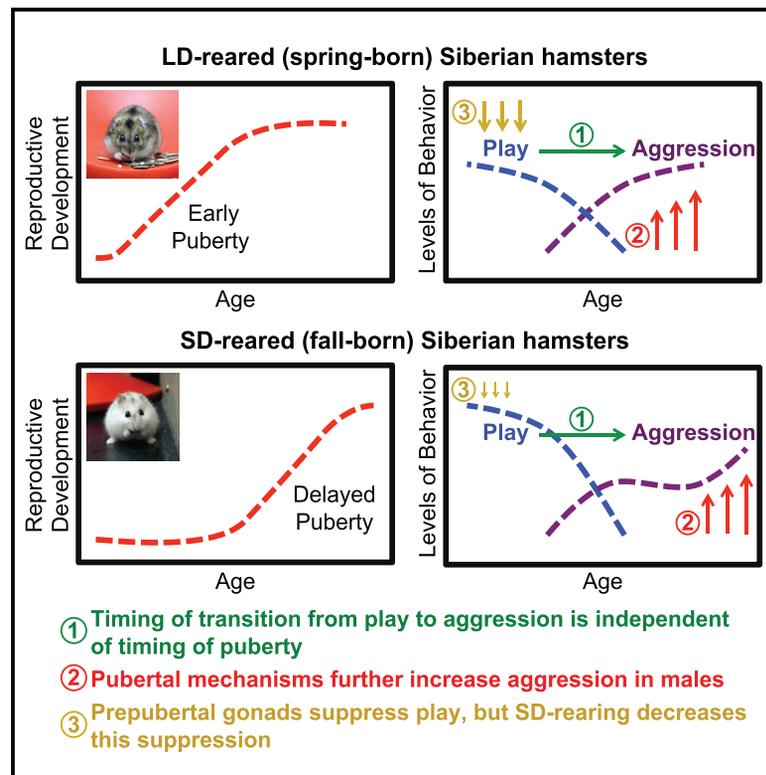


# Current Biology

## Dissociation of Puberty and Adolescent Social Development in a Seasonally Breeding Species

### Graphical Abstract



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### In Brief

Paul et al. leverage natural adaptations of a seasonal species to investigate the role of pubertal hormones in adolescent social development. The authors find that puberty-independent mechanisms regulate the *timing* of adolescent social development, whereas prepubertal and adult gonadal hormones modulate *levels* of age-appropriate social behaviors.

### Highlights

- *Timing* of the adolescent transition from play to aggression is puberty independent
- Puberty-dependent mechanisms regulate *levels* of aggression in males
- Prepubertal gonadal hormones suppress *levels* of juvenile play behavior

# Dissociation of Puberty and Adolescent Social Development in a Seasonally Breeding Species

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## SUMMARY

Alongside the development of sexual characteristics and reproductive competence, adolescents undergo marked cognitive, social, and emotional development [1]. A fundamental question is whether these changes are triggered by activation of the hypothalamic-pituitary-gonadal (HPG) axis at puberty (puberty dependent) or whether they occur independently of HPG activation (puberty independent). Dissociating puberty-dependent from puberty-independent mechanisms is difficult because puberty and adolescence typically proceed concurrently. Here, we test a new approach that leverages natural adaptations of a seasonally breeding species to dissociate pubertal status from chronological age. Siberian hamsters (*Phodopus sungorus*) reared in a long, summer-like day length (LD) exhibit rapid pubertal development, whereas those reared in a short, winter-like day length (SD) delay puberty by several months to synchronize breeding with the following spring [2, 3]. We tested whether the SD-induced delay in puberty delays the peri-adolescent decline in juvenile social play and the rise in aggression that characterizes adolescent social development in many species [4–6] and compared the results to those obtained after prepubertal gonadectomy. Neither SD rearing nor prepubertal gonadectomy altered the age at which hamsters transitioned from play to aggression; SD-reared hamsters completed this transition prior to puberty. SD rearing and prepubertal gonadectomy, however, increased levels of play in male and female juveniles, implicating a previously unknown role for prepubertal gonadal hormones in juvenile social behavior. Levels of aggression were also impacted (decreased) in SD-reared and gonadectomized males. These data demonstrate that puberty-independent mechanisms regulate the timing of adolescent social development, while prepubertal and adult gonadal hormones modulate levels of age-appropriate social behaviors.

