J. Physiol. (1986), **380**, *pp.* 239–256
With 9 text-figures
Printed in Great Britain

REGULATION OF THE MILK EJECTION REFLEX IN THE RAT

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(*Received 2 January 1986*)

SUMMARY

1. Extracellular recordings were made from neurones in or near the supraoptic nucleus in suckled lactating rats under urethane anaesthesia to investigate the mechanism by which the firing of oxytocin cells is synchronized during reflex milk ejection.

2. Cells synaptically driven but not antidromically activated by neural stalk stimulation, which thus probably receive an afferent input from supraoptic neurones, were classified as 'regular' or 'bursters' on the basis of their spontaneous electrical activity. The majority (twelve out of eighteen) of synaptically excited cells (o.d.+) were bursters and the majority of inhibited (o.d.−) cells (eleven out of nineteen) were regular, but only one o.d.+ burster showed any change of activity (inhibition) before milk ejection.

3. Putative oxytocin cells in suckled lactating rats showed a firing pattern between milk-ejection bursts which could not be distinguished from that of putative oxytocin cells in male animals. The mode interspike interval between milk ejections was 47·1 ± 3·1 ms (mean ± S.E. of mean) compared with 47·3 ± 3·3 ms in male rats, and fewer than 1·4 % of interspike intervals were less than 20 ms in duration. By contrast, within milk-ejection bursts 40 % of interspike intervals were in the range 8–20 ms.

4. Short trains (10 or 20) of pulses applied to the neural stalk at regular (5 min) intervals, in an attempt to simulate the initial part of the milk ejection burst, failed to trigger bursts. In only 2 of 150 tests was the interval between train and milk-ejection burst less than 10 s, and after the pulse train all but one cell showed reduced activity for 1–3 s. The trains of pulses were however not without effect: they significantly ($P<0·01$) enhanced the chance of a milk-ejection burst occurring within the next 2·5 min.

5. Our observation that pulse trains do not trigger bursts suggests that local positive feed-back mechanisms are not responsible for orchestrating the activation of oxytocin cells during the milk-ejection reflex. Moreover, because spontaneous firing pattern is the same in lactating and non-lactating rats, we found no evidence that the anatomical changes in the synaptic organization within the supraoptic nuclei in lactation have any influence on the firing of oxytocin cells. It is likely, however, since pulse trains alter the timing of milk ejections, that oxytocin released locally in the region of the supraoptic nucleus can influence reflex milk ejection. The

effect may involve a temporary change in the membrane properties of oxytocin cells, and occurs over a longer time scale than is usually associated with neurotransmitter action.

INTRODUCTION

When a lactating rat is suckled by a litter of hungry pups, oxytocin is released in pulses at intervals of 5–15 min for 2–3 h. Each pulse of oxytocin is a consequence of an approximately synchronous activation of probably all the oxytocin cells in the supraoptic and paraventricular nuclei of the hypothalamus which project to the neural lobe; this activation consists of a high-frequency (40–80 Hz) burst of action potentials lasting 1–3 s (Lincoln & Wakerley, 1974, 1975; Summerlee & Lincoln, 1981). The mechanism by which this activation is synchronized throughout the population of oxytocin cells is not established, but several hypotheses have been proposed. First, the presence of dye coupling between oxytocin cells observed *in vitro* has indicated that oxytocin cells may be linked electrotonically during reflex milk ejection (Cobbett, Smithson & Hatton, 1986). Secondly, the incidence of soma–soma apposition between oxytocin neurones is greatly increased during lactation, and this has led to the suggestion that ephaptic interaction may contribute to burst synchronization (Hatton, Perlmutter, Salm & Tweedle, 1984). Thirdly, some oxytocin-containing synapses have been found to make contact with oxytocin cell bodies (Theodosis, 1985) which suggests that there may be a degree of mutual synaptic excitation between oxytocin neurones; in support of this hypothesis it has recently been shown that intraventricular injections of oxytocin lead to an increase in the activity of oxytocin cells and a facilitation of the milk-ejection reflex (Freund-Mercier & Richard, 1984). Finally, the demonstration that supraoptic neurones project to the hypothalamic region adjacent and dorsal to the supraoptic nucleus (Leng, 1982; Mason, Ho & Hatton, 1984), which in turn contains neurones which project extensively into the nucleus (Hatton, Ho & Mason, 1983), has led to the hypothesis that this area contains 'interneurones' which gate the milk-ejection reflex (Leng & Dyball, 1983).

The first three of these hypotheses imply that, in the lactating rat at the time of milk ejection, the oxytocin cells are directly linked in a positive feed-back system. The fourth hypothesis implies that the link is indirect, via interneurones which may 'make or break' a positive feed-back circuit. We have attempted to test these hypotheses in two series of experiments. In the first series we recorded, during reflex milk ejection, the electrical activity of cells immediately adjacent to the supraoptic nucleus after first characterizing the orthodromic effects of neural stalk stimulation on them. In the second series of experiments we sought evidence of any difference in spontaneous firing patterns of oxytocin cells during suckling but between milk ejections, between these and similar cells from male rats, and examined the influence of repeated brief trains of electrical stimuli applied to the neural stalk while recording the electrical activity of oxytocin neurones. Some of the results have been communicated previously to the Physiological Society (Dyball & Leng, 1986).

METHODS

The experiments were performed on thirty-eight lactating Wistar rats, body weight 300–350 g. At days 8–12 of lactation, each rat was separated overnight from all but one pup of her litter. On the following morning, the mother rat was anaesthetized with urethane (ethyl carbamate; 1·1 g/kg body wt; I.P.). Catheters were inserted into the jugular vein, into a femoral artery to record blood pressure, and into an inguinal mammary gland to record intramammary pressure (Lincoln & Wakerley, 1974). The rat was placed in a stereotaxic frame and two holes were drilled into the skull to allow stereotaxic placement of a stimulating electrode (concentric bipolar, type SNEX 100; Rhodes Medical Instruments, CA, U.S.A.) upon the neural stalk, and a glass micropipette (tip resistance 15–30 MΩ, filled with 0·5 M-Na acetate) vertically into the region of the right supraoptic nucleus. At the end of surgery the rat was given an I.V. injection of propranolol (250 μg/kg body wt; this dose increases the probability that the milk-ejection reflex will occur in the anaesthetized rat: Juss & Wakerley, 1981). The position of the stimulating electrode was adjusted in steps of 0·1 mm below a depth of 8·5 mm until the increase in intramammary pressure evoked by a train of 90 shocks (50 Hz; 0·75 mA peak-to-peak; matched biphasic pulses; 2 ms pulse width) was maximal, and produced a response similar to that evoked by I.V. injection of 0·25–0·5 mu oxytocin (Syntocinon; Sandoz).

