THE EFFECT OF ANAESTHESIA ON ENDOCRINE RESPONSES TO OLFATORY STIMULI IN FEMALE MICE

K. BROWN-GRANT*

Department of Human Anatomy, South Parks Road, Oxford

(Received 15th November 1965)

Summary. The incidence of the Bruce effect (block of pregnancy in newly-mated female mice exposed to the smell of male mice of another strain) is significantly lowered in mice anaesthetized with 'Avertin' (tri-bromethanol and amylene hydrate) 10 to 12 days before mating. Pentobarbitone anaesthesia may have a similar effect. 'Avertin', but not pentobarbitone anaesthesia, also prevents the appearance of the Whitten effect. The use of 'Avertin' in the investigation of neuro-endocrine effects involving the olfactory system is not advisable.

INTRODUCTION

Olfactory stimuli are known to influence the secretion of pituitary gonadotrophins in the female mouse (Parkes & Bruce, 1961), and recent neuro-anatomical studies have shown that there is a relatively direct projection of the olfactory system to the anterior hypothalamus (Powell, Cowan & Raisman, 1963). In the course of a study with Dr T. P. S. Powell and Dr W. M. Cowan in which we were attempting to determine the effects of lesions in various parts of the olfactory system on the Bruce effect (the failure of implantation when newly-mated female mice are exposed to the smell of male mice other than the stud male in the first 3 days of pregnancy) it became apparent that animals anaesthetized and submitted to blank operations were failing to show the pregnancy block effect in a high proportion of cases. The effect of anaesthesia alone on pregnancy block induced by exposure to the smell of strange males was, therefore, investigated.

MATERIALS AND METHODS

The mice used were young adult, virgin females (8 to 10 weeks of age) of the Parkes albino strain bred in the laboratory colony from animals obtained originally from the National Institute for Medical Research, Mill Hill. They were housed in groups of eight in metal boxes, $13 \times 7 \times 4\frac{1}{2}$ in. deep with wire grid lids, for 10 to 12 days before two or three males of the same strain were placed in the box. The females were examined daily in the morning thereafter for the presence of a vaginal plug. The mated animals were isolated singly for 24 hr after the finding of a plug and then exposed to the smell of strange males for

* Locke Research Fellow of the Royal Society.
3 days by placing them singly in metal boxes 9 × 7 × 3½ in. deep with a perforated metal lid. During the preceding 12 hr these boxes, containing paper towels, but no sawdust or other bedding, had been occupied by four adult males of the CBA strain. The females were moved morning and evening for 3 days into new cages freshly soiled in the same way by male mice. This procedure is based on the method for producing pregnancy block described by Parkes & Bruce (1962). Vaginal smears were taken daily in the morning for 7 days beginning on Day 1 of pregnancy, that is 24 hr after the finding of a vaginal plug and on the morning of the day that the animals were first placed in the soiled box. Pregnancy block was diagnosed by the return of a fully cornified vaginal smear during this period and was confirmed by the examination of the uterus at post-mortem between the 10th and 14th day after mating. Failure to block the pregnancy was confirmed by the presence of embryos in the uterus at post-mortem or by the birth of a litter. No animal diagnosed as blocked, from examination of the smears, was pregnant at post-mortem or gave birth to a litter. Only two out of more than seventy animals classed as unblocked were not pregnant at post-mortem and one other failed to give birth to a litter. These animals were assumed to have been pseudo-pregnant. Control animals and animals anaesthetized 10 to 12 days before were exposed concurrently in all experiments.

