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Individual Differences in the Maternal Behavior of Male Mice: No Evidence for a Relationship to Circulating Testosterone Levels[1]

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Maternal behavior toward newborn pups and endogenous levels of testosterone (T) in peripheral plasma were measured in individual adult male mice. Separate groups of animals that either retrieved, ignored, or killed pups were found not to differ with respect to plasma T levels, body weights, or relative weights of testes, seminal vesicles, and adrenals. Furthermore, animals do not exhibit changes in T following tactile or nontactile interactions with pups.

When first presented with newborn young, male mice show one of three strikingly different behavioral responses: pup retrieval, pup killing, or complete indifference (Gandelman, 1973). The presence or absence of testosterone (T) in adult life dramatically influences maternal behavior, since castration of adult males decreases the proportion of animals that exhibit pup killing and increases the number of animals that retrieve young (Gandelman and vom Saal, 1975). Moreover, virgin female mice and neonatally castrated male mice, which typically retrieve newborn young, kill pups following adult T exposure, with high doses of T being more effective than low doses (Davis and Gandelman, 1972; Gandelman and vom Saal, 1976). Plasma T levels in male mice are also known to vary considerably from one animal to another. Bartke *et al.*, (1973) reported a range of plasma T concentrations from less than 0.3 to 44.4 ng/ml in random-bred male mice, whereas Lucas and Abraham (1972) reported that pools of plasma from adult male mice of various inbred mouse strains showed a range from 0.6 to 21.9 ng of T/ml. In view of the large differ-

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ences in maternal behavior and plasma T levels in male mice, we hypothesized that there may be a relationship between circulating T levels and the behaviors exhibited toward young which are influenced by this hormone. The following study tested this hypothesis by measuring circulating T levels and maternal behavior in male mice. In addition, we examined whether or not male mice exhibit changes in T following tactile or nontactile exposure to pups.

METHOD

One-hundred ninety-six male Rockland–Swiss (R-S) albino mice, 60–65 days of age, were individually housed in 22 × 22 × 13-cm stainless steel cages, the floors of which were covered with pine shavings. The mice had *ad libitum* access to food (Purina Mouse Chow) and water and were maintained on a 12: 12-hr light/dark cycle with the lights on at 0700 hr. Behavioral testing and blood collections were conducted between 1000 and 1200 hr.

After being isolated for 24 hr, 116 animals were tested for their response to newborn pups. This was accomplished by placing three 1-day-old R–S pups into each animal's cage for a period of 10 min. The pups were distributed in the corners (1 pup/corner) of the cage furthest from the area occupied by the male. Each animal was assigned to one of the following groups on the basis of its behavior toward the pups: (1) Maternal, retrieved one or all pups; (2) Kill, killed one or all pups; (3) Ignore, did not retrieve or kill pups. In addition, the latency to retrieve or kill the first pup as well as the number of pups retrieved or killed were recorded. The remaining 80 animals were equally divided into two additional groups. One group of animals had their cages divided in halves by a wire-mesh partition. On the day of testing, the animals of this group (Group PE) were exposed to pups that were placed on the side of the partition opposite the living quarters of the adults for 10 min. The purpose of including this group was to assess whether or not plasma T levels might change following nontactile exposure to young. The remaining group of animals (Group NPE) was not exposed to young or tested for maternal behavior. This group was included to provide a measure of resting T levels. We adopted the following procedure to examine further the possibility that T would be altered following a brief exposure to young. Blood samples were collected from half of the animals in each group with a heparinized syringe, by cardiac puncture without anesthesia, immediately following the behavioral test or pup exposure, whereas blood samples from the remaining half of the animals in each group were collected in the same manner 24 hr following testing or pup exposure. (The collection of blood samples from the animals of Group NPE was conducted at the same times, i.e., either 24 or 48 hr following isolation.) Following blood collection, the animals were sacrificed by cervical dislocation; body weight and the weights of the adrenals, testes, and seminal vesicles were recorded. Plasma T levels were determined by radioimmunoassay without chromatographic separation (Bartke *et al.*, 1973).

