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Differential Effects of Neonatal Testosterone Treatment on Aggression in Two Selection Lines of Mice

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COMPAAN, J. C., A. J. H. DE RUITER, J. M. KOOLHAAS, G. A. VAN OORTMERSSEN AND B. BOHUS. *Differential effects of neonatal testosterone treatment on aggression in two selection lines of mice.* PHYSIOL BEHAV **51**(1) 7–10, 1992.— Selection lines of mice, artificially selected for aggression based upon the attack latency score (ALS), were used. In order to determine the relative contribution of neonatal testosterone (T) in the development of aggression, we vary the plasma-T level in males of both selection lines on the day of birth. At 14 weeks the ALS was measured. Neonatal T treatment results in a reduction of aggression in the long attack latency (LAL) line, whereas aggressive behaviour of the short attack latency (SAL) line is not affected. Both selection lines show reduction in testicular weight, although the total amount of T-producing Leydig cells was not affected. Neonatal T may cause a permanent reduction in aggressive behaviour in the LAL line only, probably due to differential appearance of critical periods. It is suggested that the difference in aggressive behaviour between SAL and LAL selection lines is due to a prenatally determined difference in neonatal T sensitivity of the brain.

Aggression Testosterone Ontogeny Sexual differentiation

IN wild populations of house mice (*Mus musculus domesticus*) in the Netherlands, there exists a bimodal distribution of aggressive behaviour within the population. As shown in earlier experiments, we found that this distribution has a genetic basis (22,23). In our laboratory, these mice were artificially selected further for aggression, based upon the attack latency score (ALS) (21). Three selection lines are available now, one for short attack latency (SAL) and another for long attack latency (LAL), and a randomly bred control line. The two extremes differ in a variety of behavioural test situations, and thereby display a totally different strategy of coping with social and nonsocial environmental challenge, designated as active and passive coping (4–7). The success of this selection further supports the significance of genetic factors in social (aggressive) behaviour.

In the present study, we will concentrate on the difference in aggressive behaviour only. Numerous studies show that plasma testosterone (T) facilitates aggressive behaviour in both male and female animals at adult age (3, 8, 11). In ontogeny, the onset of fighting behaviour in male mice coincides with the first prepuberal rise in T level (1,18). Castration neonatally, prepuberally or at an adult age, reduces intermale aggression, whereas T replacement restores this behaviour (2,24). In rats, a positive correlation between baseline T level and aggression was reported (29). In addition, SAL males have a higher baseline plasma T level than LAL-type males of the unselected-control line (24). Furthermore, van Oortmerssen et al. (24) reported that SAL males show a higher response following T replacement after castration around the onset of puberty. The authors suggested that the difference in responsiveness to T is probably due to a differential sensitivity of the central nervous system (CNS) to T. The differential sensitivity may be caused by differences in T level during prepuberal phases of life. A short rise in plasma T occurs in male rats (12) and mice (19) immediately after birth. Studies from the late sixties (9, 10, 13, 14) revealed that this neonatal T surge is involved in a permanent organization of the CNS resulting in higher responsiveness to T, and thereby the activation of aggression in adulthood of both male and female animals (organization-activation model). Neonatal androgen exposure is necessary to further sensitize the CNS to the aggression-eliciting property of T later on [(2, 27, 28) for review: (16)].

Hence, it is conceivable that a variation in neonatal T level is an important causal factor in the differential development of aggressive behaviour and/or in CNS T sensitivity in these selection lines. Accordingly, we vary in this experiment the plasma T level immediately after birth in order to study the interaction of neonatal T and genetic factors in the development of aggressive behaviour using two selection lines of wild house mice. Therefore, we injected male mice of both selection lines with testosterone propionate (TP) on the day of birth, and investigated their aggressive behaviour in adulthood.

METHOD

Animals

Mice (*Mus musculus domesticus*) bred in our laboratory were used. They were artificially selected for either long attack la-

tency (LAL) or short attack latency (SAL) (generations: LAL 14–16; SAL 37–38). Parents were housed in small Plexiglas cages (17 × 11 × 13 cm), with sawdust bedding in animal rooms with a controlled light/dark cycle (L:D = 12:12; lights off at 12:30 a.m.) and temperature (19–21°C). Standard lab chow and water was available ad lib. On the morning after the night of birth (day 0), all pups were treated with testosterone or vehicle. At three-weeks postpartum, the young animals were separated from the parents. At an age of 6–8 weeks, the individual males were housed with a normal female in standard cages (17 × 11 × 13 cm). Aggressive behaviour was tested at the age of 14 weeks.

