Published in final edited form as:

Behav Brain Res. 2010 April 2; 208(2): 528–534. doi:10.1016/j.bbr.2009.12.038.

Effects of photoperiod and experience on aggressive behavior in female California mice

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Abstract

Aggressive behavior among females is observed in many species, but the mechanisms of this behavior have historically been understudied. In many species of rodents, winter-like short day photoperiods induce increased aggression levels compared to summer-like long day photoperiods. Recent reports in hamsters show that short days also increase aggression in females. We examined the effects of photoperiod on aggression in female California mice, and for the first time compare brain activity of aggression-tested female rodents under different photoperiods. We observed that female California mice were more aggressive when housed in short days versus long days. Intriguingly, we also observed that under long days female attack latency decreases with repeated testing in resident intruder tests. These data suggest that winner effects that have been described in males may also occur in females. We also used the expression of phosphorylated extracellular signal-regulated kinases (pERK) in the brain to estimate brain activity during aggression tests. pERK can alter neuronal activity in the short term and in the long term can act as a transcription factor. Using immunoblot analyses we observed that aggression-induced pERK expression in the female bed nucleus of the stria terminalis (BNST) and medial amygdala occurs under both long and short days. Thus, the mechanisms controlling increased aggression under short days are still unclear and additional study is needed.

Introduction

Aggressive behavior is observed in many species, but the mechanisms of aggressive behavior in female vertebrates have historically been understudied (Floody 1983). Aggressive behavior is often used by females competing for social status (Sterck et al. 1997), food resources (Kinahan and Pillay 2008) or shelter (Heinsohn et al. 2005). In biparental species, competition for males that provide high quality parental care may lead to increased aggression among females (Sandell 1998; Gill et al. 2007). Despite the relatively wide use of aggressive behaviors by females, relatively little is known of the mechanisms controlling these behaviors. In contrast, male aggression has been studied much more intensively (Nelson and Trainor 2007), primarily in the context of resident-intruder tests. A network of brain regions regulating male-male offensive aggression has been studied in a wide array of rodent species. In most male rodents aggressive behaviors are driven by olfactory cues (DaVanzo et al. 1983), which are processed by the medial amygdala (MEA) and sent to several hypothalamic and limbic brain areas (Canteras et al. 1995). Indirect markers of neuronal activation such as c-fos have been used to

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show that the bed nucleus of the stria terminalis (BNST), lateral septum (LAS), medial amygdala (MEA), ventromedial hypothalamus (VMH), and periaqueductal gray are activated during aggressive encounters (Kollack-Walker and Newman 1995; Veenema et al. 2003; Gobrogge et al. 2007). In contrast to males, only a few studies have examined brain networks associated with female-female aggression.

The most comprehensive study of brain activity during female-female aggression was conducted in California mice (Davis and Marler 2004). Female California mice are more aggressive during diestrus (Davis and Marler 2003), and in general female-female aggression tests stimulate a greater increase in c-fos in the brain during diestrus. In particular, BNST, LAS, MEA, VMH, and piriform cortex showed increased c-fos following resident-intruder tests (Davis and Marler 2004). This pattern of activity corresponds well with activity patterns observed during male-male resident-intruder tests (Goodson 2005; Nelson and Trainor 2007). In general, neural networks of aggression have been studied under invariant conditions, and it is unclear how neural circuits regulating aggression function in different environmental conditions. One exception is that maternal aggression towards males has been well studied. Although maternal aggression may represent a combination of offensive and defensive aggression (Blanchard et al. 2003), many of the brain areas activated during male-male and female-female resident-intruder tests are activated during maternal aggression (Lonstein and Gammie 2002; Gammie 2005).

