within the dentate gyrus.

Discussion

The present study investigated the pubertal development of the HPA axis and the role of glucocorticoids in the

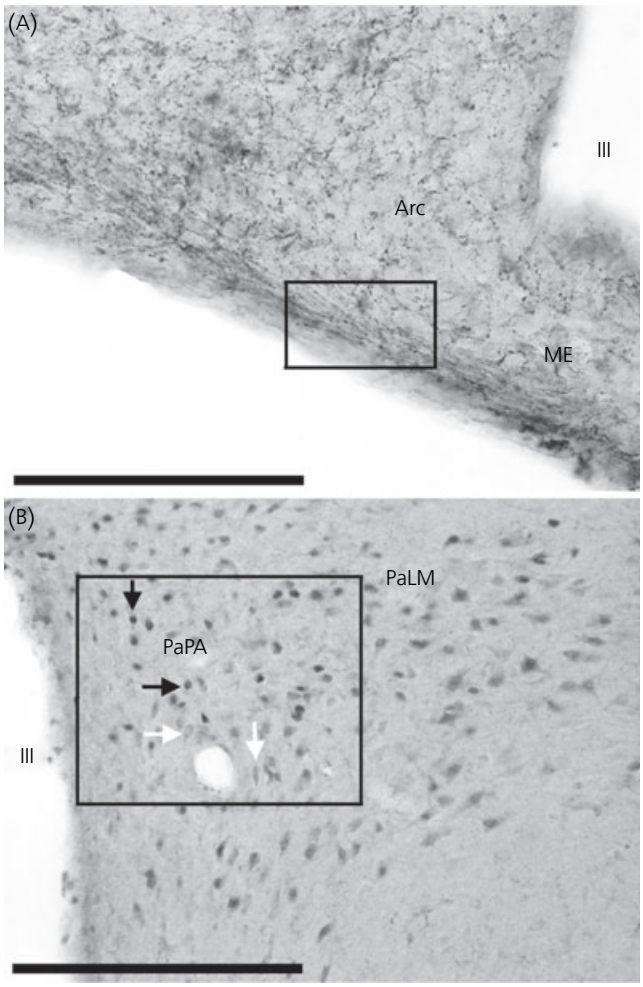

FIG. 2. Photomicrographs taken at ×20 of (A) corticotrophin-releasing hormone-immunoreactive fibres projecting to the median eminence (ME) of a male hamster on postnatal day 70 (P-70) and (B) corticosteroid type II-α receptor immunoreactivity within the paraventricular hypothalamic nucleus (PVN) of a vehicle-treated female hamster on P-28. Boxes shown represent the area where immunoreactivity was quantified. Arc, Arcuate hypothalamic nucleus; III, third ventricle; PaLM, lateral magnocellular subdivision of the PVN; PaPA, anterior parvicellular subdivision of the PVN. Black arrows point to cells with nuclear immunolabelling. White arrows point to cells without nuclear immunolabelling. Scale bars = 200 μm.

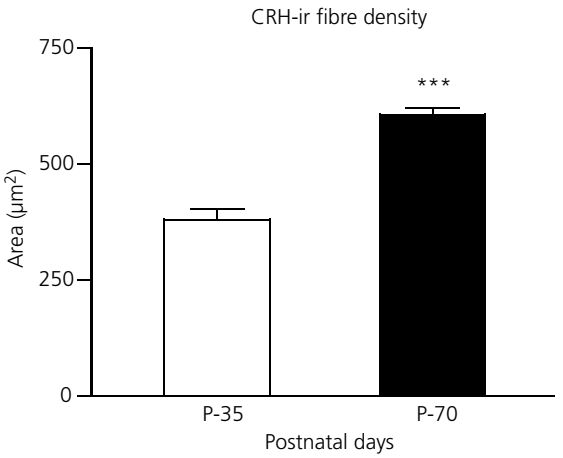

FIG. 3. The density of corticotrophin-releasing hormone (CRH) immunoreactive (-ir) fibres projecting into the median eminence in animals sacrificed on postnatal day 35 (P-35) or P-70. In golden hamsters, these time points correspond to early puberty and adulthood. Fibre densities were measured using NIH image software. (Student's t-test, two-tailed, ***$P < 0.001$).