Treatment

On day 0, between 11:00 and 12:00 a.m, a complete litter was injected subcutaneously (SC) in the neck area with either 20 μl testosterone propionate (TP) dissolved in oil (Organon: Neo-Hombreol U.R. RVG00045) or just ground nut oil. Either a low (lTP = 0.64 μg) or a high dose TP (hTP = 324.0 μg) was administered. This neonatal treatment resulted in three different groups in both selection lines: oil (SAL: n = 37; LAL: n = 25), lTP (SAL: n = 31; LAL: n = 26) and hTP (SAL: n = 25; LAL: n = 20).

Behavioural Testing

As a measure of aggressive behaviour the attack latency score (ALS) was used (21). This ALS is the mean of the attack latencies scored on three consecutive days, between 1:00 and 3:00 p.m. Each experimental animal was confronted with a standard, gonadally intact, male albino mouse opponent (MAS-GRO). The test was terminated immediately after an attack occurred, or, in the absence of any attack, after 10 minutes. For those animals which did not fight at all the ALS is 600 s.

Histology

Four weeks after behaviourial testing, the animals were weighed and sacrificed. The testes were removed, cleaned and weighed. The mean weight of the two testes per individual was calculated (testicular weight = TW). According to de Ruiter et al. (26), we measured the T production at adult age using a morphometric analysis of testicular Leydig cells. These cells were stained enzymohistochemically for Δ^5-3β-hydroxy steroid dehydrogenase (3β-HSD). Using a computer image analyzing system (IBAS), we calculated the area percentage (AREA %) and the total amount of Leydig cells in a testis.

Statistics

A two-way analysis of variance was performed on the aggression data and testicular weights. These were of a two (SAL and LAL males) by three (oil, lTP, and hTP neonatal treatment) factorial design. Another ANOVA was performed on the histological data of a two (SAL and LAL males) by two (oil and hTP) factorial design. Significant treatment effects between groups were determined with post hoc Dunnett's test (20).

RESULTS

Aggressive Behaviour

The mean attack latencies (ALS) of adult SAL and LAL males neonatally treated with oil, a low (lTP) or a high dose of testosterone propionate (hTP) are presented in Fig. 1. A two-way analysis of variance reveals significant effects of selection lines ($p<0.001$), and of neonatal T treatment ($p<0.05$) and also a significant interaction ($p<0.05$) between selection line and neonatal treatment exists. The neonatally oil-treated animals did not differ from nontreated males in both selection lines (data not shown). Neonatally oil-treated SAL (ALS = 23.0 ± 5.1 s) and LAL males (ALS = 366.2 ± 45.8 s) show a significant difference in latency to attack the opponent ($p<0.001$). Moreover, all SAL males did attack the albino, whereas no more than 68.0% of LAL males show fighting behaviour within 10 minutes. Neonatally hTP-treated LAL males (LAL-hTP) attack significantly slower (ALS = 498.2 ± 35.1 s) compared to males treated with either oil or lTP (ALS = 360.8 ± 44.3 s) of the same selection line ($p<0.05$). Less than half of this LAL-hTP group displays fighting behaviour (40.0%). In contrast, the already short ALS of SAL males remains unaffected after a neonatal T injection (Fig. 1). Each male of this selection line attacks the albino, irrespective of neonatal treatment. Thus neonatal hTP treatment reduces the aggressive behaviour in LAL males, whereas aggression in SAL males is not affected.

