

Strain differences in pubertal development of male rats' penile reflexes

by *Toru R. Saito, Naoki Moritani & Makoto Katsuyama*

Behavioral Neuroscience Laboratory, Graduate School of Veterinary Medicine, Nippon Veterinary and Animal Science University, Musashino, Tokyo 180-8602, Japan. Correspondence: Toru R. Saito, DVM, MSc, PhD, DJCLAM, Behavioral Neuroscience Laboratory, Graduate School of Veterinary Medicine, Nippon Veterinary and Animal Science University, Kyonan-cho 1-7-1, Musashino-shi, Tokyo 180-8602, Japan. Tel & Fax: +81-422-31-4571. E-mail: trsaito@nvau.ac.jp.

Summary

The penile reflexes in outbred Wistar, SD and inbred Fischer strain male rats aged from 23 to 62 days were observed for 15 min following sheath retraction. Plasma Luteinizing hormone (LH) and testosterone levels were measured at 23, 37, 51 and 70 days of age by RIA. All Wistar and SD rats displayed erections, cups and flips before plasma LH and testosterone levels peaked at 51 days of age, whereas in Fischer strain males, erections, flips, but not cups began to be seen around peak levels of these hormones at 51 days of age. The pubertal development of penile reflexes in Fischer strain males was slower than that in Wistar and SD stocks. The present study is the first to clarify the strain differences in pubertal development of male rats' penile reflexes.

Introduction

When normal male rats are held in the supine position with the penile sheath retracted, they display erections, cups and flips of the penis (Hart 1968). Castration reduces these ex copula penile responses, whereas testosterone or dihydrotestosterone, but not estradiol replacement therapy restores performance to precastration levels (Davidson *et al.* 1978, Gray *et al.* 1980). This finding is consistent with the androgen sensitivity, and estrogen insensitivity, of the penis and the striated penile muscles.

Sachs *et al.* (1979) investigated the ontogeny of sexual reflex in male rats and the relation of reflex development to the development of the penis, of copulatory behavior, and of testosterone secretion.

The results indicated that penile responses and copulatory behavior began to be expressed well before testosterone levels peaked, at about 9 ng/ml, at 50 days of age. Furthermore, the penile response potential developed concurrently with copulatory behavior in these rats.

The question addressed here was whether strain difference would be seen in the pubertal development of male rat's penile reflexes. Commercial breeders state that the age of first mating with females in Fischer strain males is later than Wistar and SD strains (personal communication).

The aim of the present study was to determine the pubertal development of penile reflexes and plasma the concentrations of hormones (LH, testosterone) in Fischer strain male rats, and to compare these data with males of Wistar and SD stocks.

Materials and Methods

Subjects

Pregnant rats of Wistar (Crj: Wistar) and SD (Crj: CD (SD)) stocks and the Fischer (F344/Ducrj) strain were obtained from Charles River Japan, Inc. (Kanagawa, Japan) nine days before parturition. The animals were housed in individual wooden cages and kept at $24 \pm 2^\circ$ C in a light-controlled room (14 hr. light, 10 hr. dark). Food (Oriental MF, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were always available. The day of parturition was designated as day 0 of lactation and the number of pups per

litter was adjusted to eight within 2 days of birth. Litter mates were weaned at 21 days of age and randomly assigned to one of 2 groups. One group was tested for penile reflexes (N=21) and one was bled for LH and testosterone quantification (N=60). Upon weaning, the rats were earpunched for identification and housed, 5 to 7 per cage, in hanging wire mesh cages by group assignment.

Penile reflex test

Penile reflexes began to appear when the rats were tested at 23 days of age and testing continued every 3 days between 1600 and 1700 until the males were 62 days old. Penile reflexes were tested after a method developed by Hart (1968) and modified by Sachs & Garinello (1978). Briefly, the male was placed on his back with the anterior portion of his body loosely enclosed with

a belt around his midsection. The penile sheath was retracted to the extent possible and held in position with a thin dowel (Figure 1). Males were tested for 15 min following the initiation of reflexes or 30 min in the event no reflexes occurred. Erections, cups and flips were recorded on an event recorder as they occurred. Erections are extensions and / or distensions of the penis. Cups are intense erections during which the glands of the penis flares so that it is more trumpet-shaped than tubular. Flips are a dorsal flexion of the penis. For comparison of penile reflexes in Wistar and SD rats, a record was made of the number of each type of reflex and the reflex latency, being the elapsed time from sheath retraction to the first display of erection, was measured on reaching the age of 49 days.