Aggression is also affected by environmental factors such as photoperiod. Studies on hamsters (Jasnow et al. 2002; Caldwell and Albers 2004; Demas et al. 2004) and *Peromyscus* (Trainor et al. 2007b; Trainor et al. 2008) show that in resident-intruder tests that male aggression is increased during winter like short days. Female-female aggression in Syrian (Fleming et al. 1988) and Siberian (Scotti et al. 2007) hamsters is also increased under short days. In hamsters and *Peromyscus*, studies using c-fos as an indirect marker of neuronal activity found no differences in c-fos expression in several brain regions between long day and short day aggression tested males (Kramer et al. 2008; Trainor et al. 2008), even though short day males were more aggressive. We recently used measurements of phosphorylated extracellular signal-regulated kinase (pERK, also known as mitogen activated protein kinase or pMAPK) as an alternative marker of brain activity (Trainor et al. 2010). In its active state, pERK can immediately alter neuronal excitability (Selcher et al. 2003) and over time can affect brain function acting as a transcription factor (Valjent et al. 2001). Following resident-intruder tests, pERK expression in the BNST increased when males were housed in short days but not long days (Trainor et al. 2010). This result suggested that pERK could be a useful marker for identifying effects of photoperiod on brain activity. Based on these results, we hypothesized that pERK might also be a useful marker for examining circuits of aggression in females.

In experiment 1 we used immunohistochemistry to identify the location of pERK positive cells in the social behavior network of female California mice housed in long days and short days. In experiment 2 we examined the effect of photoperiod on aggressive behaviors in females tested in three consecutive resident-intruder aggression tests. The rationale for this experiment was that multiple testing would allow for averaging of behavior across the estrous cycle. However, we found that the effect of repeated testing differentially affected long day and short day females. Finally, in experiment 3 we examined the effect of photoperiod on aggressive behavior in females tested in a single resident-intruder aggression test. We then used immunoblots to quantify pERK expression in the BNST and MEA. Based on previous results in males, we hypothesized that aggression testing would increase pERK expression in the BNST and MEA in short day females but not long day females.

Methods

Animals and Behavioral Testing

Adult female California mice aged 3–6 months were bred in our laboratory colony. Mice were housed in clear polypropylene cages provided with Carefresh bedding and cotton nestlets. Harlan Teklad 2016 food and water were provided ad libitum. All testing procedures were approved by the UC Davis Institutional Animal Care and Use Committee. Three days before resident-intruder aggression or control testing, each female was individually housed. In both long day and short day light cycles lights were turned off at 1400 Pacific Standard Time (PST). All behavioral tests were conducted under dim red light between 14:30 and 17:00 PST. Resident-intruder aggression tests were conducted by introducing an unfamiliar group-housed female intruder into the resident's home cage. All intruders were unrelated to residents and housed in long days. It is possible that different results could have been obtained had we tested short day females with intruders housed in short days. Using an infrared camera, aggressive behavior was observed for 10 minutes after which the intruder was removed. In control tests the lid of the cage was removed and replaced to simulate the handling during a resident-intruder test. Animals were maintained in accordance with the recommendations of the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*.

Experiment 1: Effect of Photoperiod on Diestrus Females and Immunohistochemical Detection of pERK

A total of 34 virgin female mice were randomly assigned to be housed in long days (16 L:8 D) or short days (8 L:16 D) for 8 weeks. Females were group housed with 2–3 mice per cage. Estrous cycle stage was determined on the morning of testing (08:00–12:00 PST) by vaginal lavage (30 ul of saline) on the day of behavior testing. Diestrus was identified by the presence of leukocytes (Becker et al. 2005). Immediately after aggression tests, each resident mouse was anesthetized with isoflurane and rapidly decapitated. The brain was quickly removed and fixed overnight at 4° C in 5% acrolein in phosphate saline buffer (PBS). Each brain was then transferred to 25% sucrose in PBS for 24 hr, frozen on dry ice, and stored at –40° C for pERK immunohistochemistry.