## RESULTS

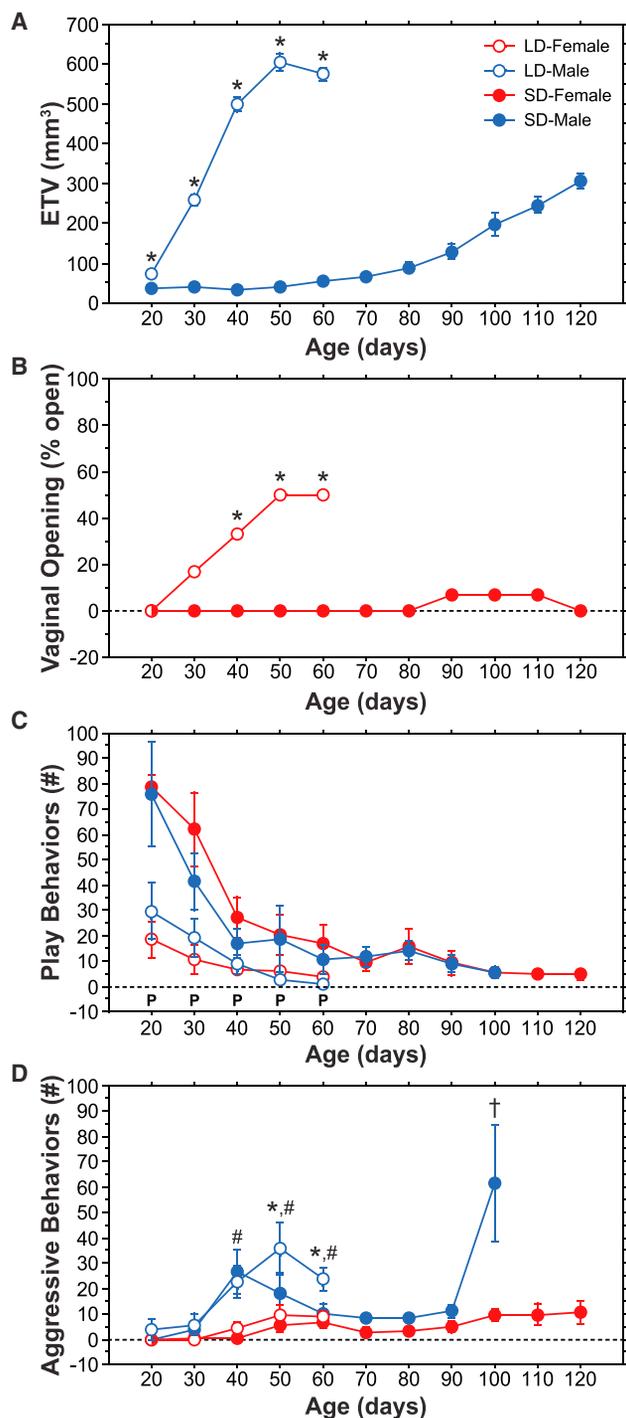
### Play Behavior in Siberian Hamsters

Play behavior of Siberian hamsters (*Phodopus sungorus*) has not previously been described. We found that Siberian hamsters exhibited similar social play behaviors to those of laboratory rats (*Rattus norvegicus*), Syrian hamsters (*Mesocricetus auratus*), and Djungarian hamsters (*Phodopus campbelli*) [7–9]. These include pounces (lunges toward the playmate's face or nape), pins (one animal on top, holding its playmate in the supine position), and boxing (both animals standing on their hind legs pushing or batting each other with their forepaws). Offensive aggressive behaviors in Siberian hamsters were typified by rapid strikes or bites typically directed toward the rump and genitals and vigorous chases that usually ended with the subordinate animal in the supine defensive posture (as in [10, 11]). Unlike during playful pins, the dominant aggressor did not sustain contact during this supine posture. Playful and aggressive interactions were easily discriminated in Siberian hamsters by considering the target of attacks (face or head versus rump or genitals), the vigor of attacks (aggressive attacks are much more rapid and raucous), the duration of contact during pins, and the presence or absence of accompanying vocalizations (playful interactions are typically silent, whereas aggression is accompanied by long, audible calls).

### SD Rearing Dissociates Puberty and Adolescent Social Development

To test whether a short day length (SD)-induced delay in puberty delays the peri-adolescent transition from juvenile social play to adult aggression, we reared male and female Siberian hamsters under long day length (LD; 14 hr light/day, 14L) or SD (10L) conditions and assessed play, aggression, and reproductive measures (estimated testis volume [ETV] or vaginal opening [VO]) every 10 days from postnatal day 20 (P20) to either P60 (LD groups) or P120 (SD groups). Measures of LD-reared hamsters were not taken past P60 because the transition from play to aggression was expected to (and did) occur by this age in LD groups.

Figure 1 depicts the reproductive and social development of LD- and SD-reared male and female Siberian hamsters. As in previous studies [2, 3], SD rearing delayed reproductive development of Siberian hamsters by 2–3 months (in males) or more (in females) compared to LD-reared hamsters (Figures 1A and 1B). The mean onset of puberty occurred at P30 for



**Figure 1. SD Rearing Dissociates Reproductive and Social Development in Siberian Hamsters**

Figure360► For a Figure360 author presentation of Figure 1, see the figure legend at <https://doi.org/10.1016/j.cub.2018.02.030>.

