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THE ROLE OF THE SEPTUM IN THE CONTROL OF THE MILK EJECTION REFLEX IN THE RAT: EFFECTS OF LESIONS AND ELECTRICAL STIMULATION

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SUMMARY

1. Experiments were undertaken to determine the role of the septum on the afferent control of the milk ejection reflex in lactating rats.

2. Massive septal lesions were produced by passing radio-frequency current through lesioning electrodes. Intramammary pressure recordings during suckling showed no significant alterations either in the frequency of milk ejections or in their amplitude and time course.

3. Electrophysiological recordings of identified oxytocin-secreting neurones in supraoptic nuclei of septal-lesioned rats engaged in suckling showed that the pattern of background electrical activity and of the high frequency discharges at milk ejection were normal.

4. The weight of litters from rats lesioned on the third day post-partum increased in a way parallel to that of control litters up to the fifteenth post-natal day.

5. Electrical stimulation was applied bilaterally to the lateral septum in trains of long duration (20–55 min) at varying frequencies. Frequencies of 5 and 10 Hz interrupted the reflex during the period of stimulation. At 1 Hz, milk ejections were not interrupted but the intervals between successive milk ejections were significantly increased in comparison to the intervals before stimulation.

6. Electrical stimulation applied to the septum in short trains of 1 or 3 min at 5 and 10 Hz significantly delayed the appearance of the subsequent milk ejection. At 1 Hz, no effect was observed.

7. Septal stimulation at 1 Hz for 20 min or more did not significantly alter the electrocorticogram during the period of stimulation. Stimulation at 5 Hz for the same period of time always desynchronized the e.e.g. for several minutes after the cessation of stimulation.

8. It is concluded that the septum is not essential for the onset and the maintenance of reflex milk ejections during lactation. The results suggest, however, that in the normal non-anaesthetized animal, septal activation could modulate the frequency of milk ejections.

INTRODUCTION

The final central nervous mechanisms leading to milk ejection in response to suckling are now well established in the lactating rat. In this species, milk ejections occur intermittently and are induced by a pulsatile release of oxytocin following a

brief and synchronous activation of all oxytocin-secreting neurones in the supraoptic and paraventricular nuclei of the hypothalamus (Wakerley & Lincoln 1973; Lincoln & Wakerley, 1974). The functional organization of the afferent pathways controlling this system, however, is still far from being clear. On the one hand, a permanent excitatory input appears to be necessary since, in the rat, the pups must suckle continuously for the reflex to take place. On the other hand, the intermittent nature of the reflex suggests the participation of specific inhibitory mechanisms, the site and nature of which are unknown (for a review, see Poulain & Wakerley, 1982).

Several studies point to a possible participation of the limbic system in the central inhibition of oxytocin release during lactation, in the rat and in other species. Milk ejection can be inhibited by cortical influences (Aulsebrook & Holland, 1969) and by stress and emotional disturbances (Newton & Newton, 1948; Cross, 1955; Peeters, Stormorken & Vanschoubroek, 1960; Taleisnik & Deis, 1964) and it can be conditioned to visual and auditory signals (Peeters *et al.* 1960; Cleverley & Folley, 1970). In the rat, no reflex milk ejection occurs during arousal or paradoxical sleep (Voloschin & Tramezzani 1979; Lincoln, Hentzen, Hin, Van der Schoot, Clarke & Summerlee, 1980). Furthermore, lesions of various limbic areas disturb maternal behaviour and lactation (Stutinsky & Terminn, 1965; Slotnick, 1967; Cruz & Beyer, 1972; Slotnick & Nigrosh, 1975; Fleischer & Slotnick, 1978).

The septum, by virtue of its extensive and reciprocal connexions within the limbic system, constitutes an area of interaction between telencephalic limbic structures and the hypothalamus (see Raisman, 1966; Swanson & Cowan, 1976). It sends projections from the medial septum-diagonal band complex to the hippocampus, and, in turn, receives afferents from the hippocampus to its lateral nuclei. A similar pattern of reciprocal connexions exists between the bed nucleus of the stria terminalis and the amygdala. All septal nuclei are inter-connected with the preoptic area and the hypothalamus, via the medial forebrain bundle; more specifically, the septum appears to project both to the paraventricular and the supraoptic nuclei of the hypothalamus (Powell & Rorie, 1967; Tangapregassom, Tangapregassom, Soulairac & Soulairac, 1974; Zaborsky, Leranth, Makara & Palkovits, 1975; Garris, 1979; Tribollet & Dreifuss, 1981). The septum could thus be thought of as a last relay in the afferent pathway conveying limbic influences to the magnocellular system.

Recently, using electrophysiological means, we confirmed that septal cells do indeed project directly to the ipsilateral supraoptic nucleus (Poulain, Lebrun & Vincent, 1981), and we showed that electrical stimulation of the septum inhibited the general activity of identified oxytocin-secreting cells in the same nucleus (Poulain, Ellendorff & Vincent, 1980). These results, therefore, support the idea that the septum has an inhibitory action on the oxytocin system. However, we could find no systematic correlation between the electrical activity of septal neurones and the different phases of the milk ejection reflex (Lebrun & Poulain, 1982). The present investigation was undertaken, therefore, to define more fully the significance of the septal-hypothalamic connexions to the release of oxytocin during lactation. The experiments involved lesions and stimulation of the septum and were conducted on lactating rats, a species in which the intramammary pressure and the electrical activity of oxytocin-secreting cells display highly characteristic variations at milk ejection, and can thus serve as direct means to appreciate the consequence of

experimental manipulations on the reflex. Some aspects of these experiments have been reported in abstract form (Poulain, Theodosis & Lebrun, 1982).

METHODS

All experiments were performed on lactating rats of the Wistar strain (280–350 g birth weight). On the day of birth, all the litters were reduced to ten pups. The animals were housed in individual cages and maintained in a controlled environment with a 14 h light–10 h dark schedule. Food and water were available *ad libitum*.