Experiment 2: Effect of Photoperiod Across Multiple Resident-Intruder Tests

To avoid the stressful effects of handling (for vaginal lavage) prior to resident-intruder aggression tests, we conducted a second study in which females were tested 3 times on successive days. The rationale for testing on consecutive days is that this would ensure that our sample of aggressive behavior would cover multiple stages of the estrous cycle. A total of 18 virgin females were randomly assigned to be housed in long days or short days (2–3 mice per cage). After 8 weeks, each female was individually housed for three days, and resident-intruder aggression or control tests were conducted as described above. A different intruder was used for each resident during each of the three aggression tests. Immediately after each test, each female was anesthetized with isoflurane and rapidly decapitated. Vaginal lavage was conducted as described above post mortem. Interestingly, similar to male California mice, females were not observed to be reproductively suppressed in short days and continued to cycle through proestrus and estrus (Table 1). Originally, we planned to examine expression of pERK in punch samples of the BNST and MEA of these animals. However, due to unexpected changes in behavior across the three tests (see results), we conducted an additional experiment in which females were tested only once (Experiment 3).

Experiment 3: Expression of PhosphoERK and Total ERK Following Resident-Intruder Tests

Sixteen females were randomly assigned to long days or short days for eight weeks. Each female was either tested in a single resident-intruder test or control test as described above.

Immediately after testing, each mouse was anesthetized with isoflurane and rapidly decapitated. The brain was rapidly removed and a brain matrix was used to collect 2 mm coronal sections (Trainor et al. 2003). A slice starting at the optic chiasm and ending 2 mm anterior contained the BNST. A second slice starting at the optic chiasm and ending 2 mm posterior contained the MEA. Sections were cooled on a freezing plate and bilateral 1 mm punch samples of each brain area were collected and frozen on dry ice. Samples were stored at -80°C until processed for western blot analyses.

Immunohistochemistry

In experiment 1, it appears that handling from vaginal lavage reduced the expression of aggressive behaviors, so only a small subset of brains were examined with immunohistochemistry to identify brain regions expressing pERK. Brains were sectioned at $40\text{ }\mu\text{m}$ on a microtome. Sections were stored in cryoprotectant (50% phosphate buffer, 30% sucrose, 1% polyvinylpyrrolidone, 30% ethylene glycol) at -20°C . Sections were then washed 3 times in PBS and incubated in 1% sodium borohydride in PBS for 10 min. Sections were then blocked in 10% normal goat serum and 0.3% hydrogen peroxide in PBS for 20 min. Sections were then incubated in primary pERK (#4370, Cell Signaling, Danvers, MA, concentration 1:250 dissolved in 2% normal goat serum and 0.5% triton X (TX) in PBS overnight at 4°C on an orbital shaker. The specificity of this antibody in male *Peromyscus* is described by Trainor et al. (2009). The sections were then washed three times in PBS before transferring to biotinylated goat anti-rabbit antibody in 2% normal goat serum in PBS TX (Vector Laboratories, Burlingame, CA, 1:500) for 2 hr. Sections were washed 3 times in PBS and incubated in avidin-biotin complex (ABC Elite Kit, Vector Laboratories) for 30 min. Sections were washed in PBS and developed in nickel-enhanced diaminobenzidine (Vector Laboratories) for 2 minutes. Sections were then mounted, dehydrated, and coverslipped. Slides were analyzed using a Zeiss Axioimager equipped with an AxioCam MRC camera. Representative photomicrographs of each of the following brain areas were identified using a mouse brain atlas (Paxinos and Franklin 2002): bed nucleus of the stria terminalis (BNST, bregma 0.02), basolateral amygdala (BLA, bregma -1.22), central nucleus of the amygdala (CEA, bregma -1.22), ventromedial hypothalamus (VMH, bregma -1.58) and medial amygdala (MEA, bregma -1.77).

Estradiol Enzyme Immunoassay

We measured estradiol using an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). When assay concentrations for serial dilutions of a California mouse plasma pool were compared with standards, computed regression lines did not differ in slope ($P > 0.2$). The sensitivity of this assay is 6.6 pg/mL and the intra-assay coefficient of variation was 14.3%. We measured estradiol in the 18 females tested in aggression tests for experiment 2 as well as 9 females tested in control tests (5 long day, 4 short day).

