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Sexual incentive motivation and male and female copulatory behavior in female rats given androgen from postnatal day 20

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ABSTRACT

Masculinization and feminization of rat sexual behavior has been supposed to occur during a short postnatal period. However, much data have made it evident that these processes may continue until adolescence. In the present study, we evaluated whether androgen treatment of females from postnatal day 20 and onwards could alter sexual motivation and behavior in a male direction. Juveniles were ovariectomized on day 20 and concurrently implanted with Silastic capsules containing either testosterone or dihydrotestosterone. Controls were implanted with an empty capsule. Tests for sexual incentive motivation and male sexual behavior were performed every fifth day when the females were between 50 and 75 days of age. At day 80, a test for female sexual behavior was performed. Females treated with testosterone approached a female sexual incentive far more than a male incentive, showing that sexual motivation had been changed in a male-like direction. Dihydrotestosterone had a similar, albeit smaller, effect. Females implanted with an empty capsule approached both incentives equally. Testosterone produced a high level of mounting behavior, whereas intromission-like behavioral patterns were rare and ejaculation-like behavior was absent. In the test for female sexual behavior, the testosterone-treated animals displayed a relatively high lordosis quotient, far above that displayed in females implanted with dihydrotestosterone or an empty capsule. It is concluded that treatment with an aromatizable androgen during the peripubertal-adolescent period masculinizes sexual motivation and partly sexual behavior. A non-aromatizable androgen weakly masculinize sexual motivation without enhancing male sexual behavior. It appears that simultaneous actions on androgen and estrogen receptors are needed for significant masculinization during the period studied here. Since the testosterone-treated females displayed lordosis, sexual behavior was not defeminized. In sum, these results suggest that sexual differentiation continues well into the peripubertal and adolescent periods.

1. Introduction

Some individuals are attracted to and engage in sexual behavior with members of their own sex, while others are attracted to the opposite sex. The factors that determine the preference for the same sex have fascinated scientists for a long time, whereas the causes of preference for the opposite sex have been largely ignored. However, studies in rodents have suggested that gonadal hormones determine both kind of preferences. Originally, it was thought that these hormones have an organizational effect on the central nervous system in such a way that perinatal exposure to androgens or estrogens masculinizes future sexual behavior whereas the absence of gonadal hormones will lead to female sexual behavior when adult (reviewed in [1]). In males, aromatization of testicular androgens within target areas in the brain appears to be essential for masculinization (reviewed in [2]). The locally produced estrogens act on the estrogen receptor α (ERα), whereas actions at the estrogen receptor β (ERβ) are not needed [3].

In females, gonadal hormones were considered unnecessary for feminization of brain and behavior. However, ovariectomy on postnatal day 1 leads to reduced paracopulatory behaviors in hormone-primed females at a series of tests starting when the females were 90 days old [4]. Likewise, females treated with the estrogen receptor antagonist tamoxifen on postnatal days 1 – 5 show reduced sexual behavior when treated with estradiol (E) + progesterone (P) as adults [5]. Furthermore,

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mice lacking aromatase display reduced female sexual behavior and do not approach olfactory stimuli from a sexually active male [6]. E treatment between postnatal days 15 and 25 restored behavior, whereas treatment between postnatal days 5 and 15 did not [7]. There are also data showing that perinatal treatment with testosterone (T) or E cause permanent defeminization, whereas treatment with the non-aromatizable androgen DHT does not [8-13]. In sum, the role of neonatal gonadal hormones for feminization of adult sexual behavior does not seem to be entirely clear.

Data concerning masculinization of females are contradictory. For example, E administered shortly after birth enhances male behavior patterns in adulthood [10, 11] and produces a preference for approaching other females [13]. However, lack of effect of early E treatment has also been reported [12]. Likewise, the effects of perinatal T treatment on masculinization of females are conflicting. Some studies report enhanced mounting (e.g. [14, 15]) whereas others fail to find an effect of perinatal T (e.g. [16, 17]). Thus, the effects of neonatal treatment with E or T on masculinization in female rats are as unclear as those on feminization. One possible explanation for the conflicting results reported may be that neonatal hormone exposure is not enough neither for masculinization nor for feminization. Indeed, several observations suggest that the organizational actions of gonadal hormones may extend into the peripubertal period and perhaps even later.

In female rats, the onset of puberty is generally marked by vaginal opening, occurring between postnatal days 32 and 34. This event is associated with the first ovulation, and consequently with high levels of estrogens (reviewed in [18]). The period following puberty, ranging between 35 and 60 days of age, has been named adolescence [19, 20]. It is characterized by profound neurobiological and behavioral changes (e.g. [21]), and may be considered a kind of sensitive period ([22] and references therein). Indeed, steroid hormones are involved in structural modifications in brain areas important for sexual behavior during adolescence [23]. The consequences of this for sexual behavior has been evaluated in only a handful of studies.

The absence of ovarian hormones during the peripubertal and adolescent periods does not seem to affect sexual behavior in female rats. Ovariectomized females given E from postnatal day 30 until day 90 were not different from control females when tested for female sexual behavior after a period of T treatment. However, in tests with a sexually receptive female as partner, the group treated with E during puberty and adolescence showed a far higher frequency of mounting and intromission-like behaviors than controls. In tests for sexual approach behavior, these females approached other females more than they approached males [24]. Thus, peripubertal and adolescent exposure to E masculinized sexual behaviors without any associated defeminization.