(1) Septal lesions and evolution of the body weight of the mothers and their litters

The daily weight of three groups of lactating rats and their respective litters were recorded every morning from the day of parturition to the fifteenth day post-partum. One group, which served as control, consisted of rats that underwent no further experimental manipulation. A second group was comprised of rats that were anaesthetized with one single I.P. injection of sodium pentobarbitone (50 mg/kg) on the third day post-partum. A third group included rats that were anaesthetized in a similar fashion and on the same day as the rats of the previous group, and then underwent septal lesions. For this purpose, the animals were fixed in a stereotaxic frame (Narishige Instruments) and bilateral burr holes of 1–2 mm in diameter were drilled through the skull. Two monopolar electrodes, 0·5 mm in diameter, insulated with Insl-X except for 0·5 mm at the tip, were lowered down to the septum on each side of the mid line at a 15° angle in a frontal plane in order to avoid the sagittal sinus. Their final position was adjusted to: anteroposterior, 8·0 mm; lateral, 0·5 mm; depth, 5·5 mm. Lesions of the septum were produced by passing a radio-frequency current of 2 mA for 20–30 s through the electrodes, the earth contact being made through the stereotaxic frame. After lesioning, the electrodes were removed, the burr holes were filled with dental cement, the skin on the skull sutured, and the animals were given an I.M. injection of antibiotic (Terramycine, 5 mg/kg). All animals that had been anaesthetized were returned to their cages with their young when they had recovered from anaesthesia.

(2) Septal lesions and recording of the intrammary pressure

These experiments were performed on rats on days 9–11 of lactation. On the evening prior to the experiments, all but one of the pups were separated from their mother to ensure full mammary glands. In the morning, they were anaesthetized with one I.P. injection of urethane (1·1 g/kg). A silicone catheter (Silastic) was inserted into the right jugular vein for any I.V. injection of oxytocin (Syntocinon). A plastic cannula (Portex PP 50) was introduced in the teat duct of an inguinal mammary gland and connected to a pressure transducer (Statham) to obtain a continuous recording of the intramammary pressure on a pen recorder (Racia Tetra 75). The animals were fixed in a stereotaxic frame and two lesioning electrodes were placed bilaterally in the septum in a manner similar to that described above. The electrodes were then fixed on the skull with dental cement. No further manipulations were made until 3 h after the beginning of anaesthesia. At this time, several I.V. bolus injections of oxytocin, ranging from 0·1 to 1·0 mu., were given to calibrate the rise in intramammary pressure with the amount of oxytocin injected, and to allow estimation of the amount of oxytocin released during the natural milk ejections. Such injections were repeated from time to time during the experimental session.

In one group of animals, bilateral lesions of the septum were made as described above in (*1*). After recording the intramammary pressure for 30 min, nine or ten pups were allowed to suckle. In a second group of animals, the septal lesions were made only after a sequence of several reflex milk ejections induced by suckling had occurred. In the two groups, the intramammary pressure was recorded continuously for the next four hours of suckling.

(3) Septal lesions and electrophysiological recordings of oxytocin-secreting neurones

Rats on days 9–11 of lactation were anaesthetized, prepared for intramammary pressure recording, and placed in a stereotaxic frame as described above in (*2*). After the surface of the brain was exposed and the sagittal sinus ligatured, a bipolar concentric stimulating electrode (Rhodes Medical Instrument) was introduced via a dorsal approach into the pituitary–stalk for antidromic identification of neurosecretory cells (Poulain *et al.* 1980). Bilateral lesions of the septum were then

made as described in (*1*), and the lesioning electrodes were removed. Three hours later, extracellular recordings of action potentials in the suprapotic nucleus were obtained with glass micropipettes connected to a conventional electrophysiological apparatus as described previously (Poulain *et al.* 1980). Once the electrode was placed in the nucleus, as seen by the shape of the evoked potential upon electrical stimulation of the stalk, nine or ten pups were applied to the nipples of the rat. Cells were then identified according to their response to the suckling stimulus (Poulain, Wakerley & Dyball, 1977). Analysis of the characteristics of the electrical activity were performed off-line from tape recordings using a PDP-8E computer (Digital Equipment).

(*4*) *Electrical stimulation of the septum during suckling*

Animals on days 9–11 of lactation, anaesthetized and prepared for intramammary pressure recording as described in (*2*), were placed in a stereotaxic frame and two bipolar stimulating electrodes were lowered down to the septum in a manner similar to that used for the lesioning electrodes in (*1*). In a few animals, two silver ball electrodes were placed on the surface of the parietal cortex to record the electrocorticogram. Three hours after the beginning of anaesthesia, nine or ten pups were allowed to suckle, and after 5–6 reflex milk ejections had occurred, biphasic pulses (width, 1 ms; amplitude, 0·5 mA; Devices) were applied using trains of different frequencies and durations.

(*5*) *Histological controls*

At the end of each experiment, animals were killed with an overdose of anaesthetic and perfused with formalin. In experiments involving bilateral septal lesions, the lesions included a large region that extended rostrocaudally between planes 6·2 and 8·4 (De Groot's atlas, 1959), and affected the lateral septal nuclei, the medial septum and the triangular and septo-fimbrial nuclei. The fibre tracts passing through or associated with the septum (fornix, fimbria septo-hippocampal commissure, stria medullaris) were also destroyed. At the end of each experiment involving electrophysiological recordings and stimulation, a small lesion was made at the tip of the electrodes by passing a direct current, thus enabling examination of their localization (blue spot method).

RESULTS

Septal lesions and the milk ejection reflex

The effects of septal lesions on the milk ejection reflex were studied: (i) by recording the intramammary pressure during suckling; (ii) by recording the electrical activity of oxytocin-secreting neurones, and (iii) by following the growth of litters whose mothers had been lesioned on the third day post-partum.

(*i*) *Intramammary pressure changes.* The variations in intramammary pressure in response to suckling were analysed in three groups of anaesthetized lactating mothers. A control group underwent no lesioning. In a second group, septal lesions were made 2–3 h before suckling began; in a third group, septal lesions were made only after several reflex milk ejections had occurred, so that each animal could serve as its own control. When suckling did induce the reflex, milk ejections were easily recognized by an abrupt rise in intramammary pressure that reached 15–30 mmHg, and lasted 15–25 s (Fig. 1). Such milk ejections were equivalent to those produced by I.V. injections of 0·2–1·0 mu. oxytocin.