Single units were recorded extracellularly, using conventional techniques, at a depth of 7·5 mm or more from the dorsal surface of the brain, 2 mm lateral to the mid line and 7·0–7·5 mm anterior to the interaural line (according to the coordinates of Pellegrino, Pellegrino & Cushman, 1979). The response of each unit to single shocks presented to the neural stalk was examined. Units characterized as supraoptic neurones were antidromically activated by stimuli at 1 mA or less and satisfied the collision test; these were invariably found at depths exceeding 8·5 mm. The remaining neurones in this study, classed as 'lateral hypothalamic neurones', could not be antidromically activated by stimuli of 5 mA peak-to-peak, and were located at depths of 7·5–8·5 mm. Spontaneous electrical activity and the orthodromic effects of neural stalk stimulation were studied on-line using a Commodore PET microcomputer. Interspike interval histograms were constructed from 8 min of spontaneous activity in the absence of any neural stalk stimulation. Post-stimulus time histograms were constructed of the responses to 300 stimuli presented at 1 Hz at an intensity of 0·75 mA or less. Spike wave forms were averaged for analysis using a Nicolet model 4094 digital oscilloscope.

Following Blount & Leng (1985), cells with a modal interspike interval of less than 20 ms were classed as 'bursters' and cells with a modal interspike interval of 20 ms and a spontaneous firing rate exceeding 2 Hz as 'regular' cells, with the exception of a small number of neurones that fired in a clearly phasic or oscillatory pattern (Leng, 1982). Unclassified cells fired at 2 Hz with modes exceeding 20 ms or with no clearly defined mode. Cells were classed as orthodromically excited (o.d. +) or orthodromically inhibited (o.d. −) if their post-stimulus time histograms revealed a clear initial excitation or inhibition respectively within 40 ms of the stimulus pulse.

Not less than an hour after the end of surgery, between eight and ten pups were applied to the uncannulated nipples, and then until the end of the experiment at least eight pups were kept suckling. Reflex milk ejections were detected by the characteristic rise in intramammary pressure (Lincoln & Wakerley, 1974) usually followed by an equally characteristic behavioural reaction from the suckling pups. Supraoptic cells which displayed a brisk activation preceding each observed reflex milk ejection were thus identified as oxytocin cells. The effects of neural stalk stimulation on the reflex were studied only during recordings from oxytocin cells. Stimuli were presented at 50 Hz with an intensity of 0·75 mA in trains of ten or twenty pulses with different intervals between trains.

RESULTS

Interneurones adjacent to the supraoptic nucleus

In experiments on virgin female rats, Blount & Leng (1985) found an association between the spontaneous firing patterns of lateral hypothalamic neurones and their orthodromic responses to neurohypophysial stimuli: they reported that most of the cells classed as bursters are orthodromically excited from the neural stalk, while most

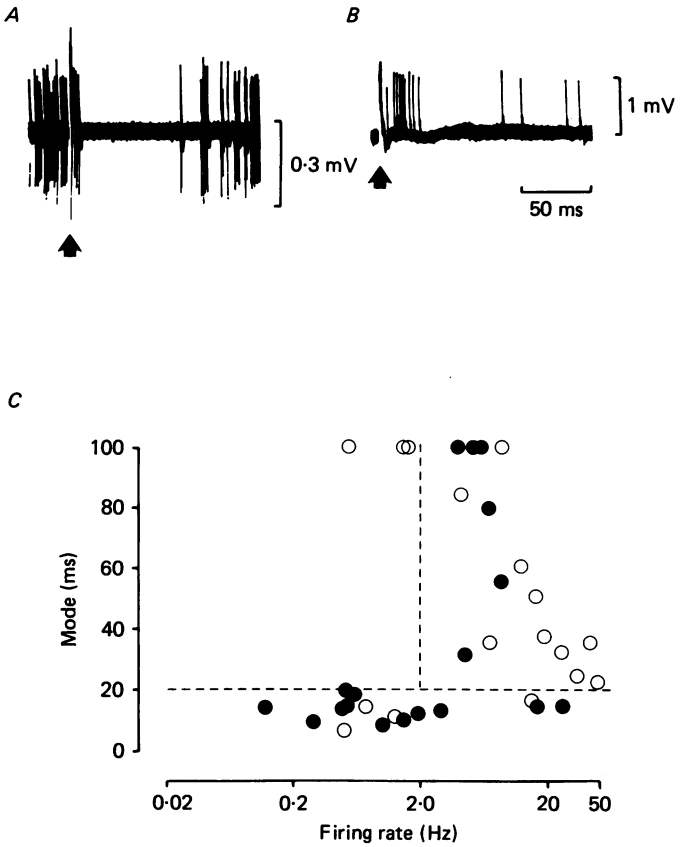

Fig. 1. *A* and *B*, photographs taken from the face of a storage oscilloscope during an experiment to show (*A*) orthodromic inhibition and (*B*) orthodromic excitation in two lateral hypothalamic neurones following stimuli applied to the neural stalk (arrows). Each photograph comprises twenty superimposed sweeps of the oscilloscope trace. *C*, modal interspike interval plotted against mean firing rate for thirty-seven lateral hypothalamic neurones classed as either o.d.+ (○) or o.d.− (●) from the neural stalk. The dashed lines indicate the criteria for classifying cells as 'regular' or 'bursters'. Cells below the horizontal dashed line were classed as bursters; such cells, despite a low average firing rate, characteristically fired with brief, high-frequency bursts of action potentials. Cells above this line and to the right of the vertical dashed line were classed as regular; despite their relatively high average firing rate, their modal interspike intervals were long. Most of the o.d.+ cells are bursters and most of the o.d.− cells are regular.

regular cells are orthodromically inhibited (Fig. 1 *A* and *B*). In the present study, in a sample of thirty-seven spontaneously active cells classed as either o.d.+ or o.d.−, twelve of eighteen o.d.+ cells were classed as bursters compared with four of nineteen o.d.− cells, and eleven of the nineteen o.d.− cells were classed as regular compared with four of the o.d.+ cells (Fig. 1*C*; $P < 0{\cdot}01$, χ^2 test).

