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Measuring of the postcopulatory departure in male rats

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Technical communication

Abstract. Spontaneous departure from the female was observed in male rats during testing of instrumental sexual responses. Male rats were tested in an apparatus consisting of two compartments connected by a runway composed of four parallel corridors. The final corridor of the runway and the goal compartment as well as the goal compartment and the start compartment were connected by one-way doors enabling the male to run only in one direction. An incentive female was tethered in the goal compartment. After contact with the female, lasting up to the end of a mount bout, or after exploration of the goal compartment, if the copulatory behaviour was not displayed, the male spontaneously passed to the start compartment and a new run started thereafter. The male thus performed the runs through the apparatus without any intervention from the experimenter.

Key words: male rats, instrumental responses, sexual behaviour, postcopulatory departure

In a previous study (Beck 1971) it was observed during testing of instrumental responses with sexual reinforcement in male rats that after a copulatory event the male departed from the female and returned to a compartment equipped with a lever. During these tests the male was placed in a compartment equipped with a lever and separated from the stimulus female by a guillotine door. The instrumental response consisted of six lever presses and was reinforced by opening the door, permitting contact between the animals. During training, the male was forced back into the compartment equipped with the lever by the experimenter after each contact with the female. Consequently, the male learned to return spontaneously to this compartment after each contact with the female. A similar phenomenon was observed during a study of the postejaculatory behaviour of male rats (Beck 1985). In this study each male was tested with the same stimulus female in two experimental situations in alternate sessions: when free access to the female was allowed, and during testing of the instrumental responses with sexual reinforcement. The results of this study indicated that during testing of the instrumental responses, the males returned spontaneously to the compartment equipped with the bar after each ejaculation while during the tests when free access to the female was allowed they tended to remain in the proximity of the female. In both these studies, the departure from the female after copulatory or ejaculatory events was interpreted as avoidance of interruption of the postcopulatory genital grooming which seems to be an element of the consummatory act due to the direct genital stimulation (Beach 1956). More recently (Beck and Biały 1993), however, such postcopulatory departure was observed also when the instrumental responses were reinforced by contact with the female lasting up to the end of a mount bout. Mount bout is defined as a sequence of mounts (one or more), with or without intromission, uninterrupted by any behaviour (other then genital autogrooming) not oriented toward the female (Sachs and Barfield 1970). In such a situation, this reaction cannot be explained in the way proposed above, as no interruption of the consummatory act ever took place. The departure of the male rat from the female after the mount bout was also observed by Larsson (1973).

Identification of the mechanism responsible for this postcopulatory departure reaction seemed to be important for better understanding of the behavioural process underlying the sexual behaviour of the male rat. The purpose of the present study was to elaborate the method allowing an analysis of this reaction. To that purpose instrumental responses with sexual reinforcement were tested in male rats in an apparatus in which the goal compartment and the start compartment of the runway were connected by one-way door which allowed such an analysis.

Eight male and eight female Wistar rats were used. They were maintained on food and water *ad lib.* and a reversed 12L:12D cycle. The animals were 180 days old at the beginning of the study. The males were housed 2 per cage with a wire mesh partition preventing direct contact between them. The females were housed 3-4 per standard laboratory cage.

The females were spayed by bilateral ovariectomy under chloral hydratum (360 mg/kg) anaesthesia. The oestrus was induced by sc. injection of 25 μg of estradiol benzoate (Oestradiolum benzoicum JELFA Jelenia Góra SA) and 500 μg of progesterone (Progesteronum JELFA Jelenia Góra SA) 48 and 3-6 h before each session respectively. After this treatment all females were sexually receptive and displayed the symptoms of behavioural oestrus.

Two kinds of apparatus, constructed of hardboard, were used. During training the apparatus (Fig.1A) consisted of start and goal compartments of equal dimensions (25 x 25 x 20 cm) connected by a runway. The runway was composed of four parallel corridors (12.5 x 25 x 50 cm). The final corridor of the runway and goal compartment as well as the goal compartment and start compartment were connected by one-way doors enabling the male to run in one direction only. During the experimental sessions the apparatus (Fig.1B) consisted of a start compartment (12.5 x 75 x 20 cm) and a goal compartment (25 x 37.5 x 20 cm) connected by a runway. The runway in this apparatus was composed of three parallel corridors (12.5 x 25 x 50 cm). The final corridor of the runway and the goal compartment, as well as the goal compartment and the start compartment, were connected by one-way doors. To register the passing of the rat, electric switches were attached at the proximal end of the first corridor of the runway, and on both one-way doors. The males were trained to run through the maze from the start compartment to the goal compartment where the incentive estrous female was tethered (Beck 1995). The contact with this female lasted up to the end of the mount bout. The male was then transferred to the start compartment. After 3-4 experimental sessions, the male was trained to open the door between the goal compartment and the start compartment. To this aim the door between

