Effects of Social Stimuli on Testosterone, Aggression, and Fighting Behavior in Male Golden Hamsters (*Mesocricetus auratus*)

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Abstract:

Testosterone has long been implicated in aggression. However, evidence for its role is contentious. This study examined the influence of testosterone on aggression but also aimed to further explore the effect of previous social stimuli on the outcome of male-male fights. Experimental males were exposed to animal stimuli (males, diestrus females, or estrous females) through a mesh barrier, and then testosterone levels were measured. The following day, control males with no prior exposure fought experimental males, and fighting behavior was recorded. It was hypothesized that exposure to social stimuli, especially exposure to estrous females, would induce testosterone surges in males. These surges would increase aggression in fights, thereby improving fighting ability in males with prior exposure. It was predicted that higher aggression would translate into experimental males winning significantly more fights than their control counterparts. However, results were rather ambiguous. A significant testosterone surge in males was only found after exposure to diestrus females. Males exposed to estrous females exhibited nearly significant testosterone surges, whereas males exposed to other males showed no significant changes in testosterone. The significant testosterone rise in males exposed to diestrus females did seem to increase aggression during fights compared to males with only male exposure. However, this did not predict the outcomes of the fights as expected.

I. Introduction:

The importance of aggressive behavior in animals cannot be understated: it can have wide-reaching effects, from dominance hierarchy status to mating and

communication (Albers et al., 2002). Aggression has been studied extensively in many animal species, including hamsters, rats, mice, prairie voles, humans, nonhuman primates, birds, and fish (Payne, 1974; Trainor et al., 2004). Aggression can be observed in both social and sexual encounters. For instance, when two male golden hamsters (*Mesocricetus auratus*) are placed within a contained area, they will almost always engage in a fight. In addition, unreceptive females will fight males approaching them for sexual contact. Specifically in golden hamsters, females and males are both quite aggressive, and females usually win fights with males (Grelk et al., 1974).

Aggression is a continually evolving area of scientific research: untangling the dominating mechanisms that mediate it has proven difficult. Various studies have indicated a myriad of behaviors and mechanisms by which aggression is mediated, chief among them being olfactory, auditory, visual, and postural cues. Hormones and other neurochemicals have also been strongly implicated in aggression (Albers et al., 2002). Hormonal and neurochemical mediation of aggression is one of the most contentious areas of study. In general, the following hormones and neurotransmitters have been indicated as possible mediators of aggressive behavior: testosterone, estrogen, cortisol, vasopressin, serotonin, and dopamine (Gleason et al., 2009; Grelk et al., 1974; Mehta and Josephs, 2010). However, results are often contradictory, most notably in research on the role of testosterone. Payne (1974) found that castrated male golden hamsters became significantly more aggressive after injection with androgens. Similarly, Drickamer and Vandenbergh (1973) found that after the ovaries of adult female golden hamsters are removed, status in the dominance hierarchy, generally a correlate of aggression, is positively correlated with injections of testosterone propionate. In addition, it is worth

mentioning that female golden hamsters represent an interesting case in models of aggression. In most species, females are less aggressive than males. Researchers often use this sexual dimorphism to support the hypothesis of testosterone's aggression mediating effects. However, female golden hamsters, despite having lower testosterone than male golden hamsters, are generally considered just as aggressive as males, a contradiction to the testosterone mediated hypothesis of aggression (Grelk et al., 1974). This has prompted some researchers to suggest that estrogen may be a mediator of aggression in animals. Although Drickamer and Vandenbergh (1973) did not find evidence for this, Vandenbergh (1971) did find partial support for this hypothesis. Vandenbergh (1971) conducted an experiment in which the gonads of both male and female golden hamsters were removed. In males, removal of the gonads resulted in decreased aggression, and both testosterone and estrogen administration led to a rise in aggression. Contrastingly, females exhibited no change in aggression as a result of gonadectomy and ensuing testosterone or estrogen injections had no effect on aggression. Therefore, Vandenbergh suggested that aggression is controlled differently in males and females (Vandenbergh, 1971).

Furthermore, there are a number of additional studies that refute the positive effects of testosterone on aggression. Both Jasnow et al. (2000) and Garrett and Campbell (1980) found that testosterone levels were negatively correlated with aggression in Siberian hamsters and golden hamsters, respectively. However, in both studies, testosterone levels were moderated by changes in day length, not castration (Garrett and Campbell, 1980; Jasnow et al., 2000). Whitsett (1975) found that castrated males did not show a difference in aggressive behaviors compared to non-castrated

males. A number of other researchers have also suggested that testosterone may mediate aggression, but it is only one of a number of factors that interact to generate varying levels. For example, Trainor et al. (2004) suggested that aggression in male California mice is mediated by the interaction of testosterone and prior experience. Interestingly, Mehta and Josephs (2010) have found evidence that testosterone and cortisol interact to control aggression in humans.

