Effect of Castration on Rabbits Housed in Littermate Pairs
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Published online: 04 Jun 2010.
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Conventional solitary caging for research rabbits precludes many postural and social behaviors. Several studies that have investigated enrichment strategies for rabbit does have found intact males usually too aggressive for paired or group housing. This study investigated the possibilities that male siblings will remain compatible if never separated from birth and that castration will improve compatibility among young sibling male rabbits. As a side benefit of pair housing for all rabbits, the larger cages (modified dog cages) allowed rest and other postures not possible in conventional single cages. In addition, there was no evidence of the stereotypies observed among individually housed rabbits. Castrated, post-weanling, male, Flemish Giant × Giant Chinchilla rabbit littermates demonstrated a significantly higher level of living together compatibly than did the intact controls. All rabbits chose to rest in close proximity or contact, although this preference was stronger in castrated males. During puberty, intact males displayed more serious aggression. We conclude that young adult littermate rabbits can live compatibly as pairs and that castration improves compatibility among males. Paired rabbits exhibit many postures and behaviors that are not possible in the small, solitary cages currently listed as minimum recommendations.

Conventional research settings often house rabbits in single, barren cages, possibly without adequate environmental enrichment (Guide for the Care and Use of Laboratory Animals [hereafter, the Guide]; Institute of Laboratory Animal Resources, 1996). We encourage careful review of rabbit social, postural, and behavioral preferences in future editions of the Guide. The Guide recommends a minimum floor
area of 4.0 ft$^2$ (122 cm$^2$) and a height of 14 in. (35.5 cm) for medium to large rabbits (4–5.4 kg); commercially available steel cages just meet this minimum. Such cages do not permit many common natural postures or one typical hop. The Guide presumes that “social animals should be housed in physical contact with conspecifics” (p. 37) but suggests no criteria for which species are considered social. The prevalence of commercial rabbit cages for single housing suggests that this consideration of social environment for laboratory rabbits is rarely met.

Several authors who have studied paired and group housing for female rabbits have documented their seeking the company of conspecifics (Brooks et al., 1993; Held, Turner, & Wootton, 1995; Huls, Brooks, & Bean-Knudsen, 1991; Love, 1994; Stauffacher, 1992). Although no data have been published (Love & Hammond, 1991), this preference has also been reported in castrated adult male rabbits. Aggression among intact adult males, however, is widely cited as a reason to not attempt group housing (Love, 1994). In this study, we investigated whether aggression was a serious problem among sibling males who had never been separated and, if so, whether castration prevents the development of aggression.

Compatible social housing makes the conversion of large cages and floor pens for rabbits more economically viable in limited facilities than large enclosures for single animals. Larger enclosures could provide more opportunities for species-typical postures and behaviors and promote greater physical and psychological well-being. Larger cages may also reduce common stereotypic behaviors as well as provide greater opportunities for social interaction and a greater physical complexity.

Both our own and other facilities house many rabbits for antibody-production protocols. Antibody-producing rabbits may be housed for weeks or months with only occasional injections or blood collections. Nothing inherent in antibody-production protocols requires solitary or confined housing (Turner, Held, Hirst, Billinghurst, & Wootton, 1997; Whary, Peper, Borkowski, Lawrence, & Ferguson, 1993). For years our institution has routinely housed antibody-production goats on pasture in compatible groups. The large numbers of rabbits used worldwide on antibody-production projects and the nature and length of these projects make antibody-production rabbits prime targets for refinement of husbandry practices.

METHOD

Animals

Twenty-four postweanling, littermate, specific-pathogen-free Flemish Giant × Giant Chinchilla male rabbits and 4 littermate females of the same breed were housed in same-sex pairs in one room. These sibling pairs were kept together from birth for
this project. Rabbits were transported from the breeding colony to the research facility at 12 weeks of age. Pairs arrived in irregular intervals over the course of 2 years and so were not all observed in the same time period. While still in the breeding colony, each rabbit was tattooed inside the left ear for identification. No pair was separated other than during castration surgery (10–20 min) or during antibody-production and injection procedures (5–10 min).