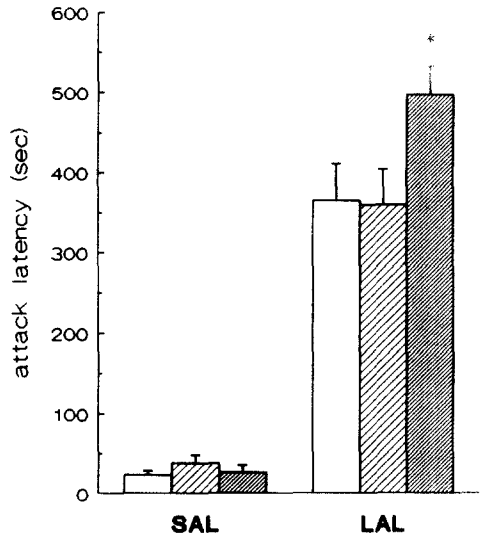

FIG. 1. Mean attack latency (ALT + SEM) of males, neonatally treated with oil (open bars), low dose TP (lightly hatched bars) or high dose TP (heavily hatched bars) (*$p<0.05$).

Histology

Testicular weight (TW) at adult age is reduced in both selection lines after the TP injection on day 0. Table 1 depicts mean absolute (TW) and relative (rTW) testicular weight of neonatally treated SAL and LAL males at adult age. A two-way ANOVA reveals significant effects of selection lines ($p<0.001$), and of neonatal T treatment ($p<0.01$) on both TW and rTW. A significant reduction in TW ($p<0.05$; Dunnett's test) of LAL-hTP compared to all other treated groups was observed. Furthermore, in SAL males, neonatal hTP results in a significant reduction ($p<0.05$) of rTW compared to neonatally oil-treated SAL males.

Just in the oil and hTP groups, we measured the T production (Table 1), because hTP only significantly affected aggression neonatally in the LAL line, and testicular weight in both selection lines. ANOVA reveals a significant treatment effect ($p<0.05$) and interaction with selection line ($p<0.05$) in the AREA % of Leydig cells. In the neonatally hTP-treated LAL males a higher AREA % of Leydig cells was found compared to the neonatally oil-treated males ($p<0.05$). A significant differ-

TABLE 1

MEAN TESTICULAR WEIGHT (±SEM) AND LEYDIG CELLS AT ADULT AGE OF LAL AND SAL MICE NEONATALLY TREATED WITH TP OR OIL

	Testicular Weight		Leydig Cells	
Neonatal Treatment	Absolute (mg)	Relative (mg/g body weight)	Area (%)	Amount (mg)
SAL				
oil	94.8 ± 4.0	4.37 ± 0.14	3.81 ± 0.32	3.72 ± 0.34
lTP	84.2 ± 2.8	4.04 ± 0.15	—	—
hTP	86.7 ± 2.9	3.88 ± 0.14†	3.91 ± 0.17	3.32 ± 0.17
LAL				
oil	84.2 ± 3.1	3.69 ± 0.19‡	2.99 ± 0.36	2.68 ± 0.31‡
lTP	75.8 ± 5.1	3.54 ± 0.19	—	—
hTP	64.2 ± 4.2*	2.78 ± 0.19*	4.36 ± 0.36†	2.98 ± 0.28

†Significant treatment effect ($p<0.01$; †$p<0.05$).
‡Significant selection line effect ($p<0.05$).

ence ($p<0.05$) was reached in the amount of Leydig cells between LAL and SAL males neonatally oil treated. However, no significant treatment effect was reached in the total amount of Leydig cells, due to a lower TW after neonatally hTP.

DISCUSSION

The males of the selection lines used in the present study were genetically selected for aggression based upon either a short or a long ALS. After a neonatal oil treatment, this difference in aggression is maintained. However, enhancing the concentration of the neonatally circulating T results in a higher ALS in the LAL line only, i.e., a further reduction in aggressive behaviour at an adult age, whereas aggressive behaviour of the SAL selection line remains unaffected. The results are in accordance with the reduction in masculine sexual behaviour as observed in male rats after high TP levels induced on day 3 postnatally (34) or perinatal exposure to high levels of androgen (25).