The anaesthetic agent used for the original lesion experiments was ‘Avertin’ (Tri-bromethanol 1 g and amylene hydrate 0.5 g/ml, Batch No. 3277L, manufactured by Winthrop Laboratories, New York, and obtained from Bayer Products, Surbiton-on-Thames); 0.3 ml were dissolved in 10 ml of warm 0.9% (w/v) NaCl solution. The dose given was 0.1 ml/10 g body weight intraperitoneally. The onset of full surgical anaesthesia was rapid (2 to 3 min) and the animals recovered consciousness in about 1 hr; mortality was less than 5%. The animals were mated 10 to 12 days after anaesthesia. Exactly the same procedure was followed in experiments designed to determine the effect of anaesthesia alone. The boxes of eight mice were brought from the animal house to the laboratory in the afternoon. Animals were selected at random, from each box, half being anaesthetized with ‘Avertin’ and half receiving an injection of an equal volume of warm saline as controls. In later experiments half the animals were anaesthetized with sodium pentobarbitone (‘Veterinary Nembutal’, Abbott Laboratories), the dose being 60 mg/kg body weight injected intraperitoneally. To avoid damage from the conscious control animals, control and anaesthetized groups were kept in separate boxes overnight in the laboratory and the original groups reassembled the next morning. The males were introduced into the cages 10 to 12 days later.

RESULTS

The main experimental results are presented in Table 1. The incidence of pregnancy block in normal animals (62%) is slightly lower than that reported by Parkes & Bruce (1962), but they used five males to soil each cage and only four were used in these experiments. The contrast between the normal and ‘Avertin’ treated animals is striking; only 34% of a group of animals anaesthetized with ‘Avertin’ showed a block of pregnancy. The difference between these two groups is statistically significant. The possibility that this effect may be
related to anaesthesia as such has not been excluded; the incidence of pregnancy block in a group of animals anaesthetized with 'Nembutal' was lower than, though not significantly different from, that of the control animals but was also not significantly higher than in the 'Avertin' treated group.

The effect of 'Avertin' anaesthesia is not restricted to the pregnancy block situation. Before mating the female mice are housed in groups of eight and when the males are placed in the boxes the greatest number of matings would be expected to occur on the 3rd night after the introduction of the males; this is the Whitten effect (Parkes & Bruce, 1961). The data on normal and 'Avertin' treated animals was examined to see whether or not it was consistent with the hypothesis that the matings were equally distributed between the different nights.

Table 2 presents the results and includes data obtained from both control and treated animals in which pregnancy block was produced by a different method and which were therefore not included in Table 1.

In the case of control animals and animals anaesthetized with 'Nembutal' the $\chi^2$-test indicates a highly significant deviation of the observed distribution of matings from that postulated, with a peak incidence on the 3rd night, whereas the results from the 'Avertin' treated animals are consistent with the hypothesis. It is not possible to tell from these results, because vaginal smears were not taken before mating, whether the 'Avertin' treated mice are abnormal in that the interaction between females housed together producing prolonged periods of di-oestrus has been abolished (i.e. an absence of the Lee-Boot effect), or whether the synchronizing effect of olfactory stimuli from the males has been abolished (an absence of the Whitten effect). This question was investigated in a further series of experiments carried out in part in collaboration with Dr R. L. W. Averill. Vaginal smears were taken daily for 30 days from groups of mice housed eight to a box as in the pregnancy block experiments. Half the animals were then anaesthetized with 'Avertin' as before and smears continued for a further 30 days. Examination of the individual cycle records showed that 5- to 7-day cycles predominated; during a cycle there were usually 2 or 3 days on which the smear contained no leucocytes but was composed of nucleated or fully cornified epithelial cells or a mixture of these two types of cell. All such smears were recorded as 'oestrogenized' smears for analysis of the results.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of mice</th>
<th>$\chi^2$-test versus controls</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blocked</td>
<td>Not blocked</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>29</td>
<td>18</td>
<td>$-$</td>
</tr>
<tr>
<td>'Avertin' treated</td>
<td>16</td>
<td>29</td>
<td>5-29 $&lt;0.05$</td>
</tr>
<tr>
<td>'Nembutal' treated</td>
<td>18</td>
<td>22</td>
<td>1-80 $&gt;0.10$</td>
</tr>
</tbody>
</table>