Since research on testosterone's effects on aggression has been contradictory, the forthcoming study aims to explore this topic further. Since castration and testosterone injections are less realistic compared to what occurs in nature, male golden hamsters were exposed to stimulus animals as a means of increasing testosterone. Past studies have documented testosterone surges in male rodents after exposure both to males and females (Amstislavskaya et al., 2004; Gleason et al., 2009). After exposure, males fought with control males. We hypothesized that exposure to stimulus animals, most notably receptive females, would induce testosterone surges in males, resulting in increased aggression. Higher aggression could translate into improved fighting ability, allowing males with exposure to win fights significantly more than control males.

II. Experimental Design:

Golden hamsters of the species *M. auratus* were used exclusively in the following study. Golden hamsters are an ideal model organism for these experiments: they have been used extensively as aggression models for years, and their fighting behaviors are well documented and highly stereotyped (Delville et al., 2000). The experimental paradigm consisted of two main parts: exposure to a social stimulus (day 1) and fights

(day 2). In the first part (day 1), male subjects were randomly assigned to experimental and control groups. Experimental males were exposed to stimulus animals through a wire mesh barrier in a plexiglass box, while control males were exposed to an identical, but empty, box. Following the respective treatments, within 30 min. blood samples were obtained, stored, and assayed for testosterone levels. Blood samples were also collected 5 days before the experiments began to obtain baseline testosterone levels for each male. The following day (part 2, day 2), fights were incited between paired experimental and control males, and winners were determined.

All animals used were sexually mature adults. We used three different stimuli animals as treatments: males (experiment 1), diestrus females (experiment 2), and estrous females (pilot study and experiment 3). Of the males and females used as subjects or stimuli in the experiment, some had had prior sexual (breeding) and social (fighting) experience. Specifically, in the pilot study (estrous female exposure) and experiment 1 (male exposure) all males were sexually naïve, but all had had fighting experience at least 2 months prior. In experiment 2 (diestrus female exposure), all males were sexually and socially naïve. In experiment 3 (estrous female exposure), there were 6 trials where both males within each pair had fighting experience at least 2 months prior and 2 trials where both males had sexual experience. Within male fighting pairs, males were matched for weight, age, and sexual and fighting experience, so any potential effects from these factors would cancel out. Weights and ages did not exceed differences of 10 grams and 4 months, respectively. A dim light source was used in all behavioral aspects of the experiments so as not to disrupt the hamsters' reversed nocturnal light schedule (lights off from 9 AM to 7 PM). However, when blood was collected for testosterone assays,

bright overhead lights were used. All trials and parts of trials were conducted at approximately the same time every day (between 12:30 PM and 4:00PM).

III. Materials and Methods:

a) Methods and procedures:

The exposure trials (day 1) occurred in a plexiglass cage with a lid. The cage was approximately 13 in. by 17.5 in. with a height of 9 6/8 in. and contained two identical compartments separated by a wire mesh barrier that spanned the width of the box. The trials on this testing day were comprised of two subparts (A and B). In part 1A, a control male was placed in the right compartment of an odorless, plexiglass cage alone for 10 min. After 10 min., the male was anesthetized with isoflurane, and a blood sample of no more than 0.25 mL was drawn from the saphenous vein in the hind leg. In part 1B, an experimental male was placed in a compartment in a different odorless plexiglass cage in a separate room. The male was randomly assigned to one of the two compartments each trial. The male spent 10 min. alone in the cage acclimating to the environment. Following these 10 min., a stimulus animal was placed in the other compartment for the next 10 min. Stimulus animals used in the experiments were males (experiment 1), diestrus females (experiment 2), and estrous females (pilot study and experiment 3). The animals were able to interact, touch, smell, and see each other through the wire mesh separating the compartments. After 10 min. of exposure to the stimulus animal, the stimulus was removed, and the male spent an additional 10 min. alone in the cage. After these 10 min. in isolation, the male was immediately removed from the cage. Within the next 5 min., the male was anesthetized, and a blood sample of no more than 0.25 mL was

drawn from the saphenous vein in the hind leg. Therefore, blood samples for experimental males were taken approximately 30 min. after the start of exposure to the stimulus. Prior experiments have indicated that male testosterone levels peak about 30 min. after exposure to a male or female, thus providing the rationale for the timing of taking the blood sample (Amstislavskaya et al., 2004; Gleason et al., 2009; Pfeiffer and Johnston, 1992). In addition, baseline testosterone levels for both the experimental and control males were measured 5 days before the exposure and control trials in all experiments except the pilot study (estrous female exposure). Both the control (part 1A) and experimental (part 1B) paradigms were conducted simultaneously, and upon completion of each trial the plexiglass cages were thoroughly cleaned with 50% ethanol solution. Lastly, when estrous females were used as stimuli, any females that had failed to go into lordosis during interaction with the males were promptly tested afterward to verify estrous status.

Blood samples were centrifuged to obtain the serum that was later frozen at -70°C until assayed for hormone content. Testosterone levels were measured by enzyme immunoassay (Cayman Chemical Company Testosterone EIA Kit). Serum samples were diluted (1:80) with assay buffer and ran in duplicate for each sample. The kit was previously validated with golden hamster serum, and the dilution factor assured that our samples would fall within the sensitivity range of the assay. The intra-assay and inter-assay coefficients of variation for each sample were 19.85%, and 17.93%, respectively.

