



NOTE

Physiology

Male Hatano low-avoidance rats show more active sexual behavior with lower plasma testosterone than high-avoidance rats

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ABSTRACT. Two inbred strains of Sprague-Dawley rats, known as the Hatano high- and low-avoidance animals (HAA and LAA respectively), have been selectively bred for high versus low rates of avoidance responses in a shuttle-box avoidance task. To investigate differences in the sexual behavior of Hatano rats, male HAA, LAA and SD rats were tested from 12 to 15 weeks of age. LAA rats exhibited more rapid and frequent sexual behavior than HAA or SD rats, and such differences increased with repeated sexual experience. Plasma testosterone levels tended to be lower in LAA rats than in HAA or SD rats, suggesting that active sexual behavior in LAA rats is not related to these levels. Strain differences in mating behavior between HAA and LAA rats may be caused by emotional responses to novelty.

KEY WORDS: Hatano rat, male sexual behavior, testosterone

The shuttle box is a device used for evaluating the effects of chemicals in pharmacological and toxicological studies and for testing learning behavior in animals. Hatano rats, bred from Sprague-Dawley (SD) rats, have been selectively inbred for either rapid acquisition of conditioned avoidance responses in shuttle box tasks (high-avoidance animals, HAA) or for failure to acquire this response (low-avoidance animals, LAA). The effects of chemicals on shuttle box avoidance performance using SD rats are puzzling because of the large individual variations typically observed. The selective breeding of two lines of SD rats was carried out at Hatano Research Institute to breed new animal models showing little inter-individual variation in the shuttle box avoidance task [10]. Although HAA and LAA rats were originally selected based on their behavioral pattern in the shuttle box avoidance test, characteristic differences between the two lines were observed not only in their avoidance performance but also in their emotionality and sexual maturation. For example, HAA rats show a higher stress response [1, 2] than LAA rats when exposed to aversive conditions, higher anxiety behavior [8], and greater plasma concentrations of the adrenocorticotrophic hormone (ACTH) [3]. HAA males sexually mature earlier than LAA male [14]. In addition, circulating testosterone concentration tends to be higher in HAA than in LAA rats [14]. Sperm motility is more active in HAA rats than in LAA rats, even though both breed naturally [13].

Roman high-avoidance (RHA) and Roman low-avoidance (RLA) rats, originating from Wistar rats, are famous strains that have been selected and bred for rapid versus poor avoidance performance in the shuttle box avoidance task, respectively [4]. Although RLA rats appear to be similar to LAA rats, RLA rats show higher stress response [7] and anxiety behavior [6] compared to RHA rats. Differences between the Roman and Hatano strains are presumably attributed to selection criteria [11]. During acquisition of avoidance performance, RLA rats show many freezing behaviors while LAA rats show few. Because freezing behavior is considered a defensive reaction rather than an indicator of poor learning capacity, animals showing freezing behavior were discarded from the LAA line during selective breeding. Recently, male Roman strains have been examined for copulatory behavior

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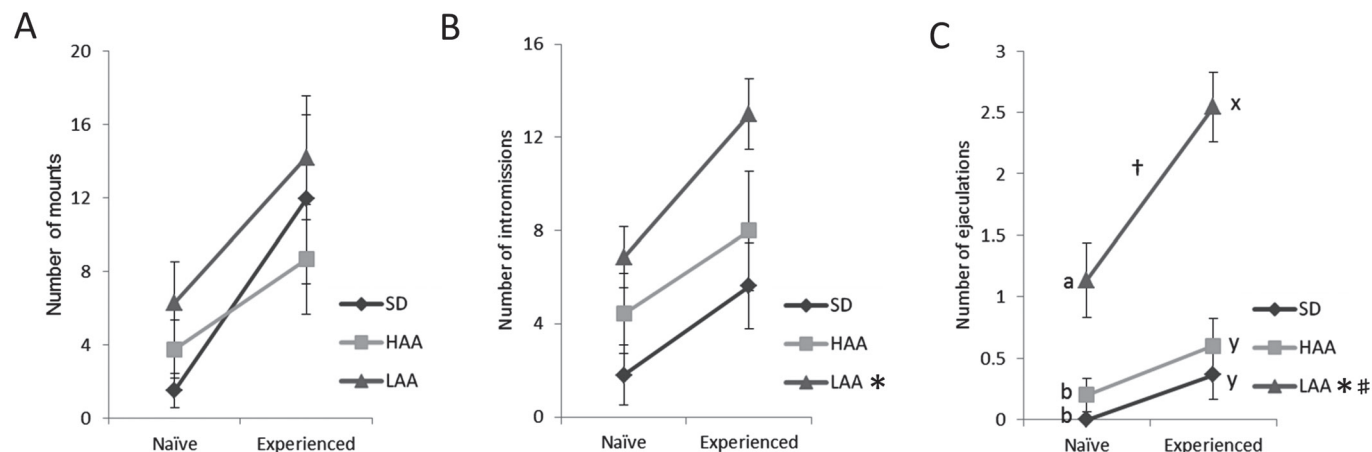


