Behavioural Processes 89 (2012) 271–277

Contents lists available at SciVerse ScienceDirect

Behavioural Processes

journal homepage: www.elsevier.com/locate/behavproc

Effect of testosterone and melatonin on social dominance and agonistic behavior in male *Tscheskia triton*

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ARTICLE INFO

Article history:
Received 21 March 2011
Received in revised form 24 October 2011
Accepted 11 December 2011

Key words:
Agonistic behavior
Castration
Testosterone
Melatonin
Photoperiod
Social dominance

ABSTRACT

Social dominance and agonistic behavior play important roles in animal societies. Melatonin and testosterone are closely related to social dominance and agonistic behavior in rodents, but interactions between both of them remain unknown. In this study we investigated the effects of testosterone and melatonin by manipulating photoperiod and castration on social dominance and agonistic behavior in male *Tscheskia triton*. Castration significantly decreases social dominance of both short- and long-day males, suggesting that testosterone benefits social dominance of males in both breeding and non-breeding seasons. In intact conditions, long-day males tended to dominate short-day males, suggesting that the effect of testosterone on social dominance was a little stronger than melatonin. However, castrated short-day males became dominant over their castrated long-day opponents meaning that high melatonin levels obviously benefit social dominance in males. Hormone implantation indicated that testosterone had no effect on non-breeding condition, but that melatonin was important during the breeding season. Our results indicate that both testosterone and melatonin are important in determining social dominance in male hamsters, and the effect of testosterone appears to be stronger than melatonin. Testosterone is responsible for aggression and social dominance in male hamsters during the breeding season, while melatonin regulates behavior during non-breeding, probably due to the different seasonal secretory patterns of the hormones.

1. Introduction

Agonistic behavior and social dominance play important roles in maintaining social stability in animal societies. Agonistic behavior is necessary for conspecifics to form social structures, for optimum population densities, and for territory and resource defense (Albers et al., 2002; Arregi et al., 2006). Social dominance is established and maintained via agonistic behavior; for example, aggressive behavior is usually observed in dominant animals and defensive and submissive behavior observed in subordinate individuals (Blanchard et al., 1993). The factors known to influence agonistic behavior and social dominance are numerous. Studies of rodents have tended to focus on photoperiod and hormones linked to photoperiod, such as gonadal hormones and melatonin in rats (van de Poll et al., 1986), mice (Compaan et al., 1992; Edwards, 1969; Kriegsfeld and Nelson, 1998; Kudryavtseva et al., 2004; Oyegbile and Marler, 2005), hamsters (Demas et al., 2004; Jasnow et al., 2000, 2002), gerbils (Christianson et al., 1972; Razzoli et al., 2003) and voles (Bowler et al., 2002; Demas et al., 1999; West and Dublin, 1984).

Photoperiod is viewed as the most important proximate factor used by seasonally breeding nontropical rodents to predict seasonal shifts and to mediate social behaviors such as agonistic behavior and social interactions (Albers et al., 2002; Nelson et al., 1990). Seasonal changes in behavior are complex and different across species. For example, short days (non-breeding condition) increases aggression in male solitary Syrian hamsters (*Mesocricetus auratus*) (Jasnow et al., 2002; Garrett and Campbell, 1980) and Siberian hamsters (*Phodopus sungorus*) (Jasnow et al., 2000) possibly to aid in food and burrow guarding, but decreases aggression in social voles during winter to avoid wasting energy (West and Dublin, 1984). Studies into mechanisms behind the effect of

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doi:10.1016/j.beproc.2011.12.010

photoperiod have focused on physiological factors such as testosterone and melatonin which also display annual cycles and seasonal variation (Albers et al., 2002; Badura and Nunez, 1989; Nelson et al., 1990; Schultz and Kay, 2003).