As has been reported by others (Tribollet, Clarke, Dreifuss & Lincoln, 1978), suckling did not always succeed in inducing the reflex. In intact animals observed for 1·5 h of suckling ($n = 21$), such failures occurred in 38% of the experiments. In five of these experiments, septal lesions were performed after 1·5 h of unsuccessful suckling, and in one case, reflex milk ejections did begin shortly thereafter (Fig. 2*A*). Septal lesions made before suckling began did not affect significantly the proportion of failures; no milk ejection was induced by suckling in 45% of the lesioned mothers

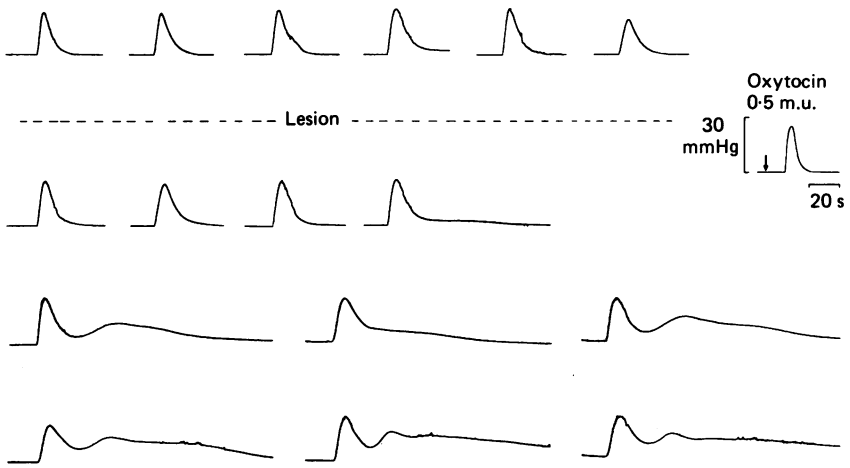

Fig. 1. Effects of bilateral septal lesions on reflex milk ejections. Intramammary pressure recordings from one lactating rat during suckling, showing from top to bottom the successive milk ejections before lesion (top six) and after lesion (below dotted line). Note the progressive increase in the variations in intramammary pressure after lesion. The response of the mammary gland to a rapid I.V. injection of 0·5 mu. oxytocin (shown on the right) was identical before lesion and at the end of the experiment.

TABLE 1. Latency from the onset of suckling to the first milk ejection and mean intervals between six successive milk ejections in normal and septal lesioned animals

		Latency of first milk ejection	Mean interval between milk ejections	
			Before lesion	After lesion
(A)	No lesion ($n = 13$)	25±26 (7–79)	7·8±3·3 (3–19)	—
(B)	Lesion before suckling ($n = 6$)	38±21 (11–60)	—	8·1±2·5 (2·6–19·5)
(C)	Lesions after milk ejections ($n = 8$)	37±32 (5–91)	6·4±2·2 (2·4–16·7)	6·8±2·9 (3·2–18·3)

Values given are the mean±S.D. (min).

($n = 11$). Finally, septal lesions made after suckling had induced several milk ejections ($n = 8$) never interrupted the reflex (Fig. 2).

In all three groups, the time taken from the beginning of suckling to the first milk ejection varied considerably from animal to animal (Fig. 2*B*), but was not significantly different whether a lesion had been made or not (Table 1). The mean interval between successive milk ejections was also similar. In particular, in animals lesioned after a few milk ejections had taken place, no variation of the interval was observed after lesion (Fig. 2, Table 2). Likewise, the amplitude and time course of the intramammary changes at milk ejection were the same in intact and lesioned animals. Usually, the mammary gland response in each animal was highly reproducible throughout the entire period of recording. However, in two rats lesioned after six milk ejections had

occurred, the ensuing milk ejections progressively increased in amplitude and duration (Fig. 1).

(*ii*) *Electrical activity of oxytocin-secreting neurones.* Electrophysiological recordings of the activity of supraoptic neurones were performed in the group of lactating rats that had been lesioned before suckling began. Twelve oxytocin- secreting cells were

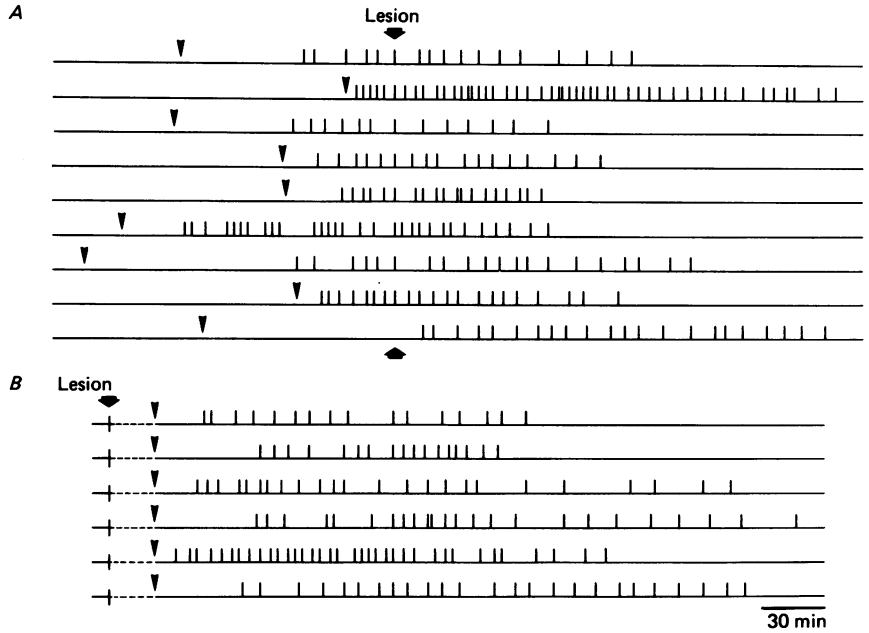

Fig. 2. Effects of bilateral septal lesions on the rhythm of milk ejections induced by suckling in anaesthetized rats. Each horizontal line represents one experiment; each vertical line corresponds to one milk ejection as seen by a rise in intramammary pressure. The small arrow heads indicate the beginning of suckling. *A*, the lesions were made after a number of milk ejections had occurred, except in one case (last line) when the lesion was made after 1·5 h of unsuccessful suckling. *B*, the lesions were made 3 h before suckling started. The patterns shown are no different from the pattern usually observed in unlesioned anaesthetized rats. Note the variability from one animal to the next in the latencies from the beginning of suckling to the first milk ejection, and in the patterns of milk ejections.

identified first by their antidromic invasion upon electrical stimulation of the neurohypophysis, then by their response at the time of milk ejection (Poulain *et al.* 1977). Septal lesions appeared to have no effect on the activity of such neurones. As in intact animals, their electrical activity was characterized by a slow irregular pattern of action potentials, with a mean firing rate of $2{\cdot}1 \pm 2{\cdot}5$ spikes/s (range 0·1–8·2 spikes/s). Twelve to fourteen seconds before each milk ejection ($n = 27$), the cells displayed a high frequency discharge of potentials with the following parameters (mean ± S.D.): duration, $3{\cdot}5 \pm 0{\cdot}7$ s (range 2·5–4·9 s); number of spikes, 54 ± 26 (range 19–117); peak firing rate over 0·5 s, $33{\cdot}2 \pm 14{\cdot}8$ spikes/s (range 8–64).