Developmental profiles of (A) mean ( $\pm$ SE) estimated testis volume (ETV), (B) percent females exhibiting vaginal opening, (C) mean ( $\pm$ SE) number of play behaviors, and (D) mean ( $\pm$ SE) number of aggressive behaviors of LD- and SD-reared male and female Siberian hamsters. “P” indicates a main effect of photoperiod within each time point ( $p < 0.02$ , ANOVA); \* indicates a significant difference between LD-reared males and SD-reared males (A and D;  $p < 0.02$ , Fisher’s protected least significant difference [PLSD]) or between LD-reared

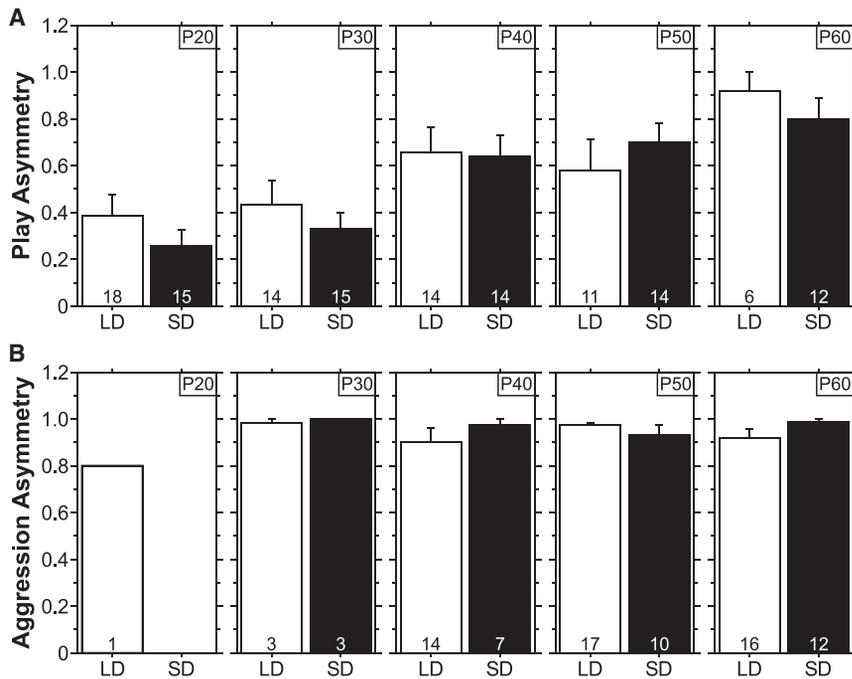
LD-reared males, P80 for SD-reared males, P52 for LD-reared females, and later than P120 for SD-reared females (only a single SD-reared female underwent vaginal opening by P120). Despite these markedly different reproductive profiles, play declined and aggression emerged at similar ages between LD- and SD-reared hamsters (Figures 1C and 1D). For SD-reared hamsters, the decline in play was completed by P40 (for SD males) or P60 (for SD females) ( $p > 0.10$ , versus final time point, Fisher’s protected least significant difference [PLSD]), and aggression emerged at P40 (for SD males) or P50 (for SD females; Figure 1D), well before the onset of puberty (P80 for SD males and  $>P120$  for SD females). Hence, this behavioral transition cannot be explained by pubertal factors indicating puberty-independent regulation.

Assessment of dyadic playful and aggressive interactions further supports this conclusion. Juvenile social play is reciprocal, with both participants engaging in the behavior [5, 12]. To assess the reciprocal/asymmetric nature of play and aggression in Siberian hamsters, we calculated an asymmetry score for which complete symmetry equals 0 (both animals exhibit the same number of behaviors) and complete asymmetry equals 1 (one animal accounts for all behaviors; see STAR Methods for the equation). As noted for other species, asymmetry was low for play in juvenile hamsters (P20 and P30 in Figure 2A). In contrast, aggression asymmetry equaled or approached 1 (Figure 2B). Play asymmetry increased from P20 to P60 as LD-reared hamsters underwent pubertal development ( $p < 0.02$ , nonparametric sign test), which is reminiscent of the increase in dominance-associated play postures in male rats across adolescence [13, 14]. Notably, SD-reared hamsters exhibited the same developmental increase in play asymmetry from P20 to P60, even though they had not yet undergone puberty ( $p < 0.007$ , nonparametric sign test). Play asymmetry did not differ between LD- and SD-reared hamsters at any age ( $p > 0.28$  at each age from P20 to P60, ANOVA), indicating that photoperiod does not alter the reciprocal nature of play.

### SD Rearing Modulates Levels of Juvenile Social Play and Adult Male Aggression

Although SD rearing did not alter the temporal profiles of play or aggression, it did impact absolute levels of these behaviors (Figures 1C and 1D;  $p < 0.04$ , main effect of photoperiod for both behavioral measures, repeated-measures ANOVA). SD-reared hamsters displayed a higher total number of play behaviors than did their LD-reared counterparts at all ages ( $p < 0.02$ , main effect of photoperiod at each age from P20 to P60, ANOVA). Once aggression emerged, the total number of aggressive behaviors increased to higher levels in LD-reared males compared to SD-reared males ( $p < 0.02$  at P50 and P60, Fisher’s PLSD). Notably, SD-reared males displayed a second increase

females and SD-reared females (B;  $p < 0.02$ , Chi square); # indicates a significant sex difference between LD-reared males and LD-reared females ( $p < 0.003$ , Fisher’s PLSD); and † indicates a significant increase in SD male aggression at P100 versus all other ages ( $p < 0.005$ , Fisher’s PLSD). Social behaviors were analyzed at the level of the pair and sample sizes were as follows: LD female = 13, LD male = 10, SD female = 8, and SD male = 7. Reproductive measures were analyzed at the level of the individual; hence, samples sizes were double those of behavioral measures.



