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PAIRED RECORDINGS FROM SUPRAOPTIC AND PARAVENTRICULAR OXYTOCIN CELLS IN SUCKLED RATS: RECRUITMENT AND SYNCHRONIZATION

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SUMMARY

1. Oxytocin cells in the paraventricular (p.v.) and contralateral supraoptic (s.o.) nuclei were pair-recorded (with two micro-electrodes) in suckled rats after being anaesthetized with urethane (1·2 g/kg), to study (a) the synchronization of their neurosecretory bursts, (b) the importance of cell recruitment and (c) their firing characteristics. The synchronization of paired bursts was determined by measuring the onset time-lag (time in milliseconds between the onset of two corresponding bursts) and the maximum firing time-lag (time in milliseconds between the two shortest interspike intervals for the corresponding bursts). For each cell, the characteristics studied were: the background activity and the frequency and amplitude (total number of spikes) of the neurosecretory bursts.

2. All paired p.v.–s.o. cells recorded were activated simultaneously 12–18 s before each milk ejection. The onset of a burst could vary either way, up to 680 ms, in relation to the other (mean onset time-lag was 206 ± 18 ms; $n = 85$) but the maximum activation periods fitted more closely, the mean maximum firing time-lag being 122 ± 14 ms ($n = 64$). Both parameters varied randomly, in duration and order (a) from one pair of cells to another, (b) from one pair of bursts to another for successive bursts of a given pair of cells and (c) independently, whether the cells were in the p.v. or the s.o. nucleus. However, in most cases, the neurosecretory burst with the highest amplitude began and reached its peak firing rate before the corresponding burst from the other cell.

3. Cell recruitment was observed when the milk ejection reflex began, for both the p.v. and the s.o. cells. The bursts of the non-responsive cells developed progressively with the reflex, but, as soon as a cell was recruited, all its successive bursts were simultaneous with those of the first-recruited oxytocin cells.

4. During a regular pattern of milk ejections, the mean background activity of sixty p.v. cells ($3·1 \pm 0·2$ spikes/s) was significantly higher than that of their s.o. counterparts ($1·9 \pm 0·2$ spikes/s). Nevertheless, the mean amplitude of the neurosecretory bursts of the sixty p.v. cells (49 ± 3 spikes) did not differ significantly from that of their s.o. counterparts (55 ± 4 spikes). There was no correlation between the background activity of oxytocin cells and the amplitude of their neurosecretory bursts for either p.v. cells or s.o. cells.

5. P.v. and s.o. oxytocin cells responded simultaneously and in a similar manner to intracerebroventricular injected drugs that enhance or inhibit the milk ejection reflex. In all cases, oxytocin cell bursts remained synchronized but the number of cells recruited sometimes fluctuated.

6. In conclusion, oxytocin cells of the four magnocellular nuclei were synchronized whatever the experimental conditions and neither the p.v. nor the s.o. nuclei prevailed over the others. For each cell, the ability to produce bursts was an intrinsic property which could be enhanced by oxytocin itself.

INTRODUCTION

The pulsatile and intermittent release of oxytocin in suckled rats has been well studied since the paper of Lincoln, Hill & Wakerley (1973). Each oxytocin pulse results from a high-frequency burst of spikes, the firing rate of which is 20- to 40-fold higher than the resting firing rate (Wakerley & Lincoln, 1973), which probably occurs in all or most oxytocin cells 12–18 s before each milk ejection (the time for the hormone to reach the mammary gland from the neurohypophysis). These neurosecretory bursts have been observed either in paraventricular (p.v.) cells (Wakerley & Lincoln, 1973; Lincoln & Wakerley, 1975; Poulain, Wakerley & Dyball, 1977), or in supraoptic (s.o.) cells (Lincoln & Wakerley, 1974, 1975; Poulain *et al.* 1977) but their synchronization has been only hinted at by: (1) the pulsatile nature of oxytocin release, which can only occur if the oxytocin cell bursts are synchronized over a sufficient pool of cells, (2) the relatively constant time-lag between the burst and the resulting milk ejection, and (3) the simultaneity of the bursts fortuitously recorded with the same micro-electrode on two close oxytocin cells (Lincoln & Wakerley, 1974).

More recently, Belin, Moos & Richard (1984), using extracellular paired recordings of p.v.–s.o. and s.o.–s.o. cells in suckled rats, have provided direct proof for the synchronization of neurosecretory bursts in oxytocin cells in the four magnocellular nuclei. However, particular results obtained during neuropharmacological studies (Freund-Mercier & Richard, 1984) have suggested either a desynchronization of the neurosecretory bursts of some oxytocin cells or a difference in their responses to drugs.

The present paper is a more extensive study of the synchronization characteristics of the neurosecretory bursts of oxytocin cells in different nuclei or of neighbouring cells in the same nucleus. The paired recordings under various experimental conditions were undertaken to determine the mechanisms whereby all the oxytocin cells fire together at milk ejection and whether they are always activated simultaneously or whether some recruitment phenomenon exists. These paired recordings also enabled a rigorous comparative study of the firing characteristics (background activity, burst amplitude) of p.v. and s.o. cells which, hitherto, had always been studied separately in different animals and even under different experimental conditions (Wakerley & Lincoln, 1973; Lincoln & Wakerley, 1974, 1975; Poulain *et al.* 1977). Such comparisons should bring out the relative importance of the functional roles of p.v. and s.o. cells during the milk ejection reflex, the meaning of the background activity and burst amplitude of oxytocin cells and the relations between these two parameters. Furthermore, drugs known to facilitate or inhibit the milk ejection pattern (a dopamine agonist and a dopamine antagonist (Moos & Richard, 1982), oxytocin and

an oxytocin antagonist (Freund-Mercier & Richard, 1984)) were used not only to test the possibility of cell desynchronization but also to compare the responsiveness of p.v. *versus* s.o. cells.

METHODS

Lactating Wistar rats (250–300 g) were taken between the eighth and the twelfth day of lactation and separated from all but one young rat 15 h before anaesthesia (urethane, 1·2 g/kg given in a single intraperitoneal injection). Local anaesthetic (xylocaine) was applied to all surgical sites. One thoracic mammary gland was cannulated to record intramammary pressure as an index of oxytocin release. A jugular vein was cannulated for the intravenous (I.V.) injection of known doses of oxytocin. The rats were then placed in a stereotaxic frame. After trephining, a microsyringe (Hamilton) slanted from front to back (26 deg from the vertical) was inserted into the third ventricle for intracerebroventricular I.C.V.) drug injections. Its exact position was confirmed by sucking up 2–3 μl of cerebrospinal fluid. A concentric bipolar steel electrode slanted from back to front (23 deg from the vertical) was inserted into the neurohypophysis and its exact position was attested by the abrupt increase in intramammary pressure following repetitive electrical stimulations (50 Hz, 8 V, 0·5 ms for 5 s).

