Testosterone Regulation of Sexual Reflexes in Spinal Male Rats

Abstract. *Castrated male rats with complete midthoracic spinal transections were maintained on exogenous testosterone; they showed intermittent clusters of genital responses consisting of erections, quick flips, and long flips of the penis when gentle pressure was constantly applied to its base. The number of these genital responses per 30-minute test was markedly influenced by withdrawal or administration of testosterone.*

The mating behavior of male rats declines rapidly subsequent to gonadectomy. All elements of the mating pattern, however, do not disappear at the same time. It is reported that the ejaculatory pattern is the first response to disappear (4 to 8 weeks for most animals), whereas appetitive responses such as investigation of the estrous female, mounting, and pelvic thrusting disappear more gradually (*1*, *2*). My results suggest that the decline in the ejaculatory response is due to the effect of withdrawal of androgens on spinal elements. The more gradual decline of appetitive responses presumably reflects the effects of withdrawal of androgens on hypothalamic or other forebrain structures.

A preliminary study showed that when a male rat with its spinal cord transected is restrained on its back and the preputial sheath is held behind the glans penis, a series of clusters of genital responses occur intermittently. Each cluster usually consists of several brief erections of the glans penis, two or three quick dorsal flips of the glans, and one to three extended, long dorsal flips of the glans (Fig. 1). The long flips are accompanied by strong ventral flexion of the pelvis. The pattern and duration of the long-flip response closely resemble the pattern and duration of an ejaculatory response of the intact rat. I have analyzed these reflexes in detail elsewhere and have suggested that the interval between the onset of response clusters (2 to 3 minutes) represents a refractory period of spinal sexual responses and that the erections and quick flip responses that occur as spinal refractoriness dissipates represent gradations of the long-flip response (*3*).

I used adult, sexually naïve, Long-Evans male rats. The animals were castrated at 120 to 131 days of age and subsequently given daily subcutaneous injections of 0.2 mg of testosterone propionate in oil (*4*). Five days after the rats were castrated a spinal transection was performed in the midthoracic region (between thoracic spinal nerves six to ten) while the animals were under barbiturate anesthesia. The spinal cord was exposed by means of a laminectomy. To be certain that the spinal cord was completely transected, I removed a segment of cord approximately 2 mm long by aspiration through a fine glass tube. The incised muscle and skin were pulled together with separate layers of simple interrupted sutures. The postoperative care consisted of expressing the urinary bladders two or three times per day and washing off any accumulated urine or feces. The flexion reflex was frequently monitored to assess the integrity and condition of the isolated spinal cord. Twelve rats which survived the surgery and postoperative period in satisfactory condition and which showed strong somatic reflexes were used as subjects.

It was judged from a preliminary study that spinal elements mediating sexual reflexes have recovered from spinal shock before 20 days. In this study tests for sexual reflexes were initiated on the 20th day subsequent to spinal transection. By this time the rats were 145- to 156-days old and had received daily injections of testosterone for 25 days. Each test for sexual reflexes consisted of placing the animal on its back in a glass cylinder for partial restraint, pushing the preputial sheath behind the glans penis and holding it in this manner for 30 minutes. The time of onset of each response cluster and the types of responses occurring within each response cluster were observed and recorded. All such 30-minute tests were conducted at 2-day intervals, and all animals were tested at 2-day intervals throughout the experiment. After the first four tests, exogenous testosterone was withdrawn from six of the rats, specified as group A, while the other six rats, specified as group B, were maintained on testosterone. The latter group thus served as a control for the possible influence of further recovery from spinal shock on sexual reflexes. Daily injections of testosterone (0.2 mg) were administered again to the animals in group A after the completion of four tests (8 days) conducted while the animals were off testosterone. Six 30-minute tests (12 days) were conducted on the animals in group A after they were again placed on testosterone. Exogenous testosterone was withdrawn from the animals in group B after the eighth 30-minute test. Six 30-minute tests (12 days) were then conducted on these animals while they were off testosterone. The schedule of hormone administration is summarized in Fig. 2. One of the animals in group A became sick after the eighth test and was killed; therefore, calculations for group A on tests 9 through 14 represent the mean of five animals rather than six.

