NEURAL FACTORS IN THE MAINTENANCE OF LACTATION IN THE RAT

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SUMMARY

The role of the peripheral innervation of mammary tissue in the maintenance of lactation has been investigated by the procedure of selective thelectomy combined with denervation of the posterior thoracic nipples.

When suckling is restricted to a single pair of nipples bilateral transection of the three adjacent nerves supplying a nipple arrests lactation completely; partial denervation is associated with a reduced level of lactational performance which is directly related to the concentration of the residual innervation.

Increase in litter size is associated with an overall increase in milk-yield up to a limit beyond which the addition of further young to the litter is without effect.

It is inferred that there is a quantitative relationship between the neural stimulus of suckling and the endocrine response of the hypothalamic-pituitary system.

INTRODUCTION

Although the importance of suckling for the maintenance of lactation in the rat is well established, few attempts have been made to elucidate the quantitative relationship between stimulus and response in the mediation of this form of neuroendocrine interaction. Inglebrecht (1935), Eayrs & Baddeley (1956) and Grosvenor (1964a, b, 1966) have shown that, in the rat, interruption of the sensory pathways linking the mammary glands with the brain by means of section of the spinal cord results in severe or total impairment of lactation, but such findings may be complicated by the coincidence of such factors as disturbance of appetite, paresis of the hind limbs, interference with visceral motor function and poor general condition most of which are usual after this operation. These effects may be minimized by interrupting the peripheral nerve supply to the mammary tissue and examining the influence of this lesser procedure on lactational performance at a time remote from that of operational trauma.

There is reason to believe from physiological (Hooker & Williams, 1940) and histological (see review by Cross, 1961) evidence that the stimuli which influence lactation arise from the sensory innervation to the nipple. Anatomical studies (Edwardson, in preparation) have shown that the six pairs of nipples normally present are respec-
tively innervated by segmental nerves centred about the lateral and ventral cutaneous branches of spinal nerves T3, T5, T7, T12 and a complex of cutaneous nerves derived from the lumbar plexus. These observations have been confirmed and extended by physiological evidence showing that a pair of nipples may be effectively denervated by bilateral section of three appropriate dorsal spinal roots centred about the main spinal nerve to the nipple (Eayrs & Edwardson, 1964). After such an operation the animal appears to be perfectly normal apart from the somatosensory deficit of the cutaneous segment surrounding the nipple.

The present paper describes experiments in which such a preparation has been used to investigate further the peripheral pathways through which the stimulus of suckling mediates its influence on lactation and also to determine the quantitative relationship between the volume of lactation and the intensity of stimulation.

**MATERIALS AND METHODS**

*Animals.* The animals used were mature female rats kept in individual cages and fed on pellet Diet 41 B and water. The third pair of thoracic nipples of each rat was totally or partially denervated by the surgical procedure described below and a post-operative period of 30 days was allowed for recovery. The rats were then mated and after parturition allowed to nurse a full-sized litter until the fifth day of lactation. All nipples except the third thoracic pair were removed under ether anaesthesia on the fifth day and the litters reduced to two young (one male and one female) which suckled this single pair of nipples for the remainder of the experiment.

*Surgical procedures.* According to preliminary anatomical studies reported elsewhere (Eayrs & Edwardson, 1964) the third thoracic pair of nipples in the rat is supplied by cutaneous branches of spinal nerve T7 with contributions from at least two adjacent spinal nerves, T6 and T8. In order to denervate the mammary tissues the several combinations of these three pairs of spinal nerves set out in Table 1 were cut bilaterally. Under anaesthesia with tribromoethanol (200 mg./kg. body weight), the intercostal nerves were approached through a mid-line incision made through the skin overlying the thoracic vertebrae followed by resection of the deep muscles of the back and the intercostal muscles. The nerves were cut about 5 mm. distal from their point of emergence from the vertebral column and the proximal stumps sutured to the erector spinae muscle to prevent regeneration. The control animals (Group A—Table 1) were similarly treated except that the intercostal nerves were not sectioned.

*Measurement of lactation.* The course of lactation was recorded for the 16 days following parturition by weighing the pups once daily. The presence or absence of milk in the stomachs of the young was also checked at the time of weighing and a note made of their general condition. The mean increase in weight of the pups surviving between the fifth and sixteenth days of lactation and the proportion of young surviving in each group provided the measures used to compare the effects of the several experimental treatments on lactation.

*Litter-size and lactation.* In further groups of intact rats an alternative method was used to modify the degree of stimulation reaching the central nervous system as a result of suckling. On the day after parturition (day 1 of lactation) the number of young was either reduced or increased, by the addition of foster-pups of the same age.
to give litters of 1, 2, 3, 4, 6, 8, 10, 12, 15, 20 and 25 young, respectively. The total increase in weight of these litters over the first 16 days of lactation was used as an index of maternal milk yields.