Testosterone is produced in the testes, is involved in spermatogenesis and reproductive behavior, and affects male agonistic behavior and social dominance in many rodents (Campbell et al., 1978; Morin and Zucker, 1978; Siegel, 1985). Male aggression and social dominance are positively correlated with testosterone levels, weakened by castration and restored by testosterone replacement in rats (*Rattus norvegicus*) and house mice (*Mus musculus*) (Brain and Haug, 1992; Compaan et al., 1992; Edwards, 1969; van de Poll et al., 1986). However, exceptions have been reported in wild seasonal breeding rodents. For example, castration does not decrease male aggression in Mongolian gerbils (*Meriones unguiculatus*) (Christianson et al., 1972) and prairie voles (*Microtus ochrogaster*) (Demas et al., 1999). Short-day conditions do not affect aggression in dusky footed wood rats (*Neotoma fuscipes*) (Caldwell et al., 1984), and even elevate aggression in Syrian and Siberian hamsters in spite of testicle regression and testosterone decline (Jasnow et al., 2000, 2002; Garrett and Campbell, 1980). Furthermore, higher testosterone replacement did not result in increased aggression in male Syrian hamsters (Romeo et al., 2003). These studies suggest that the relationship between testosterone, aggression and social dominance varies between species.

Melatonin, secreted by the pineal gland, is considered one of the triggers of seasonal physiological change in animals (Challet, 2007; Schultz and Kay, 2003). Melatonin is produced during darkness and reaches high levels at night. High levels of melatonin are maintained longer under short-day (winter) conditions, leading to many physiological changes such as "winter-like" conditioning in rodents and gonadal regression (Hazlerigg and Wagner, 2006; Jasnow et al., 2000, 2002). Melatonin has been found to promote aggression in several nocturnal rodent species such as house mice (Paterson and Vickers, 1981), Syrian hamsters (Jasnow et al., 2002) and Siberian hamsters (Demas et al., 2004). The removal of the pineal gland inhibits aggression in mice and female Syrian hamsters (Paterson and Vickers, 1981; Fleming et al., 1988); however, little is known how melatonin impacts upon male aggression and social dominance in wild rodents (currently only reported in the Syrian and Siberian hamsters, Jasnow et al., 2000, 2002). In seasonally breeding mammals, testosterone and melatonin may interact to determine social dominance patterns. In long-day photoperiod conditions (e.g. breeding seasons), the testosterone level should be high and melatonin level low, and vice versa during short-photoperiod conditions. Although the independent effects of these two components on social dominance have been investigated, their interactive effects remain unknown.

The greater long-tailed hamster (*Tscheskia triton*) is a solitary and polygamous rodent widely distributed across farmlands of northern China (Yang et al., 1996; Zhang et al., 2001a,b). It breeds from May to September each year and obvious gonad regression occurs in the non-breeding season (Yang et al., 1996). Males possess a pair of flank glands and a midventral gland for chemical communication. Females are philopatric and do not display stable mating associations with males (Song et al., 2005; Zhang et al., 1992, 2001a,b). Hamsters exhibit high aggression in the breeding and non-breeding season and winner-loser relationships are formed quickly and determine later dominant-subordinate relationships during male social interactions (Wang et al., 2006, 2009; Zhang et al., 2001b). Therefore, dominant or subordinate status in this species can be represented by the outcome of one social encounter (Pan et al., 2010). Our previous research found that the testes and testosterone is important for male aggression and dominance during the breeding season: castration reduces aggression and makes males subordinate, but testosterone replacement restores high aggression and elevates social rank (Zhang et al., 2001a). However, during the non-breeding season, when the testes of males have atrophied, aggression levels remain constant among males, suggesting that aggression is independent of testosterone and that other factors are responsible for these behaviors during times of non-breeding (Zhang et al., 2001b). Based on the literature we posited that testosterone may be responsible for the maintenance of aggression in male greater long-tailed hamsters during the breeding season and melatonin responsible during the non-breeding season. In addition, though melatonin and castration can affect aggression, their interactive effects on social dominance are unknown. Therefore, the purpose of this study was to test how testosterone and melatonin independently and interactively affect agnostic behavior and social dominance in male greater long-tailed hamsters.