The second part of the experiment (day 2) was conducted one day after the exposure/testosterone trials. In this portion, male pairs (a pair consisted of 1 experimental male and 1 control male) were allowed to fight in a neutral fighting arena

(transparent plexiglass box with lid measuring 24 in. by 24 in. with a height of 7 3/8 in.) for 5 min. Males fought in the room used for the exposure trials the previous day. Just before a fight, the males within a pair were randomly labeled on their upper backs with pieces of green or yellow tape—one piece was blank and the other had a blue dot drawn on it with an odorless Vis-à-vis pen. The male with the dotted tape was placed under an upside-down, small, transparent plexiglass container (dimensions: 5 in. by 5 in. by 5 in.) in the upper, left corner of the fighting arena. The male with the plain tape was placed under an identical container in the lower, right corner of the fighting arena. Once positioned, the males entered a 30 sec. habituation time period. Following this, the small boxes were removed, the lid was placed on top of the arena, and the males were allowed to interact and fight for 5 min. At the end of this time period, the winner and loser of the fight were recorded. The loser can be clearly identified by the following behaviors: raised tail in an upward position and actively fleeing. In addition, the fights were videotaped during experiment 1 (male exposure), experiment 2 (diestrus female exposure), and experiment 3 (estrous female exposure). Once the fights ended, the fighting arena was thoroughly cleaned with soap, water, and 50% ethanol solution. Any resulting injuries were minor, but if necessary, injuries were treated with neomycin ointment to prevent infection.

Upon completion of both days of the trials, behaviors during the fights were scored using the computer software Elan. The following behaviors were analyzed: latency to fight, latency to tail up, percentage of time spent fighting, percentage of time the winner spent chasing the loser, percentage of time the loser had his tail up, percentage of time spent interacting, number of fights per minute, number of chases per minute, and

number of tail ups per minute. See *Table 1* for explanations of these behaviors. In addition, *Figure 1* (produced by Fernández-Vargas) illustrates the general sequence of several of these behaviors diagrammatically.

Table 1: *Ethogram of the behaviors observed during the fights that were video-scored and analyzed*

Behaviors	Definitions
Latency to nose-nose	Time from the start of the trial to the first nose-to-nose interaction (noses touching or within ½ in. of each other); used for explanatory purposes in *Figure 2*, but not used for statistical analysis.
Latency to fight	Time from the start of the first nose-nose interaction to the start of the first fight.
Latency to tail up	Time from the beginning of the first fight to the time when the loser is first declared (via submissive behaviors, i.e. tail up and fleeing).
Number of fights per minute	Total number of fights (rolling fights or aggressive interactions, i.e. biting and scratching) during the trial divided by the 5 min. of the trial.
Percentage of time fighting	Percentage of time when the males are fighting with each other (rolling fights or aggressive interactions, i.e. biting and scratching). It is a function of the time from the first interaction to the end of the trial (not the whole trial).
Number of chases per minute	Total number of times the winner chases the loser divided by the 5 min. of the trial.
Percentage of time the winner chased the loser	Percentage of time when the winner is chasing the loser. It is a function of the time from the first interaction to the end of the trial (not the whole trial).
Number of tail ups per minute	Total number of times the loser raised his tail divided by the 5 min. of the trial.
Percentage of time the loser had his tail up	Percentage of time the loser displayed a sustained tail up. It is a function of the time from the first tail up to the end of the trial.
Percentage of time interacting	The sum of the percentage of time spent fighting, percentage of time the winner spent chasing the loser, percentage of time where mutual sniffing occurred, and percentage of time where one male sniffed the other but the other male did not reciprocate the sniffing. Percentage of time interacting is a function of the time from the first interaction to the end of the trial (not the whole trial).

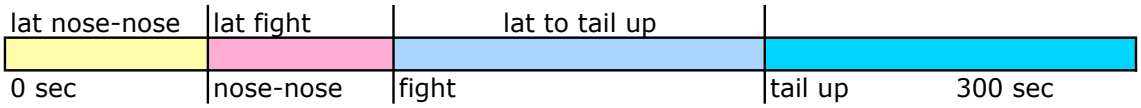

Figure 1: *Chronological order of several behaviors during fights (lat means latency)*

b) Statistical methods:

The software JMP was employed for the statistical analysis. It was used to generate ANOVA, Chi-square, Mann-Whitney U tests, t-tests, logistic regressions, and Pearson correlations. Data were first tested for normality and equal variance between distributions. If these requirements were met, parametric tests were used for analysis. If distributions were not normal and of unequal variance, then nonparametric tests were used. A probability level of 0.05 was used to determine significance. Most of the statistical analysis was performed by Fernández-Vargas.