**Figure 2. SD Rearing Does Not Alter Play Asymmetry or Its Developmental Increase across Adolescence**

Mean (+SE) asymmetry score for (A) playful and (B) aggressive interactions of LD- and SD-reared hamsters at postnatal day 20 (P20), P30, P40, P50, and P60. Play and aggression asymmetry of males and females did not differ; therefore, data were collapsed across sex. Play and aggression asymmetry did not differ between LD- and SD-reared hamsters at any age ( $p > 0.28$  for play at each age from P20 to P60,  $p > 0.13$  for aggression at each age from P30 to P60, ANOVA). Play asymmetry increased from P20 to P60 in both LD- and SD-reared hamsters ( $p < 0.02$ , nonparametric sign test). Only a single pair exhibited aggression at P20. Sample sizes (pairs per group) are indicated within the bars.

in aggression at P100 (P100 versus all other ages,  $p < 0.005$ , Fisher's PLSD), around the time of increased testicular development (see Figure 1A). Additionally, three out of seven SD-reared male pairs needed to be separated between P100 and P110 due to fighting in their home cages. Photoperiod modulation of aggression was not detected in females, for which aggression was much lower than for males ( $p < 0.003$ , LD males versus LD females at each age from P40 to P60, Fisher's PLSD) and did not differ between LD- and SD-reared hamsters ( $p > 0.52$ , LD females versus SD females at each age from P30 to P60, Fisher's PLSD). These data suggest that increased secretion of pubertal hormones promote high levels of aggression in males. Because photoperiod modulated play behavior at P20 and P30, a pubertal mechanism for altered levels of play is doubtful. In addition to delaying puberty, SD rearing suppresses gonadal function as early as P20 [3, 15], raising the possibility of a *prepubertal* gonadal mechanism.

### Prepubertal Gonadectomy Impacts Levels, but Not Developmental Timing of Social Behaviors

For testing of possible contributions of the gonads to the developmental timing and levels of play and aggression, LD-reared male and female Siberian hamsters were gonadectomized (GNX) or sham-operated (sham) on P15, and play and aggressive behaviors were assessed every 10 days from P20 to P60. GNX and sham groups were conducted concurrently with the photoperiod manipulations described above. Therefore, LD-reared hamsters from the photoperiod experiment were used as non-surgical controls (intact) for this experiment. We first assessed whether sham and intact groups could be combined into a single control group (control). Sham surgery at P15 markedly increased play behavior at P20 in both males and females (Figures 3A and 3B; sham versus intact,  $p \leq 0.05$ , main effect of surgery, ANOVA), which prevented the combining of these groups at

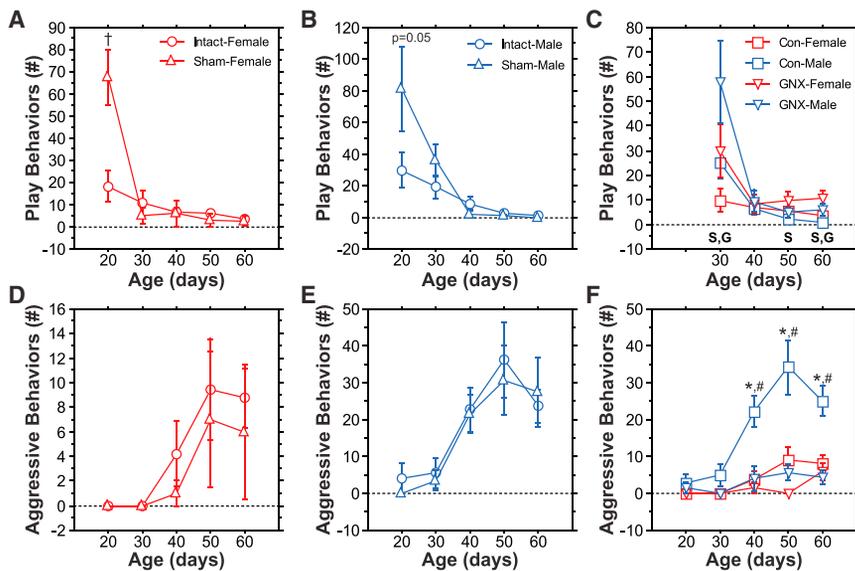
this age. This surgical effect on play was no longer evident from P30 onward and did not manifest for aggression at any age ( $p > 0.13$ , sham versus intact, P30–P60 for play and P20–P60 for aggression, Fisher's PLSD). Therefore, sham and intact groups were combined into a single control group at P30–P60 for play and at P20–P60 for aggression.

As with SD rearing, GNX did not delay the developmental decline in play (Figure 3C) or block the increase in asymmetric play interactions from P20 to P60 ( $p < 0.02$  for GNX hamsters, nonparametric sign test; Figure 4), further demonstrating puberty-independent and gonadal-independent regulation of the timing of this developmental transition. These data agree with those in rats, for which prepubertal GNX also does not alter the decline in play [16, 17]. Unlike in rats [18, 19], however, prepubertal GNX increased levels of play in Siberian hamsters at P30 ( $p < 0.004$ , main effect of GNX, ANOVA). These data, in conjunction with the play-promoting effects of SD rearing, implicate a role for the prepubertal gonads in juvenile social behavior: one in which the prepubertal gonads suppress juvenile social play and removal of this suppression, either by GNX or SD rearing, results in elevated play.