Western Blot Analyses of Extracellular Signal Regulated Kinase (ERK) and pERK

Punch samples were homogenized in $150\text{ }\mu\text{L}$ of RIPA buffer containing 1 mM of phenylmethanesulphonylfluoride (PMSF). Total protein concentrations were determined by Bradford assay and $20\text{ }\mu\text{g}$ of protein was separated by polyacrylamide gel electrophoresis. Protein was transferred to nitrocellulose membranes and blotted with pERK antibody (1:1000, #4370, Cell Signaling) then stripped and reblotted with total ERK antibody (1:1000, #4695, Cell Signaling). Antibodies were detected with horseradish peroxidase-conjugated secondary antibodies (Invitrogen) followed by development with SuperSignal West chemicals (Pierce, Rockford, IL, USA). An Alpha Innotech imaging station with FluorChem software was used to capture images. The p42 and p44 isoforms of both total ERK and pERK were quantified.

Statistical analyses

After inspecting behavior data with Q-Q plots to assess normality (Zar 1996), the number of bites was square root transformed and attack latency was log transformed. In experiment 2, a mixed model repeated measures ANOVA was used to assess effects of photoperiod on number of bites and attack latency across the three aggression tests. We used the conservative Greenhouse-Geisser correction for all within-subject comparisons. Independent t-tests were used to test for effects of photoperiod within each of the three aggression tests. We also used a Chi-square test to determine whether photoperiod influenced the likelihood that females would be in different stages of the estrous cycle. Estradiol levels were log transformed and analyzed with three-way ANOVA testing for effects of photoperiod, aggression testing, and estrus cycle. In experiment 3, pERK (normalized to total ERK) expression levels as well as total ERK band intensities were analyzed with two-way ANOVA testing for effects of photoperiod, aggression testing, and the interaction.

Results

Experiment 1: pERK Positive Cells in the Female Brain Following Aggression Tests

Although on average short day-housed females bit intruders (mean $\pm$ s.e., 2.4 $\pm$ 0.9) twice as frequently as long day housed females (1.2 $\pm$ 0.66), this difference was not statistically significant ($t_{19} = 1$, ns). Similarly, although short day housed females had shorter mean attack latencies (360 $\pm$ 82 s) than long day housed females (433 $\pm$ 70 s), this difference was not significant ($t_{19} = 1$, ns). Based on our subsequent studies in which vaginal lavage was conducted post-mortem, we believe that the low aggression levels observed in this study are most likely an effect of conducting lavage prior to testing (about 4 hours). Immunohistochemistry analyses detected expression of pERK in several brain areas related to aggressive behaviors including the MEA, BNST, VMH, anterior hypothalamus, and basolateral amygdala (Fig. 1).

Experiment 2: Effect of Photoperiod Across Multiple Resident-Intruder Tests

When females were tested in multiple aggression tests without vaginal lavage prior to testing, repeated measures ANOVA indicated that residents bit intruders significantly more in short days compared to long days (Fig. 2A, $F_{1,19} = 5.3$, $p = 0.03$). The number of bites did not change significantly across the three aggression tests and there was no test by photoperiod interaction (all p 's > 0.6). Although repeated measures ANOVA indicated there was no significant overall effect of photoperiod on attack latency ($F_{1,19} = 2.73$, $p = 0.11$), there was a nonsignificant trend for test by photoperiod interaction ($F_{1,1.49} = 2.93$, $p = 0.08$). Repeated measures ANOVA of long day mice indicated a significant decrease in attack latency across the three tests ($F_{1,1.74} = 4.09$, $p = 0.04$), whereas the same analysis of short day mice showed no significant change in attack latency across the three tests ($F_{1,1.16} = 0.67$, $p = 0.45$). Independent t-tests showed that short day females had shorter attack latencies than long day females in the first test (Fig. 2B, $t_{19} = 3.0$, $p = 0.01$) but not the second (Fig. 2C, $t_{19} = 1.5$, $p = 0.16$) and third tests (Fig. 2D, $t_{19} = 0.01$, $p = 0.99$).