The effects of administration of androgens during the peripubertal-adolescent period in female rats on T-induced, adult sexual behavior has not been evaluated. In the present experiment, we ovariectomized females on postnatal day 20 and simultaneously we initiated treatment with T. This treatment continued for the entire experiment. Tests for sexual approach behavior and for male as well as female copulatory behavior were performed between postnatal days 50 and 80.

Considering that T is easily aromatized to E, we added a group treated with the highly potent, but non-aromatizable, androgen dihydrotestosterone (DHT). This allowed us to determine whether specific stimulation of the androgen receptor has effects similar to the simultaneous stimulation of estrogen and androgen receptors brought about by T. This experiment will determine whether peripubertal – adult T treatment can masculinize or defeminize female rat sexual behavior.

2. Materials and methods

2.1. Animals

Male and female Wistar Han IGS rats (about 350 g and 250 g upon arrival, respectively) were purchased from Charles River (Sulzfeld, Germany). They were housed in same-sex pairs in Macrolon IV cages in a room with an inverted light/dark cycle (lights on 23 – 11). The temperature was 21 ± 1 °C and relative humidity was 50 ± 10%. Commercial rat pellets and tap water were available ad libitum. Some males and females were used for breeding, whereas others were used as copulation partners or sexual incentives. The males were left intact, while the latter females were ovariectomized under isoflurane anesthesia about two weeks before being used. They were given estradiol benzoate (EB), 25 μg/rat about 48 h prior to test, and progesterone, 1 mg/rat, about 4 h before. Maximum receptivity and proceptivity at the time of testing was thereby assured. This experiment was approved by the Norwegian Food Safety Authority (ID5510) and animal care was in agreement with the European Union council directive 2010/63/EU.

2.2. Procedure

About two weeks after arrival, breeding was initiated. One female and three males were put together for one week. The males were then removed, and the female was observed daily. In case of signs of pregnancy, nesting material was provided. The litter was left with the mother until weaning on postnatal day 20. On that day, the female juveniles were ovariectomized under isoflurane anesthesia. In conjunction with the ovariectomy, 10 females were implanted with a 20 mm long, empty Silastic capsule (medical grade Silastic tubing, 1.6 mm inner diameter, 3.2 mm outer diameter, Degania Silicone, Degania Bet, Israel), 9 females with a similar capsule filled with T, and 8 females received two 20 mm long capsules filled with DHT. Wounds were closed with surgical clips. The females were given postoperative buprenorphine, 0.05 mg/kg subcutaneously, every 12 h for 72 h. The male juveniles were used in other experiments.

Silastic capsules of the size used here maintain the accessory sexual glands in castrated, adult males in a state similar to that found in intact males [25]. In that sense they can be assumed to produce serum concentrations of T and DHT within the physiological range. The capsules have been shown to maintain a rather stable serum level of T for more than two months [26], i.e. for the entire duration of the present study. Likewise, DHT-filled capsules maintain the integrity of the accessory sexual glands in castrated rats for at least 24 weeks [27], suggesting serum concentrations within the physiological range. However, in the juvenile females used in the present study, the hormone levels produced by these capsules must be entirely outside of that range. This is unproblematic, since the purpose of this experiment was to determine whether these androgens could modify sexual differentiation in females during the peripubertal and adolescent periods. We will make no claim whatsoever that they may do so in physiological condition. This is a study of endocrine engineering, not of regular endocrine functions.

After ovariectomy and capsule implantation, the females were housed in pairs until postnatal day 45. From then and until the end of the experiment, they were housed singly. The animals were weighed every 10th day throughout the entire experiment.

Tests for sexual incentive motivation were performed every 5th day, from postnatal day 50 until postnatal day 75. The testing arena and procedure have been described in detail elsewhere [28, 29]. Briefly, the test environment consists of an oval arena (100 × 50 cm) with 45 cm high walls. In the long walls there are two diagonally opposed openings covered with a double wire mesh. Behind the wire mesh, an incentive animal, inaccessible to the subject in the arena, was located. Incentives in this experiment were an intact male and a sexually receptive female. Although no physical contact was possible between the incentive animals and the experimental subject, they could see, hear and smell each other. At the beginning of a test, the subject was placed in the middle of the arena. A videotracking system (Ethovision, Noldus, Wageningen, the Netherlands) determined the subject's position. A virtual area of 21 × 29 cm was defined in front of each of the two openings. The videotracking system determined the time spent in each of these areas (incentive zones) and the number of visits to them. It also calculated the

distance moved during the test, the time spent moving, and the mean velocity of movement while moving. Each test lasted 10 min. The setup was located in a dimly lit room. Light intensity on the bottom of the arena was 1 lx.

Immediately after the sexual incentive motivation test, the experimental animals were transferred to a different room for observation of copulatory behavior. The experimental female was placed in a rectangular steel sheet cage (40 × 60 cm, 40 cm high) with Plexiglas front. The floor was covered with wood shavings. Five min later, a sexually receptive female was introduced. Male behavior patterns performed by the experimental female were recorded. Mounts were counted only when pelvic thrusting was present. Intromission-like behavior was identified by the intense forward thrust at the end of a thrusting train, immediately followed by a forceful backward movement. Even though behavior patterns similar to male ejaculation have been described in females [30, 31], no such behavior was observed in the present study. The mount latency (time from the introduction of the stimulus female until first mount displayed by the experimental female was also recorded. The test lasted for 30 min.

