An Improved Chamber for the Observation and Analysis of the Sexual Behavior of the Female Rat

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MENDELSON, S. D. AND B. B. GORZALKA. An improved chamber for the observation and analysis of the sexual behavior of the female rat. PHYSIOL BEHAV 39(1) 67-71, 1987.—A new chamber designed to evaluate the sexual behavior of rats was found to have distinct advantages over chambers typically described in the literature. The new testing chamber is narrow, which maintains a male and female rat in the optimal orientation for the observer to view sexual behavior, i.e., in the side view. Moreover, the chamber consists of an upper and lower level, which allows the females an avenue of escape from the male. In Experiment 1 it was shown that use of the chamber increased the reliability of data gathered in evaluating the lordosis behavior of female rats. In Experiment 2 it was shown that the new chamber could be used to evaluate the pacing of copulation by female rats.

General Method

The Apparatus

The newly designed testing chamber, displayed photographically in Fig. 1 and schematically in Fig. 2, is constructed of Plexiglas with outside dimensions of approx-
FIG. 1. Photograph of the newly designed observation chamber (back view).

FIG. 2. Schematic drawings of the newly designed observation chamber. Approximate dimensions are in centimeters.
immediately 51×60.5×15 cm. It should be noted that the narrowness of the chamber forces animals to mount with their flanks to the observer. However, the chamber is not so narrow as to prevent a single animal from turning about freely inside. A platform 30.5 cm in length centered and set 28 cm above the floor divides the chamber into two levels. A set of ramps and a narrow landing at each end of the interior of the chamber allows the animals to move freely from one level to the other. The ramps are set at 120° angles and have thin strips of Plexiglas mounted at regular intervals to provide footholds. The floor of each level contains an inset of galvanized 1/2” hardware cloth to provide the animals with sure footholds during the display of sexual behavior. San-i-cell or other bedding material may be placed in the spaces below the hardware cloth to aid in maintaining the cleanliness of the chamber. Finally, the bottom half of the large front face of the chamber is attached by two short strips of stainless steel piano hinge. This allows easy placement and retrieval of animals, as well as access for cleaning the apparatus.

The 4 chambers employed in the present study were constructed by two workmen in a single afternoon. The construction of the 4 chambers, together, required 1.5 sheets of 4’×8’ Plexiglas, and the cost in materials for a single chamber was approximately $25 (U.S.).

**Animals and Surgery**

Sprague-Dawley rats were bred on the premises from stock originally obtained from Charles River Canada Inc., Montreal. At approximately 70 days of age, females were bilaterally ovariectomized, via bilateral lumbar incisions. Surgery was performed with animals under ether anesthesia. Animals were housed in groups of six in standard wire mesh cages, in a room maintained under a reversed 12 hr dark/12 hr light cycle at 21±1°C. Animals were allowed free access to food and water.

**Steroid Treatment**

Estradiol benzoate (EB) and progesterone (P) (Steraloids) were dissolved in warm peanut oil. All steroids were injected subcutaneously, in approximately 0.1 ml of vehicle.

**EXPERIMENT 1**

In Experiment 1, the lordosis behavior of female rats was evaluated in cylindrical glass chambers and in the newly designed chambers to determine what, if any, differences might exist between the two chambers in actual use. Of particular interest were potential differences in the reliability of evaluation, in the performance of animals, and in the times required to evaluate the animals’ behavior.

**Method**

In the first experiment, female rats were treated with 10 µg EB 48 hr and 150 µg P 4 hr prior to behavioral testing, and divided into 2 groups of 8 animals. After receiving brief instruction, two colleagues without prior experience in observing sexual behavior in rodents were asked to observe and evaluate lordosis behavior. The first naive observer evaluated the lordosis behavior of the first group of females in standard cylindrical glass chambers that measured 45 cm in height and 30 cm in diameter. Four pairs of animals were observed at one time, with each pair in a separate chamber. The responses made by each female to the first 10 mounts with pelvic thrusting by the male were evaluated. This observer then evaluated the second group of females in the newly designed chambers. In the case of the second naive observer, this procedure was reversed to eliminate any potential practice effects. In each case, lordosis behavior was evaluated concurrently by an experienced observer of lordosis behavior (the first author). The time required to evaluate the behavior of each group of 8 females was also recorded.

