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Effect of castration on social behavior and hormones in male Japanese macaques (*Macaca fuscata*)

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ABSTRACT

Population control is essential for animal welfare and human safety in free-ranging or captive settings, especially when resources are limited. As an alternative to lethal control, contraceptive methods such as castration in males can be a practical solution, because the testicles are a visual cue to determine which males have been sterilized. However, careful analyses should be carried out to ensure no disruption in the social structure of the population. Japanese macaques (*Macaca fuscata*) live in a society centered around dominance rank and matrilineal kin relationships. Testosterone and glucocorticoids have been correlated with dominance rank in humans and other species, but previous studies in Japanese macaques were inconclusive. This study aimed to investigate behavioral and physiological differences between castrated and intact male Japanese macaques, and to examine the effect of season and behavior in hormonal concentrations in intact males. Our subjects were six intact males from Jigokudani Monkey Park (Japan) and 13 castrated males from Born Free Primate Sanctuary (USA). We collected behavioral data using both focal and *ad libitum* sampling, and fecal samples for determination of testosterone (fT) and glucocorticoids (fGC) by enzyme immunoassay. We found that castrated males exhibited a social hierarchy, but not a linear hierarchy, as was the case in intact males. Castrated males were less aggressive than intact males, probably because fT concentrations were lower in the castrated males. Age was positively correlated with fGC levels, while fT concentrations were lower in old males than younger adult males. Fecal T levels correlated with both rank and atmospheric temperature. In intact males, both fGC and fT levels were elevated during the mating season. We found a negative correlation between fGC levels and the amount of grooming received. Our findings indicate that castration had a minimal impact on sociality, with season, temperature, and rank all influencing male sex steroid levels in intact males. Our study indicates that castration can be adopted as a population control mechanism without drastically altering the social relationships of males.

1. Introduction

Over the past several decades, the growing population of Japanese macaques (*Macaca fuscata*) has become a concern for local human populations due to farm crop damage [1], households, and some cases of physical attack on humans [2]. Japanese macaques are the only non-human primate species endemic to Japan, widely distributed from Shimokita peninsula (41°N) to Yakushima island (30°N). They have a despotic society, based on multimale-multifemale group formation with linear dominance ranks, and matrilineal kin relationships [3–5]. In 1972, a group of 150 monkeys from Arashiyama were translocated to a private ranch in Dilley Texas in order to control the population that had started to invade the town of Arashiyama, in the suburb of Kyoto city [6–9]. The North American researchers who began to study the monkeys named the troop "Arashiyama West". The monkeys lived in semi-free-ranging conditions where the population size increased to about 600 individuals. Since 2000 the group has been under the management of the Born Free Primate Secretary (BFPS) [10]. The large number of animals resulted in a situation where population control was initiated.

To maintain demographic stability of free-ranging and captive animal populations without harming or sacrificing animals, contraception has been applied in many species [11–15]. At BFPS, the males were vasectomized, but according to Buyukmihci [16], this method was unreliable at the site, because infants continued to be born, possibly due to mistakes in the technique. So the sanctuary decided to castrate all males of the group. Buyukmihci [16] reported no apparent short-term changes in social interactions or disruptions in the structure of the group. However, the author did not conduct behavioral observations and/or physiological studies to determine to what extent this procedure affects group dynamics, social behavior and hormonal levels.

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Testosterone, the main hormonal product of the testicles, may have an influence in male dominance rank [17–21] due to its action on musculoskeletal function [22–25] and its influence on aggressive behavior [26–30]. In addition, the testosterone-cortisol duo hypothesis states that testosterone's effect on dominance depends on cortisol, a glucocorticoid hormone associated with stress. Thus, testosterone should be related to higher status only when cortisol is low [31]. Previous studies in Japanese macaques have failed to show an association between testosterone and dominance rank [32–34], but Barret et al. [32] found that high ranking males have higher fecal cortisol levels than low ranking males. On the other hand, Abbott et al. [35] suggested that the association between rank and glucocorticoids is inconsistent and it depends on the social environment, including group stability, behavior and social support during agonistic interactions. The relationship between testosterone and cortisol is complex because acute stress results in suppressed testosterone and increased cortisol, but under non-stressful conditions, males have elevated levels of both hormones [36].

Considering the potential impact of hormones on the social structure of primates, we measured fecal testosterone (fT) and fecal glucocorticoids (fGC) from castrated and intact group-living males to investigate the effect of castration on Japanese macaques' society and to elucidate the relationship between, season, hormones and social behavior in intact males. We tested the following predictions: (1) castrated males will maintain a dominance hierarchy; (2) intact males will have higher rates of aggression than castrated males; (3) intact males will have higher fT concentrations than castrated males; (4) dominance rank will be correlated with fT levels in intact, and with fGC levels in castrated males; (5) both fGC and fT will be higher during the mating season in intact males; and (6) aggressive behavior will be associated with fT levels in intact males.