An additional test for copulatory behavior was made on postnatal day 80. Here, the partner was a sexually experienced male rather than a female as was the case in the earlier tests. This male had been allowed to perform one intromission with another female immediately before the test. This female was then withdrawn from the observation cage and replaced by an experimental female. A different male was used for each female. Female behavior patterns (lordosis, paracopulatory behaviors and rejections) were recorded until the female had received 10 mounts (including mounts associated with intromission). The time the male needed to complete the ten mounts was also recorded. This time may depend on the female's attractivity as much as on male vigor.

2.3. Data preparation and statistical analysis

Besides the variables recorded in the sexual incentive motivation test, we calculated the preference score (time spent with the female incentive / (time spent with the male incentive + time spent with the female incentive)). A score of 0.5 indicates that the female subject spent the same time with both incentives, whereas a score above 0.5 means a preference for the female incentive, and a score below 0.5 means a preference for the male incentive. The one-sample *t*-test was used for evaluating whether the observed preference score differed from 0.5, i.e. no preference. We also calculated the mean preference score obtained over the six tests. This score was analyzed with a one factor ANOVA with treatment as factor. The mean time spent with the incentives over the six tests was analyzed with two-factor ANOVA with treatment as between-subjects factor and sex of the incentive as within-groups factor. In addition, we analyzed the preference score at each of the six tests, the distance moved during the test, the time moving and the velocity of movement at each of the six tests with two-factor ANOVAs with repeated measures on one factor. The within-subjects factor was test (1 to 6) and the between-groups factor was treatment (empty capsule, T and DHT). Body weight was analyzed in a similar way. The time spent with the incentives and the number of visits to them at each test were analyzed with three-factor ANOVAs with repeated measures on two factors. The within-groups factors were incentive (male, female) and test (1 to 6) and the between groups factor was treatment (empty, T, DHT). In case of significant omnibus test, post hoc comparisons were made with Tukey's HSD test. Tests for simple main effects were used after significant interactions.

The proportion of animals displaying mounts and intromissions at each of the six tests for masculine copulatory behavior was analyzed by the chi-square test. The Bonferroni correction was used to protect *p*-values. After significant results, post hoc comparisons were made with Fisher's exact test. The number of mounts and intromissions at each test was analyzed by the Kruskal-Wallis ANOVA with Bonferroni correction. This procedure was also used for evaluating the lordosis quotient (the number of lordosis displayed / the number of mounts received), paracopulatory behaviors and rejections in the test for female copulatory behavior. It was also used for analyzing the time needed by the male to perform the ten required mounts. After a significant result, pairwise comparisons were made with Dunn's test, with *p*-values adjusted with the Bonferroni correction. A *p*-value, adjusted when appropriate, below 0.05 was considered significant.

3. Results

3.1. Body weight

As can be seen in Fig. 1, the treatments had no effect on body weights ($F_{2,24} = 0.233$, $p = 0.794$). Not surprisingly, the age of the animals had a substantial effect ($F_{4,96}$) = 1752.90, $p < 0.001$). More important, the growth was similar after all treatments, manifested as a non-significant interaction between day and treatment ($F_{8,96} = 1.12$, $p = 0.359$).

3.2. Sexual incentive motivation

In order to consider treatment effects on sexual incentive motivation to be functionally significant, it is necessary to find both increased preference score and an increase in the time spent with the corresponding incentive. An increased preference score may be the result either of reduced time spent with one of the incentives or increased time spent with the other incentive, or a mixture of both. An increased score can be interpreted as enhanced motivation only if caused by an increase in the time spent with one of the incentives. An increased score based on reduced time spent with one of the incentives cannot be interpreted as an indication of increased sexual motivation. It should rather be interpreted as increased aversion. However, an increase in time spent with one of the incentives is not sufficient by itself. It could be due to an increase in sociability, i.e. simultaneous increase in time spent with both incentives. To rule out these alternative explanations, both the preference score and the time spent with the sexual incentive must be enhanced in order to propose that sexual motivation was increased. More extensive discussion of the interpretation of data from the sexual incentive motivation test has been presented elsewhere [32, 33].

There was a significant main effect of treatment on the mean preference score ($F_{2,24} = 13.82$, $p < 0.001$). The Tukey HSD test showed that all groups differed, with the lowest score found in the group implanted with an empty capsule. Then followed the DHT group, while the highest