In a separately approved protocol, the rabbits were concurrently used for polyclonal-antibody production. When formal behavior sampling for this study was completed, compatible rabbit pairs were kept intact for the duration of their antibody-production protocol; physical examinations and food enrichments continued after the behavioral observation project had been completed. Except for one injured rabbit euthanized for health reasons, this study did not involve euthanasia of the subject animals. The remaining rabbits were eventually euthanized—under anesthesia as approved in the antibody-production protocol—for terminal large-volume blood collection.

Procedures

To minimize food competition, paired rabbits received 3 cups of pellets (Tek-Lad 15% Rabbit Diet) per day. Solitary rabbits in our facility received 1 cup of pellets per day. Rabbits received water ad libitum. They also received fresh vegetables, timothy hay, breakfast cereal, and compressed alfalfa cubes three times a week. Rabbits were weighed at each physical exam, averaging 5 kg each as young adults.

Stainless steel dog cages (127 × 100 × 100 cm = 1.27 m³ [14 ft³]) were converted for rabbit housing by covering the original coated iron-mesh dog flooring with a medium-gauge, galvanized 1-cm² wire mesh. In addition we provided a bottomless pine nest box (46 × 30 × 25 cm), a small (7-cm) piece of PVC pipe to pick up and toss or push around with the nose, a block of pine to gnaw (M length = 9 cm), one large food crock (approximately 500-mL capacity), and two water bowls. Females were housed in the same room as males.

Castration surgery was performed after 1 week of acclimation. While rabbits were hand held, isoflurane anesthesia was effected by face mask. Separate incisions were made over each testis. The tunic was either left intact (“closed” castration) or sutured closed to minimize the risk of herniation. Scrotal skin was left unsutured.

Rabbit pairs were randomly assigned to castrated or intact treatment, unless significant fighting had preceded their surgery date. This occurred once. Because this pair was assigned to be castrated, surgery was attempted that morning. A testicle in one of these bucks could not be found, thus preventing castration. They were therefore left intact and scored only as “separated for significant aggression.” To maintain experimental and control group sizes, we nonrandomly assigned another new pair to the castrated group.
Veterinary and animal care staff were numerous and often unfamiliar to the rabbits, but a single individual provided all enrichments, performed all biweekly physical examinations, and made all behavioral observations.

Behavioral Sampling

During the quiet of the late afternoon, usually between 2:00 p.m. and 5:00 p.m., animals were observed in 15-min recording sessions by one observer who was in full view of the animals. Behavioral sampling was not performed on days when antibody-production procedures or cage changes were carried out. Behavior was sampled every 10 sec according to techniques described by Martin and Bateson (1993) and recorded according to the ethogram categories in Table 1. Both rabbits of the pair were scored simultaneously. Actions were categorized as either interactive, noninteractive, or postural. Together or close is described here as a distance less than 6 in. (15.2 cm), whereas alone is a distance greater than 15.2 cm. Table 2 lists both the subcategories and the postural data. The observer provided most of the rabbits' enrichments; this food stimulus provided an opportunity to observe social and dominance interactions outside of formal sampling periods.

Rabbits were examined biweekly for wounds, scars, hair-pulling, evidence of enuration, or any other physical evidence of aggression. If suspicious signs were found, examinations were increased in frequency, and animal care staff was alerted to rabbit pairs of special concern. At the first sign of serious injury or prolonged threat (> 2 min), rabbits were separated. Only intact male rabbits required such separation. In this study, as we did not risk castrating and repairing males once aggression prompted a separation, we can only report on castration as a preventive, rather than curative, procedure.

RESULTS

Activities of rabbits during observation are summarized and quantified in Table 2. The data from observations of females are presented for interest.