IV. Results:

a) Effects of inter- and intra-sexual interactions on the outcome of male fights:

As previously described, four sets of studies were performed. These are the pilot study (exposure to an estrous female, n=10), experiment 1 (exposure to a male, n=10), experiment 2 (exposure to a diestrus female, n=10), and experiment 3 (exposure to an estrous female, n=12). Baseline testosterone levels were not measured in the pilot study (estrous female exposure), nor were the fights videotaped. Therefore, experiment 3 (estrous female exposure) was essentially a repeat of the pilot study using exactly the same methodology as the male and diestrus female experiments to allow for comparison. In the pilot study (estrous female exposure), 90% of the winners in the male-male fights

had had prior exposure to an estrous female (*Figure 2*). In experiment 1 (exposure to a male), 50% of the winners had had previous exposure to a stimulus male (*Figure 2*). In experiment 2 (diestrus female exposure), 60% of the winners had had prior exposure to a diestrus female (*Figure 2*). However, in contrast to our predictions and pilot study, in experiment 3 (estrous female exposure), 42% of the winners were experimental males

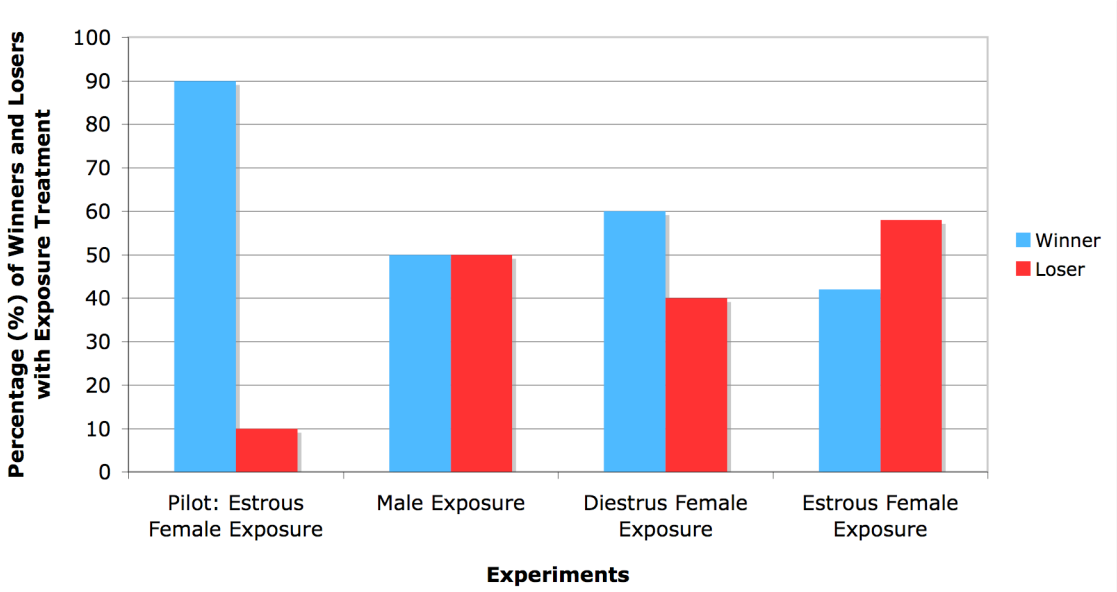

***Figure 2**: Proportion of males that became winners or losers after exposure to different stimulus animals through a wire mesh barrier*

with the prior exposure to the female (*Figure 2*). The pilot study (estrous female exposure) differed significantly from experiments 1 (exposure to a male) and 2 (exposure to a diestrus female) ($\chi^2 = 3.81$, d.f. = 1, $p = 0.05$). However, experiment 3, the repeat of the pilot study, did not differ significantly from experiments 1 and 2.

Since these results were unexpected, we pooled the data and used a logistic regression to identify if the following factors predicted the winners and losers of fights: male age ($p = 0.83$), weight ($p = 0.79$), litter size ($p = 0.76$), and sex ratio of litters ($p = 0.46$). All the results from the logistic regression were not significant. In addition, paired

t-tests for differences between weight and age in male pairs were not significant in the pilot study (weight: $t = -0.13$, d.f. = 9, $p = 0.90$; age: $t = -0.60$, d.f. = 9, $p = 0.56$), experiment 1 (weight: $t = -0.83$, d.f. = 9, $p = 0.43$; age: $t = 0.18$, d.f. = 9, $p = 0.86$), experiment 2 (weight: $t = 0.49$, d.f. = 9, $p = 0.64$; age: $t = -0.66$, d.f. = 9, $p = 0.52$), and experiment 3(weight: $t = -0.09$, d.f. = 11, $p = 0.93$; age: $t = -0.09$, d.f. = 11, $p = 0.93$).

Note that Fernández-Vargas and I usually ran the exposure trials and corresponding fights together. She generated the statistics for this section, while I produced the figure.

b) Aggression data from videos of fights:

Unfortunately, the analysis of the video data has only been partially completed. The behaviors in all the trials from experiment 1 (male exposure, n=10) and experiment 2 (diestrus female exposure, n=10) have been video-scored. The trials from experiment 3 (estrous female exposure, n=12) have not been scored yet. The fights in the pilot study (estrous female exposure) were not video taped. Therefore, the results on fighting behavior and aggression from the videos of the fights are limited to experiments 1 and 2.