**Results and Discussion**

After the evaluation, both observers were in full agreement that by maintaining the animals in the side view orientation, the improved chamber was vastly superior to the glass cylinder in allowing a clear, unimpeded view of lordosis behavior. The superiority of the improved chamber was confirmed in the finding that when using the chamber, the naive observers’ data correlated extremely well with those of the experienced observer. In the case of the standard glass cylinder, the correlation coefficient of the data of the naive and experienced observers was .403, whereas with the improved chamber, a correlation coefficient of .952 was obtained. The inter-observer reliability using the cylindrical chambers was extremely low; however, it is likely that the reliability would have been higher had all observers had prior experience.

In observing the animals in the two groups, it appeared that those in the newly designed chambers responded to the males with somewhat greater intensity and frequency. The possibility that the opportunity to escape the male might have an effect on lordosis behavior would not be unprecedented. For example, a recent report indicates that the effect of lesioning the medial preoptic area on lordosis is at least partially determined by whether or not a female is able to control her proximity to the male [11].

In the statistical analysis of the performance of the two groups, only the data recorded by the experienced observer were considered. Moreover, because each group was observed twice, i.e., once for each naive observer, only the data from the first trials were analysed. The mean lordosis quotients and standard errors of the means of the females in the cylindrical and newly designed chambers were 59±6.7 and 75±10.9, respectively. This difference, however, was not statistically significant by a Student’s t-test.

There was no difference between the two chambers with respect to the amount of time required to evaluate lordosis behavior. For both types of chambers, with four chambers of each type in use at a time, the lordosis behavior of 8 females required almost exactly 25 min to evaluate.

**EXPERIMENT 2**

Under natural conditions, where the female is free to approach or escape the male, the female rat appears to play an active role in the initiation and regulation of sequences of sexual contact with the male. The timing of approach and escape by which the female regulates sexual activity has been referred to as pacing behavior [7].

Under typical laboratory conditions, where the female is forced to remain in close proximity to the male, it is not possible to evaluate pacing behavior. However, if the female is allowed an avenue of escape from the male, then it is possible to observe and evaluate this behavior [4, 5, 6, 9]. In the second experiment we tested the assumption that in the
new chambers, the ability of the female to escape the male by running to the unoccupied level of the chamber would both precipitate and allow the evaluation of pacing behavior.

Method

Females were treated with 10 μg EB 48 hr and 500 μg P 4 hr prior to behavioral testing, and divided into 2 groups of 5 females. Each female of the first group was placed in a newly designed chamber with a single, sexually vigorous male. The second group of females served as control animals, and each female of this group was placed in a newly designed chamber with an ovariectomized stimulus-female. These stimulus-females were of roughly the same size as the males, and had not received steroid replacement therapy. The female/male and female/stimulus-female pairs were allowed to move freely in the chamber while the occurrences of two specific behaviors, leaving and returning, were recorded. Leaving was defined as the female moving to the level of the chamber unoccupied by the male/stimulus-female, whereas returning was defined as the female moving to the level occupied by the male/stimulus-female. The occurrences of these behaviors were recorded for 10 min on an Esterline Angus event recorder. An occurrence of leaving was recorded by producing a single, brief deflection of the pen, whereas returning was recorded by producing several rapid and successive deflections of the pen. Thus, an occurrence of leaving appeared on the chart as a single, narrow pen stroke, whereas an occurrence of returning appeared on the chart as a single wide pen stroke.

Results and Discussion

The steroid-primed females that had been placed with males appeared, in general, to be far more active than those in the control condition. Those placed with males displayed intense and prolonged bouts of ear-wiggling and hop-darting. These females also made frequent moves from level to level of the chamber, in what appeared to be patterns of "approach, orient, and runaway" characteristic of pacing behavior [17]. For example, one common pattern of movement among the experimental females was to run to the level occupied by the male, remain for 2 or 3 seconds, and then run away to the unoccupied level of the chamber. The female would then remain on this level, for roughly 8 to 12 seconds, before returning to the male. In other instances, the female would run away from the male for only 2 or 3 seconds, and then dart back to the male to linger for a time. In the chart records of the experimental females are frequent occurrences of closely spaced wide stroke/narrow stroke couplets, representing these rapid sequences of leaving and returning to the male.

In contrast with experimental females, control females appeared merely to wander from level to level in slow and random fashion. A number of times, the approach of the large, stimulus-female did elicit brief bouts of ear-wiggling and hop-dart behavior in control females. However, in only a few instances did control females display the "approach, orient, and runaway" pattern of behavior, and only a few wide stroke/narrow stroke couplets appear in the records of these animals.