The orthodromic effects of neural stalk stimulation observed in the present experiments occurred with latencies in the range 10–40 ms, whereas most (forty-nine of a sample of fifty-one) of the supraoptic cells were antidromically activated with latencies of 6–20 ms (mean $11{\cdot}2 \pm 0{\cdot}7$ ms, $n = 51$). The stimulus pulses used to test for orthodromic effects were at an intensity greater than the threshold for antidromic

activation of about 75% of supraoptic cells. Orthodromic effects could however be demonstrated in some cells using stimulus currents as low as 0·25 mA. The most strongly driven o.d.+ cells showed one or more spikes with a variable latency following most stimulus pulses when stimuli were presented at 1 Hz, but four such

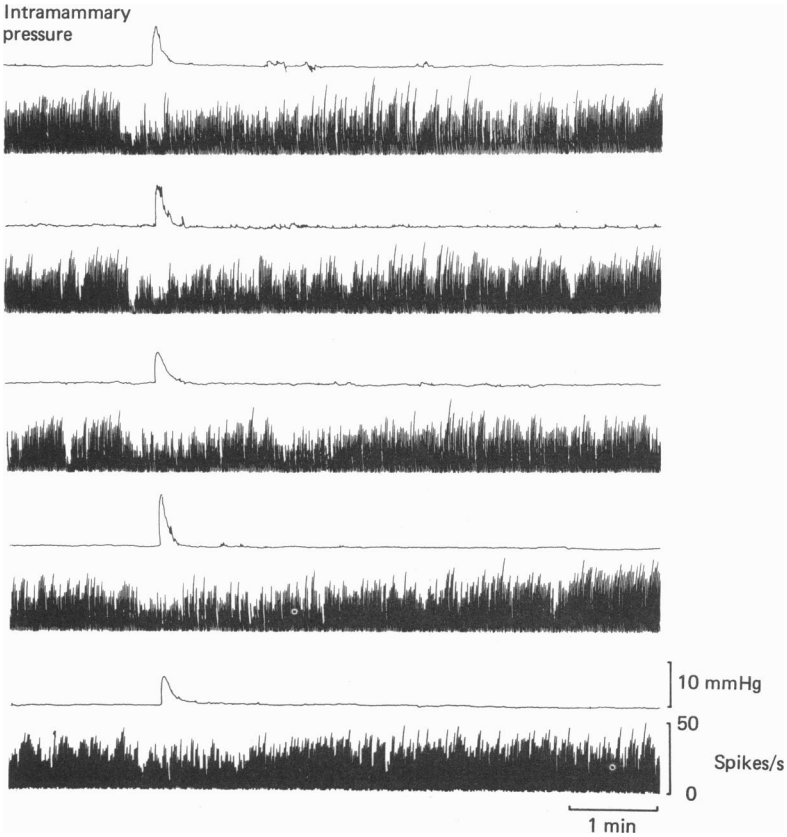

Fig. 2. Polygraph records of a lateral hypothalamic neurone during each of five reflex milk ejections. The lower trace of each record shows a rate-meter record of the firing rate of the neurone, and the upper trace shows the intramammary pressure. Each reflex milk ejection is marked by a rise in intramammary pressure similar to that evoked by I.V. injection of 0·5 mu oxytocin (not shown). Note that this cell showed a variable reduction in firing rate before each milk ejection.

cells tested with brief trains (10–20 pulses) at 50 Hz showed poor frequency following. Most of the o.d.+ cells showed one variable-latency orthodromically evoked spike for every 5–10 pulses (at 1 Hz).

Fifty lateral hypothalamic cells were recorded through a total of 117 reflex milk ejections. These included ten cells that were classed as o.d.− from the neural stalk, and seventeen that were classed as o.d.+. Nine of the o.d.+ cells and two of the o.d.− cells were classed as bursters; four of the o.d.− cells and one of the o.d.+ cells were classed as regular. One cell showed a reduction in spike discharge before each of the five milk ejections through which it was recorded (Fig. 2); this was an o.d.+ cell that was classed as a burster. The remaining forty-nine lateral hypothalamic cells

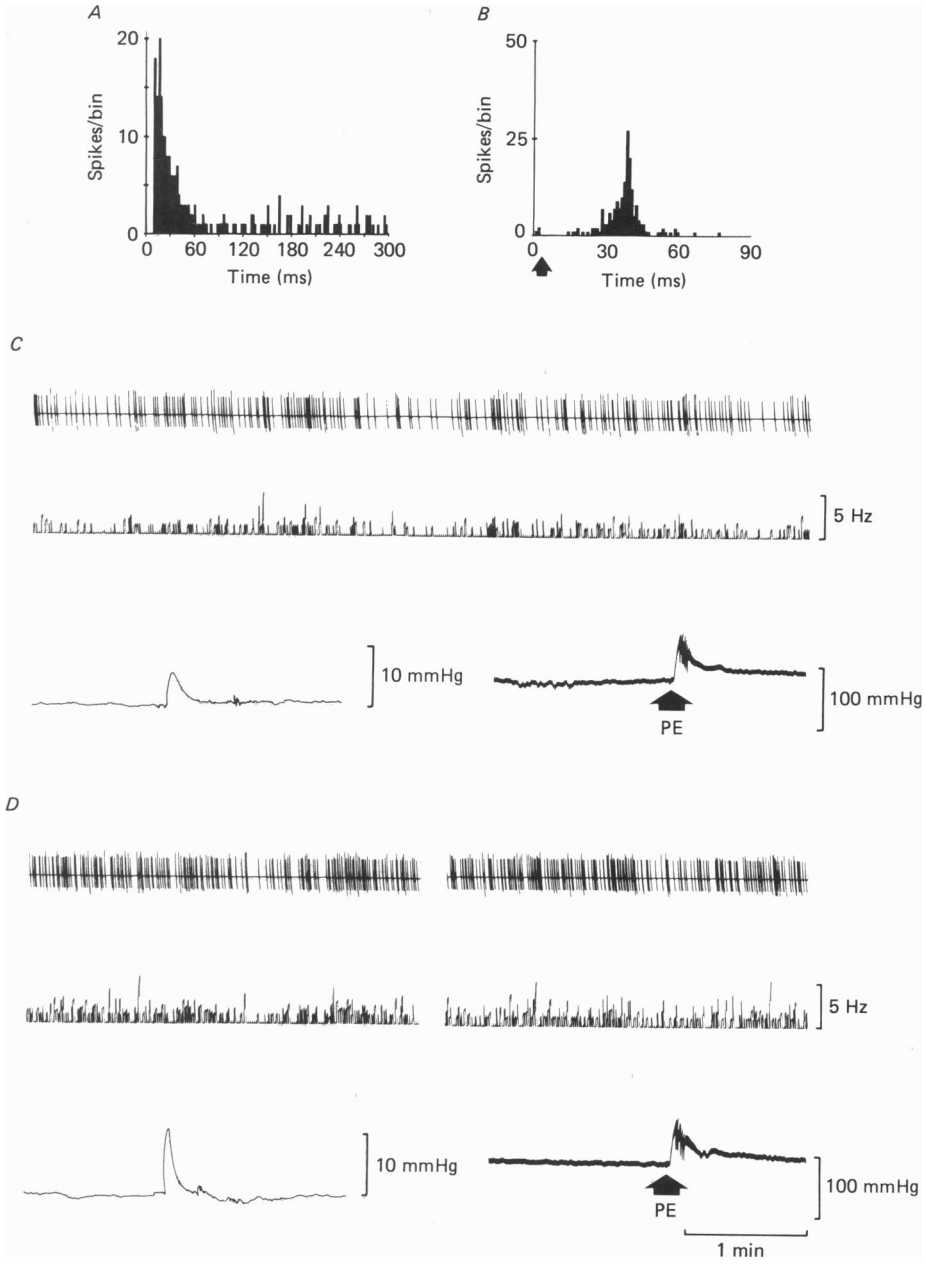

Fig. 3*A–D*. For legend see opposite.

showed no clear or consistent change in activity in the minute preceding reflex milk ejection (Figs. 3 and 4). The non-responsive cells included one cell firing with a phasic pattern (Fig. 4) similar to those reported previously in a small proportion of lateral hypothalamic neurones (Leng, 1982).