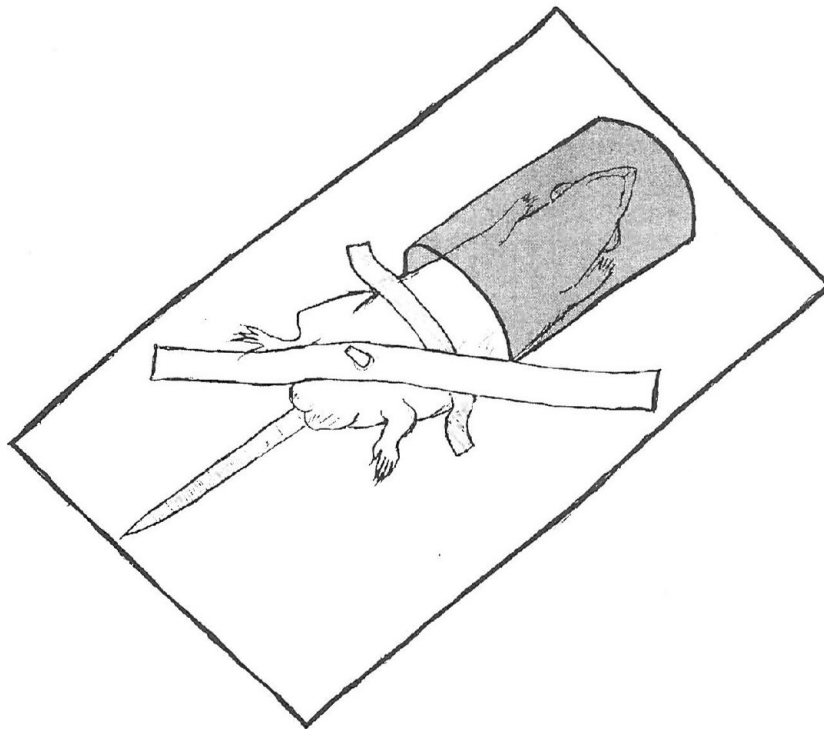


Fig. 1. Technique used in stimulating glands penis with tape

Blood sample

Five males of each stock were anesthetized with ether and blood samples were collected through the posterior vena cava with heparinized syringes between 1600 and 1700 on days 23, 37, 51 and 70 after birth. Blood collected was centrifuged at 3,000 g for 15 min for the analysis of plasma hormones. The plasma was stored at -80°C until analyzed by RIA for determination of serum LH and testosterone.

LH and testosterone assays

The serum concentration of LH was measured by RIA using the method of Furudate *et al.* (1989) with reagents provided from National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK). The standard reference used was rLH-RP-2 for LH. The intra- and inter-assay coefficients of variation were 3.5 and 5.3 respectively for LH. The testosterone content of these samples was assayed by using Coat A Count Radioimmunoassay Kit (Diagnostic Products Co., Los Angeles, USA). The Coefficient of variation was 4.5%.

Statistics

The results are expressed as means \pm s.e.m. and analyzed using the Mann-Whitney U test (Mann & Whitney 1947).

Results

Penile reflexes

The cumulative frequency curves for the development of penile reflexes in Wistar, SD and Fischer strain rats are showed in Figure 2. Erections were first seen on days 23, 26 and 47 in SD, Wistar and Fischer rats, respectively. While all of 14 males of SD and Wistar strains displayed erections by day 41, in the Fischer strain 7 of 7 males showed erections by day 62. Cups were observed after days 35 and 41 in SD and Wistar strains, respectively. All of 14 males of SD and Wistar stocks showed cups by day 47. In the Fischer strain no cups were observed during the observation period. Flips occurred on days 29, 32 and 50 in SD, Wistar and Fischer rats, respectively. All 14 males of these two stocks displayed flips by day 44, whereas only three of 7

males in the Fischer strain showed flips by day 62. The comparison of reflex latency, numbers of erections, cups and flips in Wistar and SD rats is shown in Table 1. The latency to first erection recorded in Wistar rats was significantly longer than that recorded in the SD stock ($p < 0.001$). There were no significant differences in frequencies of erections, cups and flips between the two stocks.