Prepubertal GNX decreased aggression in male hamsters ( $p < 0.004$ , GNX males versus control males at each age from P40 to P60, Fisher's PLSD) and eliminated the sex difference in this behavior ( $p > 0.59$ , GNX males versus GNX females, at each age from P40 to P60, Fisher's PLSD). Unlike SD rearing, GNX prevented the developmental rise in male aggression ( $p > 0.34$ , GNX males, main effect of age, repeated-measures ANOVA; Figure 3F).

## DISCUSSION

The present study demonstrates that adolescent social development is regulated by both puberty-dependent and puberty-independent mechanisms. Delaying puberty by rearing hamsters in a SD failed to delay the developmental transition from play to aggression. This same manipulation, however, increased play and reduced male aggression. Similarly, gonadectomy



### Figure 3. Prepubertal Gonadectomy Increases Juvenile Social Play and Decreases Adult Male Aggression

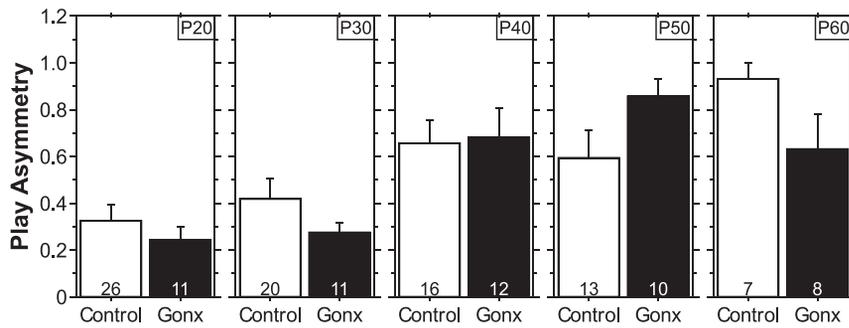
Graphs in (A), (B), (D), and (E) illustrate the mean ( $\pm$ SE) number of play and aggressive behaviors in sham-operated (sham) and unoperated (intact) LD-reared male and female hamsters; † indicates a significant difference between sham and intact female hamsters at P20 ( $p < 0.008$ , Fisher's PLSD). Because this surgical effect did not manifest at other ages for play or at any age for aggression, sham and intact groups were combined into a single control group (con) at P30–P60 for play and at P20–P60 for aggression for comparisons with gonadectomized (GNX) hamsters in (C) and (F); “G” indicates a significant main effect of GNX ( $p < 0.004$ , ANOVA); “S” indicates a significant main effect of Sex ( $p < 0.02$ , ANOVA); \* indicates a significant difference between GNX males and con males ( $p < 0.004$ , Fisher's PLSD); and # indicates a significant sex difference between con males and con females ( $p < 0.001$ , Fisher's PLSD). Sample sizes (pairs per group) were as follows: intact female = 13; intact male = 10; sham female = 3; sham male = 5; con female = 16; con male = 15; GNX female = 7; GNX male = 6.

increased play and reduced male aggression without shifting the peri-adolescent decline in play. Based on these results, we propose that (1) puberty-independent mechanisms initiate the transition from juvenile social play to adult aggression, thereby determining the *timing* of this developmental transition; (2) pubertal increases in testicular hormones synergize with this puberty-independent shift toward aggression to further increase *levels* of aggression in males; and (3) prepubertal gonadal hormones suppress *levels* of juvenile play behavior. Taken together, these data indicate that adolescent social development requires coordinated interactions between puberty-dependent and puberty-independent mechanisms.

The use of seasonal species to study such interactions has several unique advantages over gonadectomy and hormone replacement [20]. SD rearing circumvents potential confounds that can accompany gonadectomy, including early-life surgical stress [21, 22] and compensatory neuroendocrine changes after removal of gonadal steroid negative feedback [23]. Indeed, confounds from early-life surgery were evident in the present study, where sham surgery substantially, albeit transiently, increased play behavior, which prevented assessment of gonadal influences at P20. A second advantage is that unlike gonadectomy and hormone replacement, delaying puberty by SD rearing maintains endogenous hormone rhythms [24]. Finally, SD rearing more accurately mimics the prepubertal state by altering the development of the entire reproductive axis rather than just the gonads. Data in adult hamsters suggests that SD rearing most likely extends the prepubertal period of increased sensitivity to gonadal steroid negative feedback on gonadotropin secretion [23, 25, 26], thereby delaying the pubertal switch to decreased steroid-dependent gonadotropin restraint [27]. This expands the question of puberty dependence or independence to include non-gonadal levels of the hypothalamic-pituitary-gonadal (HPG) axis (e.g., pubertal changes in hypothalamic and pituitary hormones). Nevertheless, caveats must be considered when using the seasonal-species approach. It is not known whether neural

circuits that regulate behavior undergo similar changes in sensitivity to gonadal steroids during adolescence and, if so, whether photoperiod alters this process. SD housing of adult Syrian hamsters increases the steroid sensitivity of neural circuits regulating territorial aggression of females but decreases the steroid sensitivity of the neural circuits regulating the sex behavior of males and females [24, 28]. Hence, SD rearing might increase or decrease steroid sensitivity of neural circuits regulating play and/or aggression of juvenile and/or adolescent Siberian hamsters. If this occurs, behavioral development seen in the seasonal-species approach could be regulated by the combination of changes in pubertal hormones, changes in the sensitivity to these hormones, and puberty-independent changes in neural circuits. Photoperiod also regulates many seasonal traits in addition to puberty (e.g., body mass, immune function, and thermoregulation) [29, 30], and effects of SD rearing could be due to photoperiod influences on these non-reproductive systems. This is less of an issue when photoperiod does not alter the adolescent trait, as seen in the present study for the timing of the transition from play to aggression. However, experiments that demonstrate effects of SD rearing, as seen in the present experiment for levels of play and aggression, should be followed up by neuroendocrine manipulations designed to test whether these effects are due to pubertal or non-pubertal influences of photoperiod.