Social Interactions

All paired rabbits spent most of their observed time (71%) resting close together or touching. They were inactive 79% of the time, possibly because of the late afternoon time that the data were taken. Aggressive acts were rarely seen (0.1%) and were apparently decreased by castration (0.3% intact vs. 0.02% castrated).
TABLE 1
Ethogram of Selected Behaviors

Interactive behaviors

Sit/lie alone: Sitting or lying apart at a distance over 6 in. (15 cm) from cagemate.
Sit alone in or behind box: Sitting or lying within or behind nest box, effectively out of view of cagemate, humans, and other rabbits.
Sit/lie close proximity: Sitting or lying within 15 cm of cagemate but not touching other rabbit.
Sit/lie touching outside box: Rabbits are touching each other and are not within or behind nest box.
Sit/lie within or behind nest box touching: Rabbits are resting in contact with cagemate inside or behind nest box, effectively out of view of humans or other rabbits.
Eat or drink, close or touching: Rabbits eat or drink touching or within 15 cm of each other.
Sniff rabbit: Approaching, or extension or turning of head, to smell cagemate.
Allogroom: Mutual grooming by licking body or head of partner.
Aggression: includes all aspects of aggression observed during sampling: chasing, circling chase, displacement, mounting, and mount-thrusting.
Other: Includes all other observed behaviors recorded during sampling (e.g., gnawing wood, playing with toys, self-grooming).

Postures/behaviors not possible in conventional housing

Momentary axial stretch: A full-body length stretching of the back, legs, or both, with manus or pes also extended; rabbits usually rock forward, then back, with torso parallel to floor, and yawn.
Lie fully stretched: Lying in sternal or lateral recumbency with pes fully extended from hip, possibly with extended manus; these behaviors are included with together or apart resting values.
Sit on box: Sitting on top of nest box.
Hop: The only recorded ambulation of the rabbits, excluding one occurrence of something like a step; varying distances (6 in. [15 cm] or more per hop), and appears propelled with varying strength.
Rear halfway: Sitting on haunches with torso vertically erect, or leaning on an object in this position.
Rear fully: Standing on pes, haunches raised from floor, torso vertically erect.

Student’s *t* test was used to compare the behaviors of individual intact and castrated male rabbits. A significant decrease in aggression was found among castrated rabbits, *t*(20) = 2.17, *p* < .04.

Prior to their scheduled castration, one male pair was permanently separated at 13 weeks because of fighting. Three pairs of intact rabbits displayed aggression by chasing, biting, and hair pulling. One scrotal injury occurred, with resultant intestinal herniation and need for medical euthanasia. One male in a mildly aggressive pair chased his partner for a few moments. They were not separated and remained together until, as part of the antibody-production protocol, they were euthanized well past the age of puberty—at approximately 11 or 12 months. One intact pair was separated for prolonged, chasing threats. Excepting one mild chase, no castrated animals showed these aggressive behaviors. One member of a seemingly...
amiable pair with a urethra obstruction of unknown origin was euthanized 1 month after castration. There was no clear evidence of fighting or other traumatic pathogenesis.

Resting behaviors predominated during our observation period. Several active behaviors such as hopping and exploring were difficult to categorize as either prosocial or antisocial. Castrated males spent 76.6% of their time resting, eating, or grooming in close proximity and 14.7% of their time resting separately. By contrast, intact males averaged 59.6% of their time in affiliative behaviors and 24.7% of their time apart.

One rabbit in all mature pairs appeared dominant. This was qualitatively judged by frequent observation of their interactions and by one rabbit being consistently more timid, reticent, and the last to get the treats. Occasionally, one rabbit was pushed away or bullied by a gentle nip of the other’s teeth or by a scratching with the forepaws. The presence of dominant behavior first occurred during puberty and seemed to become established a few weeks after the rabbits’ arrival. Puberty is reported to begin as early as 2.5 months and to end after 7 months (Farabollini, 1987; Lehmann, 1991; Southern, 1947).

### Table 2

<table>
<thead>
<tr>
<th>Activity</th>
<th>Castrated Male(a)</th>
<th>Intact Male(b)</th>
<th>Female(c)</th>
<th>All Rabbits(d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sit/lie alone</td>
<td>11.1</td>
<td>13.03</td>
<td>7.3</td>
<td>10.6</td>
</tr>
<tr>
<td>Sit alone in nest box</td>
<td>3.6</td>
<td>11.7</td>
<td>5.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Sit/lie close</td>
<td>15.3</td>
<td>12.5</td>
<td>7.3</td>
<td>11.7</td>
</tr>
<tr>
<td>Sit/lie touching (outside box)</td>
<td>41.4</td>
<td>18.5</td>
<td>34.9</td>
<td>31.6</td>
</tr>
<tr>
<td>Sit or lie in or behind box, touching</td>
<td>11.5</td>
<td>23.2</td>
<td>18.9</td>
<td>17.9</td>
</tr>
<tr>
<td>Eat/drink close/touching</td>
<td>4.6</td>
<td>3.4</td>
<td>10.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Sniff rabbit</td>
<td>0.4</td>
<td>0.7</td>
<td>0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Allogroom</td>
<td>3.67</td>
<td>1.3</td>
<td>4.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Aggression</td>
<td>0.02</td>
<td>0.3</td>
<td>0</td>
<td>0.1</td>
</tr>
<tr>
<td>Other</td>
<td>5.4</td>
<td>10.7</td>
<td>7.9</td>
<td>8.0</td>
</tr>
</tbody>
</table>