The reduction in aggressive behaviour may be due to lower levels of adult plasma T associated with the decline in TW. Indeed, in agreement with earlier reports (15,34) a dose-dependent reduction in testicular weight was found. However, this was observed in both selection lines. Moreover, the results of the Leydig cell analysis indicate that after neonatal hTP treatment in the LAL line, the amount of T producing Leydig cells increased per unit testis tissue. Accordingly, the production of T seems to remain on the same level in both oil- and hTP-treated animals. Vanderstichele et al. (30) also showed that in neonatally androgenized male rats, although TW has been reduced, the basal steroidogenesis is maintained due to an increased synthesis per unit of testis tissue. Hence, it is not simply a decline in plasma T due to neonatal hTP treatment that causes a reduction in aggression at adult age in LAL males. The most plausible explanation is that the neonatal T treatment results in a lower androgen sensitivity of the CNS in the LAL males. This is in accordance with our previous experiments indicating that LAL males (24) and females (preliminary results: Compaan et al., 1991; in preparation) are less sensitive to a selected T dose in adulthood. The present experiment suggests that this difference in CNS sensitivity to T at adult age is possibly due to different neonatal endogenous T levels. A recent study shows that the nonaggressive LAL males indeed produce more T neonatally than SAL males (26). At an age of 23 days, this difference has already turned in favour of SAL males. Hence, a differential neonatal T production might be involved in the differentiation of aggressive behaviour at adult age.

Surprisingly, the SAL males do not react to neonatal T treatment, indicating a difference in susceptibility of the neonatal brain to T. This may be due to prenatal factors. Around day 18 postconception a T surge occurs which is involved in the sexual differentiation of the brain (32,33). The occurrence of two androgen-dependent phases is suggested: 1) a high T level prenatally organizes the CNS of developing fetuses to 2) testosterone circulating in lesser amounts neonatally (17,31). Probably the SAL CNS has been organized during the prenatal critical period, whereas the LAL CNS can still be organized neonatally. In conclusion, these results suggest that only in the LAL line an increasing T level after birth causes a reduction in aggression. This is possibly due to a downregulation of CNS androgen sensitivity. Probably the difference in adult aggressive behaviour between SAL and LAL selection lines is due to a genetically determined difference in critical periods: prenatal sensitization to T in the SAL and neonatal downregulation of androgen sensitivity in the LAL selection line. Whether this perinatal differential T surge is also involved in the development of coping strategies remains to be solved.

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REFERENCES

1. Barkley, M. S.; Goldman, B. D. A quantitative study of serum testosterone, sex accessory organ growth, and the development of intermale aggression in the mouse. Horm. Behav. 8:208–218; 1977.
2. Barkley, M. S.; Goldman, B. D. The effects of castration and silastic implants of testosterone on intermale aggression in the mouse. Horm. Behav. 9:32–48; 1977.
3. Beeman, E. A. The effect of male hormone on aggressive behavior in mice. Physiol. Zool. 20:373–405; 1947.
4. Benus, R. F.; Bohus, B.; Koolhaas, J. M.; van Oortmerssen, G. A. Behavioural strategies of aggressive and non-aggressive male mice