In order to evaluate general trends in aggression, the data from experiments 1 and 2 were first pooled and analyzed for correlations between different behaviors. Latency to tail up and percentage of time spent fighting were moderately positively correlated ($r = 0.52$, $p = 0.018$). When an extreme outlier was removed from the data set, the correlation increased strongly ($r = 0.73$, $p = 0.0004$). A moderately positive significant correlation was also identified between number of fights per minute and number of chases per minute ($r = 0.44$, $p = 0.050$). A significant negative correlation was found between percentage of time spent fighting and percentage of time spent chasing ($r = -0.62$, $p =$

0.0033). Lastly, percentage of time with tail up and percentage of time spent chasing were significantly positively correlated with each other ($r = 0.63$, $p = 0.0028$), while percentage of time with tail up and percentage of time spent fighting were significantly negatively correlated with each other ($r= -0.76$, $p = 0.0003$). No other significant correlations were observed.

The data sets from experiments 1 and 2 were compared to indicate any differences in aggression that could be attributed to treatment. When analyzing the data for differences between experiments 1 and 2, results were not broken down based upon winners with experimental treatments simply because all the resulting sample sizes were two small and variances too big to generate significant results. However, since 50% of males in experiment 1 (exposure to a male) and 60% of males in experiment 2 (exposure to a diestrus female) had had the respective experimental treatments, we surmised that the control males remaining in the data sets were roughly equivalent, given that they had all had equal exposure to an empty box as treatment. This would allow us to draw conclusions from the comparisons of the full data sets while maintaining statistical integrity. To check the accuracy of these assumptions, we broke the data sets down into winners with prior exposure and looked at the comparisons. All the resulting graphs (not presented in this paper) looked very similar to *Figures 3*, *4*, and *5* and qualitatively showed the same effects, although as predicted, the differences were not significant.

The first variable compared was the percentage of time males spent fighting. Males in experiment 1 (male exposure experiment) spent significantly more time fighting than the males in experiment 2 (diestrus female exposure experiment) ($U = 4.17$, d.f. = 1, $p = 0.041$) (*Figure 3)*.

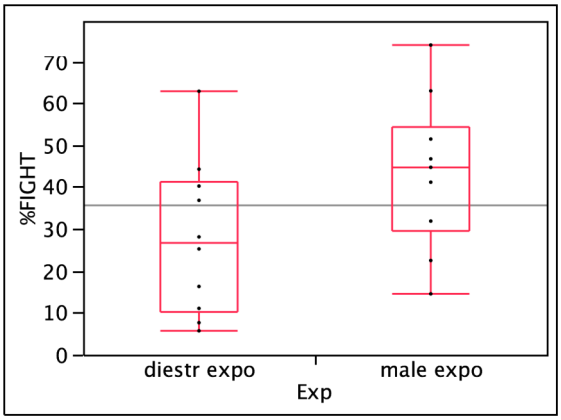

Figure 3: *Percentage of time male hamsters spent fighting in experiments 1 and 2*

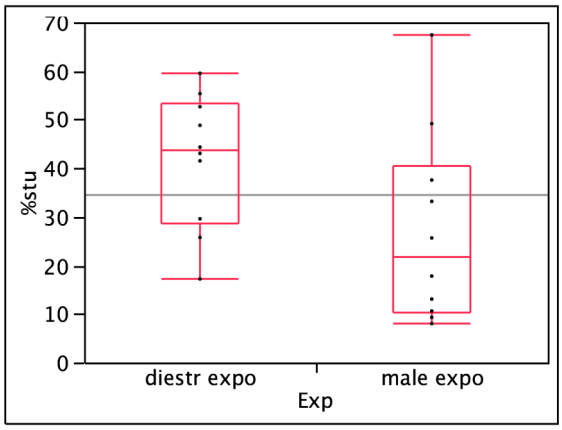

Figure 4: *Percentage of time losers' tails were raised in experiments 1 and 2 (stu is sustained tail up)*

The second variable tested was the percentage of time during the 5 min. fights that the loser's tail was raised. Qualitatively, there appears to be a difference between the two data sets (*Figure 4*), but quantitatively, the difference is only marginally significant (U = 3.57, d.f. = 1, p = 0.059). Marginally, losers in experiment 2 (diestrus female exposure) appeared to spend more time with their tails up than losers in experiment 1 (male exposure).

Lastly, the average number of chases per minute in experiments 1 and 2 were compared (*Figure 5*). Here, significant results (U = 5.14, d.f. = 1, p = 0.023) were obtained. Winners in the diestrus female exposure experiment chased losers significantly more times per minute than winners chased losers in the male exposure experiment.