One experiment produced a 'double-recording' rare enough to be worthy of note.

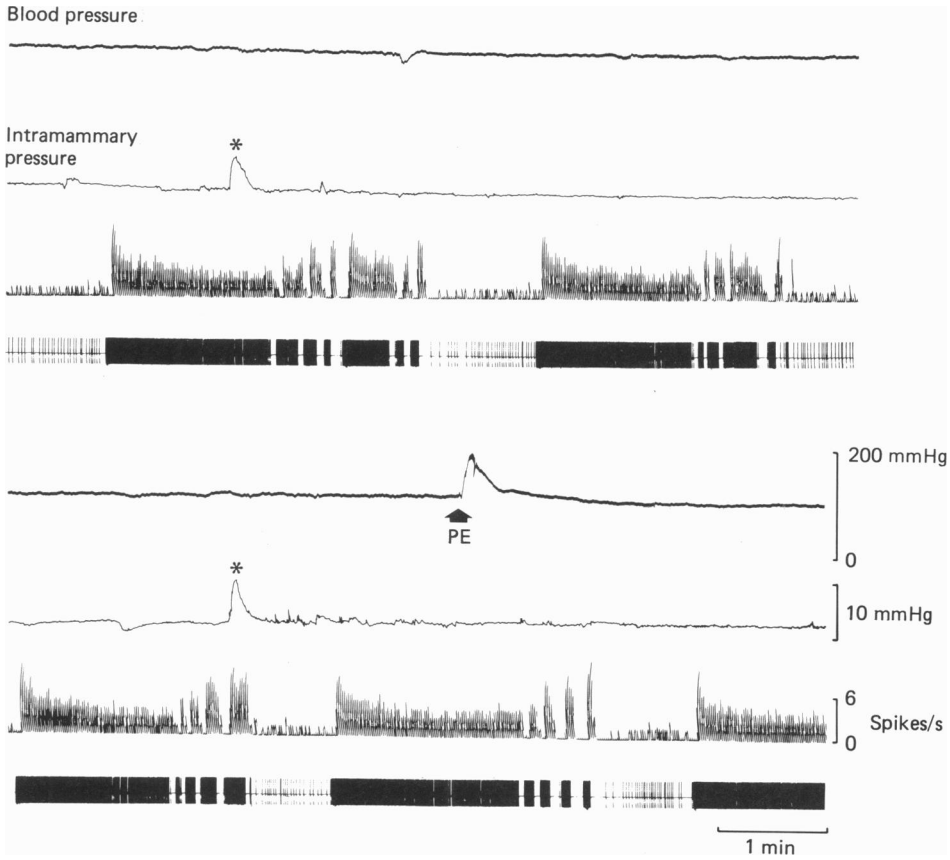

Fig. 4. Continuous polygraph record of the activity of a phasically active neurone in the lateral hypothalamus. The uppermost trace is a recording of blood pressure; I.V. injection of 10 μg phenylephrine (PE, arrow) increased blood pressure by about 80 mmHg. The second trace is a recording of intramammary pressure; two reflex milk ejections are marked with asterisks. Below this is a rate-meter record of the firing of the neurone, and at the bottom is a record in which each pen deflexion marks a single action potential. The bursting pattern of this neurone was stereotyped, and was unaffected either by reflex milk ejection or by the rise in blood pressure.

Fig. 3. *A*, interspike interval histogram from a 'burster' neurone in the lateral hypothalamus: note the very short mode. *B*, post-stimulus time histogram from the same neurone showing orthodromic excitation with a minimum latency of about 20 ms following stimuli applied to the neural stalk (arrow). *C*, polygraph record of spike discharge and rate-meter record of this neurone above an intramammary pressure record (left) and above a blood pressure record (right). This cell showed no change in activity before a reflex milk ejection, or following an I.V. injection of 10 μg phenylephrine (PE, arrow). *D*, the same cell 15 min after I.P. injection of 1 ml 1·5 M-NaCl. The spontaneous firing rate was about doubled following the injection, but still the cell showed no change in activity either preceding milk ejection or following a rise in blood pressure. Recordings from this and other lateral hypothalamic cells suggest that very few of the o.d.+ or o.d.− cells are involved in the milk-ejection reflex, and that the synaptic projection from the supraoptic nucleus to these cells conveys the effects of osmotic stimulation but not the effects of baroreceptor stimulation.

One of the recorded cells was an oxytocin cell, the other a cell that was not antidromically activated but whose activity was correlated with that of the oxytocin cell, and which was thus probably an interneurone. Spikes in the interneurone tended to precede those in the oxytocin cell (Fig. 5); of thirty instances where spikes from

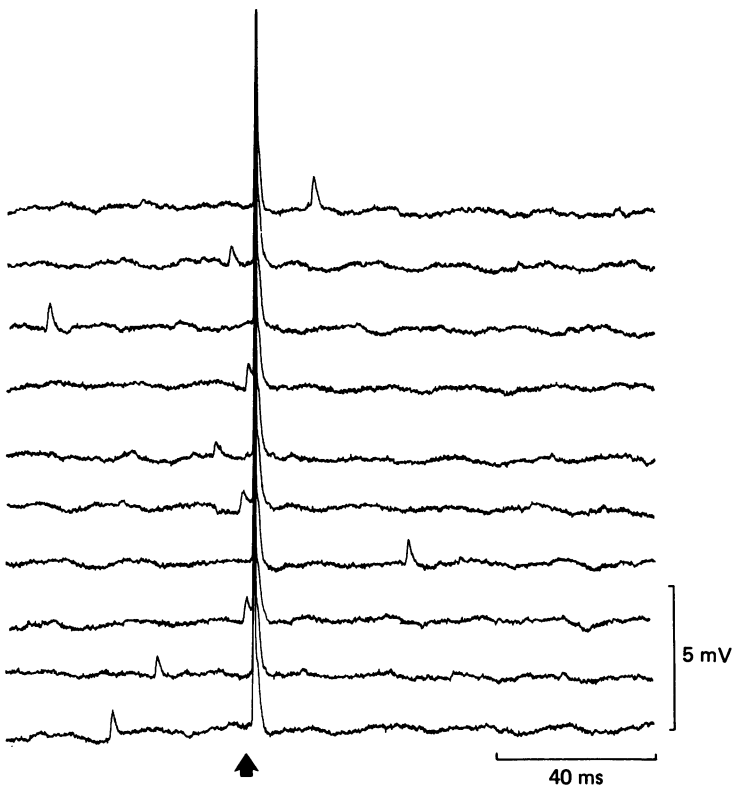

Fig. 5. Ten records, stored on a digital oscilloscope, showing action potentials from a supraoptic oxytocin neurone (large spikes, superimposed above arrow) in relation to action potentials (small spikes) from a neurone that was not antidromically activated from the neural stalk. Many more of the smaller spikes occurred in the 20 ms before an action potential in the oxytocin neurone than occurred in the following 20 ms. This suggests that the cell with the smaller spikes was driving the oxytocin neurone synaptically.