2. Materials and methods

2.1. Animals and housing conditions

Forty healthy adult greater long-tailed hamsters (>120 g) were captured in farmlands around Beijing using live-traps made of wire mesh baited with peanuts in April 2006. Hamsters were housed individually in stainless steel cages (20 cm × 20 cm × 20 cm) containing cotton nesting material for three months prior to behavioral tests to habituate to the laboratory. The room was maintained at 20 ± 2 °C with a reverse light/dark cycle. Males were housed under natural photoperiod until the beginning of experiments. Food and water were provided ad libitum. All procedures complied with guidelines for animal use and care as required by the Institute of Zoology, Chinese Academy of Sciences.

2.2. Surgical procedures

Hamsters were anesthetized deeply using sodium pentobarbital (male: 60 mg/kg). Castration was performed through bilateral scrotum incisions, the testicles were removed and the abdominal wall and incisions were closed with sutures. Hormone capsules were made from 15 mm Silastic tubing (China Medical, o.d. 2.70 mm, i.d. 2.26 mm), which was packed with 10 mm lengths of crystalline testosterone, melatonin (Sigma, St. Louis) and sealed with 2.5 mm lengths of Medical Adhesive Silicone Type A (Dow Corning) at both ends. Capsules were implanted subcutaneously in the dorsal area of the waist. The wound was sutured with sterile sutures and treated with 75% alcohol and 2% tincture of iodine.

2.3. Experimental design

Males were randomly assigned to one of two groups and housed under a short-day (n = 20, light/dark 8:16, lights on 0000 h) or long-day photoperiod (n = 20, light/dark 16:8, lights on 1600 h). Half of the animals assigned to each photoperiod were castrated. Then, all experimental hamsters were divided into four groups: long-day intact, long-day castrated, short-day intact and short-day castrated males. Castration is known to reduce testosterone in male animals, and melatonin levels are lower under short photoperiod conditions and higher in long photoperiod conditions (Jasnow et al., 2000, 2002). Thus, castration and photoperiod manipulations help us to identify the independent and interactive effects of testosterone and melatonin on social dominance in male hamsters.

2.3.1. Experiment 1: effect of testosterone on male dominance under high or low melatonin conditions

The goal of this experiment was to test the effects of testosterone on behaviors of hamsters by observing contests between

long-day intact males (low melatonin, high testosterone) and long-day castrated males (low melatonin, low testosterone) (Experiment 1a); and between short-day intact males (high melatonin, low testosterone) and short-day castrated males (high melatonin, lower testosterone) (Experiment 1b). Behavioral tests were conducted eight weeks after castration. In this experiment, intact males had higher testosterone levels than castrated males in long-day pairs, while both short-day and castration reduce the testosterone levels of short-day pairs (Demas et al., 1999; Jasnow et al., 2000). We therefore, speculated that intact long-day male hamsters would gain higher social dominance during staged encounters than castrated ones, and that this may be unbiased between short-day pairs.

2.3.2. Experiment 2: effect of melatonin on male dominance under high or low testosterone conditions

The purpose of this experiment was to observe contests between long-day intact males (low melatonin, high testosterone) and short-day intact males (high melatonin, low testosterone) (Experiment 2a), and between long-day castrated males (low melatonin, low testosterone) and short-day castrated males (high melatonin, low testosterone) (Experiment 2b). Experiment 2 was conducted two weeks after Experiment 1. In Experiment 2a, we assumed that long-day intact males had higher testosterone levels and lower melatonin levels than short-day intact males. In Experiment 2b we assumed that long-day castrated males should have the same testosterone level but lower melatonin level than short-day castrated opponents (Demas et al., 2004; Jasnow et al., 2000, 2002). We predicted that in the castration condition (low testosterone level), short-day males should be dominant over long-day ones because of higher melatonin levels. We also predicted that under the intact condition, long-day males should be dominant over short-day opponents if the effect of testosterone was stronger than melatonin, otherwise, the result should be opposite.