Downloaded from Bioscientifica.com at 10/29/2018 06:22:35PM via free access
Spontaneous pseudo-pregnancies (a period of six or more consecutive days on which leucocyte-containing smears were obtained) were observed in about 15% of mice. A convenient way of expressing this data is to calculate the number of 'oestrogenized' smears obtained as a percentage of the total number of smears examined for each group. The percentages of two groups of twenty-four mice studied over 30 days were 33% and 30% respectively; the first group consists of the mice that then received 'Avertin' as in the earlier experiments; over the next 30 days the incidence of oestrogenized smears was 32%. The second control group showed an incidence of 30% over the 30 days after saline injection. These groups did not differ before or after 'Avertin' treatment, nor did they differ from a group of seventy-five mice housed three to a box and smeared for periods of 18 to 27 days, which gave a mean value of 31% for the incidence of 'oestrogenized' smears.

Table 2
INCIDENCE OF MATINGS ON DIFFERENT NIGHTS AFTER THE INTRODUCTION OF MALES INTO BOXES CONTAINING EIGHT FEMALE MICE

<table>
<thead>
<tr>
<th>No. of matings on:</th>
<th>Night 1</th>
<th>Night 2</th>
<th>Night 3</th>
<th>Night 4</th>
<th>Night 5 or later</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>14</td>
<td>12</td>
<td>28</td>
<td>6</td>
<td>5</td>
<td>23-8</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>'Avertin' treated</td>
<td>18</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>9-15</td>
<td>$&gt;0.05$</td>
</tr>
<tr>
<td>'Nembutal' treated</td>
<td>9</td>
<td>9</td>
<td>17</td>
<td>5</td>
<td>1</td>
<td>14-8</td>
<td>$&lt;0.01$</td>
</tr>
</tbody>
</table>

The data were examined by means of the $\chi^2$-test for evidence of significant divergence from the distribution to be expected on the hypothesis that matings would be expected to be equally distributed between different nights. 'Avertin' and 'Nembutal' treated mice were anaesthetized with these drugs 10 to 12 days previously as described in the text.

DISCUSSION

Mice anaesthetized with 'Avertin' 10 to 12 days previously showed a significantly reduced incidence of pregnancy block when placed in cages soiled by strange males. In addition, the expected peak of matings on the 3rd night after the introduction of males into a cage of females (the Whitten effect) which was observed in the control mice was not seen in 'Avertin' treated mice. This effect does not appear to be related to any change in the e oestrous cycle as in a second experiment the incidence of 'oestrogenized' vaginal smears was the same before and after administrations of 'Avertin' and comparable to that of a control group housed under the same conditions.

The most likely explanation for these findings appears to be that 'Avertin' anaesthesia interferes in some way with olfactory sensitivity or discrimination; whether this is a peripheral effect on the olfactory mucosa or an effect elsewhere in the neural pathway to the hypothalamus is not known. It is also possible that the effect on pregnancy block may be a consequence of anaesthesia however produced. 'Nembutal' anaesthesia 10 to 12 days before reduces the incidence of pregnancy block but the difference from control animals is not significant; 'Nembutal' anaesthesia does not appear to modify the Whitten effect.
Anaesthesia and the Bruce effect

In experiments concerned with neuro-endocrine effects involving the olfactory system it appears that the use of ‘Avertin’, although it is a safe and convenient anaesthetic agent for small laboratory animals, is not advisable.

ACKNOWLEDGMENTS

This work was supported in part by a grant for research expenses from the Locke Fund of the Royal Society, by a grant for technical assistance from the Medical Research Council, and by a United States Air Force grant (AF EOAR 64-3) to Professor G. W. Harris, f.r.s. The valuable technical assistance of Mr M. Sherwood is gratefully acknowledged. My thanks are due to Dr R. L. W. Averill for allowing me to include in this paper data on the vaginal smear pattern of mice obtained by him.

REFERENCES