one cell occurred within 20 ms of spikes in the other cell, spikes in the interneurone occurred before spikes in the supraoptic neurone 24 times. The interneurone may therefore have been afferent to the oxytocin cell, but it was unaffected during reflex milk ejection.

Thus the great majority of cells recorded in the hypothalamic area immediately dorsal to the supraoptic nucleus do not appear to be involved in the milk-ejection reflex. Furthermore, most of these cells (twenty-four out of twenty-six) were unaffected by I.V. injection of 10 μg phenylephrine (Figs. 3 and 4); this stimulus raised blood pressure by 30–50 mmHg from means of 100–120 mmHg, and as has been

reported previously, it also silences vasopressin cells but has relatively little effect upon oxytocin cells (Harris, 1979). The two responsive cells were a regular o.d.− cell that was excited following phenylephrine and an o.d.+ bursting cell that was inhibited.

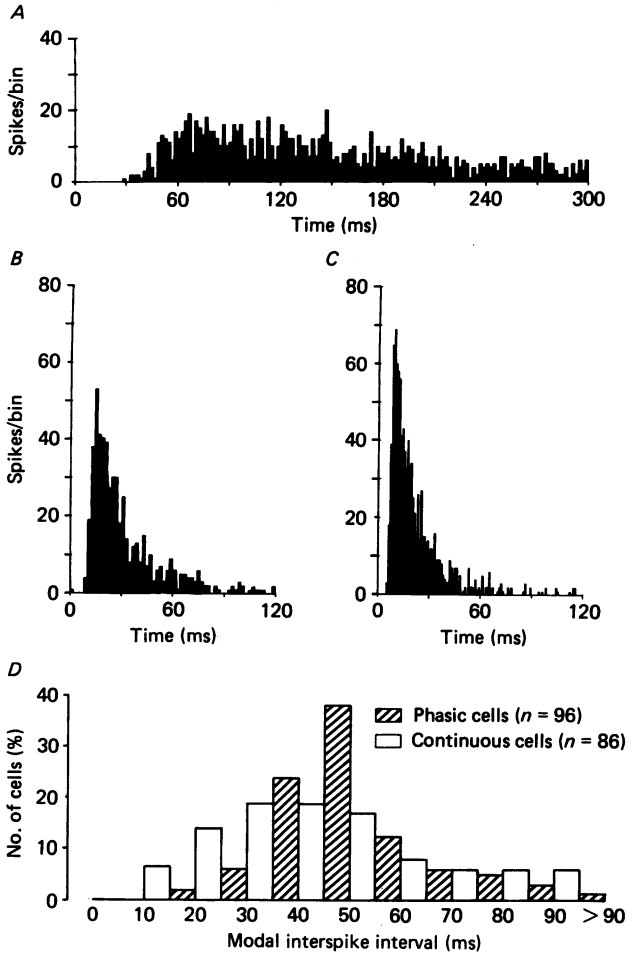

Fig. 6. *A*, *B* and *C*, interspike interval histograms of the activity of oxytocin cells in a suckled, lactating rat, between milk ejections (*A*) and during milk-ejection bursts (*B* and *C*). The histograms in *A* and *B* are from the same cell; the cell illustrated in *C* was not spontaneously active. Note that during milk-ejection bursts (but not between bursts) short interspike intervals are very common; the modal interspike intervals of the histograms illustrated in *B* and *C* are at 14 and 9 ms respectively. *D*, histogram showing the distributions of modal interspike intervals of continuous (putative oxytocin) and phasic (putative vasopressin) cells in the supraoptic nucleus of non-lactating (male) rats.

Discharge characteristics of oxytocin cells

Interspike interval histograms were constructed for eighteen identified, continuously active oxytocin cells while eight to ten pups were suckling but between milk ejections (Fig. 6*A*). The histograms showed no differences in the interval distributions

between these neurones and putative oxytocin neurones recorded from male rats. The modal interspike interval values of continuously active (putative oxytocin) neurones and of phasically active (putative vasopressin) neurones from male rats are distributed approximately normally (Fig. 6*D*), with a mean mode for continuous cells of