2. Methods

2.1. Study site and subjects

Subjects were 6 free-ranging intact male Japanese macaques (mean age ± SD = 18.84 ± 2.23 years), including 4 adults (from 7 to 19 years old) and 2 old individuals (≥ 20 years old) living at the Jigokudani Monkey Park (JMP), Japan (36°43′58″N, 138°27′46″E), and 13 semi-free ranging castrated males (mean age ± SD = 19.04 ± 6.09 years), including 9 adults and 4 old individuals, from Arashiyama West (AW), living at the Born Free Primate Sanctuary (BFPS) in south Texas (28°35′16.8″N, 99°10′33.6″W). For both locations, male subjects were selected randomly from each age category among individuals that could be identified and cross-checked with the database records. Age categories were based on the classification proposed by Hamada and Yamamoto [37] and Chalmers et al. [38]

At JMP, focal males could be identified by physical features such as scars, nose shape and birth marks. The group size was about 288 individuals, including 90 adult females (> 5 years old), 68 adult males (estimated to be > 7 years old), 14 sub-adult males (estimated to be 5–7 years old), 74 juveniles (from 1 to 4 years old) and 36 infants (< 1 year old). The troop maintains a home range, which varies seasonally from 1.23 km^2 to 1.69 km^2, with winter being the smallest and summer the largest [39]. There is a hot spring pool (40 m^2 and about 40 cm deep) available to the monkeys, with water temperature of approximately 40 °C. Tourists are not allowed to feed the monkeys or to enter the hot spring. Food is provided to the group by JMP staff at 09:00, 12:00 and 15:00 in the form of about 20 kg of wheat grains and a small amount of soybeans per day. The monkeys also consume natural foods in the mountains and spend the nights up in the mountains outside the park. Daily maximum and minimum temperatures were recorded by thermometer (Ishihara Ondokei Seisakusho Y.K., Tokyo, Japan) every day. In spring, temperature varied from − 4 to 27 °C, and in winter from − 7 to 12 °C. We also recorded the daily number of visitors to the park.

At BFPS, focal animals were identified by facial tattoos. Our subjects lived in a large enclosure (22.66 ha) surrounded by an electrified fence. The group size was about 233 monkeys, including 83 adult females (estimated to be > 5 years old), 145 males (estimated to be > 7 years old), and five juveniles (from 1 to 4 years old). Within the enclosure, the monkeys had naturally fissioned into three sub-groups. A small one consisting of about 50 monkeys, a large group consisting of about 153 monkeys, and a peripheral group formed by approximately 30 adult males. Of the 13 study subjects, 5 males belonged to the large group and 8 males were peripheral to that group. In order to control the population, all males had been castrated once their testes had descended (for more details, see [16]). The enclosure is comprised of thick thorny brush and honey mesquite. The area is semi-arid with an average rainfall of about 558.8 mm/year. The monkeys roam freely within the enclosure and make use of three ponds. There are also five water troughs, located at different locations in the enclosure. The monkeys are fed fruits, vegetables and commercial monkey diet (Labdiet® Fiber-Plus® Monkey Diet 5049) daily at 7:00 and at 13:00. Tourists are not allowed in the sanctuary. We recorded daily maximum and minimum temperature using the available online database, AccuWeather. The temperature varied between 22 and 39 °C. This research adhered to the Primate Society of Japan's (PSJ) principles for the ethical treatment of nonhuman primates. All manipulations of the subjects were approved (research clearance no. 2015 − 127) by the Primate Research Institute (PRI) Ethics Committee of Kyoto University and conformed to the PRI's Guidelines for Care and Use of Nonhuman Primates.

2.2. Data collection

All fecal samples were collected immediately after defecation and kept in a cooler bag until transferred to a freezer (− 20 °C) within 2 h after collection. We discarded any sample contaminated with water or urine.

To collect behavioral data, RSCT used focal instantaneous sampling [40] recording on the minute during a 30 min session (three sessions/week/subject) to determine the rates of aggressive events (*e.g.*, avoidance, supplants, threats, chases, and bites) and the time spent (continuously) in affiliative interactions (grooming, huddling). In addition, RSCT recorded agonistic interactions on an *ad libitum* basis from all individuals in sight of the focal to assist in establishing the dominance hierarchy. We combined the *ad libitum* and focal records to establish relative rank order among the subjects based upon the directionality of all agonistic and approach-avoidance interactions between dyads.

Observations on the intact male group (JMP) and castrated male group (BFPS) were collected during the non-mating season. From April to June of 2014, RSCT followed the six intact males from 9:00 to 17:00. RSCT collected fecal samples from all 6 males (N = 88, mean = 14.67 ± 4.37 per individual), along with 77 h of focal data (12.83 ± 0.41 h per individual), and 472 h of *ad libitum* data. Five males were central and one male was peripheral to the group. The terms 'central' and 'peripheral' refer to relative proximity to most of the females and infants in the group [41,42]. During July, RSCT followed the castrated males from 7:00 to 16:00, collected 17.5 h of focal data from 6 males (2.92 h ± 0.20 h per individual) and 153 h of *ad libitum* data from all 13 males. Among these six focal males, four were central and two were peripheral to the group. RSCT collected fecal samples from 13 males (N = 20, mean = 1.53 ± 0.97), five central and eight peripheral to the group. To compare seasonal variations in steroid hormones of the intact group, RSCT followed the intact males in the subsequent mating season (October to December 2014) between 9:00 and 17:00, collected fecal samples (N = 36, mean = 7.2 ± 2.49 per individual), 52.5 h (10.5 h per individual) of focal data, and 329 h of *ad libitum* data from five males (one of the males sampled in the previous season had disappeared from the group and thus was removed from the statistical