2.3.3. Experiment 3: effects of implantation of testosterone or melatonin on social dominance

The purpose of this experiment was to observe contests between intact long-day males (low melatonin, high testosterone) and intact short-day males implanted with testosterone (high melatonin, high testosterone) (Experiment 3a), and between long-day castrated males implanted melatonin (high melatonin, low testosterone) and short-day castrated males (high melatonin, low testosterone) (Experiment 3b). Following Experiment 2 we implanted testosterone capsules in short-day intact males and melatonin capsules in long-day castrated males. Experiment 3 was performed six weeks after hormone implantation. We predicted that if testosterone and melatonin positively influence social dominance, in Experiment 3a intact short-day males implanted with testosterone should be dominant over intact long-day males, and in Experiment 3b castrated long-day males implanted with melatonin should have similar social dominance to castrated short-day males.

2.3.4. Interactive effect of testosterone and melatonin based on Experiments 2 and 3

By comparing the results between Experiments 2a and 3a, the effects of implantation of testosterone on social dominance were tested. We predicted that implantation of testosterone in short-day intact males would increase social dominance in short-day intact males.

By comparing the results between Experiments 2b and 3b, the effects of implantation of melatonin on social dominance were tested. We predicted that implantation of melatonin in long-day intact males would increase social dominance in long-day intact males.

2.4. Behavioral tests

According to the three experiments we assigned two males of similar body weights (within 10% difference) as a pair. During this study some males died of natural causes and a few pairs could not be fully tested because of large differences in body weight. We consequently obtained at least nine pairs for each experiment, except Experiment 3b which was performed on six pairs. Winner-loser relationships are formed very quickly in greater long-tailed hamsters and we used a 5 min staged dyadic encounter in a neutral arena (Plexiglass box 60 cm × 40 cm × 100 cm) to record the outcome and agonistic behavior. In the arena, two screens were placed parallel with the lateral wall, reducing the intensity of aggression and providing a buffer for losing males to avoid further attack. The arena was divided into equal compartments using a removable opaque partition and individuals were placed into each compartment for an acclimatization period of 3 min. The opaque partition was then removed and individuals were allowed to freely interact for 5 min. Encounters were recorded using a digital video recorder and conducted under dim red illumination during the dark cycle (8:00–16:00 h). The arena was cleaned between trials with water and 75% ethanol.

Behavior was continuously recorded for 5 min by hand on a data sheet with a precalibrated time scale (10 s). Behaviors of 10 s or less was treated as occurring once; if the duration was greater than 10 s but less than 20 s, the act was considered to have occurred twice, and so on. The time was measured by video (Ferkin and Seamon, 1987; Zhang et al., 2001a). Behaviors were defined following previous studies (Siegel, 1985; Wang et al., 2006, 2009; Zhang et al., 2001a,b) as: *aggression*, including attack, sideway posture, biting, and chasing; *defense*, fleeing, upright, cowering, threatening, and lying on their back on the ground; *flank marking*, arching back and rubbing toward the wall. The individual with the higher attack score was defined to be the winner (Wang et al., 2006, 2009).

2.5. Statistical analyses

All analyses were conducted using SPSS v13.0 (SPSS Inc, Chicago, USA). The number of times that aggression, defense and flank marking were displayed were analyzed using a Wilcoxon matched pairs test between hamster pairs and a Mann–Whitney *U* Independent test for before and after hormone implantation for each group. The level of significance was set at $P = 0.05$.

3. Results

3.1. Experiment 1: effect of testosterone on male dominance under high or low melatonin conditions

Intact males won in most bouts: long-day intact males won nine of ten bouts and short-day intact males won seven of eight bouts (Fig. 1a and b). Intact males in both photoperiods showed more agonistic behavior than their castrated opponents. Long-day intact males exhibited more aggression ($Z = 2.145$, $P = 0.032$) and less defensive behavior ($Z = 2.397$, $P = 0.017$) than long-day castrated males (Fig. 1c). Short-day intact males exhibited more aggression ($Z = 2.173$, $P = 0.030$) and less defensive behavior ($Z = 2.243$, $P = 0.025$) than short-day castrated males (Fig. 1d). Flank marking tended to be more frequent and nearly significantly different in intact hamsters under both photoperiods (long-day: $Z = -1.785$, $P = 0.074$; short-day: $Z = -1.820$, $P = 0.069$).