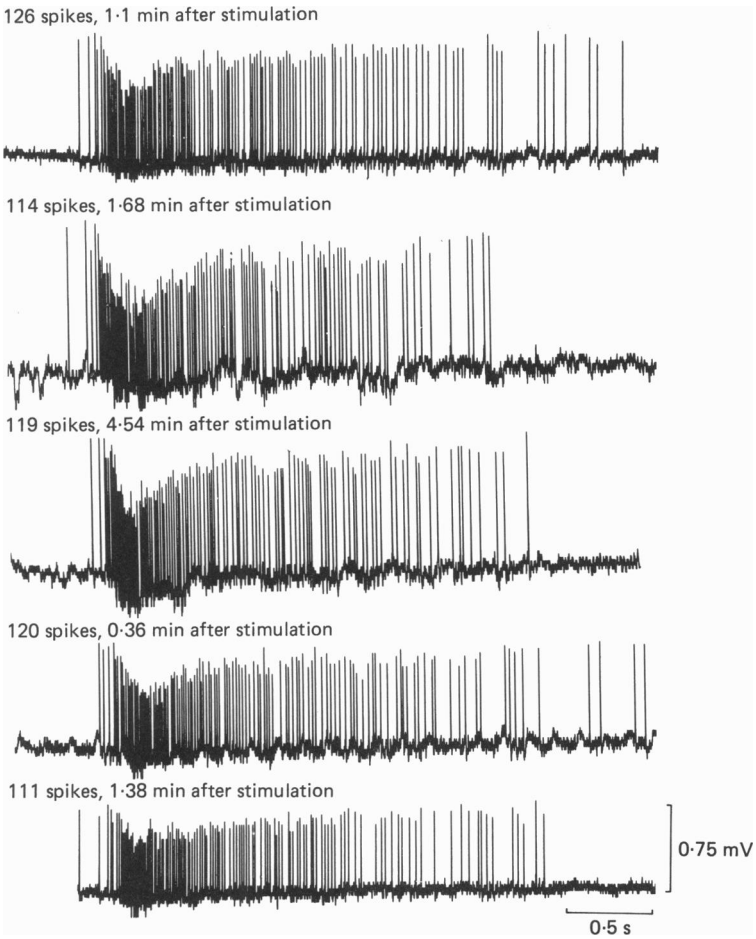

Fig. 7. Five of twelve milk-ejection bursts recorded over a 2 h period from one oxytocin cell. The pattern and duration of the milk-ejection bursts was remarkably constant over this time. The total number of spikes in each burst is given above each burst: throughout the recording this varied between 111 and 126 spikes (top and bottom traces). Short trains of stimuli (10 pulses at 50 Hz) were presented to the neural stalk throughout the period of recording. The interval between each milk-ejection burst and the preceding stimulation is also given above each trace. There was no observed correlation between this interval and the burst amplitude.

48·5 ± 2·5 ms ($n = 86$) and for phasic cells of 45·6 ± 1·5 ms ($n = 96$; data from previous experiments of Leng (1981) and Leng & Dyball (1983) using a ventral surgical approach). In the present experiments the mean modal interspike interval of twenty continuously active (2·5 Hz) oxytocin cells was 47·1 ± 3·1 ms. Although the mean firing rate of supraoptic neurones was lower in the present experiments than in the

previous experiments with male rats (this is a consistent difference between results obtained with ventral surgery and results with dorsal surgery: see Harris, 1979), the firing rates of the samples did not differ significantly. Moreover, the mode of the interspike interval is not correlated with mean firing rate in the range of firing rates (2·5–8 Hz) covered by these samples (Leng, 1981). Again in agreement with results in non-lactating rats (Leng, 1981), fewer than 25% of spikes recorded in the spontaneous activity of the oxtocin neurones in lactating rats occurred within 70 ms of the preceding spike. Of 45 620 interspike intervals recorded in 8 min segments of spontaneous activity from twenty-eight oxytocin cells, only 660 (1·4%) were less than 20 ms in duration. Thus oxytocin cells in suckled, lactating rats, in periods between reflex milk ejections, discharged in a manner indistinguishable from the activity of continuously active supraoptic neurones in male rats, and in patterns which contained very few short intervals.

By contrast, in recordings of thirty-three milk-ejection bursts from eleven oxytocin cells, modal interspike intervals were in the range 9–18 ms (Fig. 6*B* and *C*), and 1127 of 2804 (40%) interspike intervals were less than 20 ms in duration. For any individual cell the duration and total number of spikes in each milk-ejection burst were remarkably consistent (Fig. 7), and the period of maximal discharge rate occurred within a few spikes of the onset of each burst. Thus the discharge behaviour of oxytocin cells during milk-ejection bursts is highly stereotyped, but is quite uncharacteristic of the behaviour of these cells at any other time.

Influence of neural stalk stimulation on the milk-ejection reflex

In one experiment only three milk ejections were observed during a 4 h period of suckling, but each of them followed within 2 min of the first three of a series of short (5–10 pulses) trains of stimuli applied to the neural stalk at 5 min intervals. We proceeded to test whether this observation was coincidence or was a reproducible observation, by applying trains of ten or twenty pulses (at 50 Hz) to the neural stalk at 5 or 10 min intervals. From experiments on eighteen rats we observed that, of 100 milk-ejection bursts recorded within 5 min of a train of ten pulses, most (sixty-four) occurred within the first 2·5 min (significantly different from chance; $P < 0{\cdot}01$), and thirty-three out of fifty milk-ejection bursts (66%) occurred within 2·5 min after twenty pulses (Fig. 8). We found no evidence that the interval between the stimulus train and the milk-ejection burst was related to the amplitude of the milk-ejection burst.

These trains were, in every experiment, below the threshold duration for producing a detectable rise in intramammary pressure and evoked no apparent behavioural reaction from the suckling pups. In two experiments cortical electroencephalogram (e.e.g.) was recorded; trains of twenty pulses at 50 Hz had no perceptible synchronizing or desynchronizing effect on e.e.g. As trains of ninety pulses at 50 Hz produced a response similar to that produced by 0·5 mu oxytocin injected I.V., we estimated that the short trains probably released not more than 0·1 mu oxytocin. To control for the peripheral effects of this small oxytocin release, we looked at the effects on the reflex of injections of 0·1 mu oxytocin; these injections were also below the threshold dose necessary to produce a detectable intramammary pressure response. Of fifty-two milk-ejection bursts occurring within 5 min of the oxytocin injections,

