Anogenital Distance and Dominance Status in Male House Mice (Mus domesticus)

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Factors influencing the tendency to be aggressive were investigated in male house mice using a variety of paired encounters. Body size, body length, body temperature, age, and anogenital distance were measured on all males. Paired encounters were conducted using a standard mouse cage as an arena. Among 64 male mice in 224 encounters, the tendency to be dominant and win encounters was significantly correlated with anogenital distance (r = 0.36). These findings suggest that there are significant behavioral effects in male mice that could parallel the intraspecific position and related prenatal hormone effects that have been elucidated in female house mice and other rodents.

Key words: house mice, aggression, anogenital distance, intraspecific position, Mus domestica

INTRODUCTION

Among mammals, aggression and related phenomena involving mating systems, access to food resources, and establishment and maintenance of living areas have been studied in such diverse groups as ungulates (Clutton-Brock et al., 1982), mongooses (Rasa, 1986), primates (Le Bouef, 1974; Renaud, 1991), and primates (Hall, 1964; Zuckerman, 1981). Rodents have been studied very extensively with regard to aggression (Scott and Fredericksen, 1951; Barron, 1963; Lagerström, 1964; Sachts and Prove, 1964; Siegel, 1983).

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Success in aggressive encounters, resulting in differences in dominance status, has been related to reproductive success and male choice in a variety of mammals, including red deer (Cervus elaphus), Clayton-Brock et al. 1981, observed males (Raphidura estivans) Le Breuff, 1974), and house mice (Mus musculus). Fortunat et al. 1962, Döckermaier, 1963. Aggressive interactions influence other behavior, including sexual behavior (Oakeshott, 1974; Drawharry, 1981). Outcomes from dominance encounters also influence reproductive hormones, FSH and LH (Bronson et al. 1977), although endocrine changes often do not persist for as long as behavioral changes.


Intrastracheal position, another prostatic factor, is related to activity (Kinsley, 1991). Yerkes et al. 1991) and to aggression (sum. 1953). In male mice, males ranked between two males are less active (Kinsley et al. 1989) and show less investigative and less social and sexual activity, but are more aggressive than males located between a pair of partners (Yerkes et al. 1991).

There is a relationship between intrastrachal position and some combination of levels of testosterone and levels of sensitivity of the brain and reproductive organs to circulating levels of testosterone in adult male mice and gerbils. This, in turn, influences aggression and other behavior patterns as well as the functioning of reproductive organs (Carl et al. 1982; Even et al. 1982; Nitzman et al. 1982). Aggression in male mice appears to be regulated in part by the release of an endogenous substance (epinephrine or norepinephrine, as well as the formation of a specific neuroendocrine system (Sumi and Freedenberg, 1983). Edwards et al. 1983; Kamei et al. 1977; sum. 1983). Significant positive relationships between testosterone levels and aggressive components of aggression have been reported in adult male mice by a number of investigations (Bohr et al. 1979, Brosh et al. 1982, Brosh et al. 1987, Proctor et al. 1987).

Agonistic distance, a morphological trait, is affected by intrasequence variation of females, and can serve as a possible basis of the position occupied by a mouse when it is in an array. We raise the possibility here that this is also the case in males. We also raised whether other characteristics of male mice, in addition to agonistic distance, correlate with their aggressiveness and winning or losing in paired encounters. While some investigators have been unable to find such an association between body mass and dominance in male mice, others have observed a correlation between body mass and dominance or social interactions. To determine the extent of the relationship between body mass and dominance, we measured body mass and dominance in male mice. Then we asked whether the body mass of dominant mice is positively related to body mass.
Park and Andrews, 1978). If we assume body temperature is an indirect measure of general metabolic activity, then we would predict that for house mice, higher temperatures should be associated with spending more time in encounters with other males. We tested the age of males as a possible factor in determining winners in aggressive encounters, because in a variety of mammals and from our own unpublished data we know that body size increases as a positive function of age. Lastly, in the literature regarding intraspecific position effects on social and sexual behavior in rodents (Saul, 1981; Clark and Gaul, 1981), there is evidence that an ontogenetic distance in males can be affected by hormones during intraspecific development (Kinsley and Spurr, 1987; Zielinski et al., 1991) and that differences in aggressive behavior can be related to intraspecific position for male mice (from Saul, 1983; Yeh et al., 1991). We tested whether variation in ontogenetic distance, whether its proximate causes, could be related to variation in aggressive behavior in male mice. Since previous findings suggest that larger ontogenetic distances may be associated with higher levels of intraspecific exposure to antagonists in males, we predicted that males with larger ontogenetic distances would be the aggressors more often in encounters with other males.

