SEXUAL BEHAVIOUR OF MALE RATS STERILIZED BY CADMIUM

J. MADLAFousek, Z. Hliňák and J. Pařízek

Psychiatric Research Institute, Prague-Bohnice, and Institute of Physiology, Czechoslovak Academy of Sciences, Prague-Krč

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Summary. The sexual behaviour of adult male rats was studied following the injection of cadmium chloride in a dose known to induce complete testicular necrosis and permanent sterility. During the first 3 weeks after cadmium injection, most males lost their copulatory activity. This effect could be prevented by administration of androgens. Complete mounts and a normal ejaculatory behaviour pattern were resumed by all males 2 months after cadmium injection. Spontaneous restitution of copulatory activity depended on the presence of the damaged testes. A comparison of the quantitative characteristics of copulatory performance before, and more than 2 months after, cadmium revealed only slight impairment of sexual behaviour.

INTRODUCTION

Parenteral administration of a small amount of cadmium salts has been shown to produce testicular necrosis (Pařízek & Záhoř, 1956) leading to the development of castration phenomena (Pařízek, 1957). In spite of the damage to testicular tubules which persists chronically after an injection of cadmium salts, regular recovery of the accessory sex organs can be observed resulting from regeneration of the androgen-producing tissue within the testis (Pařízek, 1957, 1960). Thus, some time after cadmium administration, the gonads of treated rats become sterile organs with a purely endocrine function (Pařízek, 1960).

It is well known that surgical castration of male rats impairs their sexual behaviour. While this effect is permanent, sexual activity can be restored by exogenous testosterone (Parkes, 1966; Beach, 1970). In experiments reported in detail here, the question of how far the endocrine situation produced by cadmium in male rats is reflected in their sexual behaviour was studied. Preliminary reports of these studies have appeared elsewhere (Madlafousek, Hliňák & Pařízek, 1970).

MATERIALS AND METHODS

Male rats (Wistar substrain Konárovice), nourished on a standard laboratory diet (Ošíádalová & Pařízek, 1968) with food and water present at all times,
were kept under standard laboratory conditions with a constant lighting regimen (12 hr light/12 hr dark). Four rats were kept in each cage.

Subcutaneous injections of cadmium chloride, as in previous experiments (Pařízek, 1957), were given in the present study 19 weeks after birth. All rats received 0.04 mmol of cadmium chloride per kg body wt as a 0.02 M-solution (made isotonic with saline).

In the second experiment, one subgroup of rats received testosterone propionate in 0.2 ml oil intramuscularly in a dose of 150 μg per rat daily for 3 weeks, starting from the 1st day after cadmium injection. The control subgroup was similarly treated with the same volume of oil alone. Surgical castration was performed by the scrotal technique under ether anaesthesia.

All observations were carried out during the first 4 hr of the 12-hr dark period. Sexually inexperienced rats unused to laboratory conditions were first accustomed to an ellipsoid, perspex experimental box (with axes of 24 and 23 in., and a depth of 14 in.) during sessions without a sexual stimulus. In the following testing sessions, the male was placed in the experimental box 5 min before the stimulus female was added. Only very active (hopping and darting) females were used. These were randomly selected from a group of ovariectomized females injected with oestradiol and progesterone (Hlíňák & Madlafousek, 1969). The sexual behaviour of test females was carefully controlled during all phases of the experiments. If necessary, the stimulus female was exchanged even during the testing session to keep the sexual stimulation of the male high enough. Only those male rats were used which copulated and ejaculated in both (Exp. 1) or all three (Exp. 2) testing sessions before cadmium treatment.

Direct observation made it possible to identify (Kuehn & Beach, 1963) the following patterns of sexual behaviour: (i) the incomplete mount, during which the male did not achieve intromission; (ii) the complete mount (i.e. with intromission), in which the pelvic movements were finished by a final strong stroke; during this stroke, the penis was typically inserted into the vagina for about 0.3 sec; (iii) the ejaculatory behaviour pattern, in which intromission lasted about 2 to 3 sec while the male was standing in a characteristic posture above the female. This behaviour pattern is normally connected with ejaculation, though occasionally there may be no actual emission of an ejaculate (Davidson, 1966).