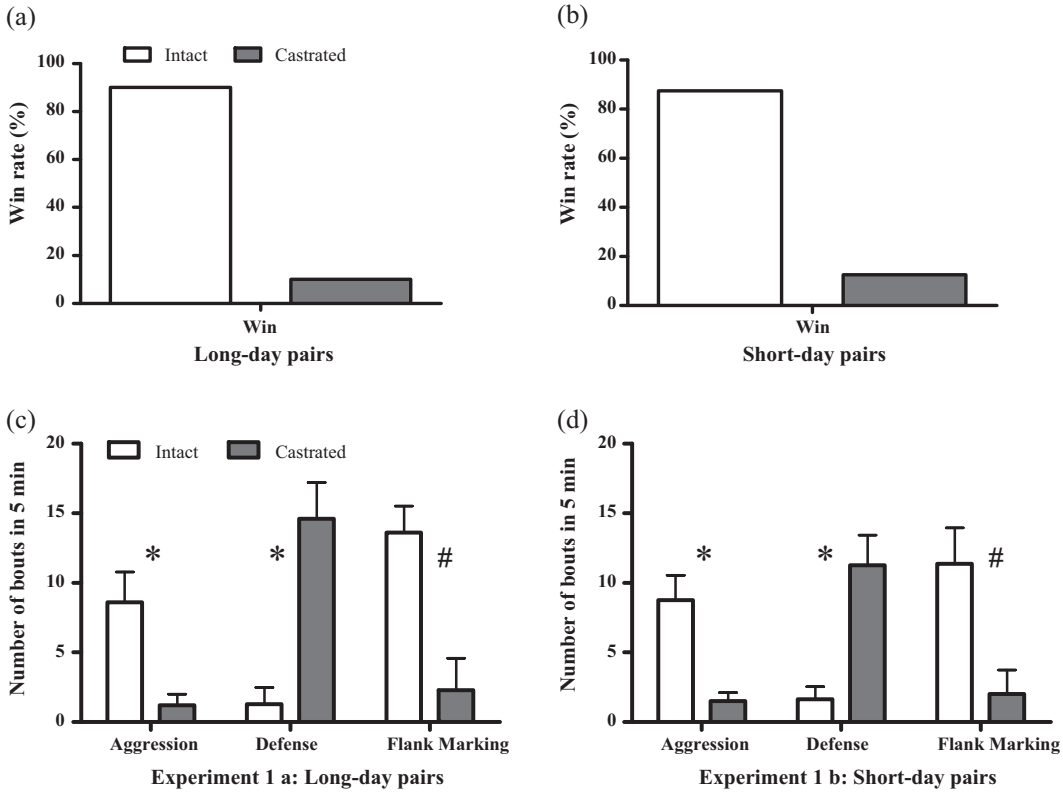

Fig. 1. Win rate (a and b) and behavior (c and d) during staged dyadic encounters between intact (white bars) and castrated (black bars) males under long- ($n = 10$) and short-day conditions ($n = 8$). *: $P < 0.05$; #: $0.05 < P < 0.1$.

3.2. Experiment 2: effect of melatonin on male dominance under high or low testosterone conditions

In encounters between long- and short-day intact males (Experiment 2a), long-day intact males showed a slightly higher win rate (six of ten bouts) against short-day intact opponents (Fig. 2a). No differences in aggression ($Z = -1.073$, $P = 0.283$), defensive behavior ($Z = -1.070$, $P = 0.285$) and flank marking ($Z = -0.833$, $P = 0.405$) were found for pairs of long- and short-day intact males (Fig. 2e).

