BRUCE EFFECT COMPETENCE IN YELLOW-LETHAL HETEROZYGOUS MICE

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(Received 24th April 1974)

Summary. The capacity of heterozygous yellow-lethal (A/a) and brown (aa) male mice to induce the Bruce effect was investigated. The proportion of SJL/J females showing a failure of implantation when exposed to males of either genotype was 80%, whereas only 20% of the control females not so exposed showed pregnancy failure. One-half of the females whose pregnancy had been blocked as a result of exposure to either the A/a or aa male mice (50% and 55%, respectively) ovulated within 7 to 8 days after the initial mating. Results are discussed in regard to the hypothesis that the neuroendocrine mechanisms involved in pregnancy blocking and synchrony of oestrus are not qualitatively different.

In several mammalian species, chemical factors called pheromones exert an important regulatory influence on reproductive, aggressive and other social behaviour patterns (Bronson, 1970). In house mice, the exposure of a recently mated female mouse to a male of a strain other than that of the stud male often results in a blocking of pregnancy and a return to oestrus. This phenomenon, first reported by Bruce (1959), has been studied and reviewed by several investigators (Bruce, 1966; Whitten, 1966; Dominic, 1966, 1969; Bronson, 1968, 1970).

Whitten (1956) reported that chemical factors in male urine influence the oestrous cycle of female mice. Grouping of female mice tended to suppress the oestrous cycle, but exposure of non-pregnant females to males or to male urine for 48 hr resulted in an induction of oestrus with the greatest number of females demonstrating oestrus on the 3rd night after exposure to the male. Females exposed to males also showed more regular oestrous cycles within a 12-day period than grouped females. This ability of the male to induce oestrus is known as the Whitten effect.

In both the Bruce and the Whitten effects, a faster return to oestrus occurs in exposed females than in control mice not so exposed. Whether the same chemical factors and/or genes are responsible for the two phenomena has not yet definitely

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been proved. It has been assumed that males capable of eliciting either reaction are capable of eliciting both, but this hypothesis has not been tested with a large number of strains.

Bartke & Wolff (1966) reported that exposure to heterozygous $A^p$ male mice did not elicit synchronous oestrus in females, but that exposure to $aa$ males did. Since the oestrous cycle of both $A^p$ and $aa$ female mice was synchronized by the presence of $aa$ and not $A^p$ mice, it was concluded that $A^p$ mice differed from $aa$ mice in the production of the odoriferous substance mediating the Whitten effect. In the present study, we investigated the pregnancy-blocking capacity of heterozygous $A^p$ and $aa$ mice. If the male pheromone responsible for pregnancy-blocking and that which induces synchrony of oestrus are identical, then it is likely that males which are incompetent to induce the Whitten effect would be incompetent to induce the Bruce effect. The present study shows that $A^p$ mice are as competent as their $aa$ littermates in blocking pregnancy.

Heterozygous $A^p$ and $aa$ male mice, 5 to 8 months of age at the time of experiment, were obtained from Dr Annabel G. Liebelt of the Medical College of Georgia. Virgin female mice of the SJL/J strain and CBA/J males were obtained from The Jackson Laboratory, Bar Harbor, Maine. Mating was encouraged by pairing two SJL/J females, about 5 months old at the time of the experiment with one CBA/J male approximately 8 months old. The females were examined for the presence of vaginal plugs each morning. Females in which vaginal plugs were found were assigned in turn to $A^p$, $aa$, or to control treatment and were placed in one side of a cage $5 \times 10 \times 5$ in., divided into two $5 \times 4.5 \times 5$ in. compartments by two impassable hardware cloth screens (grid size, $\frac{1}{4}$ in.) set $\frac{1}{2}$ in. apart from each other. The cages containing one inseminated female each were placed in a separate room. During the afternoon of the same day, either an $A^p$ or an $aa$ male was placed in the other compartment of the cage. During the afternoon of the 5th day, the males were removed. The test males were individually housed for at least 1 week in a separate room before they were used. The control females were placed in one side of the compartment and the other side was left unoccupied. Cages containing control females were then placed in a room which contained no male mice. All three rooms were on a 12-hr light/12-hr dark cycle, and no animals were housed in the treatment rooms except those being used in the study. On the 7th or 8th day following insemination, the females were killed by ether, their uteri were examined under a dissecting microscope at ×7 magnification, and the implantation sites were counted. If there were no implantation sites in the uterus, the uterus was opened and flushed with saline, the oviducts were dissected, and the ova were counted at ×25 magnification.