In the present study, results from the seasonal-species and gonadectomy approaches were convergent, but not identical. For example, the developmental rise in aggression was evident in SD-reared, but not GNX, male hamsters. This highlights an important difference between the two approaches: gonadectomy tests the role of the gonads, whereas the seasonal-species approach tests the role of *changes* in pubertal hormones. Because gonadectomy removes all gonadal hormones, whereas SD rearing maintains low levels of gonadal hormones [3], the present findings suggest that the expression of male aggression, and consequently its developmental rise, require at least some



**Figure 4. Gonadectomy Does Not Alter Play Asymmetry or Its Developmental Increase across Adolescence**

Mean (+SE) asymmetry score for playful interactions of LD-reared gonadectomized (GNX) and control hamsters at P20, P30, P40, P50, and P60. Play asymmetry of males and females did not differ; therefore, data were collapsed across sex. Play and aggression asymmetry did not differ between GNX and control hamsters at any age ( $p > 0.09$  at each age from P20 to P60, ANOVA). Play asymmetry increased from P20 to P60 in GNX and control hamsters ( $p < 0.02$ , nonparametric sign test). Sample sizes (pairs per group) are indicated within the bars.

level of gonadal hormone. However, the SD findings demonstrate that the emergence of aggression does not require changes in pubertal hormones. In males, the onset of puberty was accompanied by a further increase in aggression. Hence, the full developmental rise in aggression is brought about by synergistic actions between puberty-dependent and puberty-independent mechanisms. Prepubertal castration decreases adult aggression in some species, but not others [31]. To our knowledge, the present findings are the first to demonstrate coordinated actions between puberty-dependent and puberty-independent mechanisms within a single species.

Although gonadal steroids are known to promote aggression in numerous species [31], experiments have repeatedly shown that SD housing increases, and gonadectomy has little to no effect on, aggression in adult male and female Siberian hamsters when tested in the resident-intruder paradigm [32–36]. Instead, adrenal hormones have been proposed to regulate aggression in SD-housed Siberian hamsters [37–39] and several other seasonal species [40]. It is likely that the conflicting findings in the present study, which indicated that testicular hormones increase aggression, are due to the type of aggression examined. The present experiments tested familiar animals in a neutral setting, which assesses aggression related to social dominance, whereas previous experiments used the resident-intruder test, which assesses territorial aggression toward a stranger. Extrapolation from findings across studies suggests that gonadal hormones play a larger role in regulating social dominance than territorial aggression in Siberian hamsters.

Unexpectedly, removing prepubertal gonadal inhibition, either by SD rearing or gonadectomy, increased juvenile social play. This finding adds to the growing body of literature that challenges the widely held belief that the gonads, particularly the ovaries, are functionally quiescent during the juvenile period. The prepubertal gonads secrete measurable levels of hormones [41–49], and some evidence suggests that prepubertal ovarian hormones program sexually dimorphic adult behaviors [50–54]. The present findings demonstrate that the prepubertal ovary and testis also impact juvenile social behaviors. In rats, neither prepubertal castration nor prepubertal ovariectomy alters levels of juvenile social play [14, 17, 55, 56] (but see [57]). Discrepant findings between Siberian hamsters and rats most likely reflect a species difference and highlight the importance of using multiple, diverse animal models in biological research [58]. Further comparative studies are needed to determine whether findings in Siberian hamsters or those in laboratory rats more accurately

reflect the role of prepubertal gonadal hormones in juvenile social development across mammals, including humans.

The underlying substrates that mediate puberty-independent regulation of adolescent social development are not known. The hypothalamic-pituitary-adrenal (HPA) axis, serotonin, and vasopressin have each been proposed to regulate the transition from juvenile social play to adult aggression in Syrian hamsters [6]. HPA axis stress reactivity undergoes developmental changes during adolescence [59], and manipulations that activate type II corticosteroid receptors accelerate the transition from play to aggression in male Syrian hamsters [60, 61]. One such manipulation is social subjugation, which, in addition to increasing cortisol and adult aggressive behaviors in juvenile male Syrian hamsters [62, 63], increases serotonin innervation and decreases vasopressin content in the anterior hypothalamus [64], an area known to regulate both play and aggression in this species [65–68]. Vasopressin has long been known to regulate aggression in adult animals [69] and has recently been implicated in play [67, 70–72]. Brattleboro rats, which contain a mutation in the vasopressin gene, exhibit lower levels of play than do their wild-type littermates while maintaining the same temporal profile of play development [73], and gonadectomy markedly reduces vasopressin mRNA and/or peptide in the bed nucleus of the stria terminalis, medial amygdala, and their projection areas [74, 75]. These findings suggest a gonadal-dependent rather than puberty-independent role for vasopressin. Nevertheless, other vasopressin projections are not significantly altered by gonadal manipulations (e.g., [76, 77]), and intracranial vasopressin manipulations can have opposite effects on play depending on the brain area injected [71], raising the possibility that vasopressin has multiple roles in social development depending on its site of action.