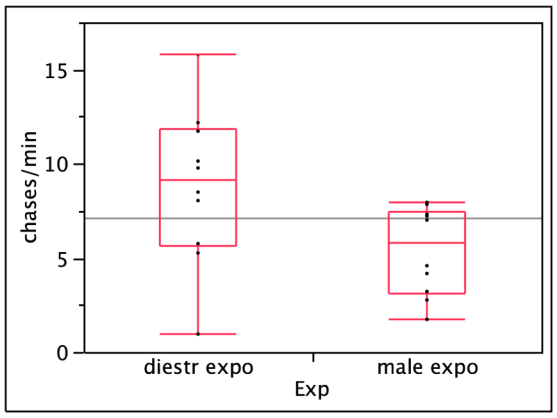

Figure 5: *Number of times per minute the winner chased the loser in experiments 1 and 2*

No other significant differences between the two data sets were found.

Note that I did all the video coding, while Fernández-Vargas did all the statistical analysis and produced all the figures in this section.

c) Testosterone levels:

We found that in experiment 1, after interacting with another male through the mesh barrier, males did not experience a significant testosterone surge in comparison to baseline nor to males that had no exposure (*Figure 6*). However, in experiment 2, we found that after diestrus female exposure, males experienced a significant surge in testosterone compared to baseline and to males that had no exposure (*Figure 6*). In fact, the interaction between treatment (baseline vs. after contact) and exposure (empty box vs. diestrus female) was significant (treatment x exposure, $F(1, 18) = 5.09$, $p = 0.037$). This indicates that testosterone levels are significantly different from baseline only after exposure to the stimulus animal. Similar results were obtained from experiment 3 (exposure to an estrous female), although they were only marginally significant (treatment x exposure, $F(1, 22) = 3.67$, $p = 0.068$).

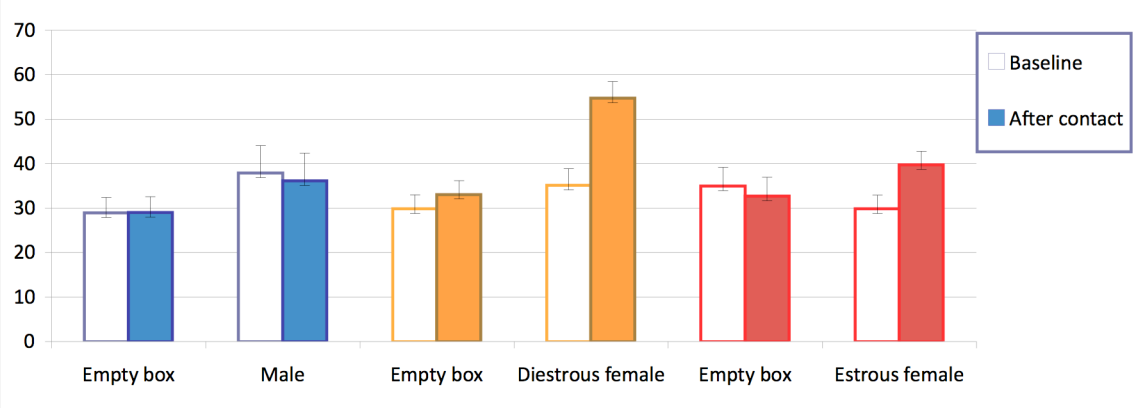

Figure 6: *Testosterone levels (pg/mL) obtained during baseline and after exposure to a stimulus animal through a mesh barrier or to an empty box (control) (y-axis is pg/mL)*

Testosterone levels were not significantly different between winners and losers in experiments 1, 2, and 3 (*Figure 7*). Although a significant increase in testosterone was

observed in males after exposure to diestrus females, this did not predict the winners in the fights the following day, thereby rejecting our initial hypothesis. Likewise, the marginally significant surge in testosterone in males after exposure to estrous females did not predict the winners of the fights.

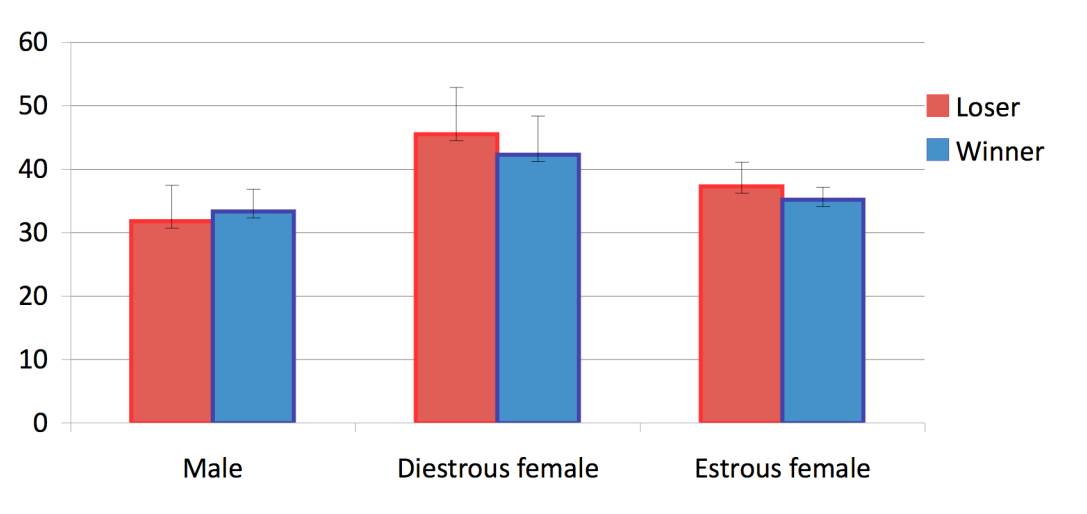

Figure 7: *Testosterone levels (pg/mL) obtained on day 1 after exposure or non-exposure of males that became winners or losers of fights on day 2 (y-axis is pg/mL)*