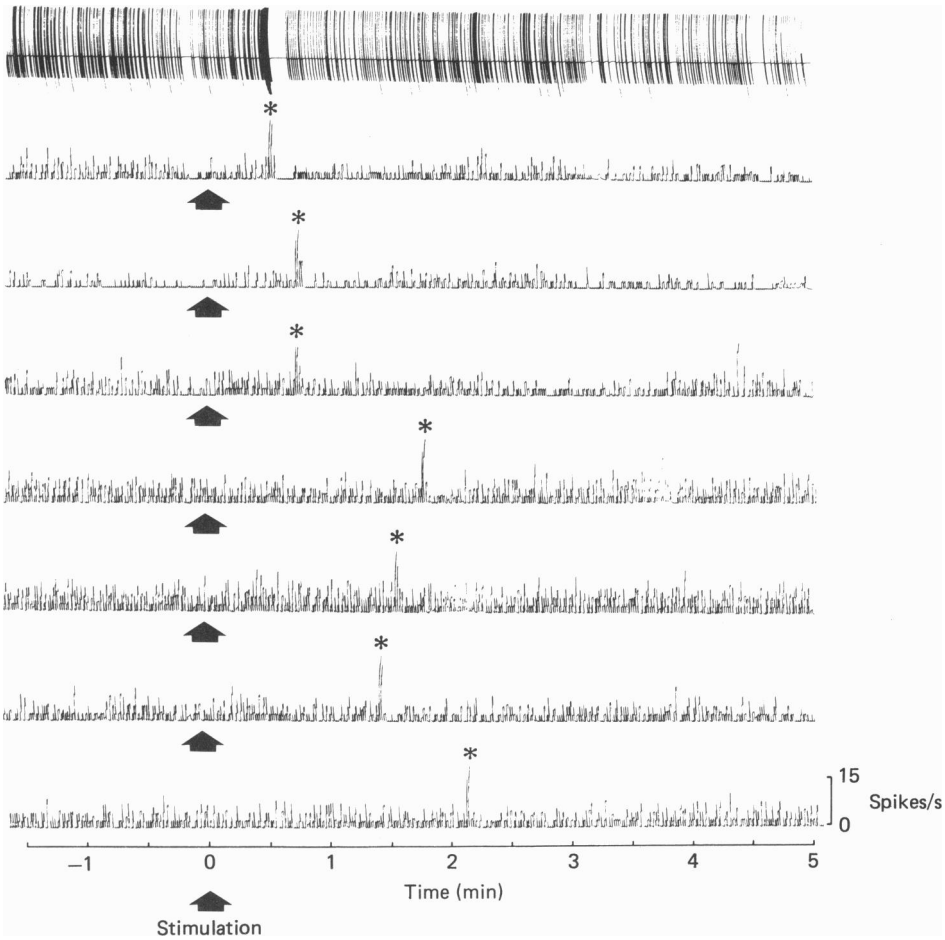

Fig. 8. Polygraph records of seven successive milk-ejection bursts recorded from one oxytocin cell. The top trace shows pen deflexions corresponding to each action potential; the lower traces show the rate-meter records corresponding to this and to subsequent records. The arrows mark the times that twenty shocks at 50 Hz were applied to the neural stalk. Each of these seven milk-ejection bursts occurred within 2·5 min of stimulation, but none were triggered immediately by the stimulation.

twenty-three (45 %) occurred within the first 2·5 min ($P < 0{\cdot}05$; Mann–Whitney U test, comparison with intervals following ten pulses).

Although neural stalk stimulation thus appeared to facilitate the milk-ejection reflex, bursts never followed immediately after a train of pulses: the shortest interval observed between a train and a milk-ejection burst was 7 s. In later experiments we also failed to 'trigger' milk-ejection bursts with pairs of stimulus trains separated by 30 s, or by brief trains (six pulses at 50 Hz) presented every 30 s. The shortest latency observed between a pulse train and a milk-ejection burst was 0·5 s: even with short intervals between trains, bursts were not triggered by the neural stalk stimulations: of eight milk-ejection bursts recorded in the periods of application of these trains, seven occurred more than 10 s after the end of a train.

Short trains of pulses to the neural stalk not only failed to trigger milk-ejection bursts, but were also associated with reduced spike activity in the 1–3 s following the train, so that not more than one or two spontaneous spikes fell within 500 ms of the end of the train. We found one exception. In one experiment, a 'double-

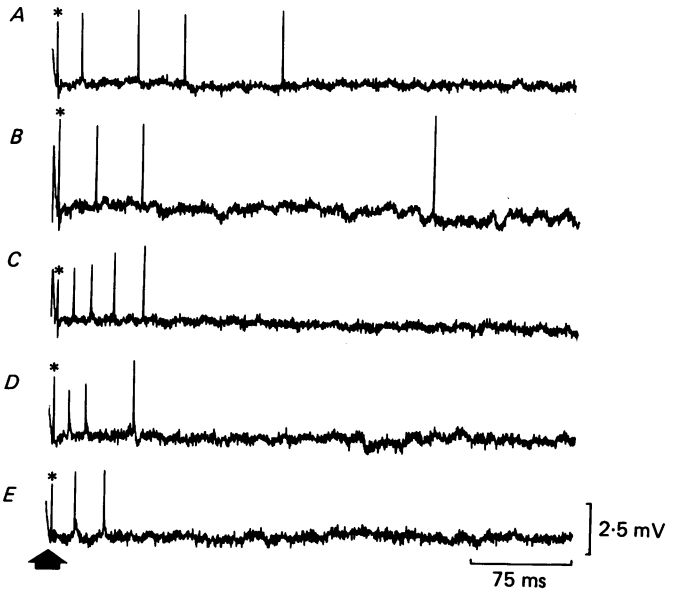

Fig. 9. Five records, stored on a digital oscilloscope, showing action potentials from a phasically active supraoptic neurone following the application of ten (*A* and *B*) or twenty (*C*, *D* and *E*) pulses to the neural stalk. In each trace the arrow marks the end of the train and the asterisk marks the last action potential evoked antidromically by the train. A brief high-frequency after-discharge followed each of these trains; no after-discharge followed trains of forty pulses (not shown).

recording' of two cells simultaneously with a single electrode was made from a pair of supraoptic cells. One of these showed periods of phasic activity characteristic of vasopressin cells, the other showed vigorous milk-ejection bursts. The phasic cell was weakly activated during five of seven reflex milk ejections, and also showed spike activity immediately following trains of shocks to the neural stalk (Fig. 9); up to six spikes occurred at about 50 Hz, following which the cell was silent for several seconds. The greatest number of spikes followed ten pulses at 50 or 100 Hz; forty pulses at 100 Hz produced one spike at most.

DISCUSSION

The present study was designed to test the hypothesis that oxytocin cells, during reflex milk ejection, are linked in a positive feed-back system; our results indicate that this hypothesis is false. In the present experiments oxytocin cells were driven antidromically at high frequencies by brief stimulus trains; no regenerative bursting activity was observed.

Lateral hypothalamic neurones are not activated during the milk-ejection reflex

Although the present study confirmed that many neurones in the lateral hypothalamus are influenced by activation of the magnocellular neurosecretory system, a surprising find was that very few of these are affected by stimuli known to influence magnocellular neuronal activity profoundly. Lateral hypothalamic neurones appear in general to respond to osmotic stimulation as a result of the synaptic influence they receive from the magnocellular system (Leng, 1982). However, osmotic stimulation produces a sustained change in the activity of the magnocellular system whereas the two stimuli examined in the present study (blood pressure changes and suckling-induced milk ejection) both induced only transient changes in the activity of the magnocellular system. Although the milk-ejection burst is a dramatic event, it lasts usually less than 2 s. The orthodromic effect upon these cells of a brief intense activation of the magnocellular system may be very slight; even lateral hypothalamic cells which are strongly orthodromically driven from the neural stalk do not follow high-frequency stimulation well.

Spontaneous patterns of activity are similar in male rats and suckled rats

A number of phenomena, observed in lactating rats but not in virgins, have been interpreted as evidence that oxytocin cells may interact differently during lactation. The glial cell processes which normally separate oxytocin cells are retracted in the lactating rat, leaving oxytocin cells in direct soma–soma apposition (Theodosis, Poulain & Vincent, 1981; Hatton & Tweedle, 1982; Theodosis & Poulain, 1982, 1984) and similar changes occur around the dendrites of magnocellular neurones, giving rise to 'bundling' of naked dendrites in the supraoptic nucleus of lactating rats (Hatton *et al* 1984). We found no electrophysiological correlate of these morphological changes in the patterns of spontaneous activity of oxytocin cells. In male rats, oxytocin cells do not show high-frequency bursts under any known circumstances: interspike intervals of 20 ms in duration are extremely rare, apparently due to a prolonged post-spike hyperpolarization mediated by a Ca^{2+}-activated K^+-conductance (Andrew & Dudek, 1984). Surprisingly, the oxytocin cells in suckled, lactating rats fired in a manner indistinguishable from the patterns observed in male rats, i.e. with a mean mode interspike interval of close to 50 ms, and very few intervals of less than 20 ms, except actually during the 1–2 s milk-ejection bursts, when modal interspike intervals were always in the range 8–20 ms. These values should be compared with those for bursting cells in the lateral hypothalamus, which have modal interspike intervals of 5–20 ms despite low mean firing rates.