(*iii*) *Growth of the litters.* These experiments were undertaken to study the long-term effects of septal lesions on milk ejection. Ten lactating rats underwent bilateral septal

lesions under brief pentobarbitone anaesthesia on the third day post-partum. At each experimental session, another lactating rat, also on her third day post-partum, was anaesthetized at the same time as the one that was lesioned, but received no further treatment. Four rats in the first group and three in the second did not recover from anaesthesia. In the other animals, no major disturbances were noted once they

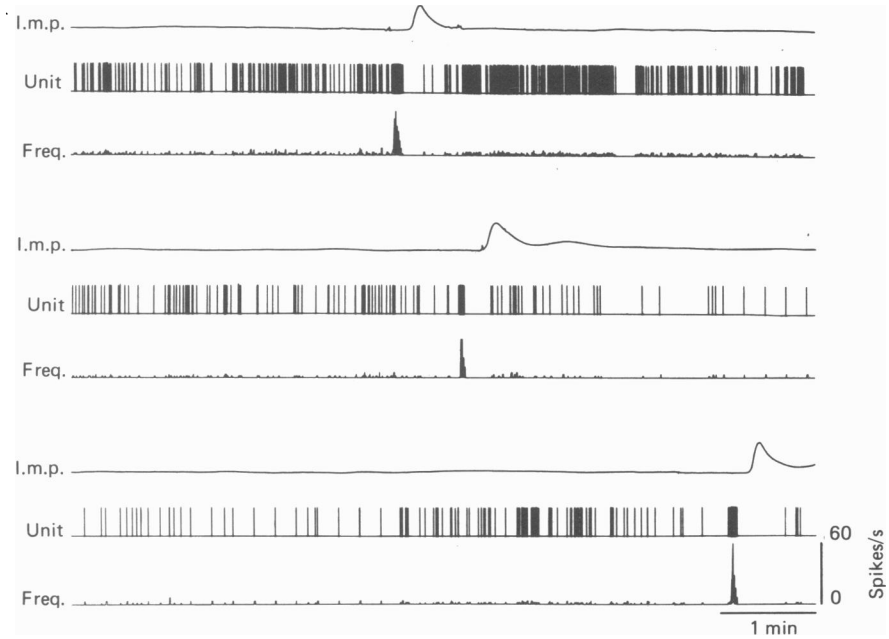

Fig. 3. Electrical activity of one oxytocin-secreting neurone recorded in the supraoptic nucleus of a lactating rat whose septum had been lesioned. The continuous polygraph record simultaneously shows the intramammary pressure (I.m.p.) and the electrical activity of the cell (Unit: each line corresponds to one action potential; Freq.: number of spikes/s). Before each milk ejection, the cell displayed a high frequency discharge of action potentials. Between milk ejections, the background activity, albeit quite variable, was low. This type of activity is indistinguishable from that recorded in unlesioned animals.

recovered from anaesthesia. By comparison with a group of ten rats that were not anaesthetized, the body weight of the experimental rats significantly declined ($P < 0{\cdot}05$, Mann–Whitney U test) on the day following anaesthesia, with no significant difference between intact (-4%) and lesioned (-6%) animals. Thereafter, the body weight of the three groups of rats fluctuated in a similar manner throughout lactation. The lesioned rats showed no sign of aggression when handled by the experimentor, and we never observed any cannibalism of the young.

The body weight of the litters was measured daily, and compared to that of a group of ten litters from normal mothers. As shown in Fig. 4, septal lesions performed in the mothers did not affect the growth of their litters. Apart from the day following anaesthesia of the mothers, the daily gain in body weight of the pups was the same in the three groups, so that the pups had grown from $10{\cdot}1 \pm 0{\cdot}1$ g/pup on the third day to $32{\cdot}5 \pm 2{\cdot}8$ g/pup on the fifteenth day after birth.

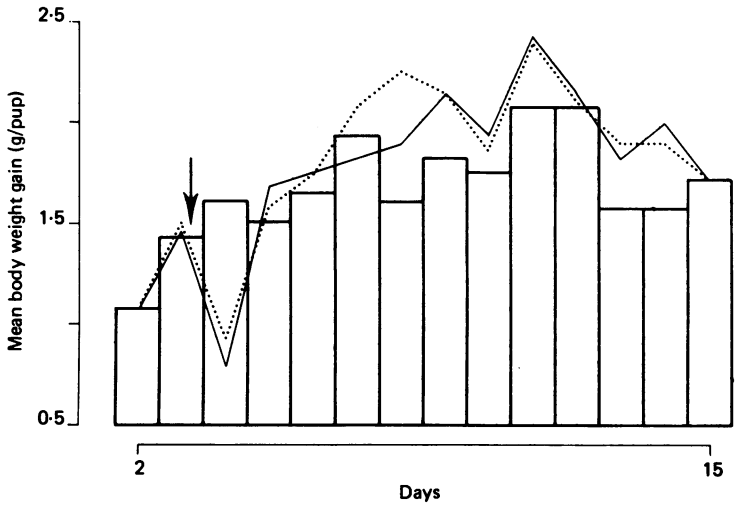

Fig. 4. Growth of litters from mothers with septal lesions. The body weight of litters from mothers lesioned under short pentobarbitone anaesthesia (continuous line), mothers submitted to short anaesthesia alone (dotted line) and control mothers (open bars) were recorded daily from day 2 to day 15 after birth. Anaesthesia and lesions were performed on day three (arrow). The gain in body weight was significantly reduced only on day 4 in the experimental groups.

Septal stimulation and the milk ejection reflex

Electrical stimulation was applied bilaterally to the lateral septum of urethane-anaesthetized rats submitted to suckling, after a number of reflex milk ejections had occurred.