LH and testosterone concentrations

The concentrations of plasma LH and testosterone in the Wistar, SD and Fischer rats on days 23, 37, 51 and 70 are shown in Figures 3 and 4, respectively. The LH levels of each strain rose gradually after day 23, showed a peak level of 0.63 - 0.92 ng / ml on day 51 and then decreased gradually. Testosterone levels began rising sharply on or before day 23, reached a maximum on day 51 (3.0 - 3.5 ng / ml) and then decreased gradually. There were significant differences in plasma levels of LH and testosterone between the three strains/stocks.

Discussion

The results of this study showed that the penile reflexes in Wistar and SD strain male rats developed prior to the time of maximum testosterone levels at 51 days of age. Our data support the previous study regarding pubertal development of penile actions in Long-Evans rats (Sachs & Meisel 1979). By contrast, Fischer strain male rats showed delayed development of penile reflexes with erections and flips, but not cups, appearing around the peak of testosterone levels at 51 days of age. The strain difference may be related to morphological and functional developments of the penis and the striated penile muscles. Mean relative weights of the penis at 23, 37, 51 and 70 days of age in Fischer strain males were similar to Wistar and SD males (unpublished data). It is considered that the perineal muscles related to penile reflexes in male rats and mice are as follows; *M. ischiocavernosus*, *M. bulbocavernosus* and *M. levator ani*. Muscle excision studies (Sachs 1982) demonstrated the role of striated penile muscles in penile reflexes. He found that the activity of the ischiocavernosus and bulbocavernosus muscles' activity

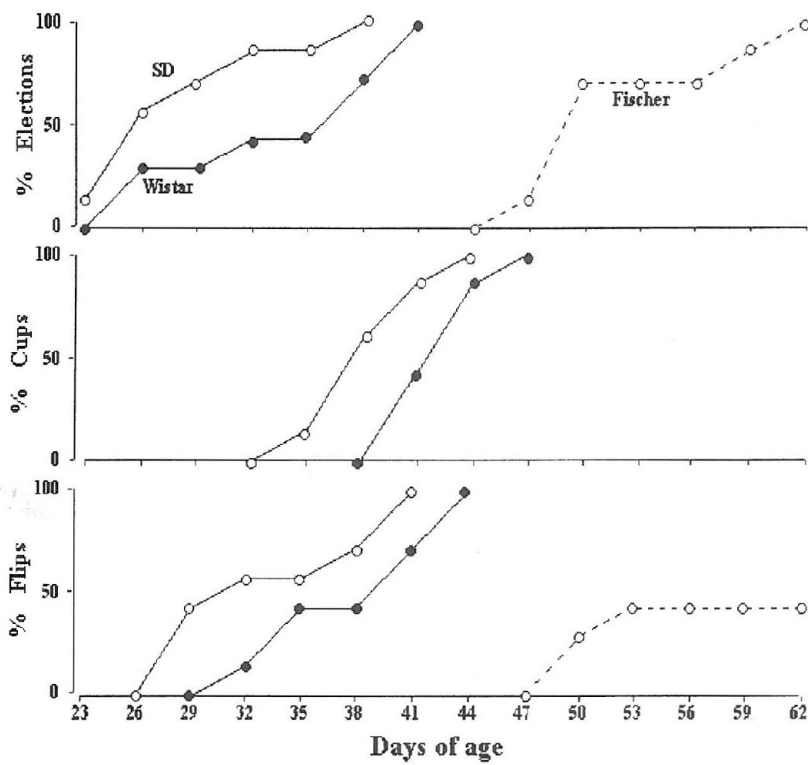


Fig. 2 Cumulative frequency curves for age of occurrence of first election, flip and cup in Wistar, SD and Fischer strain rats