The extracellular electrical activities of two neurosecretory cells in the magnocellular nuclei were recorded simultaneously using two micro-electrodes filled with 0·5 M-sodium acetate solution (8–15 MΩ impedance). One micro-electrode was placed in an s.o. nucleus and the other in the contralateral p.v. nucleus (p.v.–s.o. cells) or the other s.o. nucleus (s.o.–s.o. cells). On several occasions, two neighbouring cells in the s.o. nucleus (s.o.$_{1,2}$ cells) or in the p.v. nucleus (p.v.$_{1,2}$ cells) were picked up by the same micro-electrode. Their action potentials, recorded on magnetic tape, differed in shape and amplitude and so could be separated with a window discriminator before transcription at different speeds (1 or 125 mm/s) on a paper polygraph (Brush Gould 440).

3 h after anaesthesia, ten young rats were allowed to suckle in order to induce the milk ejection reflex. The oxytocin cells were identified by means of their antidromic response to electrical stimulation of the neurohypophysis and their characteristic activation (short high-frequency burst of spikes) 12–18 s before each reflex milk ejection.

The amplitudes of the intramammary pressure peaks during suckling were assessed on the basis of the effects of known doses of oxytocin (0·3–1 mu.) injected I.V. For each cell recorded, the following parameters were considered (using Student's paired *t* test):

(i) the background activity, expressed in spikes/s and calculated per 5 min period excluding the neurosecretory bursts and following silent periods;

(ii) the latency (in minutes) between the beginning of suckling and the first neurosecretory burst;

(iii) the interval (in minutes) between two successive neurosecretory bursts during suckling or the frequency of the neurosecretory bursts, i.e. their number per 5 min period, the reference point corresponding to the drug injection;

(iv) the amplitude of the neurosecretory bursts, i.e. the total number of spikes per burst.

For each pair of p.v.–s.o. cells recorded simultaneously, the parameters for the p.v. cell were compared to those for the corresponding s.o. cell using Student's *t* test throughout suckling, and under various experimental conditions, to assess the comparative responses of p.v. and s.o. cells.

The synchronization of two corresponding bursts for different pairs of cells was assessed from:

(i) the onset time-lag, i.e. the time in milliseconds between the onsets of two corresponding bursts;

(ii) the maximum firing time-lag, i.e. the time in milliseconds between the two shortest interspike intervals of the corresponding bursts.

Drugs

The following drugs were used in the experiments: oxytocin (Sandoz, Basel, Switzerland), at a dose of 1 ng, i.e. 1 μl of a 10^{-6} M solution; a dopamine antagonist, haloperidol (4-[4-(4-chlorophenyl)-4-hydroxy-1-piperidinyl]-1-(4-fluorophenyl)-1-butanone) (Haldol, Lebrun, Paris, France) at a dose of 5 μg, i.e. 1 μl of a 6×10^{-4} M solution; an oxytocin antagonist ([1(β-mercapto-β, β cyclopentamethylene propionic acid), 8-ornithine] vasotocin), or d(CH_2)$_5$OVT (Bankowski, Manning, Seto, Haldar & Sawyer, 1980) at a dose of 0·1 μg, i.e. 1 μl of a 10^{-4} M solution, first dissolved in 0·01

M-hydrochloric acid (1 mg in 50 μl); acetylcholine chloride (Sigma, St. Louis, U.S.A.) at a dose of 5 μg, i.e. 1 μl of a 10^{-4} M solution. For all drugs, the final dilution was made into a 0·9 % NaCl solution (w/v).