Fig. 1. Number of mounts (A), intromissions (B), and ejaculations (C) in sexually naïve (test one) and experienced (test three) copulations tests (SD: n=11, HAA: n=10, LAA: n=11). Data are mean \pm standard error of the mean (SEM). †, $P < 0.05$ sexually naïve (i.e., copulation test one) with respect to sexually experienced (i.e., copulation test three); *, $P < 0.05$ SD (Sprague-Dawley) compared to LAA (low-avoidance-animal); #, $P < 0.05$ HAA (high-avoidance-animal) compared to LAA. Different lower case letters (a vs b; x vs y) indicate significant differences ($P < 0.05$).

and RHA rats show better sexual performance than RLA rats [12]. This raises the possibility that the two Hatano rat strains may also have different copulatory behaviors. We conducted a copulatory test with male HAA and LAA rats exposed to ovariectomized rats pretreated with estradiol benzoate and progesterone and used SD rats as a reference strain. Moreover, we evaluated endocrine differences among the three rat strains by comparing their testosterone concentrations.

We used male Hatano rats maintained by sib-mating for over 25 years at the Hatano Research Institute, Food and Drug Safety Center (Hadano, Japan). Male and female SD rats were purchased from Charles River Laboratories International, Inc. (Atsugi, Japan). They were housed in polycarbonate cages (200-mm wide \times 410-mm long \times 250-mm high, three rats per cage) with same-sex animals. All animals were maintained in an air-conditioned animal room (temperature, $24 \pm 2^\circ\text{C}$; relative humidity, $55 \pm 15\%$; 12-hr light/dark schedule with lights on from 10:00hr to 22:00hr) with food (MF; Oriental Yeast Co., Tokyo, Japan) and water available *ad libitum*. All procedures were approved by the Animal Care and Use Committee of Meiji University, Japan.

All stimulus females (eight weeks old) were ovariectomized under anesthesia with isoflurane (Mylan, Osaka, Japan). They were injected with estradiol benzoate ($5 \mu\text{g}$ in 0.1 ml sesame oil; Sigma-Aldrich, St. Louis, MO, U.S.A.) 48 hr before the test and injected again with progesterone ($500 \mu\text{g}$ in 0.1 ml sesame oil; Wako, Osaka, Japan) 3 hr before the test.

Male HAA rats (n=10), LAA rats (n=11), and SD rats (n=11) were tested three times for sexual behavior from 12 to 15 weeks of age at weekly intervals. Female SD rats of the same age were used as stimuli in each test. Tests were performed between 15:00hr and 19:00hr. Male rats were moved from their home cage to a mating test cage 3 min before the stimulus female was introduced; all behavioral tests lasted 30 min. Tests were recorded by video for subsequent analysis of behavior categories by two observers unaware of the rat strains used in each test. We used average of these data for analysis. The following parameters were determined: (1) mount latency, (2) intromission latency, (3) ejaculation latency, (4) mount frequency, (5) intromission frequency, and (6) ejaculation frequency.

At the end of the experiments, animals were sacrificed by decapitation. Trunk blood was collected in heparinized tubes, centrifuged immediately, and the plasma was separated and stored at -20°C until assay. Plasma testosterone concentration was measured by a double antibody radioimmunoassay system with ^{125}I -labeled radio ligands as described previously [15]. Intra- and inter-assay coefficients of variation were 6.3 and 7.2%, respectively.

Data were analyzed using the IBM SPSS Statistics software (version 22, Chicago, IL, U.S.A.), and all results were expressed as mean \pm standard error of the mean (SEM). Behavioral variables were analyzed through an analysis of variance (ANOVA) with strain as between-subject factors, and test trial as within subject factors (repeated measure). Differences in plasma testosterone concentrations were analyzed using a one-way ANOVA, followed by Bonferroni post-hoc corrections. Latency to onset of mount, intromission, and ejaculation were analyzed using Kaplan-Meier survival analysis followed by a log-rank comparison test.