There was a pronounced decline in the total number of erections, quick flips, and long flips per 30-minute test when testosterone was withdrawn (Fig. 2). The decline appeared to be progressive. When data from the two groups were pooled for analysis of the effects of withdrawal of testosterone, the Wilcoxon matched-pairs signed-ranks test (*5*) showed that the number of erections, quick flips, and long flips for all 30-minute tests conducted while the subjects were off testosterone were significantly ($P < .05$) below the mean number of these responses for the four 30-minute tests conducted immediately before withdrawal of testosterone. In light of the possible confounding influ-

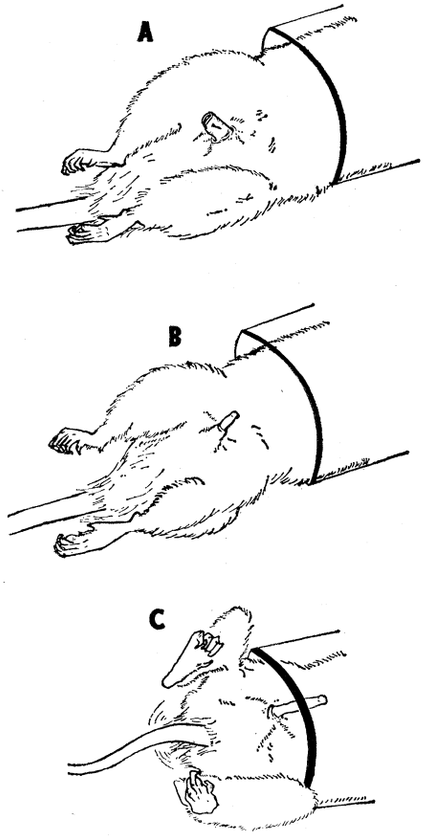

Fig. 1. Illustrations of an erection (A), quick flip (B), and long flip (C) which occur in a response cluster and which are evoked when gentle pressure is constantly applied to sides of the base of the penis. The rats are shown as they were restrained in a glass cylinder.

ence on sexual reflexes of recovery from spinal shock, the most revealing effect of withdrawal and administration of testosterone is seen in the reversal of mean number of responses for the two groups, after the eighth test, when group A was placed on testosterone and group B was taken off testosterone.

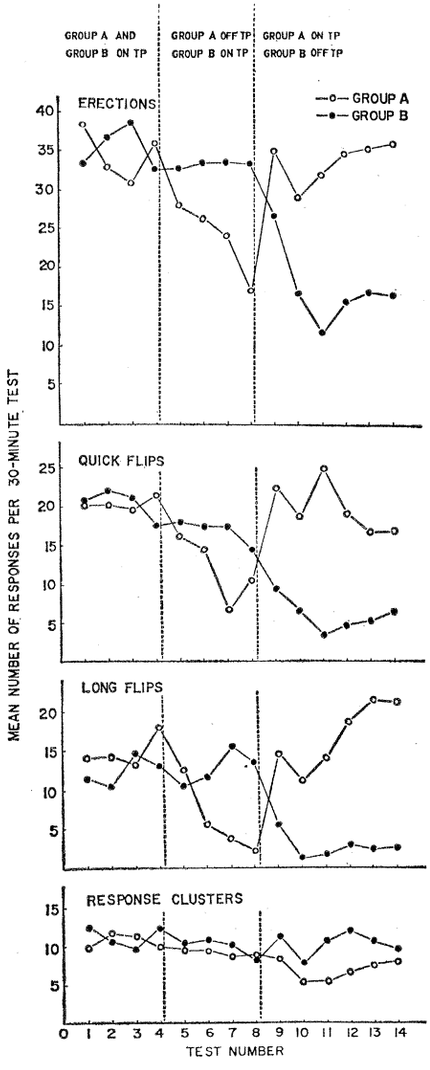

Fig. 2. Influence of withdrawal and administration of testosterone on number of erections, quick flips, long flips, and response clusters per test. Tests were conducted at 2-day intervals, and all animals were tested at 2-day intervals throughout the experiment. There were six rats with transected spinal cords in each group. When hormone withdrawal is indicated there was no injection given on the day of the last test which was conducted while the animals were on testosterone (TP). When readministration of hormone is indicated, the first injection was given 48 hours before the first test which was conducted while the animals were on testosterone. When it was withdrawn from rats in group B, the number of long flips per test fell to approximately two per test (individual range of zero to nine) on tests 10 through 14.