MATERIALS AND METHODS

Subjects and Husbandry

We used two stocks of wild house mice, each treated identically. One stock consisted of first generation laboratory progeny from mice captured at the Swine Farm of Southern Illinois University, Carbondale, Ill. The other stock was established from wild mice initially captured at Fort Collins, CO, reared in the laboratory of Dr. Sarah Leininger at Rutgers University, Newark, NJ, and crossed with an inbred laboratory strain (C3H/N). Progeny from this stock were several generations removed from the wild at the time of testing. Mice of both stocks were bred by placing a single male with a single female. Litters were weaned at 22-23 days of age and housed in individual cages until they were approximately 90 days of age. Throughout rearing and development, mice were housed in standard fiberglass polystyrene mouse cages on a bedding of wood shavings in an animal colony room at 20-23°C with overhead fluorescent lights on for 14 hr. light/10 hr. dark daily cycle. At the beginning of March 1995, 2 weeks prior to testing, all males were separated into individual cages. At this time they were moved to an enclosed ham area where they were exposed to current external ambient conditions of temperature and dewpoint. At that time the external conditions involved between 12 and 15 hr per day of daylight, temperatures ranging from lows of 3-10°C to highs of 14-23°C, and relative humidity of 40-60%. In addition to the bedding of wood shavings, each cage was given a cotton square (2.8 X 2.8 X 0.7 cm) with 0.1 ml of water made by AMC Corp., Berlins, NY, to which to make a nest. The mice were moved to the barn because of a larger field study in which they eventually were released into outdoor enclosures.

Apparatus and Procedures

Two weeks after being placed in the barn, each mouse was measured for body mass using a Pesola spring scale for body length (cm), defined as the distance from the tip of the nose to base of the tail, by holding the mouse down with a metric ruler; for body
temperatures. Using a Preciscan Model BAT-12 Digital Thermometer with a REF-3 probe, and for anemometric distance-time, we determined the minimum distance between the posterior base of the penis and the anterior tip of the anus using a rubber Calipers. Interobserver reliability values for the measurement of body mass, body length, and anemometric distance were obtained by having two individuals make each set of measurements. The Spearman rank correlation (Siegel, 1956) interobserver values were all 0.92.

We conducted the encounters in a standard opaque polycarbonate mouse cage 13 cm x 28 cm x 15 cm deep with a flat hardwood floor lid. The cage was cleaned between each encounter. Mouse measurements were made by ECD, encounters involving mice from Illinois stock were conducted by EMM, and encounters involving mice from Colorado/New Jersey stock were conducted by CC. Other than possible interactions with cage mates during early development, none of these males had any previous fighting experience.

We tested 132 males of each stock; males within each stock were divided into four groups of eight males each such that no brothers were from the same rearing cage were in the same group of eight. Thus, there were a total of eight groups of eight males. Within each group of eight males, a complete series of round robin encounters was staged for a total of seven encounters per male and 56 encounters per group. That is, each male was paired with each of the other seven males in his group. Order of testing in each group was randomized. No male was used in more than one encounter per day, and the entire encounter testing procedure was completed in a period of 10 days. En- counters lasted 10 min, or were terminated as described below when one male established clear aggressive superiority prior to the end of time period.