The present study demonstrates how natural adaptations of seasonal species can be used to investigate puberty-dependent and puberty-independent regulation of adolescent social development. Using both photoperiod and gonadal manipulations, we found that puberty-independent mechanisms regulate the timing of adolescent social development, whereas prepubertal and adult gonadal hormones modulate levels of age-appropriate social behaviors. At present, we can only speculate on the functional significance of this type of synergistic regulation for seasonal species. Perhaps decoupling of puberty (and photoperiod) from the timing of social behavior transitions allows certain developmental milestones (e.g., dispersal) to occur at a given chronological age, regardless of whether the hamster was born at the

beginning or end of the breeding season. Conversely, maintaining the link between pubertal hormones and levels of social behaviors would maintain the ability to adjust or fine-tune levels of behaviors to match the seasonal environment. Moving forward, a combination of approaches will be required to elucidate the functional significance and underlying mechanisms of puberty-dependent and puberty-independent regulation. The seasonal-species approach will provide a valuable tool to untangle the many neural, endocrine, and behavioral changes that occur during adolescence. Such experiments will begin to map puberty-dependent and puberty-independent neuroendocrine circuits and specify how they interact to regulate adolescent development.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
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  - Experimental Timeline and Groups
  - Surgeries
  - Social Interaction Tests
  - Reproductive Measures
- QUANTIFICATION AND STATISTICAL ANALYSIS

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### AUTHOR CONTRIBUTIONS

M.J.P. designed experiments. G.J.D. supervised the project and provided funding. M.J.P. and C.K.P. conducted experiments. M.J.P. and L.M.B. conducted analyses. M.J.P., G.J.D., L.M.B., and C.K.P. wrote the paper.

### DECLARATION OF INTERESTS

The authors declare no competing interests.

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## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
Siberian hamster, <i>Phodopus sungorus</i>	Local Breeding colony	N/A
Software and Algorithms		
JWatcher	<a href="http://www.jwatcher.ucla.edu">http://www.jwatcher.ucla.edu</a>	N/A
Observer XT12	Noldus Information Technology	N/A

### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Matthew Paul ([mjpaul@buffalo.edu](mailto:mjpaul@buffalo.edu)).

### EXPERIMENTAL MODEL AND SUBJECT DETAILS

Siberian hamsters (*Phodopus sungorus*) were obtained from the breeding colony of Dr. Eric Bittman at the University of Massachusetts, Amherst, which was originally derived from breeding stock supplied by Dr. Klaus Hoffmann (Munster, Germany). Male and female subjects for this experiment were gestated and reared in either a long day length (LD; 14 hr light/day) or short day length (SD; 10 hr light/day; lights off at 4pm EST for both photoperiods) and weaned at 19 days of age. At weaning, hamsters were fitted with ear tags for individual identification. Unless otherwise noted, hamsters were housed in same-sex, same-treatment pairs in polypropylene cages (28 × 17 × 12 cm) with Carefresh bedding. Ambient temperature was maintained at 22 ± 4°C. Tap water and Purina Rodent Chow (no. 5008) were available *ad libitum*. All procedures were approved by the Institutional Animal Care and Use Committee at UMass Amherst and conducted in accordance to the NIH Guidelines for the Care and Use of Laboratory Animals.

### METHOD DETAILS

#### Experimental Timeline and Groups

At 15 days of age, LD-reared hamsters underwent gonadectomy (GNX; 14 females, 12 males), sham-surgery (Sham; 6 females, 10 males), or no surgical (Intact; 26 females, 20 males) procedures. SD-reared hamsters did not undergo surgical procedures (Intact; 16 females, 18 males). Social play, aggressive behaviors, and reproductive measures were assessed every 10 (±1) days from postnatal day (P)20 to P60 (LD-reared hamsters) or P120 (SD-reared hamsters). Measures of LD-reared hamsters were not taken past P60 because puberty and the transition from play to aggression were expected to be complete (and were) by this age in LD groups. Behavioral testing and reproductive measures for all groups were conducted concurrently. Data from LD- and SD-Intact males and females were used to test the influence of photoperiod on social development (Experiment 1a), and data from all LD-reared groups (Intact, Sham, and GNX) were used to test the influence of the gonads on social development (Experiment 1b); note that the same LD-Intact hamsters were used in both experiments.

Pair-housed hamsters were checked daily for signs of fighting. Those exhibiting fighting wounds were separated into single-housing conditions and removed from further behavioral testing. This resulted in the removal of 2 male LD-Intact and 1 male LD-Sham pairs at P60 as well as 3 male SD-Intact pairs at P110; data prior to removal were included in all analyses. In a small percentage of hamsters, SD-housing fails to suppress reproductive function [78]. In the present study, this occurred in two pairs of male SD-reared hamsters, therefore, data from these hamsters were not included in analyses. Notably, these SD-reared hamsters underwent the transition from play to aggression around P40–P50 (as was true for all animals regardless of photoperiod) and exhibited high levels of aggression coincident with increased testis size (P50 for one pair and P60 for the other).