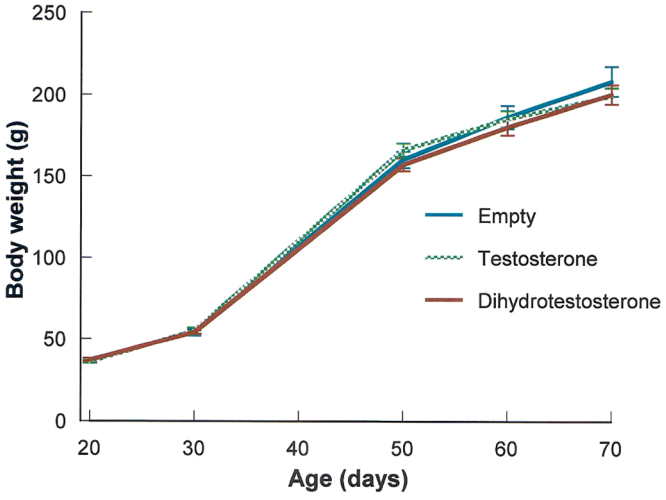

Fig. 1. Body weight from day 20 to 70 in female rats ovariectomized at postnatal day 20 and concurrently implanted with an empty Silastic capsule (Empty), a capsule filled with T or capsules filled with DHT. Weight data from postnatal day 40 were lost. Data are mean ± SEM.

score was found in the T group. The score obtained in the empty capsule group did not differ from 0.5 ($t_9 = 1.059$, $p = 0.317$), meaning that the experimental females distributed their time equally between the male and female incentive. In the DHT group, the preference score was above 0.5 ($t_7 = 3.414$, $p = 0.011$). Consequently, the females in this group preferred the female incentive over the male. This was also the case in the T group ($t_8 = 12.186$, $p < 0.001$). Data are shown in Fig. 2.

When analyzing the data from each of the six tests, we found a significant main effect of test on the preference score ($F_{5,120} = 2.927$, $p = 0.016$). However, comparisons between all tests with the Tukey HSD test failed to detect any difference. Nevertheless, as illustrated in supplementary Fig. 1, the preference score was low in all groups at the test performed on postnatal day 50. In later tests, the score oscillated around 0.5 in the group implanted with an empty capsule whereas it was consistently high in the T-group. The DHT group was intermediate at all tests. Consequently, the interaction test x treatment was non-significant ($F_{10,120} = 0.488$, $p = 0.895$).

Turning to the time spent with the incentives, ANOVA showed that there was no main effect of treatment ($F_{2,24} = 1.28$, $p = 0.296$). The main effect of incentive was significant ($F_{1,24} = 49.653$, $p < 0.001$), as was the interaction treatment x incentive ($F_{2,24} = 11.622$, $p < 0.001$). Tests for simple main effects of incentive within each treatment revealed that the females implanted with an empty capsule spent equal time close to the male and female incentive ($F_{1,24} = 0.703$, $p = 0.411$) while the T-treated females spent more time with the female incentive than with the male incentive ($F_{1,24} = 55.34$, $p < 0.001$). This was also the case with the DHT-treated females ($F_{1,24} = 14.39$, $p = 0.001$). These results are illustrated in Fig. 3.

We then analyzed the data from each test. There was no effect of test ($F_{5,120} = 1.907$, $p = 0.098$) and the interactions test x treatment, test x incentive and test x incentive x treatment were all non-significant (all $ps > 0.100$). This becomes evident upon examination of supplementary Fig. 2, in which data from each test and treatment are shown.

The mean number of visits to the incentive was unaffected by treatment ($F_{2,24} = 1.301$, $p = 0.291$). However, there was a difference between incentives ($F_{1,24} = 15.905$, $p = 0.001$). When comparing the male and female incentive in each treatment with the Tukey HSD test it

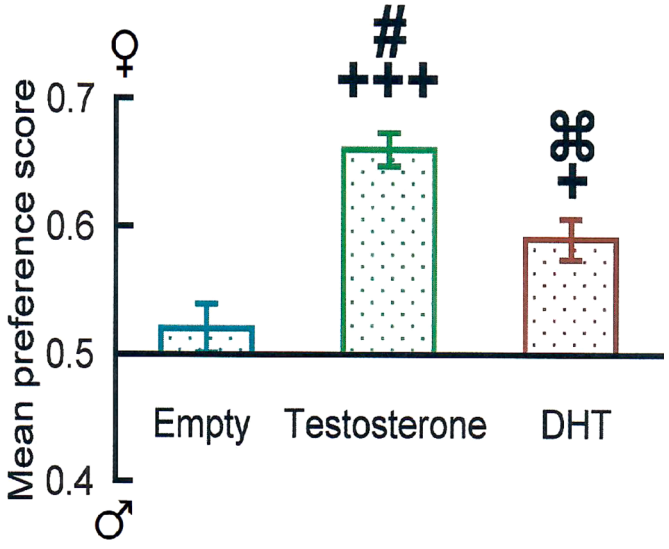

Fig. 2. Preference score average from the six tests for sexual incentive motivation performed between postnatal days 50 and 75 in female rats implanted with an empty Silastic capsule (Empty), a capsule filled with T or capsules filled with DHT. A preference score of 0.5 indicates no preference, whereas a score above 0.5 indicates preference for the female incentive. Data are mean ± SEM. +, the female incentive preferred over the male, $p < 0.05$; +++, $p < 0.001$. #, different from empty capsule, $p < 0.001$. ⌘, different from empty capsule and T, $p < 0.05$.