In encounters between long- and short-day castrated males (Experiment 2b), short-day castrated males showed a higher win rate (seven of nine bouts) against long-day castrated males (Fig. 2b). No differences in aggression ($Z = -0.914$, $P = 0.361$), defensive behavior ($Z = -1.245$, $P = 0.213$) and flank marking ($Z = -1.541$, $P = 0.123$) were found between long- and short-day castrated males (Fig. 2f).

3.3. Experiment 3: effects of implantation of testosterone or melatonin on social dominance

Long-day intact males showed a higher win rate (six of nine bouts) against short-day intact males implanted with testosterone (Fig. 2c). In Experiment 3a, flank marking behavior in long-day intact males was nearly significantly higher than opponents implanted with testosterone (flanking: $Z = -1.838$, $P = 0.066$; aggression: $Z = -0.718$, $P = 0.473$, defense: $Z = -1.190$, $P = 0.234$; Fig. 2g).

Long-day castrated males with implanted melatonin showed the same win rate (half of all six bouts) against short-day castrated males (Fig. 2d). There was no significant difference between long-day castrated males with implanted melatonin and short-day castrated males for any behavior (aggression: $Z = 0.000$, $P = 1.000$, defense: $Z = -0.105$, $P = 0.917$, flank marking: $Z = -0.210$, $P = 0.833$; Fig. 2h).

3.4. Interactive effect between testosterone and melatonin based on Experiments 2 and 3

By comparing the rates of winning encounters in Experiments 2a and 3a (Fig. 2a and c), we found that the implantation of testosterone did not increase the rate at which short-day intact males won. There was also no obvious change in agonistic behavior before and after implantation of testosterone (long-day intact males: aggression, $Z = -0.617$, $P = 0.549$; defense, $Z = -0.094$, $P = 0.968$; flank marking, $Z = -0.293$, $P = 0.780$; short-day intact males: aggression, $Z = -0.519$, $P = 0.661$; defense, $Z = -0.000$, $P = 1.000$; flank marking, $Z = -0.198$, $P = 0.905$; Fig. 2e and f).

By comparing Experiments 2b and 3b we found that implantation of melatonin into long-day castrated males increased their rate of winning encounters (Fig. 2c and d). Compared to before implantation, implantation of melatonin obviously increased social dominance in long-day castrated males and made the difference in agonistic behavior between long-day castrated males and short-day castrated males smaller (Fig. 2c and d), though no significant differences were found (long-day castrated males: aggression, $Z = -1.153$, $P = 0.328$; defense, $Z = -0.357$, $P = 0.776$; flank marking, $Z = -0.912$, $P = 0.456$; short-day castrated males: aggression, $Z = -0.362$, $P = 0.776$; defense, $Z = -1.153$, $P = 0.328$; flank marking, $Z = -1.261$, $P = 0.224$).

4. Discussion

Results from Experiment 1 show that castration significantly decreases the social dominance of both long- and short-day males,