#### Surgeries

Gonadectomies were conducted under isoflurane anesthesia. For castrations, the right and left testes and epididymes were externalized sequentially through a single incision in the abdominal wall. The testicular veins were ligated with sterile sutures, and the testes and epididymes were removed. The abdominal wall and skin were closed, and the wound was treated with antibiotic ointment. Ovariectomies were carried out in a similar manner except that two incisions were made, one on each of the animal's flanks for the left and right gonad. Hamsters receiving sham surgeries underwent identical procedures except that the testicular/ovarian veins were not ligated and the gonads were not removed. Both sham-operated and gonadectomized animals received a subcutaneous injection of buprenorphine (0.05 mg/kg) as a post-operative analgesic.

### Social Interaction Tests

Twenty-five-minute social interaction tests were conducted between 0.5 – 2.5 h after lights off under dim red light. Same-sex, same-treatment cagemate pairs were separated and subsequently reunited the following day in a fresh cage, at which point behaviors were recorded using a Sony Handycam video camera (DCR-SR85). This separation paradigm is commonly used to stimulate play behavior at the time of testing [70, 73, 79, 80]. Play and aggressive behaviors were scored from video by an observer unaware of experimental group assignments using JWatcher software (<http://www.jwatcher.ucla.edu>) or Observer XT12 (Noldus Information Technology). Play behavior was scored as the number of play attacks, boxing events, and pins. Aggression was scored as the number of offensive aggressive attacks. See text for description of behaviors, including the distinction between play and aggression. Hamsters within each testing pair were given a unique shave pattern on their dorsal skin to enable tracking of individuals during behavioral scoring. This allowed for the quantification of the proportion of behaviors exhibited by each individual, e.g., play and aggression asymmetry scores. Hamsters were shaved under light isoflurane anesthesia the day before behavior testing.

### Reproductive Measures

Reproductive development was tracked using estimated testis volume (ETV) or vaginal opening (VO). ETV and VO were recorded at the time of dorsal fur shaving (day before behavioral testing) while hamsters were under light isoflurane anesthesia. For ETV, the skin around the left testis is shaved and wet with 70% ethanol. The length and width of the left testis is then measured externally through the skin using calipers. ETV is quantified as the length  $\times$  width<sup>2</sup>, which is highly correlated with testis weight, circulating testosterone, and spermatogenesis [81–83]. VO can be used as a marker of reproductive development [84, 85], and photoperiod suppression of VO is associated with low uterine and ovarian weights [86].

### QUANTIFICATION AND STATISTICAL ANALYSIS

Play, aggression, and ETV were analyzed by repeated-measures ANOVA, with Photoperiod and Sex as independent variables for Experiment 1a and Surgery and Sex as independent variables for Experiment 1b. Photoperiod's influence on the percentage of hamsters exhibiting VO was assessed using the Chi-Square test at each age. The onset of puberty was defined as the first significant increase in mean ETV from baseline (for male groups) or mean age of vaginal opening (for female groups). Play and aggression were analyzed at the level of the pair, meaning that sample sizes for these measures equaled the number of pairs in each group (Female LD-Intact = 13; Male LD-Intact = 10; Female SD-Intact = 8; Male SD-Intact = 7; Female LD-Sham = 3; Male LD-Sham = 5; Female LD-GNX = 7; Male LD-GNX = 6). Given the low number of Sham pairs, behavioral data from Sham and Intact hamsters were combined into a single Control group for Experiment 1b where differences were not detected between these groups (see main text and Figure 3). There were recording/camera focus issues that prevented behavioral scoring in 6 of the 334 behavior tests (2 Female LD-Intact tests, 1 Male LD-Sham test, 1 Female LD-GNX test, 1 Female SD-Intact test, and 1 Male SD-Intact test). These 6 missing data points along with the 3 data points that could not be tested due to fighting between LD-reared male cagemates (see Experimental Timeline and Groups section above) were replaced with the mean of the experimental group for that time point (total of 9 data points out of 334). Mean replacement was not used for the missing P110 and P120 data points of the 3 SD-reared male cagemate pairs that were removed for fighting because this constituted 43% of Male SD-Intact pairs (3 of 7 pairs). Instead, P110 and P120 time points were not included in SD-reared male behavioral analyses.

Play and aggression asymmetry scores were calculated as: (# play/aggressive behaviors of the more active hamster - # play/aggressive behaviors of the less active hamster) / Total number of play/aggressive behaviors of the pair. If no play/aggressive behaviors were exhibited during a given test, the asymmetry score was undefined and the pair was removed from the analysis for that age. Hence, not all pairs contributed to each time point. Therefore, group differences in play and aggression asymmetry data were analyzed at each age by ANOVA, and differences between P20-30 versus P50-60 were assessed using the nonparametric sign test. Post hoc comparisons were conducted using Fisher's PLSD when the overall ANOVA yielded significant main effects or interactions. Statistical tests were conducted using Statview 5.0.1 (SAS Institute, Cary, NC), and significance was assumed when  $p < 0.05$ .