Each encounter was scored according to the outcome. If one male attacked another male five times in succession we terminated the encounter; that male was recorded as a clear winner and given a score of 5. A lower in each encounter was given a score of 0. If both males consistently initiated and engaged each other a few times during the 10 min, we scored this animal as a partial winner and gave it a score of 2. Losers in these encounters received a score of 1. When two males did not attack or distance one another during 10 min, the outcome was a draw and each male was given a score of 1. Following this scoring system existed in Lexington, 1991. An average aggression score was computed for each male at the conclusion of the seven encounters.

Analyses
All analyses were conducted on a Microvax II 1200 microcomputer using Stata II (1987). We analyzed differences between the two stocks of mice using parametric tests. Pearson product-moment correlations were used to test relationships between individual traits and level of aggression. Lastly, a stepwise multiple regression analysis was used to determine the relative importance of the five independent parameters for predicting aggression score. Results were analyzed separately for the two stocks of mice, except that a combined analysis was also run for the purpose of multiple regression.

RESULTS
The two stocks of mice differed with respect to body mass \( t = 6.2, P < 0.001 \) and body length \( t = 6.2, P < 0.001 \), but did not differ for age \( t = 1.2, P > 0.10 \).
DISCUSSION

The principal conclusion from these data is that there is a significant relationship between anogenital distance and the likelihood of being more aggressive and achieving dominance in interactions with other males. The length of the spines separating the genitalic papillae and scrotum at birth is a function of exposure to testosterone during fetal and neonatal life in mice (Dutta, 1988; Kessler et al., 1991). Animals with longer anogenital distances at birth also have considerably larger anogenital distances at adulthood (von Saal and Bronson, 1978). This latter finding suggests that the differences in anogenital distance observed in adult male mice in the present study were a consequence of differences in exposure to testosterone during sexual differentiation of the external genitals, which occurs during the latter part of prenatal and early postnatal life (Rugh, 1966).

There is a well-established relationship between exposure to testosterone during prenatal and early postnatal life and increased aggression in mice. This relationship is based on at least two lines of evidence: 1) Experimentally increasing levels of testosterone during perinatal life in female mice results in the females showing levels of aggression toward males that are similar to those seen in male-male encounters (von Saal, 1979). There is a correlation between levels of testosterone

| TABLE 1: Mean Values ± 1 SE for Five Independent Variables Measured on Two Stocks of Wild Male House Mice |
|---|---|---|---|---|
| Variable | Illinois | New Jersey | Pennsylvania |
| Body mass (g) | 19.6 ± 0.3 | 19.4 ± 0.3 | 19.5 ± 0.3 |
| Body length (cm) | 3.6 ± 0.1 | 3.7 ± 0.1 | 3.7 ± 0.1 |
| Body temperature (°C) | 36.9 ± 0.2 | 37.3 ± 0.3 | 37.5 ± 0.3 |
| Anogenital distance (cm) | 1.08 ± 0.02 | 1.20 ± 0.02 | 1.20 ± 0.02 |
| Age (days) | 123.5 ± 2.5 | 124.5 ± 2.5 | 125.0 ± 2.5 |

P = 0.20, body temperature r² = 0.42, P = 0.29, or anogenital distance r² = 0.25, P = 0.26 (Table 1).
TABLE II. Results From Stepwise Multiple Regression Analysis of Five Independent Measures for Predicting Aggression Scores in Male House Mice*

<table>
<thead>
<tr>
<th>State</th>
<th>B</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Illinois</td>
<td>0.410</td>
<td>0.66</td>
<td>0.27*</td>
</tr>
<tr>
<td>Colorado/New Jersey</td>
<td>0.379</td>
<td>0.64</td>
<td>0.14*</td>
</tr>
<tr>
<td>Combined</td>
<td>0.333</td>
<td>0.12*</td>
<td>0.77**</td>
</tr>
</tbody>
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*Significant at 0.05 level for each step and in combined analysis. Only ungenital distance was entered in the regression as a significant factor affecting aggression.