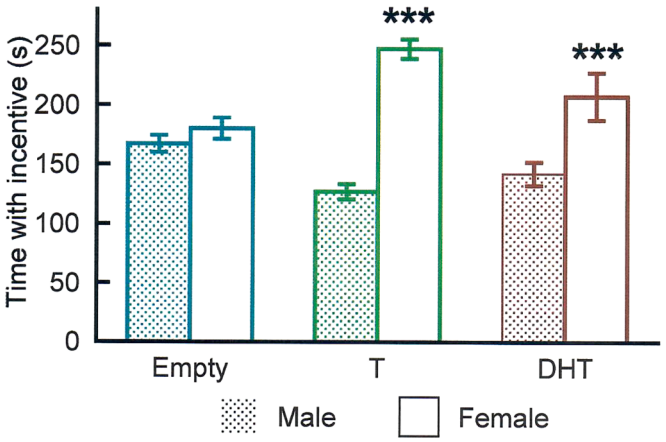

Fig 3. Mean ± SEM time spent with the incentives in female rats implanted with an empty Silastic capsule (Empty), a capsule filled with T or capsules filled with DHT. Average of the 6 tests. ***, different from the time spent in the male incentive zone in the same treatment, $p < 0.001$.

was found that the females implanted with an empty capsule made an equal number of visits to the male and female incentive ($p = 0.406$) while those implanted with T or DHT containing capsules made more visits to the female than to the male incentive ($p = 0.001$ and $p = 0.014$, respectively). These data are illustrated in Fig. 4. Supplementary Fig. 3 shows the number of visits to each of the incentives at each of the six tests. The tests differed ($F_{10,120} = 4.639$, $p = 0.001$) whereas the interactions treatment x incentive ($F_{2,24} = 1.354$, $p = 0.277$), treatment x test ($F_{10,120} = 1.904$, $p = 0.051$) and test x incentive ($F_{5,120} = 0.347$, $p = 0.884$) turned out to be non-significant. This was also the case for the three-factor interaction test x incentive x treatment ($F_{10,120} = 0.975$, $p = 0.469$).

3.3. Ambulatory activity

The hormone treatments did not affect any of the measures of ambulatory activity. Data and statistics are shown in Supplementary Fig. 4.

3.4. Copulatory behavior

The proportion of females displaying mounts differed between the

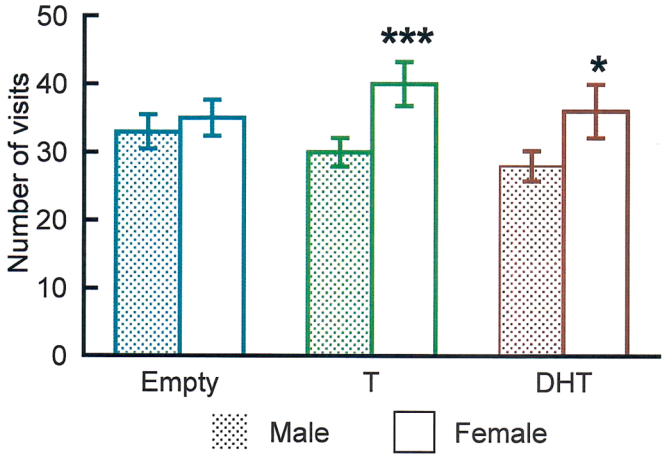

Fig. 4. Mean ± SEM number of visits to the incentives in female rats implanted with an empty Silastic capsule (Empty), a capsule filled with T or capsules filled with DHT. Average of the 6 tests. *, different from the number of visits to the male incentive zone in the same treatment, $p < 0.05$; ***, $p < 0.001$.

groups at all tests (chi-square values, all with df = 2, uncorrected *p*-values: Test at 50 days, 13.25, $p = 0.001$; test at 55 days, 20.67, $p < 0.001$; test at 60 days, 24.66, $p < 0.001$; test at 65 days, 21.03, $p < 0.001$; test at 70 days, 24.66, $p < 0.001$; test at 75 days, 21.03, $p < 0.001$; critical value for Bonferroni corrected *p* is 0.008). It was always larger in the T-group than in the group implanted with an empty capsule whereas there was no difference between the latter and the DHT-group according to the Fisher test. At the test performed on day 50, there was no difference between the females treated with T and DHT. However, these groups differed at all subsequent tests (see Fig. 5A). The number of mounts also differed between groups at all tests (values of Kruskal-Wallis *H* with 2 degrees of freedom, uncorrected *p*-values: Test at 50 days, 12.10, $p = 0.002$; test at 55 days, 17.28, $p < 0.001$; test at 60 days, 27.00, $p < 0.001$; test at 65 days, 22.74, $p < 0.001$; test at 70 days, 27.00, $p < 0.001$; test at 75 days, 26.54, $p < 0.001$; Bonferroni corrected critical *p*-value is 0.008). Dunn's test revealed that the number of mounts was larger in the T-group than in the group implanted with an empty capsule at all tests. However, there was no difference between the T- and DHT-groups at the test on day 50, although these groups differed at all later tests. Data are shown in Fig. 5B.

Even though most females in the T-group displayed mounts, few females in the group implanted with an empty capsule (one female mounted once in one of the six tests) and in the DHT-group (one female mounted in two tests, two females mounted in one of the tests) did so. Since the mount latency could be recorded only for mounting females, statistical between-groups comparison would not be meaningful.

The proportion of females displaying the intromittive behavior pattern did not vary significantly between groups after the Bonferroni correction had been applied. In fact, only 33% of the females ever displayed intromission-like behavior (data not shown).