in active shock avoidance. Behav. Processes 20:1–12; 1989.
5. Benus, R. F.; Bohus, B.; Koolhaas, J. M.; van Oortmerssen, G. A. Behavioural strategies of aggressive and non-aggressive male mice in response to inescapable shock. Behav. Processes 21:127–141; 1990.
6. Benus, R. F.; Koolhaas, J. M.; van Oortmerssen, G. A. Individual differences in behavioural reaction to a changing environment in mice and rats. Behaviour 100:105–122; 1987.
7. Benus, R. F.; Koolhaas, J. M.; van Oortmerssen, G. A. Aggression and adaptation to the light-dark cycle: Role of intrinsic and extrinsic control. Physiol. Behav. 43:131–137; 1988.
8. Brain, P. F. Hormones and aggression. vol. 1. Annual research reviews. Montreal: Eden Press; 1977.
9. Bronson, F. H.; Desjardins, C. Aggression in adult mice: Modification by neonatal injections of gonadal hormones. Science 161:705–706; 1968.
10. Bronson, F. H.; Desjardins, C. Neonatal androgen administration and adult aggressiveness in female mice. Gen. Comp. Endocrinol. 15:320–325; 1970.
11. Bronson, F. H.; Desjardins, C. Steroid hormones and aggressive behavior in mammals. In: Eleftheriou, B. E.; Scott, J. P., eds. The physiology of aggression and defeat. New York: Plenum Press; 1971:43–65.
12. Corbier, P.; Kerdelhue, B.; Picon, R.; Roffi, J. Changes in testicular weight and serum gonadotropin and testosterone levels before, during, and after birth in the perinatal rat. Endocrinology 103(6): 1985–1991; 1978.
13. Edwards, D. A. Mice: Fighting by neonatally androgenized females. Science 161:1027–1028; 1968.
14. Edwards, D. A. Early androgen stimulation and aggressive behavior in male and female mice. Physiol. Behav. 4:333–338; 1969.
15. Feigelson, M. Suppression of testicular maturation and fertility following androgen administration to neonatal male rats. Biol. Reprod. 35:1321–1332; 1986.
16. Gandelman, R. Gonadal hormones and the induction of intraspecific fighting in mice. Neurosci. Biobehav. Rev. 4:133–140; 1980.
17. Hoepfner, B. A.; Ward, I. L. Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. Behav. Neurosci. 102(1):61–65; 1988.
18. McKinney, T. D.; Desjardins, C. Postnatal development of the testis, fighting behavior, and fertility in house mice. Biol. Reprod. 9:279–294; 1973.
19. Motelica-Heino, I.; Castanier, M.; Corbier, P.; Edwards, D. A.; Roffi, J. Testosterone levels in plasma and testes of neonatal mice. J. Steroid Biochem. 31(3):283–286; 1988.
20. Myers, J. L. Fundamentals of experimental design. Boston: Allyn and Bacon Inc.; 1979.
21. Oortmerssen van, G. A.; Bakker, T. h. C. M. Artificial selection for short and long attack latencies in wild Mus musculus domesticus. Behav. Genet. 11(2):115–126; 1981.
22. Oortmerssen van, G. A.; Benus, I.; Dijk, D. J. Studies in wild house mice: Genotype-environment interactions for attack latency. Neth. J. Zool. 35:155–169; 1985.
23. Oortmerssen van, G. A.; Busser, J. Studies in wild house mice 3: Disruptive selection on aggression as a possible force in evolution. In: Brain, P. F.; Mainardi, D.; Parmigiani, S., eds. House mouse aggression: A model for understanding the evolution of social behavior. Chur: Harwood Academic Publishers; 1989:87–117.
24. Oortmerssen van, G. A.; Dijk, D. J.; Schuurman, T. Studies in wild house mice II. Testosterone and aggression. Horm. Behav. 21:139–152; 1987.
25. Pollak, E. I.; Sachs, B. D. Masculine sexual behavior and morphology: Paradoxical effects of perinatal androgen treatment in male and female rats. Behav. Biol. 13:401–411; 1975.
26. Ruiter de, A. J. H.; Koolhaas, J. M.; Keijser, J. N.; van Oortmerssen, G. A.; Bohus, B. Differential testosterone secretory capacity of the testes of aggressive and non-aggressive house mice during ontogeny. Aggress. Behav. 18:in press.
27. Saal vom, F.; Svare, B.; Gandelman, R. Time of neonatal androgen exposure influences length of testosterone treatment required to induce aggression in adult male and female mice. Behav. Biol. 17: 391–397; 1976.
28. Saal vom, F. S.; Gandelman, R.; Svare, B. Aggression in male and female mice: Evidence for changed neural sensitivity in response to neonatal but not adult androgen exposure. Physiol. Behav. 17:53–57; 1976.
29. Schuurman, T. Hormonal correlates of agonistic behavior in adult male rats. In: McConnell, P. S.; Boer, G. J.; Romijn, H. J.; van de Poll, N. E.; Corner, M. A., eds. Progress in brain research. vol. 53. Adaptive capabilities of the nervous system. Amsterdam: Elsevier; 1980:415–420.
30. Vanderstichele, H.; Eechaute, W.; Lacroix, E.; Leusen, I. Influence of neonatal androgenization on the testicular steroidogenesis in the adult rat. J. Steroid Biochem. 28(4):421–427; 1987.
31. Ward, I. L. Prenatal stress feminizes and demasculinizes the behaviour of males. Science 175:82–84; 1972.
32. Ward, I. L.; Weisz, J. Maternal stress alters plasma testosterone in fetal males. Science 207:328–329; 1980.
33. Weisz, J.; Ward, I. L. Plasma testosterone and progesterone titers of pregnant rats, their male and female fetuses, and neonatal offspring. Endocrinology 106:306–316; 1980.
34. Zadina, J. E.; Dunlap, J. L.; Gerall, A. A. Modifications induced by neonatal steroids in reproductive organs and behavior of male rats. J. Comp. Physiol. Psychol. 93:314–322; 1979.