Parkes (1926) has shown that with small litters of either one or two pups there is no lactational dioestrus in the mouse. Since vaginal dioestrus is dependent upon the release of prolactin, vaginal smears were taken daily from the 8th to 16th days from rats suckling one, two or three pups per litter.

**RESULTS**

Table 1 shows that lactation is completely arrested when suckling is confined to nipples previously deafferented by section of the three spinal nerves which supply the surrounding dermatome and that, after partial denervation, a marked correlation exists between the degree of residual innervation and the level of lactation.

Table 1. **Effect on lactation of sectioning various combinations of the main spinal nerves supplying the sensory innervation of the third thoracic pair of nipples**

<table>
<thead>
<tr>
<th>Group</th>
<th>Level of transection</th>
<th>No. of rats</th>
<th>Lactated</th>
<th>Failed</th>
<th>Mean weight increase of young, days 5-16 (g. + s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Control</td>
<td>7</td>
<td>7</td>
<td>0</td>
<td>12.67±1.10</td>
</tr>
<tr>
<td>B</td>
<td>T678</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>T7</td>
<td>12</td>
<td>5</td>
<td>7</td>
<td>7.45±0.86</td>
</tr>
<tr>
<td>D</td>
<td>T67</td>
<td>6</td>
<td>1</td>
<td>5</td>
<td>3.50</td>
</tr>
<tr>
<td>E</td>
<td>T78</td>
<td>7</td>
<td>2</td>
<td>5</td>
<td>3.97</td>
</tr>
<tr>
<td>F</td>
<td>T68</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>8.16±1.01</td>
</tr>
<tr>
<td>G</td>
<td>T6</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>10.14±0.32</td>
</tr>
<tr>
<td>H</td>
<td>T8</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td>11.0±0.60</td>
</tr>
</tbody>
</table>

**Effect of total denervation**

The level of lactation characteristic of the control rat is illustrated in Fig. 1. This shows that the smooth curve of growth of the young is severely reduced for about 72 hr. after the operation of thelectomy and reduction of the litter to two in number. During this period little milk was observed in the stomachs of the young while the glands of the does were considerably engorged. The eventual restoration of lactation and subsequent growth of the pups of this group contrasts markedly with the performance of rats in which the nipples had been denervated. No milk was ever seen in the stomachs of young suckling these animals and death from inanition followed within 5 days.

**Effect of partial denervation**

The rate of growth of the pups suckling rats in which the mammary tissue had been partially denervated showed a marked correlation with the expected degree of sensory deprivation. Thus the impairment to lactation resulting from transection of spinal nerve T7 (Group C), shown on anatomical grounds to provide the main weight of innervation to the third thoracic nipple, was substantially greater (see Table 1) than that produced by interrupting either or both of the adjacent pairs of spinal nerves (Groups G, H and F respectively).

Where the principal nerve, T7, together with one of the adjacent nerves (T6 in
Group D and T8 in Group E) were transected, lactation was depressed to the extent that most of the pups failed to survive. In those which did survive growth was severely retarded, the mean increase in weight of the young over the experimental period being about 25% of control values. The similarity between the results obtained after transection of the two pairs of adjacent nerves T6 and T8, either independently (Groups G and H) or together with T7 (Groups D and E), indicates a consistent contribution by each of these nerves to the sensory innervation of the nipple.

The direct quantitative relationship between the amount of residual innervation and performance in lactation is shown in Table 2 and Fig. 2. In the former the groups of rats representing the several experimental treatments are arranged in order from the totally denervated to the fully innervated. Analysis of variance of the mean gain in weight of each group indicated that these differed at a high level of significance (P < 0.001), the severity of the impairment being directly related to the presumed degree of denervation. A similar influence is seen with respect to the proportion of litters surviving the different experimental treatments. Figure 2, which further illustrates the relationship between the innervation of mammary tissue and lactational performance suggests that, if an adjacent nerve be regarded as providing a single unit of sensory input, then the contribution of the principal nerve is three times as great, each unit of input accounting in the conditions of the experiment for an increased weight gain of about 2 g. This follows from the finding that the slope of the line representing the weight gained by the young when plotted against the degree
The relationship between the level of lactation and the degree of residual innervation of the third thoracic pair of nipples (principal nerve = spinal nerve T7, adjacent nerve = T6 or T8)