The test for female copulatory behavior performed on postnatal day 80 showed that the treatments differed with regard to LQ ($H_2 = 18.63$, $p < 0.001$) and the number of paracopulatory behaviors ($H_2 = 10.22$, $p = 0.006$) displayed during the test (Fig. 6A and 6 B). Post hoc tests revealed that the group treated with T had higher LQ and showed more paracopulatory behaviors than the other groups. There was no difference between the groups implanted with an empty capsule and that implanted with DHT-filled capsules. The number of rejections ($H_2 = 4.38$, $p = 0.112$) as well as the time needed for receiving 10 mounts ($H_2 = 0.20$, $p = 0.905$) were unaffected by treatment; Figs. 6C and D).

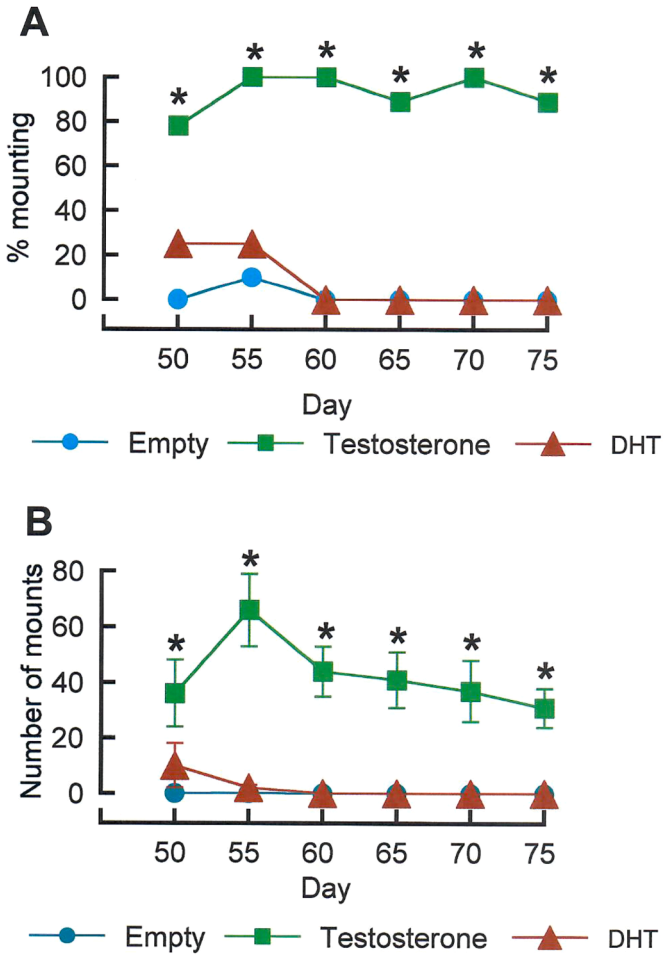

Fig. 5. Male copulatory behavior in female rats implanted with an empty Silastic capsule (Empty), a capsule filled with T or capsules filled with DHT at each of six tests performed between postnatal days 50 and 75. A. Proportion of females displaying mounts. B. Number of mounts. *, different from empty, $p < 0.05$; #, different from DHT, $p < 0.05$. Data are mean ± SEM.

4. Discussion

T treatment from weaning to adulthood made the females prefer to approach another female rather than a sexually active male. Considering that T is easily aromatized to E, and that treatment with E during the peripubertal-adolescent period also causes a preference for the female [24], this observation is not surprising. What might be surprising is the fact that DHT also made the females prefer to approach another female, albeit somewhat less than after treatment with T. This shows that stimulation of androgen receptors without any concomitant actions at estrogen receptors can masculinize sexual preferences in females. When DHT was given to adult, ovariectomized females, no effect on sexual approach behaviors could be detected [34, 35]. It appears, then, that exposure to DHT during the peripubertal and adolescent periods is necessary for effects of this steroid in the adult female.

Contrary to the lack of effect of DHT treatment in adult females, T causes a robust approach to a male in such females [34, 36]. In fact, T is as efficient as E. Furthermore, the effects of T were much reduced by concurrent administration of the estrogen antagonist MER-25. It was concluded that T needs to be aromatized to E before affecting female sexual approach behaviors [34]. These observations show that treatment with T limited to the adult period has effects completely opposite to those seen after peripubertal – adolescent treatment in the way that the former leads to a preference for approaching a male whereas the latter leads to a preference for other females. Consequently, the effects of T as well as those of DHT on sexual approach behaviors found in the present study must be attributed to actions during the peripubertal-adolescent period.

The data reported here imply that T and DHT have an organizational effect far beyond the neonatal period. The activational effect, i.e. the actions of these hormones in the adult, presumably already organized brain, is determined by their prior organizational actions. Therefore, the effect of T and DHT in our females is opposite to that found in females not exposed to these androgens during peripuberty – adolescence, as mentioned in the preceding paragraph.