There was no evidence that females were more likely to be diestrus in short days compared to long days after the third aggression test (Table 1, $\chi^2 = 0.15$, ns). Estradiol levels varied significantly across the estrous cycle (Fig. 3, $F_{1,16} = 4.0$, $p < 0.05$) and were higher in proestrus females than in diestrus females (planned comparison, $p = 0.02$). There was a nonsignificant trend for estradiol levels to be higher in proestrus females compared to estrus females (planned comparison, $p = 0.06$). There was no significant effect of photoperiod ($F_{1,21} = 1.2$, $p > 0.05$) or aggression testing ($F_{1,21} = 1.3$, $p > 0.05$).

Experiment 3: Expression of pERK Following Resident-Intruder Tests

When females were tested in a single aggression test without vaginal lavage prior to testing, females bit intruders more frequently (Fig. 4, $t_{15} = 3.87$, $p < 0.01$) and had shorter attack latencies (Fig. 4, $t_{15} = 3.79$, $p < 0.01$) when housed under short days versus long days. In punch samples of the BNST aggression testing caused a significant increase in expression of the pERK p44 isoform (Figs. 5B, 6A, $F_{1,12} = 5.22$, $p < 0.05$) and there was a nonsignificant trend for increased expression of the pERK p42 isoform (Figs. 5A, 6A, $F_{1,12} = 4.53$, $p = 0.06$). There was no effect of photoperiod or interaction between aggression testing and photoperiod for either isoform in the BNST. Similarly, analyses of punch samples from the MEA showed that aggression testing increased pERK expression of both the p42 (Fig. 5C, 6B, $F_{1,12} = 12.38$, $p < 0.01$) and p44 (Fig. 5D, 6B, $F_{1,12} = 11.64$, $p < 0.01$) isoform. There was no effect of photoperiod or interaction. There were no significant differences in the expression of total ERK (Fig. 6, Table 2, all p 's > 0.2).

Discussion

Female California mice show a similar phenotype to males in that aggression levels in resident-intruder tests are increased in short days. However, in contrast to males, increased pERK expression was induced in the BNST and MEA following aggression tests under both long days and short days. In males, aggression-induced pERK expression in the BNST was observed only under short days (Trainor et al. 2010). Our results suggest that there may be important sex differences in the neurochemical mechanisms used to generate the increased aggression phenotype observed under short days. We also observed that repeated aggression testing decreased attack latency in long day females but not long day males. Previous work in males has shown that winning aggressive encounters can increase aggressive behavior in males, including California mice (Oyegible and Marler 2005). These changes in behavior are thought to comprise part of the “winner effect”, in which the experience of winning aggressive encounters increases the probability of winning future encounters independent of intrinsic competitive ability (Dugatkin 1997). Our results suggest that winner effects may also occur in females.

Photoperiod and Circuits of Aggression in Female California Mice

As has been reported for Syrian and Siberian hamsters, we have observed that both male and female California mice show higher aggression levels when housed in short day photoperiods. However, it appears that the mechanisms underlying this environmental modulation of aggression differ in males and females. In male California mice, aggression-induced pERK in the BNST is only observed in short day mice whereas pERK in the MEA is elevated under short days in both control and aggression tested mice (Trainor et al. 2010). Based on these data, we hypothesized that only more aggressive short day female California mice would have increased aggression-induced pERK expression in the BNST and/or MEA. Our immunoblot analyses do not support this hypothesis, as aggression-induced pERK expression was observed under both long days and short days. In the BNST the p44 isoform was more consistently elevated by aggression tests whereas there was only a nonsignificant trend for an increase in the p42 isoform. In contrast, in the MEA resident-intruder testing caused a significant increase in both isoforms of pERK. It is possible that pERK in the BNST and MEA may have different effects on aggression in males and females, but we have not determined that pERK plays a causal role in regulating aggression. A related possibility is that there may be sex differences in the neurochemical pathways that regulate pERK expression. Recent data suggest there are important sex differences in the hormonal regulation of aggression in photoperiod sensitive rodents, which could affect pERK regulation.