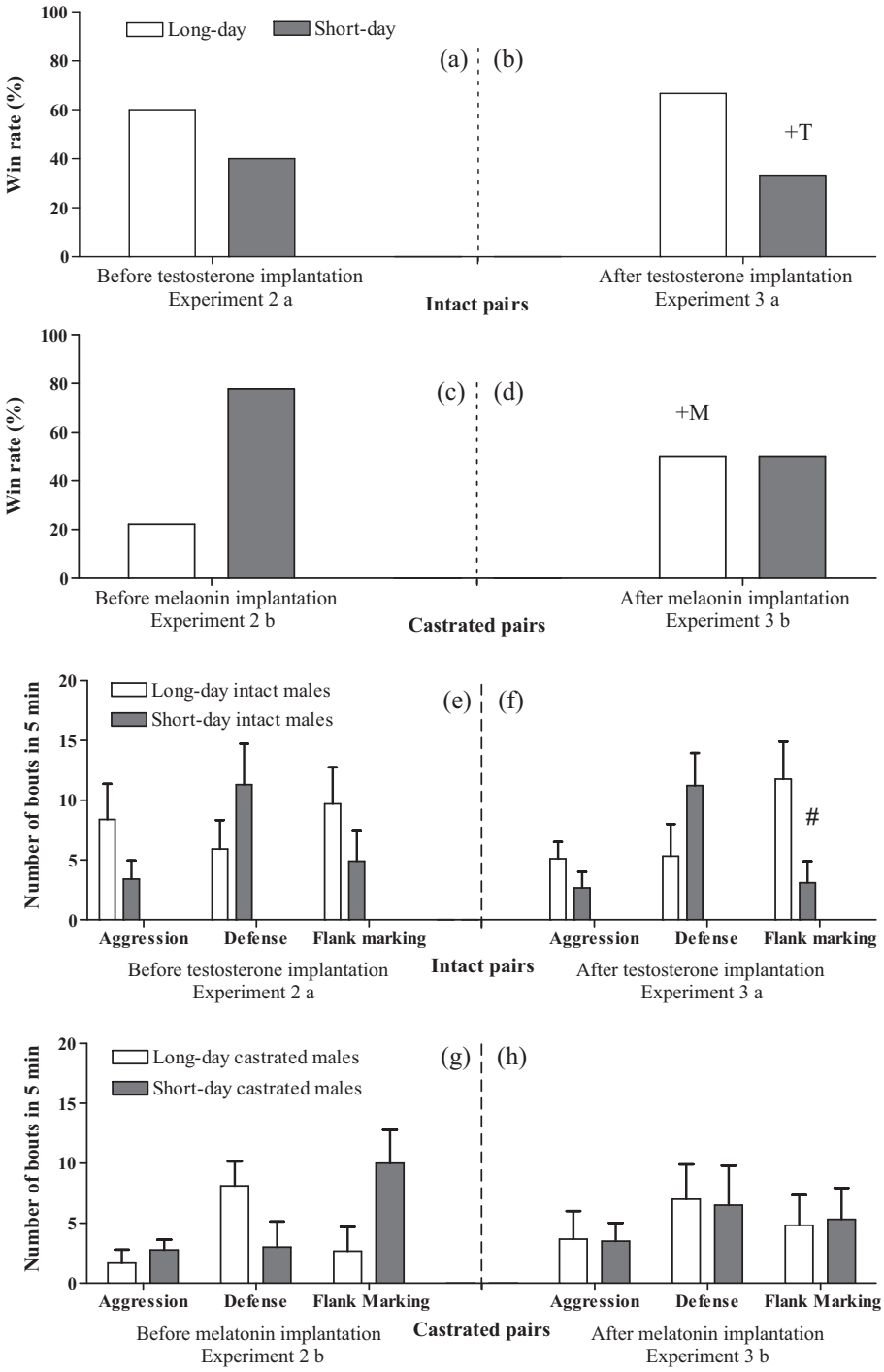

Fig. 2. Win rate (a and b) and behavior (e and f) during staged dyadic encounters between long-day and short-day intact males before ($n = 10$) and after ($n = 9$) testosterone implantation. Win rate (c and d) and behavior (g and h) during staged dyadic encounters between long-day and short-day (black bars) castrated males before ($n = 9$) and after ($n = 6$) melatonin implantation. #: $0.05 < P < 0.1$.

which suggests that testes benefit social dominance of males year round. These results support our hypothesis regarding the breeding season (long-day condition). During breeding, testosterone is essential in maintaining dominance in male rodents and castration significantly reduces testosterone levels and social dominance (Brain and Haug, 1992; Edwards, 1969; Ogawa et al., 1996). Previous work from our laboratory has shown that testosterone implantation restores dominance in castrated male greater long-tailed hamsters (Zhang et al., 2001a). The influence of testosterone on male aggression can follow two pathways, translate 5-α-dihydrotestosterone (5-α-DHT) bonding to androgen receptors or translate estradiol by aromatase bonding to estrogen receptors (Schlinger and Callard, 1990). For example, high territorial aggression in male song sparrows (*Melospiza melodia*) with basal level testosterone during non-breeding periods cannot be eliminated by castration but is limited by the implantation of an aromatase inhibitor, and reversed using extrinsic estradiol (Wingfield and Hahn, 1994; Soma and Wingfield, 2001).