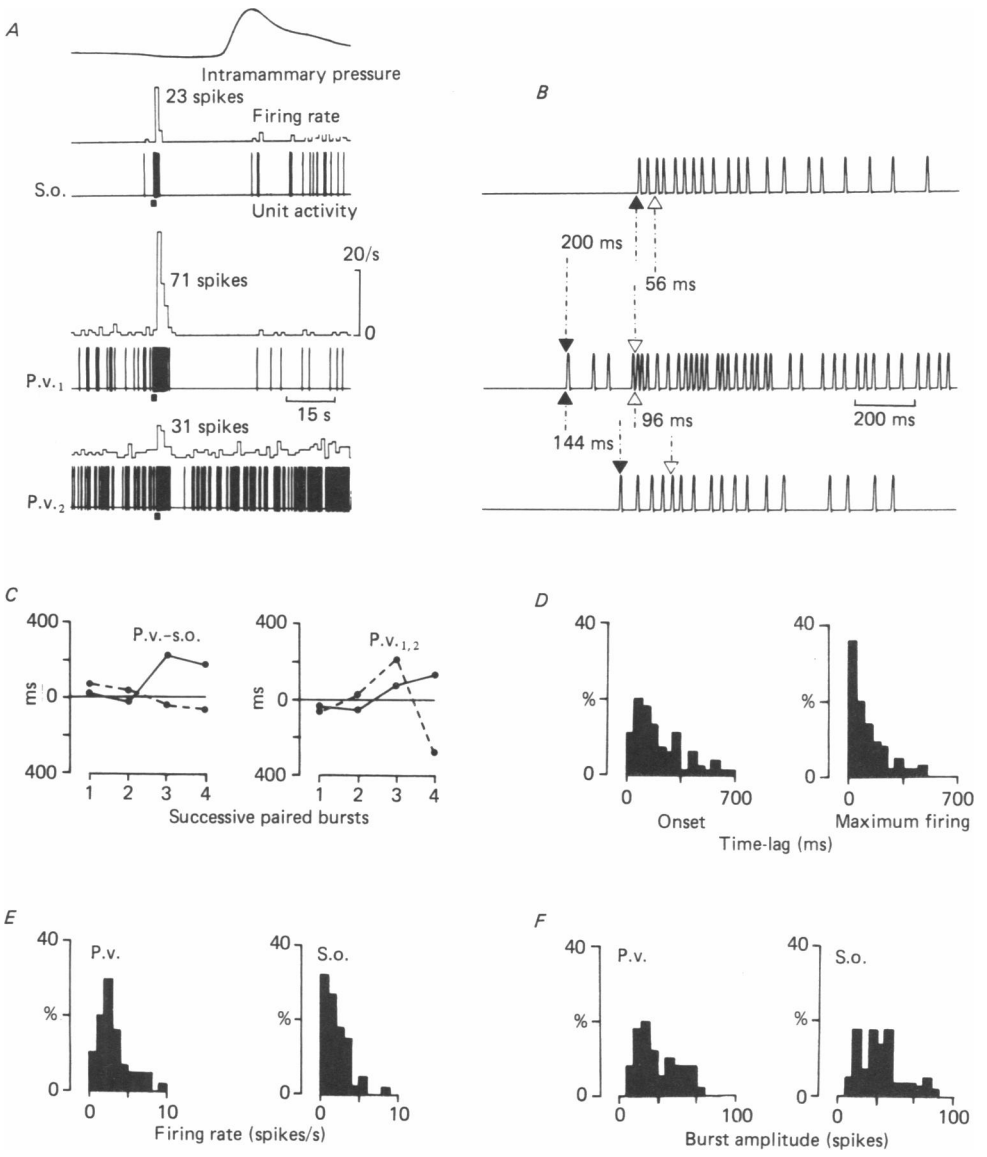
RESULTS

Characteristics of p.v.–s.o. oxytocin cells

Phenomenological study of oxytocin cell synchronization and recruitment. Seventy paired recordings were made under control conditions in fifty-five lactating rats, sixty times with paired p.v.–s.o. cells and ten times with paired s.o.–s.o. cells. On four occasions, three oxytocin cells (triplet) were recorded together, two neighbouring ones being in the same p.v. nucleus (three times) or in the same s.o. nucleus (once). Under control conditions, the cells in each unit of two or three cells recorded together for several successive milk ejections displayed a neurosecretory burst simultaneously before each increase in intramammary pressure (Fig. 1*A*).

Synchronization between corresponding neurosecretory bursts was studied using thirty-two pairs of p.v.–s.o. cells, nine pairs of s.o.–s.o. cells, three triplets of p.v.$_{1,2}$–s.o.

Fig. 1. Synchronization and firing characteristics of p.v. and s.o. oxytocin cells recorded simultaneously under control conditions. *A*, simultaneous recordings of the electrical activity of three oxytocin cells during suckling, illustrating the synchronization of their neurosecretory bursts at milk ejection. The different traces show the intramammary pressure (top), the firing rate (spikes/s) and the unit activity of the three cells, one in an s.o. nucleus and two in the same p.v. nucleus. In the latter case, the activities of the p.v cells were picked up by the same micro-electrode and separated with a window discriminator. The number beside each neurosecretory burst indicates its amplitude (total number of spikes). The filled bars indicate the portion of unit recordings expanded in *B*. All three oxytocin cells were simultaneously activated about 15 s before the increase in intramammary pressure. *B*, expanded recordings of the three neurosecretory bursts in *A* (not shown in full). The onset of each burst is indicated with filled arrowheads and the shortest interspike interval with open arrowheads. The burst of the p.v.$_1$ cell with the largest amplitude (71 spikes) began 144 ms before that of the p.v.$_2$ cell (31 spikes) and 200 ms before that of the s.o. cell (23 spikes). Maximum firing was reached first by the p.v.$_1$ cell, then by the s.o. cell (56 ms after) and finally by the p.v.$_2$ cell (96 ms after the p.v.$_1$ cell). *C*, onset and maximum firing time-lags (in ms) for successive bursts of one pair of p.v.–s.o. cells and one pair of p.v.$_{1,2}$ cells. Onset time-lag (continuous line) and maximum firing time-lag (dashed line) are plotted sequentially. The same cell (p.v. cell for the paired p.v.–s.o. cells) served in each case as the reference for the onset and maximum firing time-lag order. When the burst of the reference cell began first or reached its maximum firing first, the values are above the zero line. Both graphs show that the onset and maximum firing time-lags varied independently of one another, in duration and sometimes in order, from one pair of bursts to another. Synchronization parameters of two corresponding bursts for two neighbouring cells in the same nucleus were similar, and of the same order as those observed for two cells in different nuclei. *D*, frequency distribution histograms (%) of the onset and maximum firing time-lags. The distribution of the onset time-lags for eighty-five pairs of bursts was unimodal, with a mode at 50–100 ms; the distribution of the maximum firing time-lags for sixty-four pairs of bursts was exponential, with a mode at 0–50 ms. *E* and *F*, frequency distribution histograms of the firing rate and burst amplitude for sixty p.v. and sixty s.o. cells, pair-recorded. Distribution of the firing rate of p.v. cells was unimodal, with a mode at 2–3 spikes/s. The distribution for the s.o. cells was exponential, with a mode at 0–1 spike/s. Distribution of the p.v. cell burst amplitude was unimodal, with a mode at 30–40 spikes. The amplitude of the s.o. cell bursts was distributed mainly between 20 and 70 spikes.



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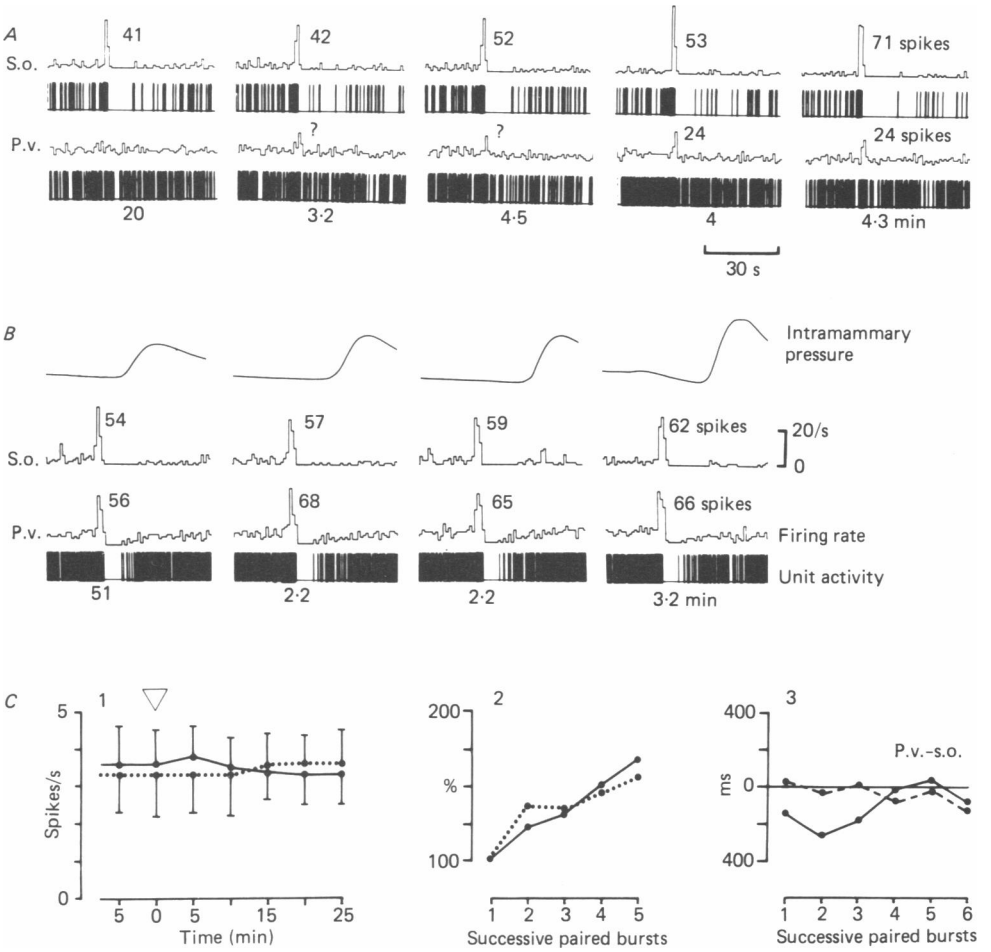
cells and one triplet of s.o.$_{1,2}$–p.v. cells. Several successive bursts were recorded in each case, so ninety-one pairs of bursts were studied to determine the onset and maximum firing time-lags. Examples are shown in Fig. 1 *B* and *C*.