\(a_n = 12\).\(b_n = 10\).\(c_n = 4\).\(d_n = 26\). This behavior is included with either “alone” or “touching,” depending on proximity of rabbits.
Whether they eventually fought or not, all rabbits spent an average of 71% of their time resting either close or touching, and 17% of time was spent alone. The most commonly recorded behavior was lying or sitting together touching (50% of the time). The prevalence of this activity suggests that the rabbits desire the close presence of a companion. They sought lying together more than the various enrichment objects provided, possibly because our observations were performed during the crepuscular rabbits' less active late-afternoon periods (Podberscek, Blackshaw, & Beattie, 1991).

Use of Space

Although use of space was not the primary focus of this research, rabbits were observed making extensive use of the large cage space and the available enrichments. None of the stereotypies identified in singly caged rabbits in small cages (e.g., licking and biting cage bars; biting food bowls, cage, and hardware; racing around in circles) were noted in the paired rabbits in larger cages. Dog-sized water bowls were placed in the original holders at a height of 20 cm. This height, comfortable for dogs, presented no problem for the rabbits unless the water levels were low. Rabbits sat up on their haunches with their forepaws on the bowl rim and leaned over it to lap the water.

The nest box provided a visual blind, a shaded area for the rabbits’ highly sensitive eyes, a climbing or escape route, and some vertical complexity in the tall cages. In addition, the technicians could use the boxes to catch the rabbits.

Our rabbits will hop when out of their enclosures. A startled rabbit may hop several feet (1–1.5 m); unstartled, a rabbit commonly hops 15 to 30 cm per hop. Rabbits in the larger cages often moved 15 cm per hop, two to three times more than in the smaller, solitary cages.

The larger cages allowed stretched-out resting postures not seen in rabbits in the 40.5-cm-high cages. The greater height also allowed rabbits to sit up and explore as well as to have room to sit on top of their nest boxes.

DISCUSSION

The Guide (Institute of Laboratory Animal Resources, 1996) bases cage-size recommendations on the presumption that social animals should generally be housed with other compatible animals. Despite this, male, and even female, rabbits are frequently caged in isolation. Castration reduces aggression among males of several species (Houpt, 1998). In this study, we documented the assumption that this is true of rabbits as well. Castration may be an important aid to meeting the performance
standard of addressing caged the social needs of animals. All rabbits in our study chose close association with their sibling; castration can markedly decrease the aggression that can interrupt this sociality.

Behaviors Observed

Stereotypic behaviors were observed, although not quantified, in several singly caged rabbits, including rabbits caged singly after separation from, or euthanasia of, their paired companions. Observed stereotypies included cage-bar and feeder licking and biting, running in circles, scratching at cage walls, and digging or pawscraping at the cage floor. These behaviors were never observed when rabbits were paired, reinforcing our belief that social housing should be provided for rabbits whenever possible.

Many behaviors suggestive of dominance were observed in the general presence of humans and were noted qualitatively rather than during formal data sessions. Dominance among male pairs appeared established at about age 4 months, within a month after arrival. Females followed a similar pattern. In most pairs, one rabbit appeared to take more interest in people, spent more time lying in the open, well-lit cage front, and was occasionally seen to take food treats away from a partner. Displacement was rare. Rates of allogrooming, often done by both members, were not used to infer dominance. Most food and resting locations were shared without apparent competition.