It might be interesting to compare the sexual incentive motivation shown by the females in the present study to that normally shown by males. Although we did not run a group of males as part of the present experiment, we have a substantial amount of data from males collected in exactly the same setup as used here. The preference score in intact male rats ranges from 0.65 – 0.78 (see, e.g. [25, 28, 37, 38]). The mean score observed in the T-treated females in the present experiment was 0.66 ± 0.01 (mean ± SEM), i.e. close to the low range of intact males. Castrated males wearing a DHT-implant of the same size as our females had a score of 0.62 [25] whereas our DHT-treated females had a score of 0.59 ± 0.02 (mean ± SEM). All preference scores mentioned here were associated with a corresponding increase in the time spent with the sexual incentive. It appears that both T and DHT have effects of about the same magnitude in our females as they have in males, reinforcing the notion that peripubertal – adolescent treatment masculinizes adult

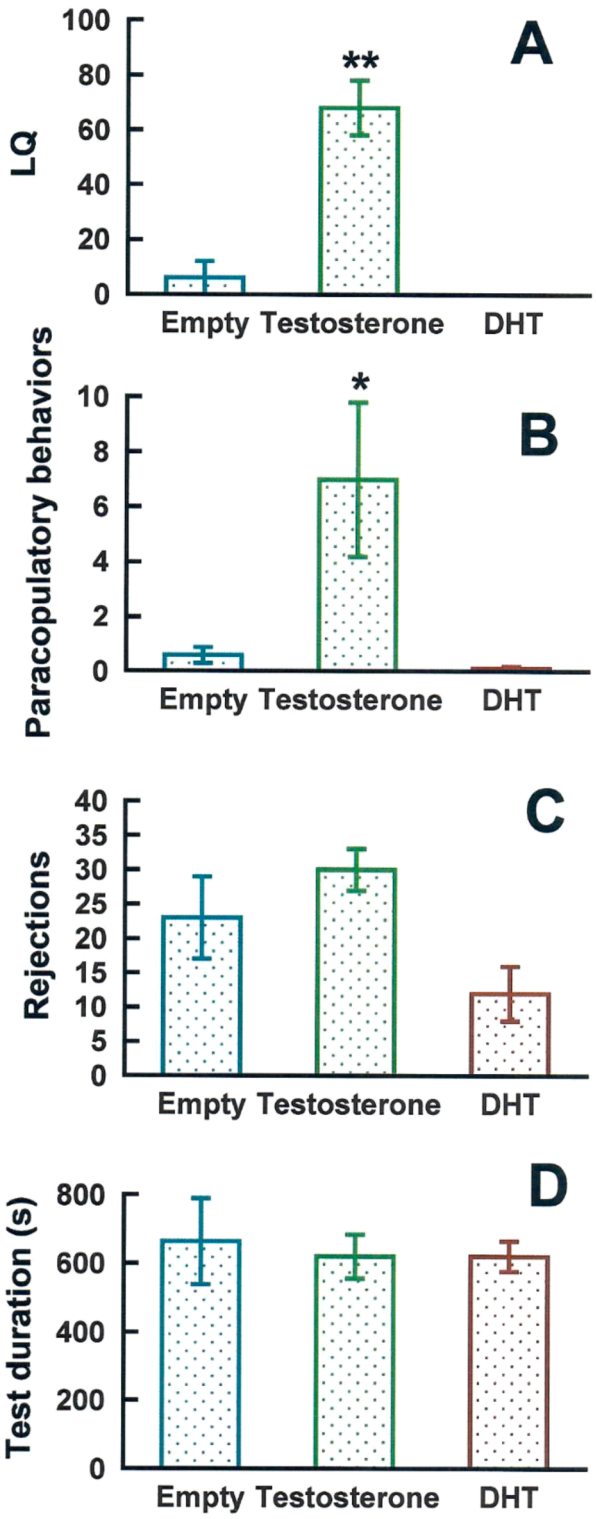

Fig. 6. Female copulatory behavior in female rats implanted with an empty Silastic capsule (Empty), a capsule filled with T or capsules filled with DHT at a test performed when the females had reached an age of 80 days. A. LQ. B. Number of paracopulatory behaviors performed during the test. C. Number of rejections displayed during the test. *, different from empty and DHT, $p < 0.05$; **, $p < 0.01$.

approach behavior.

Comparisons of male copulatory behavior between males and females are difficult because fundamental aspects of this behavior, like intromission and ejaculation, were extremely rare in our females, probably because of the lack of a penis. The only reasonably frequent male behavior pattern observed in the present study was mounting in the T-treated females. This behavior was displayed with a latency of 198 ± 60 s, which is far above what is normally observed in males. In our testing conditions, castrated males implanted with a T capsule of the same size as used in the females, have a mount latency of 21 s. Even though a statistical comparison would be inappropriate, it seems safe to conclude that male copulatory behavior in our females was less masculinized than sexual incentive motivation.

DHT affected neither male nor female copulatory behavior despite the fact that it stimulated approach to other females. This pattern of effect is quite similar to what we earlier observed in male rats, castrated as adults and then treated with DHT. These males approached a sexually receptive female more than they approached another male, yet they displayed almost no sexual behavior [25]. Incidentally, it can also be mentioned that these males approached the female incentive less than males treated with T, exactly as was the case with the females in the present experiment. It appears that the effects of DHT in females exposed to this androgen during the peripubertal-adolescent period is very similar to the effects seen in adult males. Therefore, we propose that sexual approach behavior had been masculinized by peripubertal-adolescent DHT treatment. It must also be pointed out that sexual approach behaviors are controlled by mechanisms somewhat different from those controlling copulatory behavior. For example, there is no correlation between the intensity of approach and intensity of copulation, and some drugs may affect one of these processes without altering the other (see [39] for an extensive discussion). The fact that DHT-treated females approach other females without copulating with them is just another example of this partial independence.