Frequencies of sexual behavior in sexually naïve (i.e., copulation test one) and experienced (i.e., copulation test three) males are shown in Fig. 1. Although there was a significant effect of trial in mount frequency [$F(1, 29) = 16.2, P < 0.01$] (Fig. 1A), there were no significant effects of strain [$F(2, 29) = 0.855, P = 0.436$] or trial and strain interaction [$F(2, 29) = 0.675, P = 0.517$]. These results suggest that experienced males showed more frequent mount behavior than naïve males independently of strains.

There was a significant effect of trial [$F(1, 29) = 14.9, P < 0.01$] and strain [$F(2, 29) = 5.12, P < 0.05$] for intromission frequency (Fig. 1B), but no significant interaction [$F(2, 29) = 0.5, P = 0.621$]. These results suggest that experienced males showed more intromission behavior than naïve males, and that intromission behavior was higher in LAA than in HAA or SD rats.

There were significant effects of trial on ejaculation frequency [$F(1, 29) = 23.87, P < 0.01$], strain [$F(2, 29) = 26.83, P < 0.05$] (Fig. 1C) as well as a trial and strain interaction [$F(2, 29) = 5.41, P < 0.05$]. In LAA rats, experienced males significantly increased

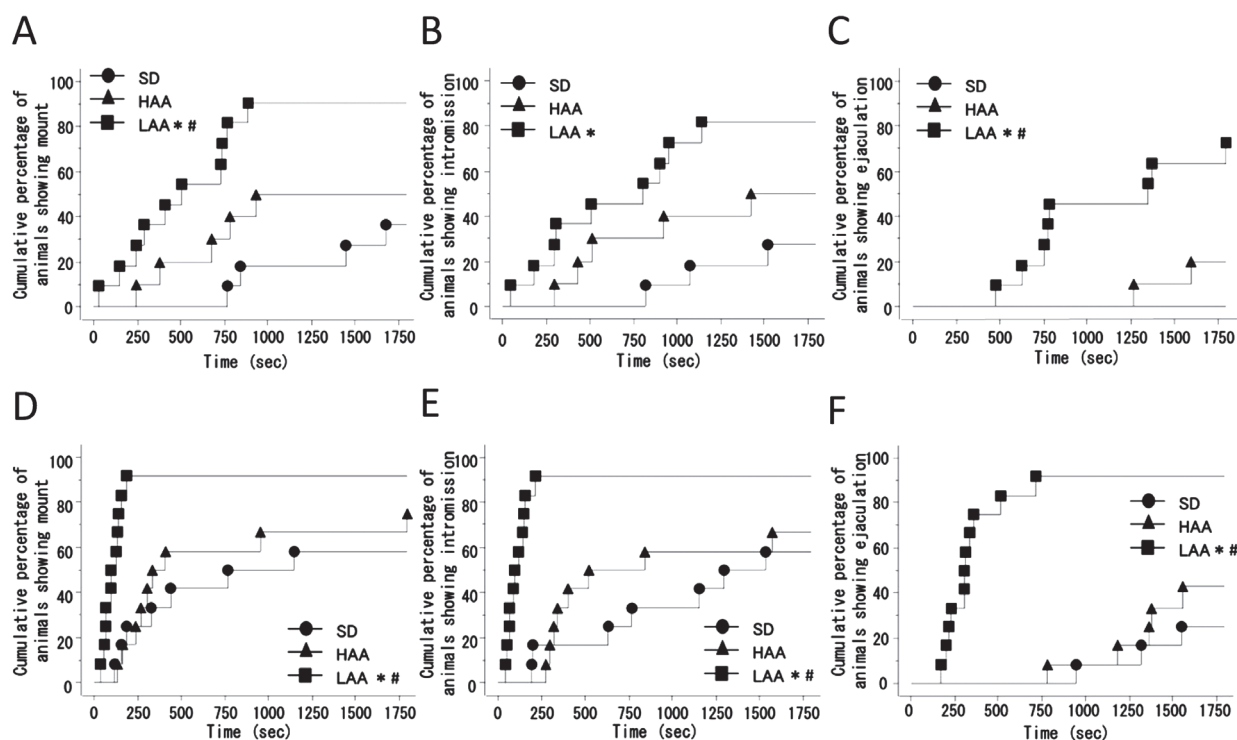


Fig. 2. Cumulative percentage of animals showing mount (A, D), cumulative percentage of animals showing intromission (B, E), cumulative percentage of animals showing ejaculation (C, F) in the sexually naïve (A–C test one; SD: n=11, HAA: n=10, LAA: n=11) and experienced (D–F test three; SD: n=12, HAA: n=12, LAA: n=12) tests, respectively. *, $P < 0.05$ SD (Sprague-Dawley) compared to LAA (low-avoidance-animal). #, $P < 0.05$ HAA (high-avoidance-animal) compared to LAA.

(Bonferroni, $P < 0.05$) their ejaculation frequency as compared with naïve males. These results suggest that ejaculation frequency was higher in LAA rats than in HAA or SD rats, and that experienced LAA males showed more ejaculation behavior than naïve LAA males.

Latency time to onset of sexual behavior in sexual naïve (i.e., copulation test one) and experienced (i.e., copulation test three) males are shown in Fig. 2. In naïve males, latency time to onset of mount (Fig. 2A), intromission (Fig. 2B), and ejaculation behaviors (Fig. 2C) were shorter in LAA rats than in HAA or SD rats. For experienced males, latency time to onset of mount (Fig. 2D), intromission (Fig. 2E), and ejaculation behaviors (Fig. 2F) were shorter in LAA rats than in HAA or SD rats.

Plasma testosterone concentrations in LAA rats (0.293 ± 0.109 ng/ml) tended to be lower than in HAA (0.463 ± 0.144 ng/ml) or SD rats (0.413 ± 0.109 ng/ml), even though the difference was not significant in a one-way ANOVA.