In one series of experiments, stimuli were given in trains of sufficiently long duration such that, under normal conditions, several milk ejections would have taken place. Trains at 10 Hz (three rats) and 5 Hz (five rats, six trials), applied for 20 min interrupted milk ejections during the entire period of stimulation (Figs. 5, 6 and 8). Except in one case (10 Hz), milk ejection recommenced with a delay of 7–27 min (mean: 15 ± 6 S.D.) following cessation of stimulation (Fig. 5). The interval during which stimulation was given was thus prolonged more than 5-fold in comparison to the mean interval before stimulation (Table 2). During that time, the mammary gland could still react to I.V. injections of oxytocin in a manner similar to that observed during reflex milk ejections. When the reflex started again, milk ejections were similar in form and amplitude to those observed before stimulation (Fig. 8).

When stimulating at 1 Hz for 20–55 min (five animals), reflex milk ejections were not interrupted, and their amplitude was not significantly altered (Fig. 5). However, their frequency decreased. For each animal, we calculated the mean interval between milk ejections occurring during stimulation, including the interval following the last ejection. A similar calculation was made for an equivalent period of time before stimulation. The mean interval thus increased from $7{\cdot}7 \pm 1{\cdot}5$ min before to $10{\cdot}9 \pm 3{\cdot}3$ min during stimulation ($P < 0{\cdot}01$, Student's paired t test). By comparison, in unstimulated animals, the mean interval for a period of time equivalent to the

TABLE 2. Effect of septal stimulation for 20 min at 5 and 10 Hz on the intervals between milk ejections

Latency of first milk ejection	Mean interval		
	Before stimulation	During stimulation	After stimulation
27±13	7·2±1·6	35·8±7·1*	10·3±2·0

Values given are the mean±S.D. (min; $n = 7$).
* Interval significantly increased at $P < 0.001$, Student's paired t test.

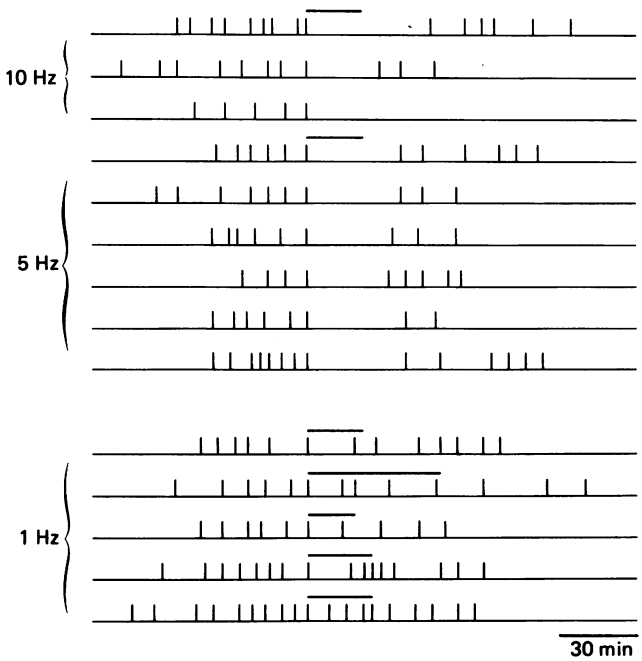

Fig. 5. Effect of electrical stimulation of the lateral septum on the milk ejection reflex in anaesthetized rats. Each horizontal line corresponds to an experiment, and each vertical line to a milk ejection. The horizontal bar indicates the duration of septal stimulation. Note the interruption of the reflex during stimulation at 10 and 5 Hz. At 1 Hz, the intervals between milk ejection tended to increase.

durations of stimulation was 8·1±3·1 min before, and 8·9±3·9 after the sixth milk ejection.

Electrical stimulation was also used in short trains applied just at the time of a milk ejection in order to study its effect on the delay to the next milk ejection. Trains of 1 min at 5 Hz (two rats, three trials) given at the end of each of a series of consecutive milk ejections tended to increase the successive intervals (Fig. 6). At 1 Hz (three animals, four trials), no effect was apparent (Fig. 4). When applied after a single milk ejection chosen at random (Fig. 7), stimulation at 10 and 5 Hz during 1 or 3 min also increased the intervals (seventeen out of twenty four cases, $P < 0.05$, Sign test), but trains of 1 Hz had no significant effect (increased intervals in nine out of fourteen cases, $P > 0.2$, Sign test).

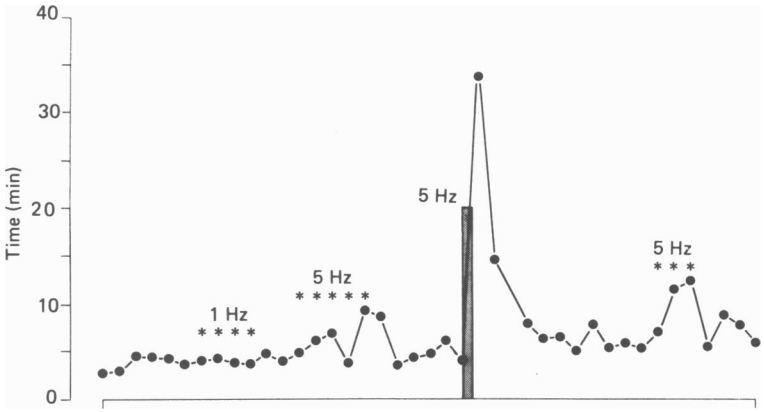

Fig. 6. Effect of septal stimulation on the milk ejection reflex. Example from one anaesthetized lactating rat. The dots represent the duration of the interval between two successive milk ejections. Stimulation at 5 Hz for 20 min (vertical bar) delayed the onset of the next milk ejection. Stimulation in trains of 1 min (asterisk) applied at the time of milk ejections had no effect at 1 Hz, but tended to increase the intervals at 5 Hz.