Measuring the onset time-lag was not possible in all cases since it was sometimes difficult to determine the first spike of the burst, especially in the fast-firing cells. Onset time-lags were measured in eighty-five out of the ninety-one pairs of bursts recorded. The mean value was 206 ± 18 ms, the range being from 0 to 680 ms and the median 152 ms. The onset time-lag distribution was unimodal, with a peak between 50 and 100 ms (Fig. 1 *D*).

The maximum firing time-lag could not be measured for bursts with the highest firing rates since, at the peak of firing, spike amplitude was so low that they merged with the background noise. The maximum firing time-lag was measured for sixty-four of the pairs of bursts whose onset time-lag was determined. The mean value was 122 ± 14 ms (the range being from 0 to 480 ms and the median 88 ms) and was significantly shorter ($P < 0{\cdot}001$) than the onset time-lag. The maximum firing time-lag distribution was exponential, with a peak for values of less than 50 ms (Fig. 1 *D*).

Both parameters varied in duration and even in order, not only from one pair of cells to another, but also from one pair of bursts to another for successive bursts of a given pair of cells. The order did not depend on whether one of the two cells was in the p.v. or in the s.o. nucleus but rather seemed to be correlated with the amplitudes of the bursts. For fifty-three pairs of cells with bursts of different amplitudes (more than 20% difference), the burst with the highest amplitude (highest firing rate and longest duration) started in most cases (forty-five out of fifty-three) and reached peak firing (thirty-five out of fifty-three) before the other corresponding burst (see example in Fig. 1 *B*). The maximum firing time-lag varied in the same way as the onset time-lag in thirty-three of the fifty-three cases. For pairs of cells with bursts of similar amplitudes, the onset and maximum firing time-lags varied equally either way (Fig. 1 *B*). Study of the synchronization of four triplets of cells showed that the onset and maximum firing time-lags for the two neighbouring cells varied in duration and in order, as for the cells in different nuclei (Fig. 1 *B* and *C*).

Fig. 2. Recruitment, synchronization and firing characteristics of paired p.v.–s.o. cells at the beginning of the milk ejection reflex. *A*, recordings of paired cells showing progressive recruitment of one of them. The different traces represent the firing rate (spikes/s) and the unit activity of one p.v. and one s.o. cell recorded simultaneously. The number beside each neurosecretory burst indicates its amplitude. The numbers under the bursts of the p.v. cell indicate the latency (in minutes) of the first burst after suckling and the interval since the previous burst. 20 min after suckling, the reflex began but only the s.o. cell displayed the first few bursts, the p.v. cell not yet having been recruited. It was only at the fourth burst of the s.o. cell that a small burst clearly emerged from the background activity of the p.v. cell at the same time as the burst from the s.o. cell. The following successive paired bursts occurred simultaneously. *B*, recordings of paired cells recruited at the very first bursts. The different traces represent the intramammary pressure, the firing rate of one s.o. cell and the firing rate and unit activity of one p.v. cell. Same legend as in *A*. The paired p.v.–s.o. cells simultaneously displayed the first neurosecretory bursts 51 min after the young began to suck. Thereafter, the successive bursts of p.v.–s.o. cells were always simultaneous and occurred at regular intervals (2–3 min) with greater and greater amplitudes. The increases in the subsequent intramammary pressure peaks were in relation to those in the burst amplitudes. *C*, evolution of firing characteristics and synchronization of paired p.v.–s.o. oxytocin cells. *C*1, mean firing rate (spike/s) of six p.v. (continuous line) and six paired s.o. (dotted line) cells. The firing rates for the p.v. and s.o. cells did not vary significantly from 5 min before to 25 min after the first neurosecretory bursts (white arrowhead). *C*2, mean burst amplitude of six p.v. (continuous line) and six paired s.o. (dotted line) cells. For each cell, the amplitude of the successive bursts is expressed as a percentage of the first burst amplitude. The amplitudes of the bursts of both p.v. and s.o. cells increased slightly and progressively, reaching, respectively, about 167% of the p.v. and 155% of the s.o. control values at the fifth burst. *C*3, onset (continuous line) and maximum firing (dashed line) time-lags for successive bursts of one pair of p.v.–s.o. cells. Same representation and legend as in Fig. 1 *C*. These parameters varied independently of one another both in duration and in order from one pair of bursts to another. The maximum firing time-lags were generally shorter than the onset time-lags.



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Neither desynchronization, *sensu stricto*, nor a cell's failure to display one of the successive bursts were observed under control conditions. However, a recruitment phenomenon occurred at the beginning of the reflex. One of the paired cells displayed bursts whereas the other failed to do so initially; the bursts of the latter developed progressively with the reflex, the first burst occurring at the same time as the third, fourth or fifth burst recorded from the cell that was activated first. Thereafter, bursts from the two cells were simultaneous (Fig. 2*A*) and the synchronization parameters were similar to those described above. The recruitment phenomenon was observed in three out of eleven paired p.v.–s.o. cell recordings and in one out of four s.o.–s.o. cell recordings. Late-recruited cells were observed in both the p.v. and the s.o. nuclei.

Comparison of p.v.–s.o. oxytocin cell firing characteristics. The firing characteristics of p.v. and s.o. cells were compared when the reflex began and during control conditions.