Note that concerning the testosterone, Fernández-Vargas collected the blood samples, ran the hormone assays, compiled the data, did the statistical analysis, and produced the figures for this section.

V. Discussion:

a) Effects of inter- and intra-sexual interactions on the outcome of male fights:

Unfortunately, the results from the fights were quite ambiguous. The pilot study (estrous female exposure) generated significant results and provided the basis for doing the additional three experiments. However, the results from the fights from these three experiments were not at all significant. Furthermore, it is noteworthy that experiment 3

(estrous female exposure), which was a repeat of the pilot study, obtained completely different results from the pilot study. In experiment 3, 42% of the winners of the fights had had prior exposure to an estrous female, whereas in the pilot study, 90% of the winners had had this prior exposure. The results from experiment 3 were unexpected and disappointing. Overall, neither in the pilot study nor in the three subsequent experiments, did differences in age or weight explain the outcomes of the fights. These were variables that we had purposely controlled. It is well known that differences in weight have an effect on determining the winners of fights (Albers et al. 2002). Even when the weights and ages of the fighters are controlled, the factor that ultimately determines the outcome of the fight still remains elusive.

b) Aggression data from videos of fights:

We developed a set of guidelines to analyze the differences in aggression between experiments 1 and 2, and the correlations served as support for these guidelines. For example, we hypothesized that if one male in a fight is significantly more aggressive, the other male will probably submit more quickly. If both males in a fight are equally aggressive, they will fight longer and have a longer latency to tail up by the loser. Therefore, there should be a significant positive correlation between latency to tail up and percentage of time spent fighting, which was found. In fights where one male is significantly more aggressive, the other male should submit more quickly and avoid any further fighting as much as possible. The winner may try to keep fighting the loser to continue asserting dominance, but the loser will constantly attempt to run away. Therefore, there should be more fights and more chases per minute as a result of all the little scuffles and running away. These variables were indeed significantly positively

correlated. Further, more fights per minute and more chases per minute should result in a lower percentage of time spent fighting and a higher percentage of time the winner spends chasing the loser. As expected, these two variables were significantly negatively correlated. This was not surprising, since the two variables are mutually exclusive of each other. In addition, a higher percentage of time spent chasing the loser should result in a higher percentage of time the loser has his tail up. As predicted, these variables were significantly positively correlated. Finally, a higher percentage of time the loser has his tail up should indicate a lower percentage of time spent fighting, since a raised tail is not observed during rolling fights. As hypothesized, this negative correlation was significant.

In keeping with these guidelines, the comparison of the video data for fights from experiments 1 and 2 provokes one important, but tenuous, conclusion. The data indicates that males with the exposure to the diestrus females spent significantly less time fighting, losers spent nearly significantly increased time with their tails up, and winners chased the losers significantly more times per minute compared to the males with the exposure to other males (*Figures 3*, *4*, and *5*). This indicates that one male in each fight from the diestrus female exposure experiment was significantly more aggressive than the other male in the fight. If one male is much more aggressive, the other male will probably give up more quickly, leading to less time fighting, more time the loser has his tail up, and more chases per minute. Since the only difference in treatment between the two males was exposure to a diestrus female, the higher aggression in one male could be attributed to this exposure. However, this didn't lead to a higher number of winners with this prior exposure, and since the data sets include all male winners (some experimental (had exposure) and some control males (no exposure)) the data could potentially have

confounding variables. Analyzing the behavior of only experimental males that won fights would generate extremely small subgroups, though, so any significant results would be questionable. Furthermore, as previously detailed, since 50% of males in experiment 1 (exposure to a male) and 60% of males in experiment 2 (exposure to a diestrus female) had had the respective experimental treatments, we surmised that the control males remaining in the data sets were roughly equivalent in numbers, and thus, would cancel out.

c) Testosterone levels:

The only treatment that led a significant change in testosterone levels was exposure to a diestrus female (*Figure 6*). It is noteworthy that this was the only experiment in which all males entirely lacked social and sexual experience. Perhaps this lack of experience allowed for the significance of these results. Further, it was surprising that exposure to an estrous female did not have quite the same effect (*Figure 6*). However, the testosterone surge in these males after exposure was marginally close to significance; lack of significance may simply have been due to small sample size or large variance. Interestingly, the testosterone surge from diestrus female exposure did appear to increase aggression, as discussed in the previous section. Unfortunately, the videos of the fights from estrous female exposure have not been scored yet, but a similar effect may be observed. Despite testosterone surges in experiments 2 (exposure to a diestrus female) and 3 (exposure to an estrous female), and the increase in aggression in the fights from experiment 2, there was no ultimate influence on outcomes of male-male fights. Correspondingly, winners and losers of fights showed no significant differences in testosterone levels (*Figure 7*).