Of note, and in contrast to our hypothesis, castration also affected dominance in males exposed to a short-day photoperiod. It

appears that testes have an important role in short-day conditions despite their regression and testosterone level depression (though we did not measure hormonal levels, the testes were observed in the abdomens of short-day intact males). A possible explanation for this is the increased sensitivity of gonadal hormone receptors during periods of non-breeding (Jasnow et al., 2000). Photoperiod and castration both influence the number and sensitivity of androgen receptors and then alter the aggression of male animals (Schwabl and Kriner, 1991; Wood and Newman, 1993). For example, blocked androgen receptors suppress territorial aggression in male European robins (*Erithacus rubecula*) in spring, but not in autumn (Schwabl and Kriner, 1991). In rats and Syrian hamsters the level of androgen receptors was down-regulated by castration and up-regulated after testosterone implantation (Wood and Newman, 1993). However, results from Experiments 2a and 3a do not support the reasoning above because testosterone implanted in short-day intact males did not alter the outcome of their conflict with long-day opponents; perhaps intact male hamsters are insensitive to testosterone under short-day conditions. An alternative explanation is that the side-effects of castration influenced or changed other factors which regulate male aggression and dominance in our experiments such as vasopressin and its receptor serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norpinephrine and dopamine (Skowsky et al., 1979; Johnson et al., 1995; Bernard and Paolino, 1974).

Results from Experiment 2 indicate that under intact conditions, long-day males show a tendency to dominate short-day males, suggesting that the effect of testosterone may be stronger than melatonin in social dominance during the breeding season (long-day conditions). However, under the castration condition, where the effect of testosterone is fully removed, the effect of melatonin on short-day males meant they tended to domiate long-day males. High melatonin levels enhance social dominance in male hamsters. It is also likely that melatonin was more important in determining male aggression and social dominance than testosterone during the non-breeding season. The effect of melatonin on aggression has been reported in several rodent species (Paterson and Vickers, 1981; Jasnow et al., 2000, 2002). It acts as a signal to trigger adrenocortical hormones such as glucocorticoids and dehydroepiandrosterone (DHEA), which regulate aggression in rodents (Soma et al., 2008).

The effects of testosterone and melatonin were further tested in Experiment 3. By comparing the results of Experiments 2a and 3a, implantation of testosterone into short-day intact males did not affect their subordinate relationship with long-day intact males (Fig. 2a and b). By comparing Experiments 2b and 3b, implantation of melatonin increased both the rate of winning and agonistic behavior in long-day castrated males (Fig. 2c, d, g and h) as compared to short-day castrated males. These results imply that testosterone mainly effects aggression and social dominance during the breeding season, and that melatonin maintains aggression during the non-breeding season and affects social dominance among males. For solitary species, aggression during breeding is necessary for controlling territory and mates for reproduction success, and during non-breeding for accessing food and occupying burrows (Yang et al., 1996; Zhang et al., 1992; Jasnow et al., 2000, 2002).

In general our results suggest that both testosterone and melatonin are important in determining social dominance in male hamsters. However, testosterone appears to be more important than melatonin whereby testosterone is most important during periods of breeding and melatonin during non-breeding. This pattern is most likely due to different secretory patterns of testosterone and melatonin seasonally. It should be pointed out that due to our small sample sizes, some of the conclusions drawn from this study, especially from Experiments 2 and 3 are weak and further work into physiological mechanisms are needed to confirm our observations.

Acknowledgments

We are grateful to the Beijing Institute of Immunoassay for technical support and Yongliang Pan, Zhenlong Xiao, Yingjuan Liu and Xiaoping Rao for assistance with experiments. Fusheng Wang and Jinhua Zhang helped capture and care for hamsters. This work was supported by grant 30370232 from the National Science Foundation of China.

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