At the beginning of suckling, six pairs of p.v.–s.o. cells with similar burst patterns, i.e. paired cells that were recruited right away at the first burst, were compared

(Fig. 2*B*). The background activity of the p.v. and s.o. cells was not affected when the reflex started. The mean values recorded 5 min after the first burst did not differ from those recorded 5 min before it: respectively $3{\cdot}4 \pm 0{\cdot}7$ spikes/s and $3{\cdot}6 \pm 0{\cdot}9$ spikes/s for the p.v. cells; $3{\cdot}5 \pm 0{\cdot}9$ spikes/s and $3{\cdot}3 \pm 1{\cdot}0$ spikes/s for the s.o. cells (Fig. 2*C*1). The evolution of the mean delay (in minutes) between successive bursts was strictly the same for the p.v. and s.o. cells since all paired cells were always activated simultaneously from the first burst on. The mean latency of the first burst after suckling began was $20 \pm 4{\cdot}5$ min and thereafter the mean values for the four consecutive burst intervals ranged from 6 to 8 min. The amplitudes of the successive bursts of the p.v. and s.o. cells increased progressively and similarly. The mean amplitude of the first burst was 30 ± 6 spikes for the p.v. cells and 43 ± 14 spikes for their s.o. counterparts. The amplitudes were generally maximal at the fifth burst and, on average, 167 % of that of the first p.v. burst and 155 % of the first s.o. one (Fig. 2*C*2). The synchronization parameters of successive pairs of bursts did not improve with time (Fig. 2*C*3). Four paired s.o.–s.o. cells were also recorded when the reflex began: their firing characteristics and the synchronization parameters of their successive paired bursts varied like those of p.v.–s.o. cells.

For the late-recruited cells, two p.v. and two s.o. cells, no difference was noted in the variation of their firing characteristics, which were, moreover, similar to those of the cells activated first except in the delay of the first burst. They displayed steady and continuous background activity, ranging from 1 to 3 spikes/s, and their burst amplitude also increased progressively with the reflex (Fig. 2*A*). No correlation was found between the absence of bursts and background activity.

During regular patterns of milk ejections, sixty pairs of p.v.–s.o. cells were recorded. In about half of the cases (thirty-four out of sixty), the p.v. cells had higher background activities (difference > 20 %) than their s.o. counterparts; in fifteen cases their basal activities were similar to and in eleven cases they were lower than those of s.o. cells. The mean value for all p.v. cells was $3{\cdot}1 \pm 0{\cdot}2$ spikes/s (range from 0·3 to 9·0 spikes/s; median 2·7 spikes/s; six cells fired at less than 1 spike/s) and differed significantly ($P < 0{\cdot}001$) from that for the s.o. cells: $1{\cdot}9 \pm 0{\cdot}2$ spikes/s (range from 0·02 to 8 spikes/s; median 1·7 spikes/s; nineteen cells fired at less than 1 spike/s). The firing rate distribution was unimodal for the p.v. cells, with a peak at 2–3 spikes/s (30 % of cells) and exponential for the s.o. cells, with the highest percentage of cells (32 %) firing at less than 1 spike/s (Fig. 1*E*). The mean amplitude of the neurosecretory bursts of the sixty p.v. cells (49 ± 3 spikes; range from 14 to 103 spikes; median 43 spikes) did not differ significantly from that of their s.o. counterparts (55 ± 4 spikes; range from 13 to 127 spikes; median 54 spikes). The difference between the amplitudes of two corresponding p.v.–s.o. bursts ranged from 0 to 78 spikes and varied one way or the other in the following proportions: for the sixty pairs of bursts in p.v.–s.o. cells, the amplitudes were higher (more than 20 % difference) in the p.v. cells nineteen times; the amplitudes were similar to those of the s.o. cells fourteen times and higher in the s.o. cells twenty-seven times. Burst amplitude distributions for p.v. and s.o. cells are shown in Fig. 1*F*. There was no close correlation between background activity and burst amplitudes either for the sixty p.v. cells (correlation coefficient, $r = 0{\cdot}15$) or for their sixty s.o. counterparts ($r = 0{\cdot}26$).

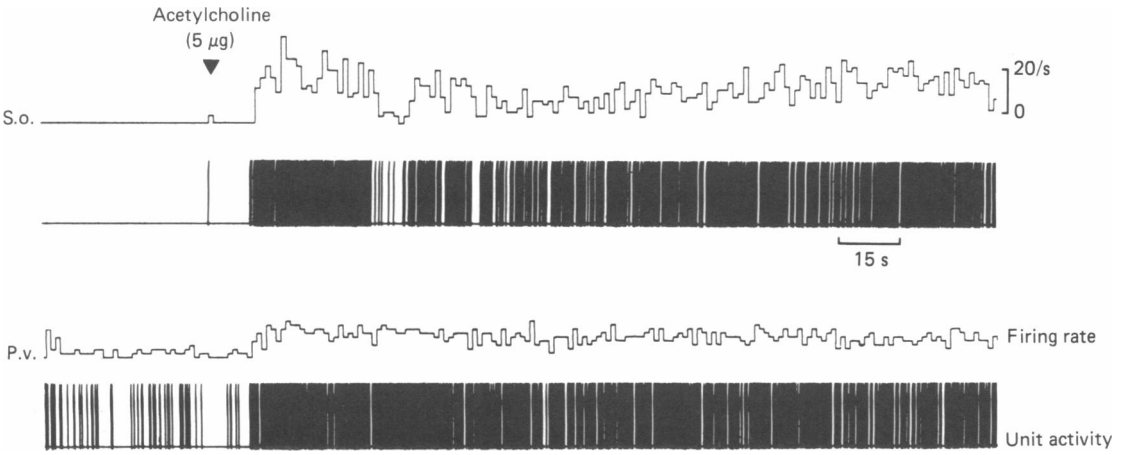

Fig. 3. Rapid and synchronous excitation of a pair of p.v.–s.o. oxytocin cells by 5 μg acetylcholine injected I.C.V. Same representation as in Fig. 2*A*. There were rapid and simultaneous increases in the firing rates of both the s.o. and the p.v. cells, 8 s after injection of the acetylcholine. The effects lasted for 10 min (not shown in full).

Effect of drugs that modify the milk ejection pattern

The firing characteristics of p.v. and s.o. cells were compared and burst synchronization was studied: (1) after triggering or activation of the milk ejection reflex by intracerebroventricular (I.C.V.) injections of oxytocin (Freund-Mercier & Richard, 1984), (2) during inhibition of the reflex following I.C.V. injection of haloperidol (Moos & Richard, 1982) or d$(CH_2)_5$OVT (Freund-Mercier & Richard, 1984).