During fetal life and immediate postnatal life, sex differences in male mice (von Saal et al., 1983; von Saal, 1979). Taken together, the testing findings show that both anatomical distance and intermediate axis were related to the levels of testosterone to which males are exposed during the perinatal period of development. Thus, we propose that the present finding of a correlation between ungenital distance and intermediate axis in adult male mice is mediated at least in part by variation in exposure to testosterone during perinatal life.

An interesting and significant aspect of our findings is the large degree of variation in ungenital distance among adult males of both stocks (Table II). We calculate the percent difference in ungenital distance (largest - smallest) for the hybrid stock × wild Colorado mice × laboratory LTMA strains used in the present study: the percent difference is 141%. An ungenital distance was statistically larger than for the wild stock strain used in Illinois (54%). This lower value is virtually identical to the value for ungenital distance at birth in wild mice derived from a founder stock of mice trapped in Alberta, Canada (157% from von Saal, unpublished observations).

Previous findings using a number of outbred and inbred laboratory mice have produced evidence for relatively little variability among mouse mice in ungenital distance at birth (Kinsley and Searle, 1987; von Saal, 1979). For example, in male Rockland-2 mice, the percent difference in ungenital distance at birth was 26% from Saal, unpublished observations, or about one half that observed in wild mice. In studies with laboratory mice, the likelihood of finding correlations between ungenital distance at birth or in adulthood and various behaviors or patterns, such as intermediate axis, is unlikely due to the low level of variability among males in ungenital distance (Kinsley and Searle, 1987; Rossler et al., 1984; von Saal, unpublished observation).

Neither body temperature nor age were significant predictors of aggressiveness, although all animals tested were adults (Table I). We also did not find any significant relationships between ungenital distance in adulthood and either measure of body size: body mass or body length or aggressiveness and body size in either stock of mice. This latter finding is interesting in that some investigators have reported a correlation between body weight and being victorious in encounters between male rodents (Barben, 1986; Laplante, 1984; Spencer and Cameron, 1983). We should note that body mass values for our mice ranged from 17 to 27 g in Table II, encompassing almost the entire range for wild stock adult mice. Thus, our prediction is that while perinatal levels of testosterone influence adult ungenital distance and aggressive events, factors other than levels of testosterone during perinatal life likely influence adult body size.

Adults as well as prenatal testosterone levels influence intermediate axis and other behavior patterns in male mice. However, testosterone is released into the blood in
male mice in pulses this occur in unpredictable intervals (Coquelet and Desjoyeaux, 1982). Thus, it is not surprising that relationships between circulating testosterone and behavior patterns such as aggression are not always found (Ovendale et al., 1979). Brain and Nowell, 1989; van Oortmerssen et al., 1987), since the pulsatile release of testosterone makes correlating blood testosterone levels in males with behavior possible only with frequent collection of blood samples via cannulae. We thus did not attempt, in the present study, to measure circulating testosterone and correlate this with a male's anal- genital distance or aggressiveness. Average levels of circulating testosterone can be estimated by measuring scrotal size in intact males (Guthoff Schiefer and Toni Zeigler, personal communications, and this will be done in future studies.

Our findings suggest two lines for future research. The high variability in anal-genital distance observed for wild mice, even at birth and in adulthood, suggests a much greater variability in testosterone levels during the prenatal period of differentiation of the external genitalia relative to variability that has been observed in laboratory stock of mice. One possible source of variability in prenatal testosterone levels—anal-genital distance at birth and adult behavior—is that the teratogenic proximity of male fetuses to male or female siblings is influencing position phenomena. Namely, males exposed to elevated levels of testosterone in utero due to being positioned between male fetuses may have the largest anal-genital distance at birth and in adulthood and they may also be the most aggressive animals. In contrast, males positioned in utero between female fetuses are exposed to the lowest levels of testosterone during fetal life and thus may have the shortest anal-genital distances at birth and in adulthood and be the least aggressive males (von Saal, 1989). A second area for future investigation involves the examination of additional correlations between anal-genital distance and other behavioral patterns in male mice, e.g., home range size (Zielinski and Van Dongen, 1981), resource defense, and reproductive success.

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