In male California mice and old field mice (*P. polionotus*), estrogens act rapidly to increase aggression in short days but not long days (Trainor et al. 2007a; Trainor et al. 2008). Rapid action of steroid hormones is usually assumed to be mediated by nongenomic pathways (Woolley 2007), which do not involve changes in transcription mediated by nuclear estrogen receptors. For example, estrogens act rapidly to phosphorylate ERK (Abraham et al. 2004). Although no study has tested whether estrogens act rapidly to regulate aggression in virgin female rodents, recent studies in hamsters suggest that the relationship between photoperiod and estrogen function is different in females. In Siberian hamsters ovariectomy does not significantly alter aggression whether females are housed in long days or short days (Scotti et al. 2007). Intriguingly, when ovariectomy was combined with exogenous estradiol implants, aggression levels were reduced. Similar results were reported in female Syrian hamsters (Gutzler et al. 2009). Thus, while rapid estrogen action has been linked to increased male aggression in short days, it appears unlikely that estrogens facilitate increased aggression during short days in females as has been observed in males. Our measurements of estradiol showed that similar to other rodents (Becker et al. 2005), estradiol levels increased during proestrus. Combined with our observations that females continue to cycle in short days, these data reinforce observations from males that reproduction in California mice is not inhibited in short days (Nelson et al. 1995; Trainor et al. 2008).

An important consideration is that to some extent the increased expression of pERK following aggression tests was caused by exposure to odors from intruders as opposed to engaging in aggressive behavior *per se*. For example, female odors have been shown to increase pERK expression in the vomeronasal system of male mice (Dudley et al. 2001). One study on male Syrian hamsters controlled for the effects of intruder stimuli by exposing control animals to a wood block that had been scent marked by an intruder (Delville et al. 2000). Males tested in aggression tests had more c-fos positive cells in the MEA and BNST versus males exposed to the scented block. Most likely, our measurements of pERK in the BNST and MEA are affected by a combination of intruder stimuli and engaging in aggressive behavior. It is unlikely that effects of photoperiod on aggression are due to differences in hormonal responses related to stress. Female California mice do respond to resident-intruder tests with an increased in corticosterone, but there is no effect of photoperiod on this response (Trainor et al. submitted).

Effects of Experience on Aggression

In experiment 2 females showed a significant decrease in attack latency between the first and third aggression tests, but only if they were housed in long days. Increased aggression after winning aggressive encounters has been observed widely across the animal kingdom in males (Beaugrand et al. 1991; Drummond and Canales 1998; Fuxjager et al. 2009), but we are not aware of any previous reports showing a similar effect in females. This change in aggressive behavior is thought to be a component of the “winner effect”, which occurs when the experience of winning increases the probability that an individual wins an aggressive contest. In males, testosterone levels are often observed to be increased after winning aggressive encounters (Wingfield et al. 1990; Oyegible and Marler 2005; Gleason et al. 2009), and this increase is necessary for future changes in aggression to occur (Trainor et al. 2004). In females, less is known about the hormonal changes associated with winning aggressive encounters. Female California mice housed in long days exhibit decreased progesterone following aggressive encounters (Davis and Marler 2003). Interestingly, aggressive challenges in an African black coucal (a species of cuckoo) also resulted in a rapid decrease in progesterone levels in females (Goymann et al. 2008). Progesterone can affect behavior rapidly (Mani 2008), raising the possibility that rapid changes in progesterone could mediate the effects of experience on aggression in females. We did not observe changes in aggression in short day mice, although

this could be due to a ceiling effect given that short day mice had aggression levels that were already very high in initial aggression tests.

It could be argued that the effects of experience on aggression in long day females would make photoperiod differences irrelevant, because aggression levels in long day females would increase by winning fights. However, for California mice gaining a territory is an important challenge as females typically disperse from their natal home range (Ribble 1992). In California mice the winner effect is only observed when a male is tested in his home cage (Fuxjager et al. 2009). These data suggest that for both males and females, initial aggressive encounters for young mice, while it is not clear whether this effect is also present in females, it is likely that initial aggressive encounters for both males and females end in losses. Thus in the absence of winning experiences, photoperiod could exert critical effects on the outcome of aggressive encounters and whether an individual (male or female) gains a territory. It is also unlikely that the effect of photoperiod on aggression in females can be explained by glucocorticoid responses. Both long day and short day female California mice exhibit increased corticosterone levels following aggression tests compared to control mice (Trainor et al. submitted).