Serious aggression was mainly seen in intact males toward the end of puberty, between 6 and 7 months. Violence, when it occurred, could either be sudden or preceded by a few days of small skirmishes. In one instance, the wounds inflicted to the scrotal area were sufficiently serious—exposing herniated intestines—to warrant euthanasia. Housing intact males together with the expectation that they will be safely separated before violence erupts seems a risky endeavor, given the serious consequences of incorrectly estimating this time interval.

Comfort and exploratory behaviors, such as allogrooming, stretching the body, stretching out fully to rest, rearing up on the hind legs to see and smell better, leaning on objects in a “leaning rear” posture, and playing in and sleeping on hay were observed in paired rabbits in larger cages either during or outside of formal observation periods. The Guide (Institute of Laboratory Animal Resources, 1996) promotes a performance standard that “at a minimum, an animal must have enough space . . . to express normal postural adjustments . . . [and] cage heights should take into account typical postures of an animal” (pp. 24–25). These behaviors are not possible for large rabbits in cages that just meet the Guide or Animal Welfare Act of 1985 standards (Animals and Animal Products, 1990) and were never observed in our conventionally housed large-breed rabbits. Other natural behaviors such as digging were not possible in conventional or larger cages.
CASTRATION IN RABBITS

Sternal or lateral recumbence with the rear legs fully extended from the hip were two important rest positions. One rabbit was measured in this position at over 25-in. long (64 cm). Our facility’s standard cages are only 20-in. (51-cm) wide and 16-in. (40-cm) high. No rabbit in our colony was seen lying across the diagonal (longest) axis of the conventional cages, the only possibility of fully stretching out. Gunn and Morton (1995) also made this observation.

Large Cages Versus Small Cages

The low-cost modification of discontinued-use cages permits both social companionship and more space for a variety of postures. Small cages may result in musculoskeletal deformation, especially of the femur, vertebral column, and ribs (Drescher & Loeffler, 1991; Stauffacher, 1992). Stauffacher observed space–time dysfunction, common stereotypies, and impaired locomotion in both large and small breeds. Cages measuring 4 ft² are unlikely to prevent these problems for medium to large rabbits.

Rearing up on hind legs with ears only partially extended, a posture not possible in conventional commercial cages, a rabbit was measured at 23-in. (58-cm) high. Here too, the discrepancy between the Guide’s (Institute of Laboratory Animal Resources, 1996) performance standard of “normal postural adjustment” and table of recommended cage-size minima is evident. Earlier editions of the Guide acknowledge that cage-size recommendations were “arbitrary” but representative of the current “best judgment of experienced animal care workers” (Animal Care Panel, 1963, p. 19). The fourth edition of the Guide actually decreases rabbit cage-height recommendations without explanation, from 40 to 35 cm. The recommendations remain unchanged (Institute of Laboratory Animal Resources, 1972). We hope future Guide committees will closely review such ethological studies of rabbits and revise cage-size recommendations accordingly.

Where protocols require maintaining rabbits for longer than 6 weeks, several authors (Gunn, 1994; Harris et al., 1996; Morton et al., 1993) recommend pair or group housing to satisfy the physical and psychological needs of domestic rabbits. Castration makes this a viable and safe refinement for housing male rabbits. The increase in affiliative behaviors and decrease in both frequency and seriousness of aggression would seem to offset the associated pain or distress of this minor surgery, especially for rabbits on long-term antibody-production protocols.

CONCLUSION

Castrated rabbits displayed more affiliative behaviors and less serious aggression than intact controls, although all rabbits sought close, frequent contact with their
siblings. Even when rates of aggressive acts are low, their seriousness can be life threatening. We caution against prolonged pairing of confined, intact male rabbits—even when raised together from birth—and encourage castration as a viable means to increase social compatibility. In enriched, paired rabbits, no stereotypic behaviors were observed. Rabbits exhibit many postures and behaviors that are not possible in the small, solitary cages currently listed in the Guide (Institute of Laboratory Animal Resources, 1996) as minimum recommendations. We encourage a careful review of rabbit social, postural, and behavioral preferences in future editions of the Guide.

ACKNOWLEDGMENT

The University Institutional Animal Care and Use Committee approved this study. The breeding colony and the experimental facilities and programs are accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care for compliance with all relevant animal care laws and policies.

REFERENCES