For quantitative description of the copulatory performance (Text-fig. 1), the following characteristics were determined (for details, see Larsson, 1956): (1) intromission latency (the time from the entrance of the female into the testing box to the first intromission), (2) ejaculation latency (the time from the first intromission to the appearance of the ejaculatory behaviour pattern), (3) mean interval between intromissions (i.e. between two consecutive complete mounts), (4) post-ejaculatory interval (the time from the ejaculatory behaviour pattern to the next complete mount), (5) intromission frequency (the number of intromissions performed before an ejaculatory behaviour pattern is achieved), (6) incomplete mount frequency (the number of incomplete mounts between the first intromission and an ejaculatory behaviour pattern), (7) incomplete mount frequency to intromission frequency ratio.
The male was considered unable to copulate on the test day if no complete mount was achieved during the 10-min period of exposure to the test female. Use of the 10-min test period made it possible to test all experimental males on the same day during the optimal first 4 hr of the dark period. Previous experience in this laboratory has shown that exposure of the male to a sexually active female for 10 min under laboratory conditions is sufficient to ascertain with high probability the male's ability to copulate. If at least one complete mount was observed during the 10-min period, the test was regarded as positive and the male's sexual behaviour was followed up to the performance of the first (Exp. 1) or second (Exp. 2) ejaculatory behaviour pattern.

**RESULTS**

The decline of copulatory activity

Eight reliably copulating and ejaculating males were selected from sixteen animals for Exp. 1. At the first testing on the 9th day after cadmium injection, most males did not exhibit complete mounts and were considered unable to copulate (Text-fig. 2). Their sexual behaviour was limited to a few incomplete mounts during the first minutes of exposure to the female. Only two males appeared to be capable of complete mounts. After a typical series of intromissions, both these animals accomplished the ejaculatory behaviour pattern.

On the 13th and 23rd day after cadmium injection, most males were unable to copulate. This corresponded with an earlier finding (Pařízek, 1960) that the weight of seminal vesicles and prostate decreased rapidly during the first 10 days after cadmium treatment and that the low weight was maintained for at least 3 weeks.

In order to test the hypothesis that the decline of copulatory behaviour after cadmium treatment was due to the destruction of testicular endocrine tissue, a second experiment was undertaken. Fourteen reliably copulating and ejaculating males were selected from forty animals and were subdivided into two groups. After cadmium treatment, seven males in one group were injected daily with testosterone. In tests during the critical 3 weeks, all the males
copulated normally (Text-fig. 3). The cadmium-treated males in the second group which received the vehicle (oil) alone without testosterone, showed a loss

Text-fig. 2. The copulatory activity of adult male rats at different times after cadmium chloride injection.

Text-fig. 3. The effect of testosterone on the copulatory activity of adult male rats treated by cadmium chloride. ●, Testosterone propionate (TP), 150 µg daily/rat; ○, oil, 0.2 ml daily/rat.

of copulatory ability in similar proportion to the cadmium-treated group in Exp. 1.

In both experiments, the data were statistically analysed in order to assess
two points: (1) for evaluation of the cadmium effect, the copulatory abilities of the same animal before, and on the 23rd day after, treatment were compared; the decline in the percentage of copulating males was tested by the McNemar test for the significance of change ($P<0.001$ for $N = 15$, taking all hormonally non-treated males from both experiments); (2) to prove the compensatory effect of testosterone, the androgen- and oil-treated groups in Exp. 2 were compared; the difference in proportions of males, copulating and non-copulating on the 23rd day after cadmium, was tested by Fisher's exact probability test ($P<0.001$).