RESULTS AND DISCUSSION

Table 1 shows the results of the behavioral tests, plasma T levels, body weights, and peripheral organ weights. The percentages of animals that retrieved, killed, or ignored newborn young are similar to those previously reported for male mice (Gandelman, 1973). The time of blood collection did not influence plasma T levels in any of the groups, thus indicating that our measurements represented resting values. As a result, we did not include this factor in the statistical analysis. As can be seen in Table 1, plasma T levels were similar for groups of males that showed striking differences in their behavior toward newborn mouse pups. The table also shows that T levels were similar for Groups PE and NPE, thus indicating that T levels were not altered by nontactile exposure to pups. Indeed, the similarity of T values among all groups [Kruskall–Wallis one-way analysis of variance (ANOVA): $H(4) = 0.21$, $P > 0.20$] in combination with the failure to observe a time of blood collection effect further confirms that the individual differences in behavior are not related to differences in circulating T, that animals do not respond to tactile or nontactile experience with young by changes in T, and that our measurements represented resting T values. Table 1 also shows that there was great individual variation in plasma T levels in all groups (range among all groups = 0.1–20.4 ng/ml), thus confirming previous reports of the highly variable nature of circulating T in the mouse (Bartke *et al.*, 1973; Lucas and Abraham, 1972). In addition, Kruskall–Wallis ANOVA tests showed nonsignificant between-groups effects for body weight [$H(4) = 4.61, P > 0.30$], relative testis weight [$H(4) = 9.37$, $P > 0.05$], relative seminal vesicle weight [$H(4) = 5.55, P > 0.20$], and relative adrenal weight [$H(4) = 6.18, P > 0.10$]. Finally, Spearman rank correlation coefficients were computed to evaluate the relationships between the behavioral measures and plasma T levels. For Group Maternal, there was no relationship between plasma T and either the latency to retrieve the first pup [$\rho\ (52) = -0.027, P > 0.20$] or the number of pups retrieved [$\rho\ (52) = 0.130, P > 0.20$]. For Group Kill, there was no relationship between T levels and either the latency to kill the first pup [$\rho\ (35) = -0.180$, $P > 0.20$] or the number of pups killed [$\rho\ (35) = 0.069$, $P > 0.20$].

The present findings indicate that the individual differences in the maternal behavior shown by male mice when first confronted with newborn young are not related to differences in the amount of circulating T, body weights, or relative organ weights. One might suspect that individual differences in male maternal behavior are related to the way in which the central nervous system responds to testosterone. However,

TABLE 1

Plasma T Levels, Body Weight, and Weights of Other Organs of Male Mice Exhibiting Different Types of Maternal Behavior[a]

Group	N	Latency to retrieve or kill first pup (sec)	Number of pups retrieved or killed	Plasma testosterone (ng/ml)	Body weight (g)	Relative testis weight (mg/100 g body wt)	Relative seminal vesicles weight (mg/100 g body wt)	Relative adrenal weight (mg/100 g body wt)
Maternal	54 (46.6)[b]	203 (34–570)	3 (1–3)	0.82 (0.14–20.4)	34.0 (26.4–40.3)	731.3 (536.0–907.9)	429.5 (309.2–602.5)	18.6 (11.8–33.3)
Kill	37 (31.9)[b]	218 (33–518)	3 (1–3)	1.08 (0.21–17.03)	34.1 (26.1–42.4)	734.6 (547.5–912.5)	412.5 (313.2–584.9)	17.6 (11.1–29.1)
Ignore	25 (21.5)[b]			1.07 (0.15–12.19)	35.1 (29.4–40.2)	739.0 (308.7–861.8)	396.3 (288.6–560.2)	16.8 (11.8–35.8)
PE	40			1.16 (0.11–10.11)	34.1 (28.2–42.0)	691.6 (549.9–884.4)	427.4 (283.5–592.3)	17.7 (7.8–26.5)
NPE	40			.80 (0.20–14.14)	33.6 (30.2–40.0)	768.0 (543.6–931.0)	451.9 (285.6–672.1)	15.1 (9.9–24.5)

[a] The number and percentage of animals that retrieved (Maternal), ignored (Ignore), or killed (Kill) newborn young and the median and range latency to retrieve or kill the first pup, number of pups retrieved or killed, circulating testosterone levels, body weights, and relative organ weights of these groups and groups that either had young placed behind a wire partition in their cage (PE) or that were not exposed to young or behavioral tests (NPE). Behavioral tests or pup exposure were 10 min in duration.

[b] Percentage.

Harding and Feder (1976a,b) recently examined this possibility for copulatory behavior in the male guinea pig and found that individual differences in behavior bore no relationship to differences in circulating T or in the uptake or metabolism of radioactive T in several brain areas.

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