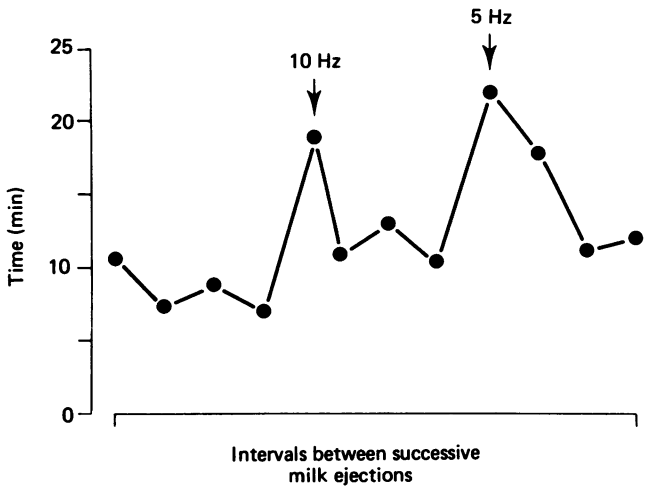

Fig. 7. Effect of bilateral septal stimulation on intervals between successive milk ejections in one urethane-anaesthetized rat. As in the preceding Figure, each dot corresponds to one interval. Stimuli, applied in trains of 3 min at the end of a milk ejection, increased the duration of the interval.

In five anaesthetized animals, the electrocorticogram was recorded from the parietal cortex during the whole period of suckling. For most of the recording period, and in particular at the time of milk ejection, all the animals presented a synchronized high amplitude e.e.g., indicative of slow-wave sleep. Stimulation at 1 Hz did not alter this pattern (Fig. 8*B*), during stimulation or thereafter. Stimulation during 1 min at 5 Hz desynchronized the e.e.g. At the end of stimulation, the e.e.g. reverted back to a pattern of slow-wave sleep (Fig. 8*C*) or remained desynchronized (Fig. 8*E*). When stimulating for 20 min at 5 Hz, the e.e.g. became desynchronized, and remained so for several minutes after the end of stimulation (Fig. 8*D*). However, by the time the next milk ejection occurred, the e.e.g. was again synchronized.

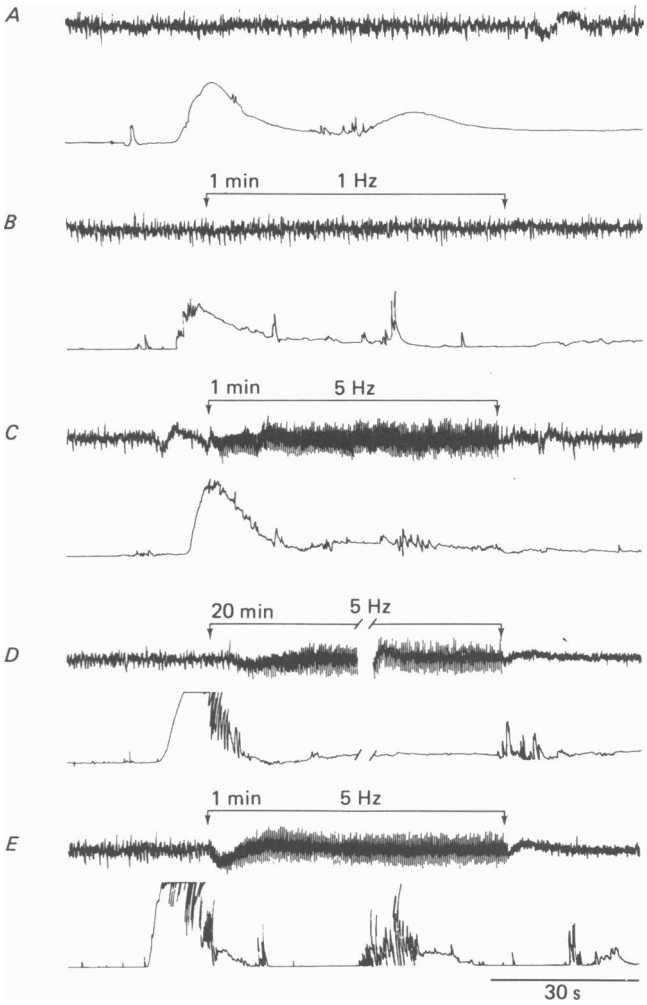

Fig. 8. Effect of septal stimulation on the electrocorticogram in an anaesthetized lactating rat during suckling. The example is from the same animal as that depicted in Fig. 6. The slow variations of the intramammary pressure characteristic of milk ejection are sometimes altered by fast variations caused by the movements of a pup on the cannulated gland. Before each milk ejection, the e.e.g. had always a high amplitude (*ca.* 100 μV), synchronized rhythm, characteristic of slow-wave sleep. In *A*, no stimulation was applied. Stimulation at 1 Hz had no effect on the e.e.g. (*B*), but at 5 Hz, it induced evoked potentials during stimulation (*C*, *D*, *E*). After stimulation, the e.e.g. reverted back to slow-wave sleep (*C*), or became desynchronized for a few minutes (*E*). Stimulation for 20 min at 5 Hz was followed by several minutes of desynchronized e.e.g.

DISCUSSION

Two main conclusions can be drawn from this study. First, the observations from our experiments involving septal lesions clearly indicate that the septum is not a structure that is essential for the expression of the milk ejection reflex. Secondly, the results obtained with electrical stimulation of the septum suggest that, although septal activity may not be essential, it could play some inhibitory role on the reflex.

The recordings of intramammary pressure and of the electrical activity of oxytocin-secreting cells during suckling permitted us to determine whether septal lesions altered the pulsatile and periodic nature of the release of oxytocin during the milk ejection reflex. The lesions obviously did not prevent the reflex from taking place, nor did they interrupt the reflex once it was set. They did not significantly alter the amplitude, time course and frequency of the milk ejections, nor did they affect the electrical activity of oxytocin-secreting neurones during suckling.

On the first day after the maternal lesions, the reduction in body-weight gain of the litters can certainly be attributed to disturbances due to anaesthesia (Fig. 4). Thereafter, the normal growth of litters from lesioned mothers indicates that oxytocin secretion and release were maintained. It also suggests that prolactin secretion was not significantly impaired, which substantiates other observations that septal lesions have no effect on basal secretion of prolactin or stress-induced prolactin release (Brown, Uhlir, Seggie, Schally & Kastin, 1974; Uhlir, Seggie & Brown, 1974). Contrary to other reports (Carlson & Thomas, 1968; Cruz & Beyer, 1972; Fleischer & Slotnick, 1978), we never observed troubles in maternal behaviour resulting in the death of the litters, but it must be noted that behavioural disturbances, and in particular the septal rage syndrome, are not systematically induced by septal lesions (see Grossman, 1976).