<table>
<thead>
<tr>
<th>Series</th>
<th>Residual innervation of the nipple</th>
<th>Coded value</th>
<th>Experimental group (see Table 1)</th>
<th>No. of animals surviving</th>
<th>% litters</th>
<th>Weight gain of young, days 5–16 (g ± s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>Complete denervation</td>
<td>0</td>
<td>B</td>
<td>8</td>
<td>0.0</td>
<td>—</td>
</tr>
<tr>
<td>I</td>
<td>1 adjacent nerve</td>
<td>1</td>
<td>D+E</td>
<td>13</td>
<td>23.1</td>
<td>4.15 ± 0.36</td>
</tr>
<tr>
<td>II</td>
<td>2 adjacent nerves</td>
<td>2</td>
<td>C</td>
<td>12</td>
<td>41.7</td>
<td>7.45 ± 0.86</td>
</tr>
<tr>
<td>III</td>
<td>Principal nerve</td>
<td>3</td>
<td>F</td>
<td>7</td>
<td>71.4</td>
<td>8.16 ± 1.01</td>
</tr>
<tr>
<td>IV</td>
<td>Principal nerve + 1 adjacent nerve</td>
<td>4</td>
<td>G+H</td>
<td>11</td>
<td>100.0</td>
<td>10.61 ± 0.35</td>
</tr>
<tr>
<td>V</td>
<td>Complete innervation. Principal nerve + 2 adjacent nerves</td>
<td>5</td>
<td>A</td>
<td>7</td>
<td>100.0</td>
<td>12.67 ± 1.10</td>
</tr>
</tbody>
</table>

Fig. 2. Mean weight gain of the young between the fifth and sixteenth day of lactation plotted against the degree of residual innervation (an arbitrary coded value given to each group in order of increasing residual innervation, see Table 2). The regression line has been fitted and the standard error of each mean is indicated by the vertical lines.

of residual innervation \((b = 1.97 ± 0.08)\) is highly significant \((P < 0.001)\) and shows no material departure from linearity provided that the latter is given the arbitrarily coded values ranging from 1 to 5 shown in Table 2.

**Effect on establishment of lactation**

The relative effects of denervation, subtotal thelectomy and reduction of litter size on the initiation of a second lactation was examined by remating the animals of these experimental groups. At parturition the litters were reduced to two young. Nearly all the young in each group died within 3 or 4 days irrespective of the degree of residual innervation. That this effect could be attributed to the reduced number of nipples.
rather than to the influence of the earlier procedure of denervation is suggested by the finding that only two out of six second litters born to the sham-operated control group (Group A) and one out of five litters born to a group of virgin females previously thelectomized except for the lower pair of thoracic nipples survived until the 16th day of age and even then were considerably retarded in growth.

![Graph showing relationship between litter size and milk yield.](image)

**Effect of varying litter size**

The relationship between litter size and milk yield, as measured by the mean gain in the weight of the whole litter, showed that an increase in the number of pups was associated with a significant ($P < 0.001$) increase in the weight of the litter during the experimental period of 16 days. This increase was not, however, proportional to the number of young added, for a significant ($P < 0.001$) departure from linearity by the regression relating litter size to weight gained showed that the increment to milk yield is reduced for each additional pup up to the number of 10 or 12 (Fig. 3). Total milk yield was maximal with this number of pups, further increase being without significant effect.

The mean gain in weight of individual pups was on the whole inversely related to the size of litter (Fig. 3) though there was no statistically significant difference between groups containing 2, 3, 4 and 6 animals/litter. Litters consisting of a single pup must be excepted from this observation, however, for the weight gained by these animals was but 50% of that of the young of litters containing two rats and similar in magnitude to that measured in litters of 15 young or more. Consistent with this finding, in all four animals suckling one pup per litter vaginal smears showed a return
Nervous system and lactation

of the oestrus cycle during lactation. This contrasted with the groups containing 2 and 3 pups/litter where only 1 out of 6 and 0 out of 7 animals respectively showed vaginal cornification before the 16th day of lactation.