The milk-ejection activation of oxytocin cells is not a regenerative burst

Other phenomena, not unique to lactation, point to the existence of a neuroanatomical network with, in theory at least, the capacity to couple oxytocin cells. Gap junctions occur between a limited number of supraoptic neurones (Andrew, MacVicar, Dudek & Hatton, 1981; Dudek, Andrew, MacVicar, Snow & Taylor, 1983), both between oxytocin cells and between vasopressin cells (Cobbett *et al.* 1986), and a small number of synapses onto oxytocin cells have been shown to contain oxytocin (Theodosis, 1985).

Previous studies have sought, and failed to find, electrophysiological evidence for extensive excitatory interactions between supraoptic neurones in non-lactating rats (Leng, 1981; Leng & Dyball, 1983). The present results demonstrate that in the suckled, lactating rat, when reflex milk ejections are occurring regularly, there is still no sign that milk-ejection bursts can be triggered by imposing a brief high-frequency activation on the whole population of oxytocin cells. Thus the significance of the morphological changes in the supraoptic nucleus during lactation, and that of the presence of a limited degree of electrical coupling, remain uncertain.

The failure to trigger bursts in oxytocin cells is in marked contrast to the behaviour of vasopressin cells. During osmotic stimulation, vasopressin cells fire 'phasically', with low-frequency (6–12 Hz) bursts of 20–40 s duration separated by silent periods of similar duration (Brimble & Dyball, 1977). These bursts can be triggered by antidromic stimuli (Dreifuss, Tribollet, Baertschi & Lincoln, 1976); a full burst can be triggered by even a single stimulus pulse to the neural stalk (Buckley & Leng, 1981). Thus the difference between the bursting activity of vasopressin cells and that of oxytocin cells is not merely quantitative. Bursts in vasopressin cells are endogenous to vasopressin cells although they may be modulated by afferent inputs; bursts in oxytocin cells do not appear to be endogenous to oxytocin cells or even to the supraoptic nucleus, although, perversely, as we report here, their behaviour may be modulated by changes in the activity within the nucleus.

The only evidence that stimulation of the neural stalk had persisting excitatory effects came from one recording of a phasic cell. Almost all phasic cells are apparently vasopressin cells (Poulain, Wakerley & Dyball 1977; Brimble & Dyball 1977; Yamashita, Inenaga, Kawata & Sano, 1983), although there have been reports that some phasic cells are, as this was, activated during reflex milk ejection (Lincoln & Wakerley, 1974; Summerlee, 1982). The behaviour of this cell following pulse trains (Fig. 9) was consistent with ephaptic or synaptic excitation resulting from activation of neighbouring oxytocin cells. It is striking that stimulation of the neural stalk had no persisting excitatory effects upon any of the cells clearly identified as oxytocin cells.

Facilitatory effect of neural stalk stimulation on the milk-ejection reflex

Despite the failure of short trains of pulses to trigger reflex milk ejection the present results do support a facilitatory role for endogenous oxytocin in the milk-ejection reflex. Richard and co-workers have recently shown that small quantities of oxytocin injected into the third ventricle increase the spontaneous activity of oxytocin cells in suckled, lactating rats (Belin, Moos & Richard, 1984) and increase the frequency and amplitude of milk-ejection bursts (Freund-Mercier & Richard, 1984). A similar increase in burst frequency and amplitude can also be evoked by neural stalk stimulation (Negoro, Uchide, Honda & Higuchi, 1985). Experiments *in vitro* indicate that oxytocin is released in the vicinity of the supraoptic nucleus following stimulation with raised extracellular K^+ (Chapman, Hatton, Ho, Mason & Robinson, 1983) or following administration of an oxytocin agonist (Moos, Freund-Mercier, Guerne, Guerne, Stoeckel & Richard, 1984). Oxytocin cells in the supraoptic nucleus have axon collaterals that appear to terminate within the hypothalamus (Mason, Ho & Hatton, 1984), and electrophysiological evidence in the present and previous studies

(Leng, 1983; Hatton *et al.* 1983) suggests that there is extensive interaction between neurones in the supraoptic nucleus and lateral hypothalamic neurones. The present results, like those of Negoro *et al.* (1985), indicate that the activity of oxytocin cells can indeed modify the timing of the milk-ejection reflex. How this influence is mediated remains unclear, but it is apparently exerted over a relatively long time scale. Our experiments did not, however, reveal any effect of the pulse train on e.e.g. so that it is unlikely that the change in the pattern of milk ejection is mediated indirectly via a change in arousal.

The present studies appear to argue against a positive feed-back coupling of oxytocin cells during suckling-induced reflex milk ejection, whether a direct coupling or an indirect coupling via interneurones as was proposed by Leng & Dyball (1983). The simplest conclusion would be that the milk ejection is co-ordinated further back in the afferent pathway: Bruni & Perumal (1984) have described an extensive network of small fibres that pervade the supraoptic nucleus, making multiple synaptic contacts that may be the source of an approximately synchronous synaptic activation, but there remain two problems with this interpretation. First, the results of the present study and those of Freund-Mercier & Richard (1984) suggest that the activity of the oxytocin cells themselves can modify the reflex. Secondly, the patterns of spontaneous activity recorded extracellularly from oxytocin cells suggest that action potentials are normally followed by a period of hyperpolarization which limits the frequency at which they can fire in response to synaptic input. This was confirmed by intracellular recordings from supraoptic neurones *in vitro* which show that action potentials are indeed normally followed by a hyperpolarization resulting from a Ca^{2+}-activated K^+ current (Andrew & Dudek, 1984). One explanation may be that while the afferent stimulus for reflex milk ejection is co-ordinated elsewhere, the stimulus is nevertheless 'gated' in the hypothalamus and probably close to the supraoptic nucleus, that this gating may involve a temporary change in the membrane properties of oxytocin cells to enable them to fire at the high frequencies involved in the milk ejection burst, and that oxytocin is involved in this gating mechanism.

We are grateful for the technical assistance provided by R. Bunting. We also thank Drs R. J. Bicknell, P. Cobbett, R. G. Dyer, R. Grossman, D. J. S. Siranathsinghi and K. Shibuki for their helpful criticism of an earlier draft of this manuscript.

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