From the present observations, as well as from those of our earlier study where it was seen that there was no systematic correlation between the pattern of milk ejection, and the patterns of electrical activity of septal cells (Lebrun & Poulain, 1982), it is clear that the septal nuclei, despite their connexions with the magnocellular system, cannot be responsible for a tonic inhibition that would explain the intervals between successive milk ejections. Likewise, the intermittent high frequency discharge of action potentials occurring in oxytocin-secreting neurones at milk ejection cannot be due to an intermittent removal of septal inhibition, or to a sudden activation of oxytocin cells by septal structures. Furthermore, our data suggest that other limbic structures interconnected with the septum do not intervene in a essential manner, either via their role on septal activity, or through their pathways to the hypothalamus that traverse the septal area. Conversely, septal influences on these structures do not appear to be necessary for the maintenance of the reflex.

Nonetheless, even though the septum may not be fundamental, it could intervene as an inhibitory structure modulating the milk ejection reflex. The effects of septal lesions are compatible with such an hypothesis. When the reflex occurs, we may reasonably suppose that inhibitory influences are minimal. Destruction of an inhibitory structure external to the main pathway should therefore be of little consequence, as was generally the case in the lesion experiments.

However, the most suggestive arguments that the septum may have some inhibitory role, derive from our experiments involving electrical stimulation of the septum. During stimulation, the normal reactivity of the mammary gland to I.V. injection of oxytocin eliminates the possibility of a direct inhibitory effect on the gland, and suggests that stimulation acted centrally to inhibit the oxytocin-secreting neurones. Under normal conditions, the periodic and pulsatile release of oxytocin is achieved through the recruitment of the whole population of oxytocin cells into a synchronous burst of electrical activity that occurs intermittently. The inhibition

induced by septal stimulation seemed to act more on the periodicity than on the intensity of the neuronal activation since stimulation either increased the intervals between milk ejections or, above a certain threshold in frequency and duration, interrupted the reflex. On the other hand, milk ejections, when they occurred, were little affected in their time course and amplitude. It is unlikely, therefore, that septal stimulation reduced the number of cells recruited into activation, or inhibited the high-frequency discharges in a significant way, for, in both cases, the resulting reduction in oxytocin release would have reduced the amplitude of the milk ejections.

Although it is difficult to establish whether the observed effects are due to the activation of the septum, or of some associated circuit, septal activation itself may be partly responsible for an inhibition of the reflex. Of particular interest to the milk ejection reflex is the activity of the septum in relation to the hippocampal electroencephalographic theta rhythm that occurs during arousal or paradoxical sleep (Green & Arduini, 1954; Petsche, Stumpf & Gogolak, 1962; Gogolak, Stumpf, Petsche & Sterc, 1968; Morales, Roig, Monti, Macadar & Budelli, 1971; McLennan & Miller, 1974). In the lactating rat, the milk ejection reflex never occurs during arousal or paradoxical sleep (Voloschin & Tramezzani, 1979; Lincoln *et al.* 1980). It is noteworthy that, in our experiments, the most striking inhibitory effects were obtained with a frequency of stimulation close to that of the hippocampal theta rhythm (3–7 Hz), and capable of producing cortical arousal. In a previous experiment, we also observed two cases of increased septal activity clearly correlated with the onset of cortical arousal, during which time there was no milk ejection (Lebrun & Poulain, 1982). It is likely, then, that in the normal rat, the inhibitory action of the septum would be manifest only when the septum is activated, in particular in situations of arousal associated with attention, noxious stimuli, or emotional disturbances, circumstances known to inhibit the reflex.

Our experiments do not allow us to determine the site of such an inhibitory action. The existence of direct projections from septal neurones to the magnocellular nuclei makes it possible that the septum acts directly on these nuclei. Indeed, electrical stimulation of the septum does inhibit the background activity of oxytocin-secreting cells (Poulain *et al.* 1980). However, although there is some relation between the background activity and the intensity of the high-frequency discharges (Lincoln & Wakerley, 1975), there is no evidence that it has any influence on their periodicity. Moreover, an exclusively direct action of the septum on the magnocellular cells could only be considered if we had any evidence that the mechanisms underlying the synchronization and the periodicity of neuronal firing were taking place entirely within the magnocellular nuclei. Thus, alternatively to, or in addition to, direct effects, we must also consider the possibility that the septum acts at a different, yet undetermined, level on the afferent pathway.

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REFERENCES

AULSEBROOK, L. H. & HOLLAND, R. C. (1969). Central inhibition of oxytocin release. *Am. J. Physiol.* **216**, 830–842.

BROWN, G. M., UHLIR, I. V., SEGGIE, J., SCHALLY, A. V. & KASTIN, A. J. (1974). Effect of septal lesions on plasma levels of MSH, corticosterone, GH and prolactin before and after exposure to novel environment: role of MSH in the septal syndrome. *Endocrinology* **94**, 583–587.

CARLSON, N. R. & THOMAS, G. J. (1968). Maternal behavior of mice with limbic lesions. *J. comp. physiol. Psychol.* **66**, 731–737.

CLEVERLEY, J. & FOLLEY, S. J. (1970). The blood levels of oxytocin during machine milking in cows with some observations on its half-life in the circulation. *J. Endocr.* **46**, 347–361.

CROSS, B. A. (1955). Neurohormonal mechanisms in emotional inhibition of milk ejection. *J. Endocr.* **12**, 29–37.

CRUZ, M. L. & BEYER, C. (1972). Effects of septal lesions on maternal behavior and lactation in the rabbit. *Physiol. Behav.* **9**, 361–365.

DE GROOT, J. (1959). *The Rat Forebrain in Stereotoxic Coordinates.* Amsterdam: North Holland.

FLEISCHER, S. & SLOTNIK, B. M. (1978). Disruption of maternal behavior in rats with lesions of the septal area. *Physiol. Behav.* **21**, 189–200.

GARRIS, D. R. (1979). Direct septo-hypothalamic projections in the rat. *Neurosci. Lett.* **13**, 83–90.

GOGOLAK, G., STUMPF, Ch., PETSCHE, H. & STERC, J. (1968). The firing pattern of septal neurons and the form of the hippocampal theta wave. *Brain Res.* **7**, 201–207.