DISCUSSION

It is now generally accepted that, in the rat, the release of both galactopoietic and galactokinetic hormones from the pituitary gland is regulated by the stimulus of suckling. While the neural pathways by which this stimulus influences the hypothalamus have been little studied it is known that the pathway enters the spinal cord by the dorsal roots (Eayrs & Baddeley, 1956; Eayrs & Edwardson, 1964) and that the autonomic nervous system does not seem to be involved (Bacq, 1932). The present findings demonstrate that, when this pathway is interrupted peripherally, not only is there a failure of lactation similar to that observed after section of the spinal cord (Inglebrecht, 1935; Eayrs & Baddeley, 1956) but also that, as a result of partial denervation, a direct relationship is found between the amount of residual innervation and the response of the pituitary gland as measured by the level of lactational performance. The suppression of lactation observed when suckling is confined to totally denervated nipples does not appear to be due to a local effect, such as the interruption of a secretomotor supply to the gland, for when the other (innervated) nipples also were suckled during the first 5 days of lactation, milk was readily obtained from the denervated glands and it was only after the removal of these nipples that lactation ceased. It seems likely, therefore, that the impairment to lactation observed after partial denervation of the nipples reflects a reduced intensity of stimulation reaching the hypothalamic-hypophysial system and the consequent reduction in the release of hormones affecting the whole mammary system. These results stand in marked contrast to the report of Cardin (1950) that, when some of the mammary glands of the bitch were denervated by section of the dorsal roots, there occurred a failure of lactation only in the denervated glands. This failure was observed even when the other nipples were successfully suckled and indicates the existence of important local mechanisms for the control of milk secretion and ejection in this species. The sheep (Denamur & Martinet, 1959a, b, 1960) and the goat (Tverskof, 1958; Linzell, 1963) likewise differ from the rat in so far as there is no impairment of either milk secretion or of milk ejection after denervation of the udder.

The present results help to throw some light on discrepancies in reports on the effects of central and peripheral lesions involving the sensory pathways mediating lactation, for although both Inglebrecht (1935) and Eayrs & Baddeley (1956) reported total cessation of lactation after lesions to the spinal cord, Grosvenor (1964a, b, 1966) observed that lactation continued in these circumstances although at a reduced level. The disagreement between these results has been explained by Grosvenor (1964a) on the basis of the difference in the age of the pups used in the several studies, the older and more vigorous pups in his experiment being better able to withstand the effects of temporary inanition during the first few days after spinal transection when lactation is most severely depressed. The inability, shown in the present experiments, of newborn pups to establish lactation when only one pair of nipples is available for suckling is consistent with this explanation but an alternative or supplementary
reason is suggested by the demonstration of a quantitative relationship between stimulus and response. Since the rostral pelvic mammary glands in the rat are innervated by spinal nerves T11, T12 and T13 (Edwardson, 1966) spinal transections at the level of the 13th (Grosvenor, 1964a, b) or even the 11th thoracic vertebrae (Grosvenor, 1966) may not have been sufficiently anterior to remove the component of innervation arising from the most rostral of these nerves. Furthermore, while in Grosvenor's experiments the galactophores of the nipples anterior to the point of spinal transection were ligatured, the nipples themselves were left available for suckling. It has recently been found (Edwardson, in preparation) that at least a portion of the innervation to the nipples passes, not by way of the galactophores, but superficially through the corium and it is possible that stimuli arising from these nipples may have reached the central nervous system. In either instance, lactation at a reduced level might be expected in the light of the present findings.

The results obtained from varying the size of the litters are also in good agreement with the view that the volume of stimuli reaching the hypothalamo-hypophysial system may control the level of lactation. As the size of the litter is increased, the associated increase in the weight of the litter must represent a substantial increase in milk yield since the maximum rate of growth of the pups is obtained with a small litter of two or three animals and with larger litters it may be assumed that all the milk produced by the mother is removed. Maximum milk production is attained when the doe is suckled by at least ten young and any further increase in litter size results in a reduced growth of the individual pups. With the standardized litters of six pups commonly used in studies of lactation milk yields are at only 75% of the maximum possible level and litters of larger size would seem to be more suitable in such cases. When the litter contained only one pup it is clear that the impairment of lactation was associated with a reduced secretion of prolactin as shown by the absence of a persistent, dioestrous vaginal smear. Two young were sufficient to maintain a high level of milk secretion and prevent the return of oestrus as judged by the vaginal smear, provided that the full complement of nipples was suckled. This contrasts with the observation that lactation was severely impaired when only a single pair of nipples was available to two newborn young even when the innervation of the nipples was intact. Since two young will grow quite normally when given access to two nipples from the fifth day it must be presumed that the increased size and vigour of the older pups is responsible in some way for this difference. It is possible that two young suckling all 12 nipples during the first few days of lactation can obtain sufficient milk for normal growth, and that eventually their increased vigour in suckling provides sufficient stimulation to maintain further lactation. By contrast, when two newborn young suckle from only two glands a shortage of milk may result in weakening of the pups and in a further decline in milk secretion.

No attempt has been made in this study to differentiate between the processes of milk secretion and milk ejection. The results of replacement therapy with oxytocin and various adenohypophysial hormones will be reported in detail elsewhere (Edwardson, in preparation) and will show that the failure of both processes is involved in the suppression of lactation which follows somatosensory deprivation of the nipples in the rat.
Nervous system and lactation

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