The restitution of copulatory activity

Previous studies (Pařízek, 1957, 1960) have shown that about 10 weeks after cadmium treatment, a recovery in the weight of the seminal vesicles and prostate can be observed. This is due to the restitution of endocrine function in the testes; surgical castration leads to further loss of weight in these organs. The present experiments on sexual behaviour show that all animals in both experiments exhibited copulatory activity when tested on the 70th day after cadmium treatment (Text-figs. 2 and 3). The number of copulating males increased significantly from the 23rd day to the 70th day after cadmium treatment (for both experiments $P<0.001$, McNemar test for the significance of change). The difference between the 33rd day and the 70th day (Exp. 2) was also statistically significant ($P<0.005$). All these males exhibited the behavioural ejaculatory pattern which chance inspection showed to be accompanied by the production of plugs.

The restitution of copulatory ability was reliable and enduring. Five males from Exp. 1 (three animals from the original group had died earlier) were re-tested on the 210th day after cadmium treatment: all exhibited complete mounts and the ejaculatory behaviour pattern. One week after surgical removal of their necrotic testes, these five males did not show any sexual activity (even incomplete mounts were not observed.) This effect of surgical castration seemed to be permanent (see right side of Text-fig. 2).

The copulatory performance after restitution

An attempt was made to assess whether the restitution of the sexual behaviour pattern accompanying recovery of testicular endocrine function was complete or whether some impairment of the copulatory performance could still be detected by the available quantitative measurements. The quantitative characteristics of the copulatory performance of each male in the last test before cadmium treatment were compared with those found on the 70th day after cadmium injection. Statistical analysis used the $t$ test for paired scores. While no differences were found amongst the three groups studied (the group from Exp. 1 and both groups from Exp. 2), all data were summarized for the twenty-two cadmium-treated male rats from both experiments.

As shown in Table 1, the copulatory performance before, and on the 70th day after, cadmium treatment was not different in most quantitative characteristics. While this negative result seems to be invalidated by the high variability of data, a closer inspection shows the factual sources of the variability. For
example, the group mean of the intromission latency after cadmium treatment was longer than before treatment, although in almost half of the animals the intromission latency was a little shorter after treatment than before treatment; the longer group mean and higher variability was accounted for by a few animals whose intromission latency on the 70th day after cadmium treatment was substantially prolonged. A similar finding holds for the ejaculation latency and the mean interval between intromissions. The small and non-significant changes in frequencies of incomplete and complete mounts (incomplete mount frequency was higher and intromission frequency smaller after treatment) resulted in a statistically significant rise of the ratio between the incomplete mount frequency and the intromission frequency. The most consistent change was prolongation of the post-ejaculatory interval. It should be noted that both these statistically significant differences represent physiologically small changes when compared with the changes known to be produced by partial sexual exhaustion (Larsson, 1956). The data permit the conclusion that in spite of the changes induced by cadmium treatment, the copulatory performance of male rats on the 70th day after treatment shows only small changes which could be interpreted as weakened copulatory ability.

**DISCUSSION**

It is a well-known fact that surgical castration of male animals results in a decline of their sexual behaviour. A detailed analysis of this phenomenon was given recently by Davidson (1966), Larsson (1966) and Beach (1970). From these, as well as from a great number of previous studies (for review see Parkes, 1966; Beach, 1970), the following facts should be summarized before discussing the data found in this study.

### Table 1

**Copulatory performance of male rats* before, and 10 weeks after, the injection of cadmium**

<table>
<thead>
<tr>
<th>Characteristics of the sexual performance</th>
<th>Before treatment (Mean ± S.D.)</th>
<th>After treatment (Mean ± S.D.)</th>
<th>Statistical significance†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Intromission latency (sec)</td>
<td>61.5 ± 71.4</td>
<td>138 ± 196</td>
<td>NS</td>
</tr>
<tr>
<td>2. Ejaculation latency (sec)</td>
<td>382 ± 234</td>
<td>425 ± 289</td>
<td>NS</td>
</tr>
<tr>
<td>3. Mean interval between intromissions (sec)</td>
<td>40.6 ± 20.2</td>
<td>53.9 ± 35.5</td>
<td>NS</td>
</tr>
<tr>
<td>4. Post-ejaculatory interval (sec)‡</td>
<td>346 ± 52</td>
<td>389 ± 34</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>5. Intromission frequency</td>
<td>10.5 ± 3.8</td>
<td>9.1 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>6. Incomplete mount frequency</td>
<td>3.8 ± 3.4</td>
<td>4.8 ± 4.0</td>
<td>NS</td>
</tr>
<tr>
<td>7. Incomplete mount frequency/intromission frequency</td>
<td>0.35 ± 0.29</td>
<td>0.68 ± 0.41</td>
<td>P &lt; 0.05</td>
</tr>
</tbody>
</table>