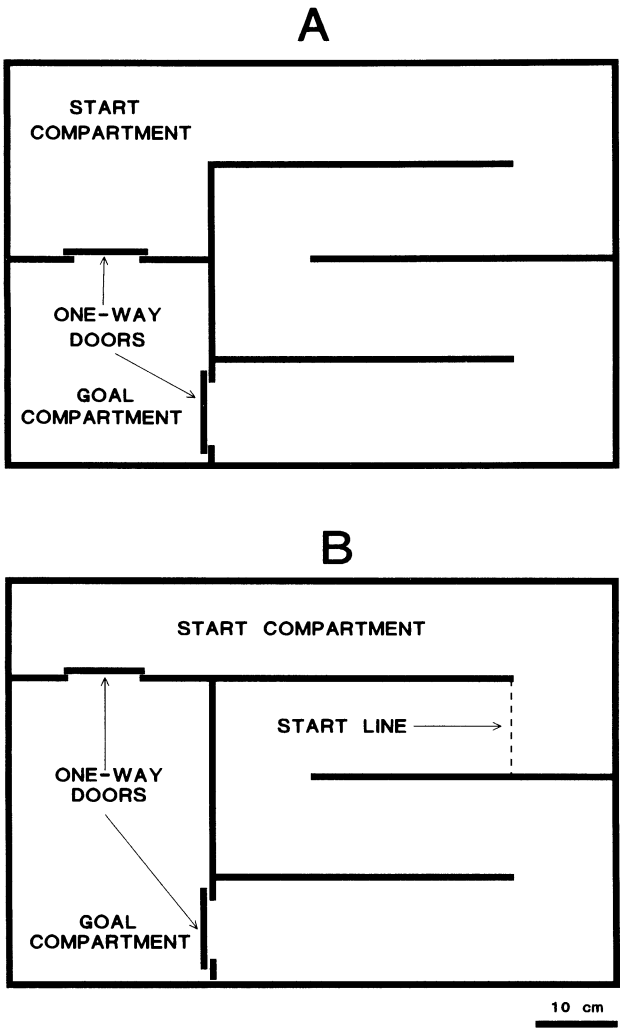

Fig. 1. A, the apparatus used during the training; B, the apparatus used during the test.

both compartments was opened by the experimenter in front of the rat after the end of the mount bout, while the rat explored the goal compartment. The rat learned this task after 1 or 2 trials. As a result, he spontaneously passed from the goal compartment to the start compartment after the end of the mount bout, and the new run could start without the intervention of the experimenter. As after copulation or ejaculation the male always tended to rest at the end of the first corridor near to the entrance to the second corridor, this corridor was treated as part of the start compartment during the experimental sessions.

The sessions were performed two times per week. Each session ended at the first intromission after the first ejaculation of the rat, or if ejaculation was not observed, after 15 min. Eight to eleven sessions were performed with each subject.

At the onset of the experimental session, the subject was placed in the goal compartment. The copulatory behaviour was then displayed in 0-41% of cases (mean 22 ± 4.6%) while in the remaining cases the subject passed to the start compartment after only a short (about 1 s) exploration of the goal compartment. Exploratory behaviour was observed also after the runs performed between the ejaculation and first postejaculatory intromission. Figure 2 shows a series of single frames from a videorecording illustrating the subject's behaviour during the test. In one case, the male did not depart from the female and remained in the goal compartment until ejaculation had been achieved. In one case during a session consisting of 10 runs only precopulatory behaviour (body or genital investigation) as well as exploratory behaviour were displayed.

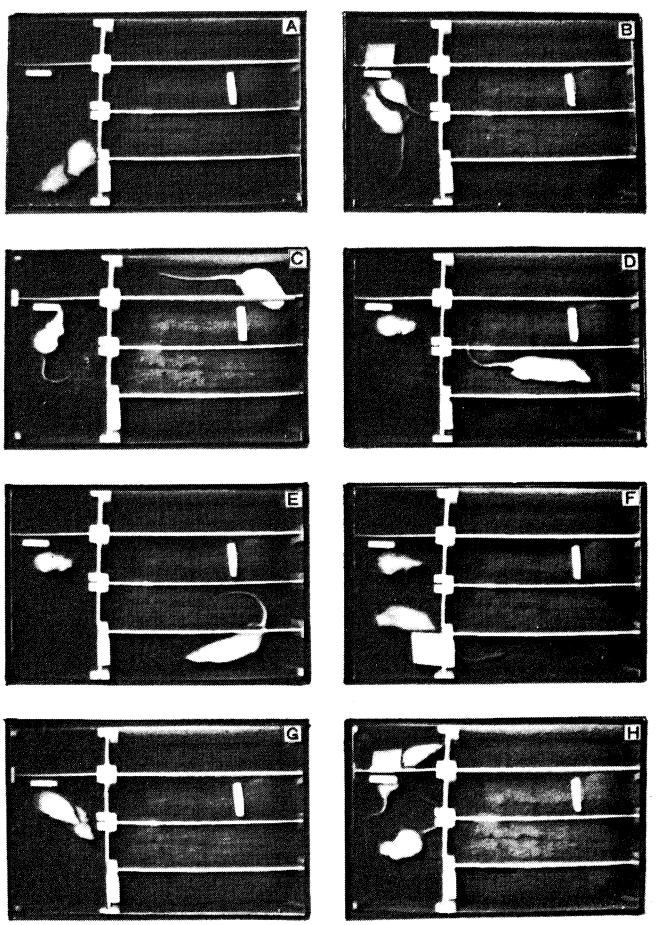

Fig. 2. A series of single frames from a videorecording illustrating behaviour of the subjects during the test. A, copulation; B, passing from the goal compartment to the start compartment; C, male in start compartment; D-E, run through the runway; F, entering the start compartment; G, copulation; H, passing from the goal compartment to the start compartment.

The method described in this study allows a precise analysis of the behavioural process responsible for the postcopulatory departure observed in male rats during testing of instrumental sexual responses. The identification of this mechanism, however, requires further studies. The results of this study indicate only that this behaviour is not a result of the action of some aversive drive related to sexual behaviour, as it was observed not only after the copulatory events, but also during the trials when no copulatory behaviour was displayed. The existence of such aversive drive in female rats was suggested by Peirce and Nuttal (1961). Moreover, these results indicate that this departure reaction was always displayed after the end of mount bout.

In conclusion, the method described above allows precise analysis of the postcopulatory departure reaction observed during testing of instrumental sexual responses in male rats. Moreover, it can be used for testing of instrumental sexual responses in male rats without the presence of an experimenter.

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