It was hypothesized that exposure to at least one type of stimulus animal would cause a surge in testosterone, increasing aggression and thereby affecting the outcome of male-male fights. However, the results do not show any clear, robust connection between testosterone levels, aggression, and fighting ability in male golden hamsters. There are several plausible explanations for the lack of correlation between the results. First, testosterone may influence aggression, but the surges in testosterone in experiments 1 (male exposure), 2 (diestrus female exposure), and 3 (estrous female exposure) may not have been great enough to produce widespread effects. All the testosterone changes after exposure to stimuli were not significant except in the diestrus female exposure experiment, indicating that most of the males experienced a relatively small testosterone surge, if any. Since the changes in testosterone did not predict the winners of the fights, the necessary increase in testosterone to measurably increase aggression and influence the winners may be much higher. In many experiments that test the influence of testosterone on aggression, males are castrated and given controlled injections of testosterone (Payne, 1974). These injections can produce higher, controlled testosterone levels than those resulting from the exposure experiences in this experiment. This could explain why other studies have indicated that testosterone has a stronger effect on aggression. Hence, these results may just indicate that exposure to stimulus animals through a mesh barrier is not adequate to raise testosterone to a necessary level to significantly change the aggression and fighting ability of a male. Further, males may require physical interactions with stimulus animals before testosterone increases enough to produce an observable effect. For instance, Gleason et al. (2009) suggests that a higher level of physical interaction, such as fighting, rather than simple exposure through a

partition, is necessary to induce a rise in testosterone in males after exposure to male stimuli. However, Pfeiffer and Johnston (1992) reported that even when males were allowed to interact without a barrier and fights ensued, no significant changes in androgen levels were observed. In addition, a study with house mice reported that exposure to an estrous female through a partition was able to elicit a significant testosterone surge in males (Gleason et al., 2009). This result is not consistent with our results from experiment 3 (estrous female exposure).

Another possibility is that testosterone may influence aggression, but the effects were not robust enough to significantly affect the outcome of a fight. The results from the diestrus female exposure experiments conform well to this hypothesis. As Trainor et al. (2004) suggested, testosterone might interact with several other factors, such as prior experience, to influence aggression and the outcome of male-male fights. Furthermore, the time frame of this experiment may be to blame for the ambiguous results. Conducting the fights one day after the exposure trials may allow the effects of testosterone on aggression to decline before the fights. An experiment in which the fights occurred the same day as the exposure trials could possibly produce different results. However, Gleason et al. (2009) have suggested that testosterone may affect long term brain structure and/or chemistry, which implies that the effects from elevated testosterone are long lasting and do not extinguish after less than one day. Further, testosterone's long term effects may take more than one day to be realized (Gleason et al., 2009).

The postulated long term effects of testosterone may be achieved by influencing the release of other hormones and neurotransmitters that have been implicated in aggression (Gleason et al., 2009). For instance, as mentioned earlier, some researchers

have proposed that testosterone and cortisol, a hormone associated with stress and antisocial behavior, interact to produce or inhibit aggressive behavior. A study in humans suggests that, only when cortisol is low, does a rise in testosterone correlate with increased social dominance. When cortisol is high, testosterone's promotion of dominance may be inhibited or reversed (Mehta and Josephs, 2010). Others have conjectured that testosterone influences aggression via reward pathways in the brain by influencing the release of dopamine (Gleason et al., 2009). Finally, a number of studies have provided evidence that both serotonin and arginine-vasopressin are involved in aggression. It has been suggested that they interact antagonistically with each other in the hypothalamus to influence aggression. Serotonin seems to have an inhibitory effect on aggression, whereas arginine-vasopressin promotes aggression (Ferris et al., 1999; Ferris et al., 1997). However, mechanisms by which testosterone could influence this system are unknown.

VI. General Conclusions and Future Directions:

Exposure to diestrus or estrous females through a mesh barrier may increase testosterone in males (as opposed to exposure to a male through a barrier) and observably promote aggression, at least in the case of exposure to a diestrus female. However, this effect does not seem to impact aggression strongly enough to win fights. Hopefully more light can be shed on this effect once the fights from the estrous female exposure experiment are analyzed. Future experiments should explore the effects of different amounts of time between the exposure treatments and fights on aggressive behavior and outcomes of fights. Experiments exploring the interaction of different experiences with

testosterone on aggression could help elucidate the robustness of testosterone's effects. In addition, research on the roles of other purported hormones in aggression will yield more valuable insight into this area. Furthermore, the correlations and guidelines generated by the analysis of the videotaped fights could be useful for researchers studying aggression as well.

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