* Data from twenty-two male rats.
† Differences in the copulatory performance 'before treatment' versus 'after treatment' were evaluated by the t test for paired scores.
‡ Data from fourteen males only (Exp. 2).
(a) There is a high individual variability in the behavioural effects of surgical castration. Whereas some males lose their copulatory activity during the 1st week after castration, the copulatory activity of other individuals does not seem to be affected for several weeks or even months after removal of the gonads. Studies on larger groups of rats (Davidson, 1966; Larsson, 1966) suggest that the percentage of copulating males declines gradually and progressively with time after castration.

(b) Behavioural effects of surgical castration can be prevented in all individuals by administration of androgenic hormones.

(c) Without the treatment with exogenous androgens, only about 10% of male rats exhibit complete mounts and an ejaculatory behaviour pattern 10 weeks after surgical removal of the testes (Davidson, 1966; Larsson, 1966).

(d) No spontaneous persistent restitution of copulatory activity was observed in males which lost their copulatory activity after surgical castration.

In the present study, the consequences of cadmium injection closely resembled those of surgical castration. However, spontaneous and persistent restitution of copulatory behaviour did appear in cadmium-treated rats so that 10 weeks after cadmium injection, all males were copulating again. The reappearance of complete mounts was consistently accompanied by an ejaculatory behaviour pattern.

The decline of sexual behaviour in a situation where the testes had been completely destroyed by cadmium with resultant atrophy of the androgen-dependent accessory sex organs (Pařízek, 1960) was to be expected. The behavioural effects of cadmium administration resemble atrophy of the accessory sex organs (Pařízek, 1957) in that they can be completely prevented by administration of androgenic hormones. The decisive factor for all these reactions would appear to be androgen deficiency, although a possible additional rôle of some extra-testicular and/or general toxic effects cannot be fully excluded. Gunn, Gould & Anderson (1970) recently concluded that testosterone treatment can prevent the “inhibitory effect of cadmium on mating” 6 to 9 days after cadmium injection, as indirectly assessed by a method for the detection of spermatozoa in vaginal smears.

The direct observation and quantitative evaluation of sexual performance, including the ejaculatory pattern, seem to be the method of choice in a situation where behavioural effects of antifertility agents are studied, especially those known to affect the spermiogenic function of the gonads. This approach has shown that all the elements of the copulatory behaviour of male rats reappeared 2 months after cadmium treatment, i.e. when all seminiferous tubules had disappeared and the male gonads had been converted to small, yellowish organs with a purely endocrine function (Pařízek, 1960).

The reappearance in the present study of copulatory activities corresponds well with the regeneration of testicular endocrine tissue and the restitution of testicular androgen secretion described previously (Pařízek, 1957). Surgical removal of the testes, which had been damaged by cadmium, resulted not only in a decrease in the weight of the accessory sex organs to castration levels (Pařízek, 1957), but also in the immediate loss of sexual behaviour. It is concluded that several weeks after cadmium injection, the remnants of the testes
again produce androgens in amounts sufficient to restore sexual behaviour. In this connection, it should be noted that daily injections of 50 to 75 µg of testosterone propionate are sufficient to sustain the sexual activity of surgically castrated male rats (Beach & Holz-Tucker, 1949).

In spite of dramatic changes induced by cadmium and in spite of a long period of sexual inactivity (Folman & Drori, 1969), differences in the quantitative characteristics of copulatory performance at the beginning and end of the experiments was surprisingly small. It can be concluded that the complete and persistent loss of fertility of male rats, known to be produced by cadmium, is not accompanied by a substantial and permanent impairment of male sexual behaviour.

REFERENCES