First, acetylcholine was used to compare the diffusion latencies of the I.C.V.-injected drugs in the p.v. and the s.o. nuclei. This drug, applied by microiontophoresis, is known to increase the background activity of neurosecretory cells (Moss, Urban & Cross, 1972) and to induce the release of neurohypophysial hormones (Pickford, 1960). Four pairs of p.v.–s.o. cells were recorded after injection of 5 μg acetylcholine. The background activities of the p.v. and the s.o. cells increased simultaneously less than 10 s after injection (Fig. 3). This firing activation lasted for between 8 and 13 min and led in some cases to an increase in intramammary pressure (latency some 25 s) equivalent to that elicited by between 0·3 and 1 mu. of oxytocin injected I.V.

Effect of oxytocin. Twelve pairs of p.v.–s.o. cells were recorded when suckling began. In two cases, oxytocin was injected during the recruitment period, after the non-responsive cells (one p.v. and one s.o.) had failed to display the first two to three bursts. As illustrated in Fig. 4*A*, after oxytocin injection, the non-responsive cells were recruited right away and displayed bursts simultaneously with the first-recruited cells. For both cells, the amplitude of the successive bursts increased progressively; the variations in the amplitude of the milk ejections were related not only to these variations in burst amplitude but also to the number of cells recruited. In the other ten cases, oxytocin was injected when the reflex did not occur within more than 1 h of suckling. In all ten cases, oxytocin facilitated the occurrence of the reflex and all paired p.v.–s.o. cells recorded were recruited right away, displaying the first and

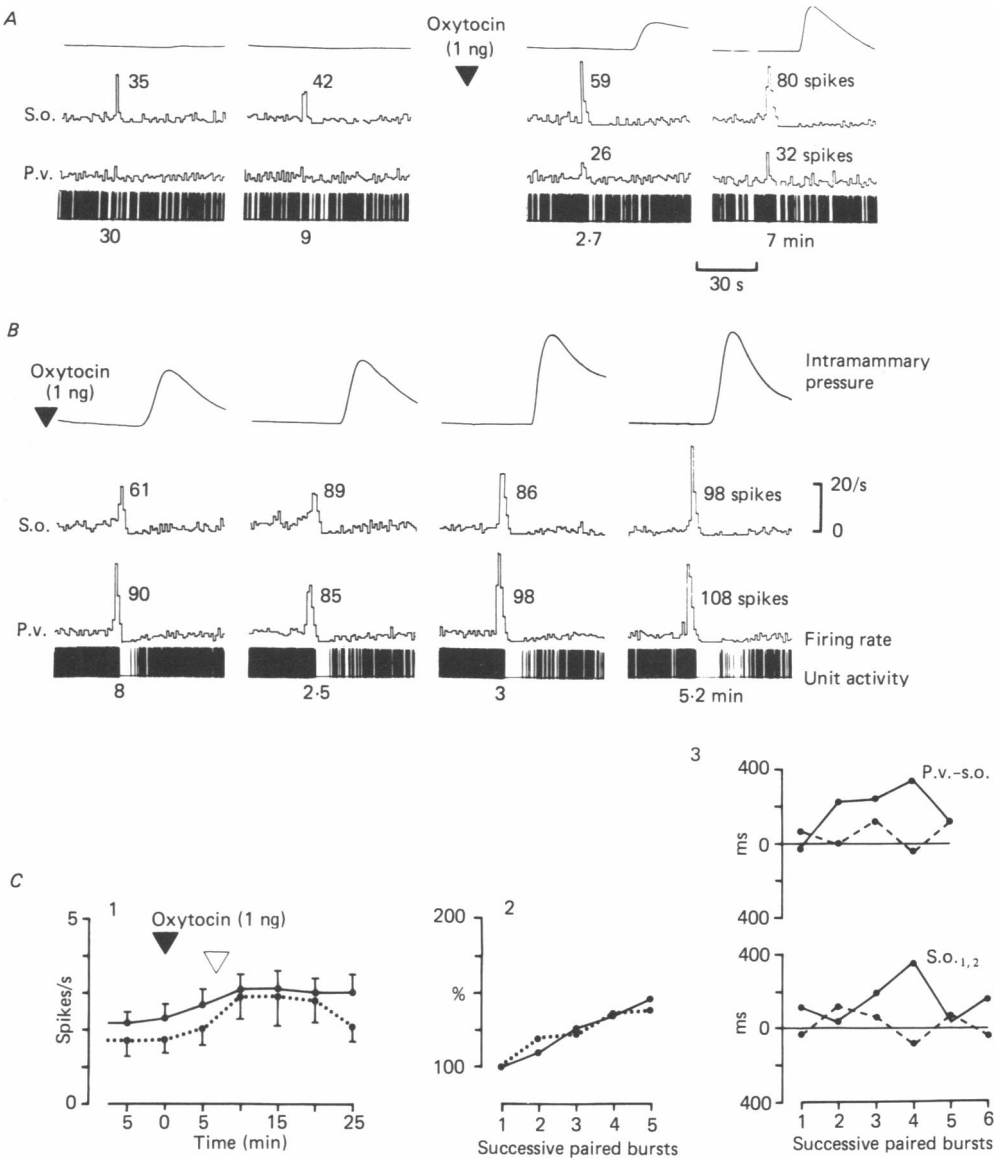

Fig. 4. For legend see opposite.

following bursts simultaneously (Fig. 4*B*). Seven of the ten pairs of p.v.–s.o. cells were recorded for more than 35 min. Their background activities increased slightly and progressively after oxytocin injection. The mean values 5 min before and 20 min after oxytocin injection were respectively $2{\cdot}3 \pm 0{\cdot}3$ and $3{\cdot}0 \pm 0{\cdot}4$ spikes/s for the p.v. cells, and $1{\cdot}7 \pm 0{\cdot}3$ and $2{\cdot}8 \pm 0{\cdot}6$ spikes/s for their s.o. counterparts. Although the background activity evolution was parallel and very similar for the cells of both nuclei (Fig. 4*C*1), only the increase observed for the s.o. cells was significant ($P < 0{\cdot}05$). The p.v.–s.o. cell bursting patterns were similar. The mean latency for the first bursts after

I.C.V. oxytocin injection was $6{\cdot}8 \pm 1{\cdot}8$ min and thereafter the mean values for the four consecutive burst intervals varied from 3 to 6 min. The amplitudes of successive bursts increased progressively and similarly in both the p.v. and the s.o. cells. The mean values for the first bursts were 43 ± 10 spikes for the p.v. cells and 41 ± 5 spikes for the s.o. cells. The maximum values, recorded at the fifth bursts, were, on average, 143 % of the p.v. and 132 % of the s.o. control values (Fig. 4*C*2). The synchronization parameters for successive pairs of bursts did not improve with time and were similar to those under control conditions (Fig. 4*C*3). Three triplets of s.o.$_{1,2}$–p.v. cells were also recorded when the reflex began after I.C.V. injection of oxytocin. In each case, for the two neighbouring cells the synchronization parameters varied in duration and in order like those of two cells in different nuclei (Fig. 4*C*3).