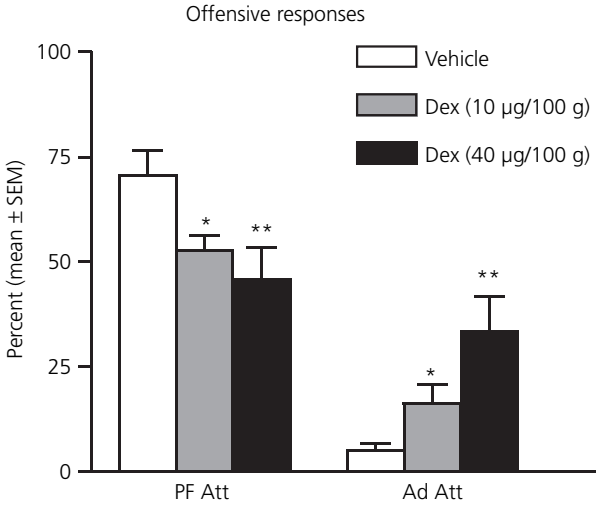

FIG. 4. Male golden hamsters were treated daily with dexamethasone (Dex, 10 or 40 μg/100 g) or vehicle (propylene glycol) from postnatal day 31 (P-31) P-36. On P-37, all animals were tested for offensive responses in the presence of a smaller and younger intruder. The percentage of attacks that either directed at the face and cheeks (PF Att) or belly/rear (Ad Att) were compared between treatment groups. (ANOVA, *$P < 0.05$, **$P < 0.01$).

development of agonistic behaviour. First, we found that post-restraint cortisol levels increased during puberty indicating that this is an important period for the development of stress responsiveness. Second, a two-fold increase in the density of CRH-ir fibres projecting from the PVN into the median eminence was observed between juvenile and adult hamsters. In a third phase of the study, we treated juvenile hamsters with Dex to understand the effects of increased corticosteroid receptor activation on the development of agonistic behaviour. Dex-treated animals showed an accelerated transition from play fighting to adult aggression, suggesting that corticosteroid receptor activation controls the maturation of agonistic behaviour. In all, this study shows that pubertal changes in glucocorticoid levels influence the maturation of social behaviour.

Across various intervals in puberty cortisol levels increased following restraint stress. Previous studies have shown that baseline and postdefeat cortisol levels also increase during puberty in male golden hamsters (8). Our new data show that increases in poststress cortisol levels are not specific to social defeat and suggest a general increase in stress responsiveness during puberty that may help to determine the consequences of a stressor. For example, social defeat during early puberty results in an accelerated development of agonistic behaviours and transient decreases in risk-assessment behaviours (2, 8, 14). By contrast, socially defeated adults show a long-lasting inhibition of offensive aggression known as conditioned defeat (28, 29). The pubertal increase in stress responsiveness may explain the age-dependent consequences of social stress in male golden hamsters.

TABLE 1. Corticosteroid Type II-α Receptor Immunocreactivity in Female Golden Hamsters Treated with Dexamethasone (40 μg/100 g), Cortisol (40 μg/100 g) or Vehicle (Propylene Glycol) on Postnatal Day 26 (P-26) or P-27.

	Paraventricular hypothalamic nucleus			Dentate gyrus		
	Cells	Nuclei	Pecent	Cells	Nuclei	Pecent
Vehicle	362.8 ± 24.0	189.3 ± 30.4	48.75 ± 7.5	106.3 ± 10.4	99.2 ± 10.1	6.7 ± 4.6
Dexamethasone	365.3 ± 13.9	246.0 ± 12.3	32.6 ± 1.1	104.5 ± 23.7	101.2 ± 25.3	3.5 ± 2.2
Cortisol	301.5 ± 54.5	235.8 ± 44.3	23.9 ± 4.3	113.1 ± 11.1	108.3 ± 8.9	4.1 ± 1.9
ANOVA	$P > 0.1$	$P > 0.1$	$P < 0.001$	$P > 0.1$	$P > 0.1$	$P > 0.1$

Presented are the number of cells, nuclei and percentage of cells without nuclear labelling (mean ± SEM) for each treatment group. Receptor immunoreactivity was quantified within the paraventricular hypothalamic nucleus and the dentate gyrus. (ANOVA, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to vehicle).

When considering the HPA axis during puberty, it is important to recognise similarities and differences between species. The steady increase in basal cortisol levels that we assayed in golden hamsters have also been reported in other species such as tree shrews and humans (7, 9–11). By contrast, basal glucocorticoid levels do not increase during puberty in rats (4–6). The stress responses of pubertal rats can be characterised by prolonged ACTH release, but do not differ from adult stress responses in terms of peak values. Importantly, pubertal changes in HPA activity of rats are unrelated to changes in the HPG axis because prepubertal males treated with adult levels of testosterone fail to show adult stress responses (6).

The current study shows that pubertal changes in HPA activity in golden hamsters are not limited to cortisol levels. Analysis of CRH immunocytochemistry showed that the density of CRH-ir fibres projecting into the median eminence also increased by two-fold from early puberty to adulthood. It is likely that enhanced CRH-IR is a result of increased CRH mRNA. It is also likely that increased CRH-IR is related to increased ACTH release from the anterior pituitary in golden hamsters. These data suggest that all elements of the HPA axis increase during puberty in male golden hamsters. Future studies will address these issues.