T stimulated mounting of other females, coincident with its effects on sexual approach to the female incentive. There are many reports of T-stimulated mounting in adult female rats (e.g. [40] and references therein). When low doses of T are used, mounting is stimulated without any concurrent stimulation of lordosis behavior, and lordosis may be enhanced by E without any concurrent effect on mounting [41]. In the present study, T enhanced receptivity as well as mounting. Furthermore, a substantial amount of paracopulatory behaviors were displayed by the T-treated females. This is remarkable, since these behaviors are only displayed after rather high doses of estradiol. When low doses of estradiol are used, progesterone is needed for the display of paracopulatory behavior [42, 43]. Thus, the fact that the complete female copulatory behavior pattern was shown by the T-treated females in the absence of progesterone clearly demonstrates that the peripubertal – adolescent hormone treatment did not defeminize copulatory behavior.

There are many studies showing that large doses of T in adult females efficiently stimulates female copulatory behavior (e.g. [44, 45]), probably because of aromatization. Indeed, there are data showing that the effect of T can be blocked by estrogen receptor antagonists [46, 47] or an aromatase inhibitor [48]. As is often the case in the field, there are also contradictory observations. The aromatase inhibitor fadrozole failed to reduce the stimulatory effect of T on Fos responses in sexually relevant brain structures [49]. There are also older data suggesting a role for the androgen receptor in female sexual behavior. Long-term treatment with large doses of DHT stimulated lordosis in females ovariectomized as adults [50]. This effect was not replicated here, since DHT had no effect neither on male nor on female copulatory behavior patterns. It may also be mentioned that treatment with large doses of DHT around the same time as EB is given to ovariectomized females will lead to a considerable inhibition of lordosis behavior (e.g. [51, 52] and references therein). To the contrary, when DHT is given shortly before behavioral tests in EB primed females, lordosis is facilitated [53]. Thus, the effects of DHT on female sexual behavior are contradictory, ranging from stimulation

through no effect to inhibition, depending on the timing of hormone administration, dose, and probably also on the age at which treatment was performed.

A relevant question here is whether unmanipulated females ever are exposed to the amounts of DHT employed in the studies mentioned above. We have already manifested that the amount used in the present study is far above any possible physiological level. Nevertheless, the enzyme responsible for DHT production, 5α–reductase, is widely distributed in the brain of females, and there is no sex difference [54]. However, the serum T concentration is far lower in females than in males (e.g. [55, 56]), thereby limiting the availability of substrate for the 5α-reductases in females. Despite the modest T concentration in females, the concentration of DHT in hypothalamic tissue is similar in males and females during the period between 20 and 60 days of age [57]. Even though actions of DHT at central androgen receptors may be of limited importance in the female, this hormone has been shown to enhance the expression of 5α-reductase. This enzyme is necessary for the production of 5α-reduced progesterone derivatives [58, 59], some of which have been shown to affect female sexual behavior [see [60] and references therein). Whether such actions of DHT have any importance for the results reported here is uncertain, but it would be premature to exclude the possibility that DHT has some physiological function in females.

The present study shows that DHT, in supraphysiological doses, somewhat affects female sexual motivation. Perhaps prolonged overproduction of DHT, due to some pathological condition, could alter the direction of female sexual preferences. In fact, DHT implant into female rats on postnatal day 21 has become a rodent model of the polycystic ovary syndrome in women [61]. This shows that DHT has effects in female reproductive organs, at least. Although this model is extensively used [62], no data concerning sexual motivation in these DHT treated female rats have been reported. However, sexual dysfunction is more common in infertile women diagnosed with the polycystic ovary syndrome than in women suffering infertility from other causes [63]. Desire, arousal, orgasm and lubrication were all reduced. Insofar as the polycystic ovary syndrome may be associated with elevated androgen production starting already before puberty and continuing even during menopause [64-66], these observations suggest that androgen exposure during a period similar to the one in the present study may have adverse effects in humans. Another study found that women diagnosed with polycystic ovary syndrome are more attracted to other women than healthy controls are [67]. This observation coincides with the results of the present study.

5. Conclusion

Present data show that treatment with T or DHT from postnatal day 20 and on leads the female to prefer to approach another female rather than a male. Sexual approach behavior was masculinized. Since T was more efficient than DHT, it may be concluded that the simultaneous action at both estrogen and androgen receptors is more efficient than isolated action on the androgen receptor. Copulatory behavior was masculinized since abundant mounts were displayed, but not defeminized because lordosis also was displayed. The LQ was quite high, particularly when considering that no progesterone was administered. These observations show that exposure to androgens long after the neonatal period changes female rat sexual behavior in a male direction. It is most important to note that the females were exposed to substantial amounts of androgens during the post-weaning, peripubertal and adolescent periods. Similar amounts could never be found in unmanipulated females. Here we report what androgens can do to females during this period, not what they are normally doing.

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Declaration of competing Interest

The authors declare that there are no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.physbeh.2021.113460.

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