All animals showed the change characteristic of their respective groups, and the probability is less than .01 of this reversal occurring by chance. There was no appreciable effect on the number of response clusters per test (Fig. 2). Nor was there any detectable change in the latency to the first response cluster or in the intervals between response clusters which could be attributed to withdrawal or administration of testosterone. Thus there was a decline in the number of genital responses per cluster (and hence a decline in the duration of the cluster), but not in the timing mechanism controlling the onset of a response cluster.

It could be argued that the decline in sexual reflexes in the spinal animals following withdrawal of androgen is a reflection of a decreased sensitivity of genital sensory receptors, since it has been reported that genital papillae on the glans penis of the male rat decrease in size and number subsequent to castration (*6*). Two facts argue against this contention: (i) there is an occasional male rat which shows a complete ejaculatory pattern several months after castration (*2*); (ii) a complete mating response can be evoked in castrated male rats with hypothalamic implantation of testosterone in amounts too small to affect genital morphology (*7*).

The role that the sexual reflexes, which can be evoked from spinal rats, play in mating behavior of the intact male rat is uncertain. Assuming they have some function in copulation, it appears as though the decline of ejaculatory and, possibly, intromission behavior in the male rat following castration may be due to the influence of withdrawal of gonadal androgens on spinal neurons mediating the sexual reflexes. The more gradual decline in appetitive responses, such as investigation of the female genitalia, mounting, and pelvic thrusting, is probably a reflection of the effect of withdrawal of gonadal androgens on hypothalamic or other forebrain structures. This is suggested by studies which show an abolishment of mating behavior in male rats (which could not be attributed to impairment of gonadal androgen output) following hypothalamic lesions (*8*) and by a study by Davidson showing a resumption of mating activity in castrated male rats caused by the implantation of testosterone into the hypothalamus (*7*).

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References and Notes

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4. Preliminary observations showed that in some male rats with transected spinal cords seminiferous tubules of the testicles undergo marked degeneration. Since there was some question whether intersitial cells (and hence androgen secretion) were also affected, all animals were castrated and administered a replacement dosage of androgen considered to be well above that required to maintain normal mating behavior of castrates. The daily dose of 0.2 mg of testosterone propionate is two to four times that estimated to be an adequate replacement dosage (*1*).
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Prevention of Induced Atherosclerosis by Peroxidase

Abstract. *Hepatocatalase peroxidase, an active peroxidase-oxidase subunit isolated from beef-liver catalase, prevents cholesterol deposition and aortic atherosclerosis in cholesterol-fed rabbits and has no apparent toxicity or undesirable side effects. No allergic or immunological reactions have been observed. The participation of this enzymatic subunit in homeostatic control mechanisms and its potential pharmacological value in the control of human atherosclerosis are suggested.*

The peroxidatic properties of catalase were recognized as early as 1936 by Keilin and Hartree (*1*). These authors postulated that "the physiological function of catalase would be mainly peroxidatic and only in exceptional cases a catalatic one" (*2*). However, in spite of the early recognition of its possible metabolic significance, little information on the peroxidatic activity of catalase has been gained over the years, owing to technical difficulties arising from the interfering action of its catalatic activity which is approximately 1000 times higher than its peroxidatic activity.

We have recently isolated a molecular subunit of beef hepatocatalase which exhibits high enzymatic activity as a peroxidase-oxidase and is essentially free of catalatic action (*3*). The hepatocatalase peroxidase subunit (HCP)