To evaluate sexual behavior in the Hatano strains (HAA and LAA rats), we conducted copulation tests of male HAA, LAA and SD rats exposed to ovariectomized females pretreated with estradiol benzoate and progesterone. There were clear differences in sexual behavior between LAA and HAA rats. LAA rats exhibited more rapid sexual behavior, such as mount, intromission and ejaculation. The frequencies of these behaviors were greater in LAA rats than in HAA or SD rats. The sexual behavior of HAA rats was comparable to that of SD rat (i.e., the standard strain showing normal sexual behavior), indicating that LAA rats naturally display hyper-sexual behavior.

Male sexual behavior in vertebrate species is dependent on testosterone secreted by Leydig cells in the testes [9]. However, plasma testosterone levels tended to be lower in LAA rats than in HAA or SD rats. Therefore, testosterone is not responsible for different sexual behaviors in Hatano rat strains. Another possible reason for active sexual behavior is an increased number of androgen receptors in the medial preoptic area [5], thus we would also need to compare the distribution and hormonal regulation of androgen receptors mRNAs in the medial preoptic area of HAA and LAA rats.

A copulatory behavior study on male Roman strains has shown that sexual behaviors are less active in RLA rats than in RHA or SD rats [12]. The distinct copulatory patterns of RLA and RHA rats may be related to their different coping styles and novelty seeking traits, because RLA rats are more emotional and display more freezing behavior than RHA rats. Studies on Roman and Hatano strains suggest that sexual behavior in LAA rats is more active but RLA rats are less active than in SD rats, the sexual behaviors of HAA rats and RHA rats are similar to those of SD rats. Differences between Roman and Hatano strains are presumably attributed to the selection criterion of low avoidance rate (i.e. RLA rats show many freezing behaviors but LAA rats show few freezing behaviors during acquisition of avoidance performance) [11].

A previous study has also suggested that male HAA rats are more predisposed to high anxiety than LAA rats [8]. In the third copulation test in this study, mount frequency in HAA rats increased to levels similar to LAA rats. This result suggests that HAA

rats required at least three trials before displaying active mating behavior. Once the effect of sexual novelty is overcome through repeated sexual experience, HAA rats show similar active mount behavior to LAA rats. However, ejaculation behavior in HAA rats was poorer than that in LAA rats, even at the third copulation test.

From the results of this study, we found that male LAA rats showed more active sexual behavior but lower plasma testosterone levels than male HAA rats. Strain differences in sexual behavior tended to increase with repeated sexual experience. Finally, differences in sexual behavior between LAA and HAA rats may be caused by different emotional responses to novelty.

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