When general activity was evaluated simply in terms of movements from level to level, the experimental females were found to be significantly more active than control females, with averages of 35.6±5.1 and 14.4±2.1 level changes made by each group, respectively, t(8)=3.8, p<0.006. A significant difference was also found between the two groups in their patterns of leaving and returning to the level occupied by the male/stimulus-female. The chart records of the experimental females contained significantly more wide stroke/narrow stroke couplets than those of the control stimulus-female, with averages of 13.8±3.5 and 0.8±0.5 couplets, respectively. t(8)=3.66, p<0.007. For this analysis, a couplet was defined as a wide stroke and a narrow stroke that occurred within 4 sec of each other on the event recorder chart.

In Experiment 2, both males and females were allowed to wander through the chamber in a "free pursuit" mode. However, by placing shock grids in the ramp landings at each end of the chamber (see Fig. 1), the male can easily be trained to remain in either the top or bottom level of the chamber. Under these conditions, the female is in complete control of the pace of copulation, and parameters such as the latency of the female's approach to the male, and the amount of time the female spends with the male can easily be measured and evaluated.

GENERAL DISCUSSION

In the present study we have described a new kind of observation chamber that offers distinct advantages over the standard chamber. One advantage is that in some parameters, use of the new chamber enhances the reliability of data. For example, in Experiment 1 it was shown that when using the new chamber, naive observers of sexual behavior were able to perform nearly as well as an experienced observer in evaluating the lordosis response. Another advantage is that use of the new chamber increases the number of behavioral parameters that can be measured. In the standard chamber, the male and female are forced to remain in close proximity to each other, which prevents the female from pacing the progress of copulation. However, as shown in Experiment 2, the ability of the female to escape the male in the new chamber precipitated pacing behavior. Moreover, and no less importantly, the simple bi-level design of the chamber allowed the components of pacing behavior to be defined and recorded in an exceedingly straightforward manner. Of course, it must be noted with caution that what has been interpreted as pacing behavior in the present experiment may not be fully comparable to pacing behavior observed under natural conditions, or in the devices that have been designed by others.

Not all studies on the sexual behavior of rodents have been performed with simple restrictive chambers. For example, in one elegant study females were trained to lever press for access to males. Differences in the latencies of the females to press for males under certain conditions led to the conclusion that the female rat takes an active role in pacing the progress of copulation [2]. Other excellent studies have taken place in the wild, or under elaborate semi-natural conditions in laboratories. The results of these studies have provided further basis for the definition of new parameters of sexual behavior, such as pacing behavior [3, 7]. In several cases, special testing chambers have been devised to more easily evaluate pacing behavior in a laboratory setting. Experimenters have interconnected 2 or 3 separate chambers and, by allowing the female to move freely from chamber to chamber, have been able to evaluate pacing behavior [4, 5, 6, 9]. However, to the best of our knowledge, these testing devices were not specifically designed to maintain the animals in the optimal side view orientation. Presumably, the
reliability of data from devices consisting of several interconnected cylindrical or cubical chambers would be no greater than the reliability from a single, standard chamber. It must be mentioned that in some ways the new chamber also may be somewhat inadequate. For example, whereas the hopping and darting that characterizes female proceptive behavior can be noted in the new chamber, the dimensions of the chamber clearly do not allow the full expression of this behavior. We have also observed that very large rats can appear somewhat crowded on the relatively short upper and lower levels of the chamber. It is possible that increasing the length of these levels from 30.5 cm to 40 cm may ameliorate these problems to some degree.

An initial concern was that the design of the chamber would allow a female to remain stationary on one of the landings between levels, where the male could not mount her. However, this problem did not occur in the present study. This may have been due in part to the fact that a female on a landing always remained in reach of the male. Indeed, in several instances we observed a male gently using his teeth on the scruff of a female's neck to pull her off the landing up or down to his level. We note that neck biting by the male has been reported to occur during sexual activity in wild rats [3]. However, to the best of our knowledge, this particular behavior by male rats has not previously been reported and may deserve further attention.

In conclusion, we have presented a new chamber for the evaluation of sexual behavior with distinct advantages over other chambers that have been described in the literature. The new chamber can be used to analyze pacing behavior and increase ease and reliability in evaluating lordosis behavior of female rats. Of course, the chamber also provides an excellent environment in which to evaluate the sexual behavior of the male rat, and may be useful in the analysis of other animal behaviors, such as aggression, play, maternal behavior, place preference, and spatial learning. Finally, we note that the chamber would be very easily standardized, and extremely well suited for computer and/or video analysis of behavior.

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REFERENCES