Table 1. Comparison of penile reflexes (Mean \pm s.e.m.) between Wistar and SD strain rats on day 49

Strain	n	B.W.	Latency (Sec.)	Frequency		
				Erections	Cups	Flips
Wistar	7	224.0 \pm 6.8	652.3 \pm 66.7	16.1 \pm 4.0	8.9 \pm 2.7	4.0 \pm 2.4
SD	7	227.0 \pm 6.3	280.3 \pm 60.7	25.0 \pm 1.7	11.0 \pm 3.1	5.9 \pm 2.0

Mann-Whitney U test: # $p < 0.001$

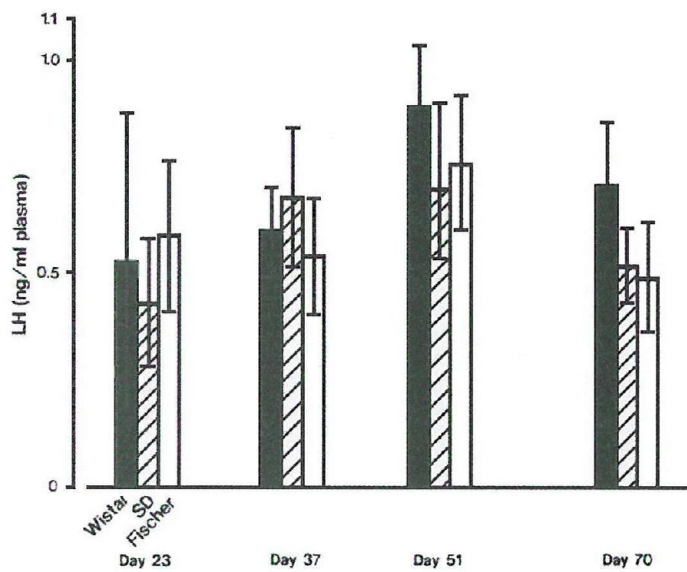


Fig. 3 Plasma LH levels in Wistar, SD and Fischer strain rats

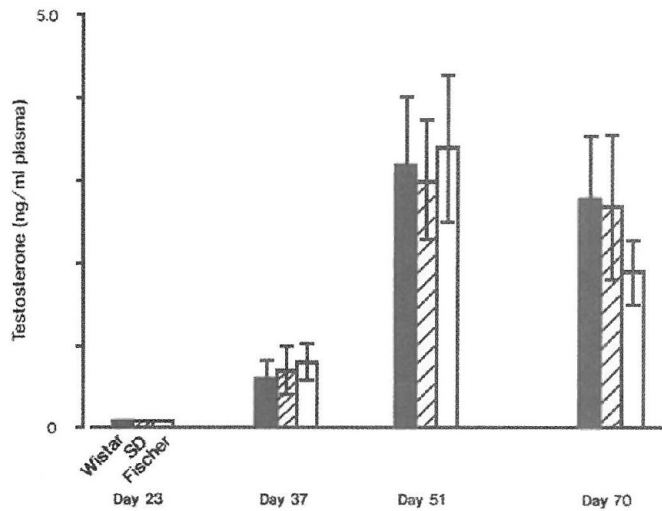


Fig. 4 Plasma testosterone levels in Wistar, SD and Fischer strain rats

predominated during erections, the ischiocavernosus muscle activity predominated during erections and flips, and the bulbocavernosus muscle activity predominated during cups. Fischer strain males displayed no cups throughout the course of our experiments suggesting that the development of the bulbocavernosus muscle may be delayed, compared to that of the ischiocavernosus muscle. The pubertal development of penile reflexes in intact and spinally transected male rats was investigated by Meisel & Sachs (1980). They found that spinal transection advanced the onset of penile erections and flips by about 10 days relative to control males. The occurrence of erections and flips in Fischer strain males was significantly later than Wistar and SD strain males suggesting that the role of spinal inhibition in the control of penile reflexes may be different from Wistar and SD

strain males. Further studies will be necessary in order to clarify these points in Fischer strain males.

In conclusion, the results of this study are the first to show the strain differences in pubertal development of male rats' penile reflexes.

Acknowledgments

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