The results of the experiment are summarized in Table 1. Whereas 86% of the control females showed implantation sites, only 20% and 23% of those females exposed to $A^p$ or $aa$ males, respectively, were pregnant. There was no significant difference between $A^p$ and $aa$ males in the ability to block implantation ($\chi^2 = 0.02$, N.S.). The difference in the number of pregnancies between control females and females exposed to males was highly significant ($\chi^2 = 8.30$, $P < 0.005$), indicating that both types of males were capable of inducing the
Bruce effect. The numbers of implantation sites in the three groups were subjected to analysis of variance, the results of which indicated no significant differences among the groups \( F = 1.23, P < 0.25 \).

Of twenty females whose pregnancy was blocked by the presence of \( A^\alpha \) male mice, ten (50%) ovulated, while eleven out of seventeen (65%) of those exposed to \( aa \) males did so. There were only three control females with a spontaneous failure of implantation and in only one of these were ova found in either the oviducts or the uterus.

Clearly, the chance for successful implantation in inseminated female mice was significantly reduced by exposure to either \( A^\alpha \) or \( aa \) mice, and there was no difference between the two genotypes in the effectiveness of the induced block to pregnancy. This appears to be the first time that the Bruce effect competence of \( A^\alpha \) heterozygous mice has been explicitly tested. If the data of Bartke & Wolff (1966) are valid, then \( A^\alpha \) heterozygous mice represent a genotype in which the ability to block pregnancy is present, whereas the ability to synchronize oestrus is absent. This would suggest that the pheromone(s) involved in the Bruce effect is either qualitatively or quantitatively different from that

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<th>Table 1. Effect of exposure to yellow (( A^\alpha )) and brown (( aa )) male mice on implantation and ovulation in SJL/J female mice</th>
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<tr>
<td>( \text{No. of females with implantations/} )</td>
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<td>( \text{no. inseminated} )</td>
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<td>( A^\alpha ) Male</td>
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<td>( aa ) Male</td>
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* Numbers too few for percentage to be meaningful.

involved in the Whitten effect. The claim that \( A^\alpha \) heterozygous males lack the ability to synchronize oestrus (Bartke & Wolff, 1966) has, however, been challenged by Whitten (1969). This investigator repeated the comparison of \( A^\alpha \) and \( aa \) males, and found that the peak of mating in females paired with \( A^\alpha \) males came on the 3rd night, the usual indicator of synchronous induction of oestrus. Whitten (1969) has pointed out that in the experiment of Bartke & Wolff (1966), the ability to produce the oestrus-inducing pheromone was confounded with the ability to copulate, since multiple matings (two females to a male) were used. Since different genotypes have variable recovery times after ejaculation, ranging from 1 to 74 hr (McGill & Blight, 1963), the multiple mating procedure does not provide the optimal test for pheromonal synchrony of oestrus unless the male is able to copulate more than once within a few hours. Assuming that these criticisms are correct and that Whitten's data more accurately describe the competence of \( A^\alpha \) mice with respect to the Whitten effect, we can only conclude that \( A^\alpha \) mice, like most other strains tested, are able to induce both the Bruce and Whitten effects. Only one strain, C57BL/6J, is known to give rise to males which are incompetent to block pregnancy (Chapman & Whitten, 1969; S. Kessler, S. Blum and R. Kakihana, unpublished
data), but are capable of synchronizing oestrus in females (Bronson, 1968). Whether the same neuroendocrine mechanisms are involved in blocking pregnancy and in inducing oestrus remains to be determined. The further investigation of C57BL/6J mice may shed light on the neuroendocrine bases of these two pheromonal phenomena.

This work was supported by grants from the National Science Foundation (NSF GB 31099) and from the National Institute of Alcohol Abuse and Alcoholism (AA 0498).

REFERENCES