GREEN, J. D. & ARDUINI, A. A. (1954). Hippocampal electrical activity in arousal. *J. Neurophysiol.* **17**, 533–557.

GROSSMAN, S. P. (1976). Behavioural functions of the septum: a re-analysis. In *The Septal Nuclei*, ed. DE FRANCE, J. F., pp. 361–422. New York: Plenum Press.

LEBRUN, C. J. & POULAIN, D. A. (1982). Electrical activity of septal neurones during suckling and the milk ejection reflex in the lactating rat. *Exp. Brain Res.* **47**, 203–208.

LINCOLN, D. W., HENTZEN, K., HIN, T., VAN DER SCHOOT, P., CLARKE, G. & SUMMERLEE, A. J. S. (1980). Sleep: a prerequisite for reflex milk ejection in the rat. *Exp. Brain Res.* **38**, 151–162.

LINCOLN, D. W. & WAKERLEY, J. B. (1974). Electrophysiological evidence for the activation of supraoptic neurones during the release of oxytocin. *J. Physiol.* **242**, 533–554.

LINCOLN, D. W. & WAKERLEY, J. B. (1975). Factors governing the periodic activation of supraoptic and paraventricular neurosecretory cells during suckling in the rat. *J. Physiol.* **250**, 443–461.

MCLENNAN, H. & MILLER, J. J. (1974). The hippocampal control of neuronal discharges in the septum of the rat. *J. Physiol.* **237**, 607–624.

MORALES, F. R., ROIG, J. A., MONTI, J. M., MACADAR, O. & BUDELLI, R. (1971). Septal unit activity and hippocampal EEG during the sleep-wakefulness cycle of the rat. *Physiol. Behav.* **6**, 563–567.

NEWTON, M. & NEWTON, N. R. (1948). The let-down reflex in human lactation. *J. Paediat.* **33**, 698–704.

PEETERS, G., STORMORKEN, H. & VANSCHOUBROEK, F. (1960). The effect of different stimuli on milk ejection and diuresis in the lactating cow. *J. Endocr.* **20**, 163–172.

PETSCHE, H., STUMPF, Ch. & GOGOLAK, G. (1962). The significance of the rabbit septum as a relay station between the midbrain and the hippocampus: I. The control of hippocampus arousal activity by the septum cells. *Electroenceph. clin. Neurophysiol.* **14**, 202–211.

POULAIN, D. A., ELLENDORFF, F. & VINCENT, J. D. (1980). Septal connections with identified oxytocin and vasopressin neurones in the supraoptic nucleus of the rat. An electrophysiological investigation. *Neurosci.* **5**, 379–387.

POULAIN, D. A., LEBRUN, C. J. & VINCENT, J. D. (1981). Electrophysiological evidence for connections between septal neurones and the supraoptic nucleus of the hypothalamus of the rat. *Exp. Brain Res.* **42**, 260–268.

POULAIN, D. A., THEODOSIS, D. T. & LEBRUN, C. J. (1982). Septal lesions do not disrupt the milk ejection reflex in the rat. *J. Physiol. Paris*, **77**, 10B.

POULAIN, D. A. & WAKERLEY, J. B. (1982). Electrophysiology of hypothalamic magnocellular neurones secreting oxytocin and vasopressin. *Neurosci.* **7**, 773–808.

POULAIN, D. A., WAKERLEY, J. B. & DYBALL, R. E. J. (1977). Electrophysiological differentiation of oxytocin- and vasopressin-secreting neurones. *Proc. R. Soc. Lond.* B **196**, 367–384.

POWELL, E. W. & RORIE, D. K. (1967). Septal projections to nuclei functioning in oxytocin release. *Am. J. Anat.* **120**, 605–610.

RAISMAN, G. (1966). The connections of the septum. *Brain* **89**, 317–348.

SLOTNICK, B. M. (1967). Disturbances of maternal behavior in rats following lesions of the cingulate cortex. *Behaviour* **29**, 204–236.

SLOTNICK, B. M. & NIGROSH, B. (1975). Maternal behavior of mice with cingulate cortical, amygdala, or septal lesions. *J. comp. physiol. Psychol.* **88**, 118–127.

STUTINSKY, F. & TERMINN, Y. (1965). Effects des lésions du complexe amygdalien sur le réflexe d'éjection de lait chez la rate. *J. Physiol., Paris* **57**, 279–280.

SWANSON, L. W. & COWAN, W. M. (1976). Autoradiographic studies of the development and connections of the septal area in the rat. In *The Septal Nuclei*, ed. DEFRANCE, J. F., pp. 37–64. New York: Plenum Press.

TALEISNIK, S. & DEIS, R. P. (1964). Influence of cerebral cortex inhibition on oxytocin release induced by stressful stimuli. *Am. J. Physiol.* **207**, 1394–1398.

TANGAPREGASSOM, A. M., TANGAPREGASSOM, M. J., SOULAIRAC, A. & SOULAIRAC, M. L. (1974). Effets des lésions septales sur l'ultrastructure du noyau supraoptique. *Ann. Endocr.* **35**, 149–152.

TRIBOLLET, E., CLARKE, G., DREIFUSS, J. J. & LINCOLN, D. W. (1978). The role of central adrenergic receptors in the reflex release of oxytocin. *Brain Res.* **142**, 69–84.

TRIBOLLET, E. & DREIFUSS, J. J. (1981). Localization of neurones projecting to the hypothalamic paraventricular nucleus area of the rat: a horseradish peroxidase study. *Neurosci.* **6**, 1315–1328.

UHLIR, I., SEGGIE, J. & BROWN, G. M. (1974). The effect of septal lesions on the threshold of adrenal stress response. *Neuroendocrinology* **14**, 351–355.

VOLOSCHIN, L. M. & TRAMEZZANI, J. H. (1979). The neural input of the milk ejection reflex in the hypothalamus. *Endocrinology* **92**, 973–983.

WAKERLEY, J. B. & LINCOLN, D. W. (1973). The milk ejection reflex of the rat: a 20- to 40-fold acceleration in the firing of paraventricular neurones during oxytocin release. *J. Endocr.* **57**, 477–493.

ZABORSKY, L., LERANTH, CS., MAKARA, G. B. & PALKOVITS, M. (1975). Quantitative studies on the supraoptic nucleus in the rat. II. Afferent fiber connections. *Exp. Brain Res.* **22**, 525–540.