Importantly, the present study shows that pubertal changes in plasma glucocorticoids levels are related to behavioural development. Repeated treatment with Dex accelerated the development of agonistic behaviour. Animals that received daily injections of Dex during the first week of puberty performed a lower percentage of play-fighting responses and a higher percentage of adult attacks than vehicle-treated animals. These effects were dose-dependent. Animals from all treatment groups showed similar levels of attack frequency and latency, contact time, and pins. The lack of difference in these other variables indicates that the effects of Dex are specific to behavioural development.

Studies focusing on the role of the HPG axis in the development of aggressive behaviour have produced surprising results. In both rats and golden hamsters, castration during puberty does not alter the development of agonistic behaviour (15, 30, 31). In previous reports from our laboratory, repeated social stress accelerated the transition from play fighting to adult aggression, yet subjugated juveniles have lower testosterone levels than nonstressed controls (8). These findings are of interest because puberty is a period commonly characterised by dramatic increases in androgen levels and a large number of studies have shown testosterone to be critical for male aggression during adulthood. The lack of involvement of the gonadal steroids in pubertal changes in behaviour has led some authors to hypothesise that these maturational processes are regulated by nonsteroidal mechanisms (32). Data from the current study show that aggressive behaviour is influenced by another type of hormone (i.e. glucocorticoids).

Previously, stress has been shown to affect the development of agonistic behaviours. Male golden hamsters that are repeatedly subjugated during puberty show an accelerated transition from play fighting to adult aggression (2, 14). Social subjugation is a stressor to which juveniles are incapable of fully habituating (8, 14). As such, social defeat during early puberty causes a daily increase in plasma cortisol levels and subsequent activation of corticosteroid receptors. Because the behavioural consequences of social subjugation and repeated Dex treatment during puberty are similar, it is likely that the maturation of agonistic behaviour is controlled by corticosteroid receptors. Future studies will investigate this topic in more detail.

The analysis of the immunoreactivity for corticosteroid receptors type II showed differences between brain areas. Compared to the vehicle, Dex and cortisol injections resulted in significantly higher proportion of nuclear immunolabelling within the parvicellular subdivision of the PVN. This activation by treatment with Dex and cortisol was not observed throughout the brain. For example, no group differences in nuclear labelling were observed within the dentate gyrus. Consequently, our data suggest that cortisol and Dex were capable of activating corticosteroid type II receptors in some parts of the brain. However, it is important to note that the animals used for this study were not adrenalectomised, but were immature female hamsters. These animals were used for this study because they have very low plasma cortisol levels (3), although high enough to activate corticosteroid type II receptors in the dentate gyrus. Possibly, corticosteroid type II receptors in this area are more responsive to low plasma levels of cortisol.

In rats and mice, there are data suggesting that Dex does not cross the blood–brain barrier (33–35). Rats treated with Dex show no increase in nuclear corticosteroid receptor binding within the hippocampus (33). On the other hand, Dex treatment causes a significant increase in corticosteroid binding in the anterior pituitary gland of rats (34). Dex penetration through blood–brain barrier is enhanced in mice

with a knockout for the mrd1A p-glycoprotein gene (35). It is important to note that the primary glucocorticoid in rats and mice is corticosterone whereas it is cortisol in hamsters. Cortisol has three hydroxyl groups compared to the two hydroxyl groups on the carbon rings of corticosterone. This indicates that the two molecules have different chemical properties. Cortisol poorly crosses the blood–brain barrier in mice (36). A large number of vertebrate species use cortisol as their primary glucocorticoid, such as hamsters (8), guinea pigs (37), pigs (38), new and old world primates (39) and fish (40, 41). However, there are no existing studies on the blood–brain barrier in these species. Dex is similar to cortisol in that it also has three hydroxy groups on its main carbon chain. As such, Dex may more readily cross the blood–brain barrier in species where cortisol is the predominant glucocorticoid. Our data show that Dex is indeed capable of activating corticosteroid receptors within the brains of golden hamsters, suggesting species differences in the blood–brain barrier.

In conclusion, the present study shows that puberty can be described as a period of increasing HPA activity in male golden hamsters. During this time, both basal and poststress cortisol levels increased significantly. The changes in cortisol levels were coupled with a two-fold increase in the density of CRH-ir fibres projecting into the median eminence. The pubertal changes in the HPA axis of hamster differ from previous reports in other species, such as rats, and highlight the importance of considering species differences with respect to the development of stress responsiveness. Repeated treatment with Dex resulted in an accelerated behavioural development. The data show, for the first time, that glucocorticoids are important for the maturation of agonistic behaviour. The implications of these data are broad because the HPA axis undergoes pubertal changes in a number of species. As such, it is unlikely that the effects of glucocorticoids on the maturation of social behaviours are limited to golden hamsters.

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