Conclusions

Like males, females show higher aggression levels when housed in short days versus long days. However, it appears that different mechanisms are used to achieve this phenotype. Our immunoblot analyses on females showed that aggression-induced pERK expression in the BNST is observed in both long and short days. In contrast, for males aggression-induced pERK expression in the BNST is observed only in short days. These results resemble findings on the mechanisms regulating parental behavior in prairie voles (De Vries and Boyle 1998). Both male and female prairie voles exhibit parental behaviors, yet males have more AVP mRNA than females after pups are born (Wang et al. 1994a). Furthermore, vasopressin promotes parental behavior in males but has no effect on females (Wang et al. 1994b). Thus, a sex difference in AVP function appears to contribute to expression of parental behavior in both males and females. Similarly, like males, we observed that female California mice exhibit shorter attack latencies after winning aggressive encounters, yet previous studies show that females have a very different hormonal profile than males after winning aggressive encounters. Future studies will be needed to determine the mechanisms used by females to translate information on photoperiod and winning experiences into changes in aggressive behavior.

Acknowledgments

The authors thank Katie Crean, Michael Steinman, and Julie VanWesterhuzen for technical help. This work supported by a President's Undergraduate Fellowship award to A.L.S and grants from the UC Davis Hellman Fellows Program and R01 MH85069 to B.C.T.

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Figure 1.
Photomicrographs of phosphorylated extracellular signal regulated kinase (pERK) immunoreactivity in the BNST (A), MEA (B), VMH (C), and BLA (D). Abbreviations: a.c., anterior commissure; f, fornix; o.t., optic tract; e.c. external capsule. Scale bars = 1 mm.

Figure 2.

Short days increased the average number of bites across three resident-intruder tests (A). Repeated measures analyses of variance showed that attack latency significantly decreased across the three tests in long day mice but not short day mice (B). Note that individual dots represent individual data points and lines indicate medians. * $p < 0.05$.

Figure 3. Estradiol levels in females from experiment 2 (n=4–6 per group). Estradiol levels were significantly higher in proestrus females than diestrus females. Among diestrus females, estradiol levels were higher in short days. † $p < 0.05$.

Figure 4.

Effects of photoperiod on aggression in a single aggression test. Females bit intruders more frequently and had shorter attack latencies when housed in short days. * $P < 0.05$.

Figure 5.

Effects of aggression testing on pERK activation (relative to total ERK activation) in the BNST (A, B) and MEA (C, D). Aggression testing significantly increased expression of the p42 isoform in the BNST (A) and MEA (C). Aggression testing significantly increased expression of the p44 isoform in the MEA (D) whereas there was a nonsignificant trend in the BNST (B). * $P < 0.05$ effect of aggression testing, + $p = 0.055$ effect of aggression testing.

Figure 6. Representative images from western blot analysis of protein extracted from BNST (A) and MEA (B) punch samples. A = aggression, C = control.

Table 1

Estrous cycle stages in third aggression test of experiment 2

	diestrus	proestrus	estrus
Long day	6	2	2
Short day	6	2	3

Table 2

Band intensities for total ERK expression in BNST and MEA (mean $\pm$ s.e).

	Long days		Short days	
	Control	Aggression	Control	Aggression
BST p42	5267 $\pm$.712	5273 $\pm$ 1245	5371 $\pm$ 748	5864 $\pm$ 1193
BST p44	2061 $\pm$ 160	2664 $\pm$ 424	2140 $\pm$ 311	3163 $\pm$ 281
MEA p42	5720 $\pm$ 1047	5451 $\pm$ 897	5875 $\pm$ 1344	6575 $\pm$ 536
MEA p44	1778 $\pm$ 193	2003 $\pm$ 130	1712 $\pm$ 310	1985 $\pm$ 131