Nine pairs of p.v.–s.o. cells were recorded when a well established reflex was activated by I.C.V. injection of 1 ng oxytocin (see Fig. 5). Their respective background activities increased progressively and similarly: from $2{\cdot}1 \pm 0{\cdot}4$ spikes/s (5 min before injection) to $3{\cdot}5 \pm 0{\cdot}5$ spikes/s (20 min later) for the p.v. cells (significant difference, $P < 0{\cdot}02$) and from $1{\cdot}7 \pm 0{\cdot}5$ to $2{\cdot}9 \pm 0{\cdot}6$ spikes/s for the s.o. cells ($P < 0{\cdot}05$) (Fig. 6*A*1). The burst frequencies rose identically for 15 min in both the p.v. and the s.o. cells, with a maximum being reached 5–10 min after injection (about three times higher than under control conditions) (Fig. 6*A*2). The amplitude of the bursts increased in the same way in both the p.v. and the s.o. cells for more than 20 min.

Fig. 4. Recruitment, synchronization and firing characteristics of paired p.v.–s.o. cells at the beginning of the milk ejection reflex after facilitation by an I.C.V. injection of 1 ng oxytocin. *A*, recordings of paired cells showing facilitation of cell recruitment by oxytocin. Same representation as in Fig. 2*B*. 30 min after suckling, the reflex began but the first two bursts occurred only in the s.o. cell, no activation emerging from the background activity of the p.v. cell. No rise in intramammary pressure was seen after either of these two bursts. After the oxytocin injection, the p.v. cell displayed bursts simultaneously with the s.o. cell, with progressively greater amplitudes. The increases in subsequent intramammary pressure peaks resulted not only from those of burst amplitudes but also from the further recruitment of oxytocin cells. *B*, recordings of paired cells with similar bursting patterns when the reflex began after injection of oxytocin. Same legend as in Fig. 2*B*, except that the number below the first neurosecretory burst indicates the latency (in minutes) since the injection of oxytocin. Both cells simultaneously displayed the first neurosecretory bursts 8 min after oxytocin injection. Thereafter, the successive bursts of the p.v.–s.o. cells were always simultaneous and occurred at regular intervals (2–5 min) with progressively greater amplitudes. The increases in the subsequent intramammary pressure peaks were in relation to the increases in the burst amplitudes. *C*, evolution of firing characteristics and synchronization of paired p.v.–s.o. oxytocin cells. Same legend as in Fig. 2*C*. *C*1, mean firing rate (spikes/s) of seven p.v. (continuous line) and seven paired s.o. (dashed line) cells. The mean firing rates increased slightly and progressively in both the p.v. and the s.o. cells after the oxytocin injection. The mean latency for the occurrence of the first bursts (white arrowhead) after the injection was about 7 min. *C*2, mean burst amplitude of the seven p.v. (continuous line) and seven paired s.o. (dashed line) cells. The amplitudes of both the p.v. and s.o. cell bursts increased slightly and progressively to reach about 143 % of the p.v. and 132 % of the s.o. control values at the fifth burst. *C*3, onset (continuous line) and maximum firing (dashed line) time-lags for successive bursts of one pair of p.v.–s.o. cells and one pair of s.o.$_{1,2}$ cells. Same representation and legend as in Fig. 1*C*. Both parameters varied independently of one another, both in duration and in order from one pair of bursts to another. The maximum firing time-lags were generally shorter than the onset time-lags.

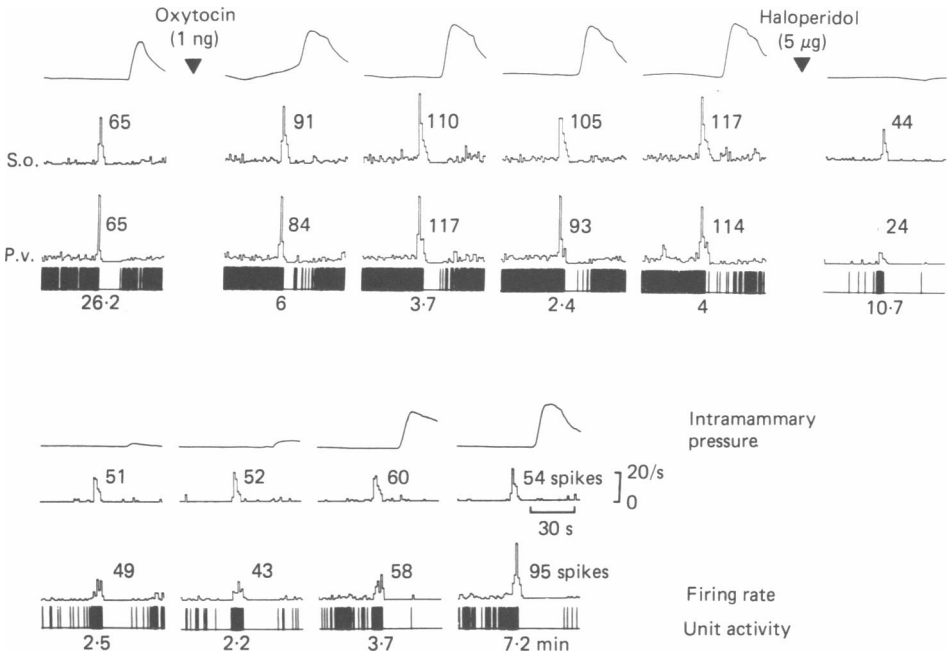

Fig. 5. Responses of a pair of p.v. and s.o. oxytocin cells after I.C.V. injections of oxytocin and haloperidol 15 min later. Same representation as in Fig. 2*B*. Injection of 1 ng oxytocin I.C.V. activated the milk ejection reflex by increasing the frequency and amplitude of the neurosecretory bursts of both the s.o. and the p.v. cells. The interval between successive bursts was shortened (from 26·2 to 2·4 min) and bursts occurred simultaneously in the p.v. and s.o. cells at each milk ejection. The increase in burst amplitude was parallel and of the same order for both cells (from 65 to more than 110 spikes). 5 μg haloperidol injected I.C.V. 15 min later, when the facilitatory effect of oxytocin was maximum, inhibited the milk ejection reflex. The background activity of both cells decreased. The first bursts occurring in both oxytocin cells after haloperidol injection were delayed by about 10 min. Their amplitudes were greatly reduced, being even less than the control values, and were not sufficient to induce any release of oxytocin detectable through intramammary pressure. The following lower bursts occurred more frequently and always simultaneously in both oxytocin cells. The burst amplitudes became progressively greater in a parallel way in the s.o. and the p.v. cells despite light individual variations.

The maximum amplitude was recorded 5–15 min after oxytocin injection and corresponded to 203 and 202% of the control values for the p.v. and the s.o. cells respectively (Fig. 6*A*2). Individual cell variations were slight and were not confined to any one nucleus. As regards the synchronization of the successive paired bursts, the onset and maximum firing time-lags were in the same range both before and after I.C.V. injection of oxytocin (Fig. 6*A*3). For three pairs of s.o.–s.o. cells and one triplet of p.v.$_{1,2}$–s.o. cells, the synchronization parameters of the successive corresponding bursts of s.o.–s.o. cells and of neighbouring p.v. cells (Fig. 6*A*3) were in the same range both before and after injection of oxytocin.

Effect of haloperidol or d(CH$_2$)$_5$OVT. Haloperidol and d(CH$_2$)$_5$OVT inhibited the milk ejection reflex, decreasing both the frequency and the amplitude of bursts.

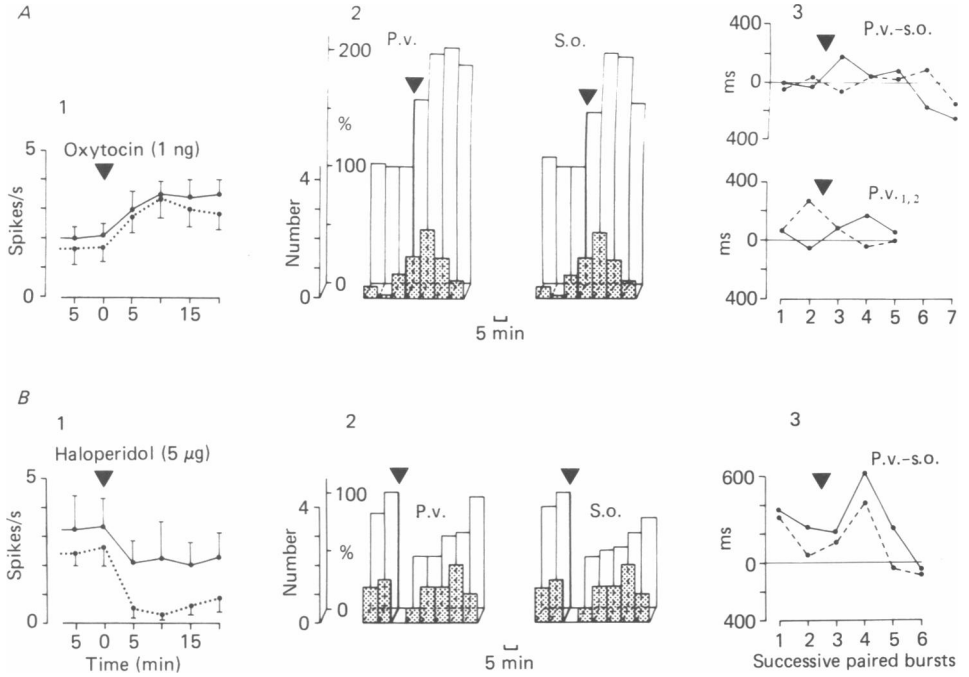

Fig. 6. Similar responses of p.v. and s.o. oxytocin cells during facilitation of the milk ejection reflex with oxytocin and during its inhibition with haloperidol. *A*, facilitation of the reflex with 1 ng oxytocin injected I.C.V. *A*1, mean background activities of nine p.v. cells (continuous line) and nine paired s.o. cells (dotted line). After oxytocin injection, the increase in background activity for both cells was progressive and similar. *A*2, frequency and amplitude of bursts in successive 5 min periods for the nine p.v. and nine paired s.o. cells. The shaded columns indicate the mean number of neurosecretory bursts and the open columns indicate the mean amplitude of the bursts expressed as a percentage of the amplitude of the burst(s) recorded for the 5 min before injection. After injection of the oxytocin the increase in burst frequency was strictly the same for the p.v. and the s.o. cells, the maximum burst frequency being reached 10 min after injection. The increase in burst amplitude was parallel and of the same range (about 200% of control values, 10–15 min after injection) in both the p.v. and the s.o. cells. *A*3, onset (continuous line) and maximum firing (dashed line) time-lags for successive bursts of one pair of p.v.–s.o. cells and one pair of $p.v._{1,2}$ cells. Same representation and legend as in Fig. 1*C*. The parameters were similar before and after oxytocin injection both for the two cells located in different nuclei and for the two neighbouring cells in the same nucleus. *B*, inhibition of the reflex with 5 μg haloperidol injected I.C.V. *B*1, mean background activities of four p.v. cells (continuous line) and four paired s.o. cells (dotted line). After haloperidol injection, the decrease in background activity was simultaneous in both the p.v. and the s.o. cells. In these experiments, the decrease in firing of the s.o. cells was more pronounced than that of the p.v. cells. *B*2, frequency and amplitude of bursts for successive 5 min periods for the four p.v. and four paired s.o. cells. Haloperidol inhibited the p.v. and s.o. cells in a similar way. For 5 min after haloperidol injection, no burst was recorded from either the p.v. or the s.o. cells. Thereafter, the frequency of bursts followed strictly the same pattern of evolution in both p.v. and s.o. cells. The amplitudes of the bursts occurring after injection were initially very low (40% of control values in both cases). They then increased progressively and in a similar way for both p.v. and s.o. cells. *B*3, onset and maximum firing time-lags for successive bursts of one pair of p.v.–s.o. cells. The synchronization parameters were similar before and after haloperidol injection.

However, for each pair of cells, the extent and duration of these inhibitions varied from one cell to the other: some cells were more inhibited and did not display bursts whereas their counterparts did.

Eight pairs of p.v.–s.o. cells were recorded during inhibition following I.C.V. injection of 5 μg haloperidol and four pairs of cells were recorded after I.C.V. injection of 0·1 μg d$(CH_2)_5$OVT. Injections were carried out either under control conditions or after the milk ejection reflex had been facilitated with 1 ng oxytocin (see Figs. 5 and 7). The paired p.v.–s.o. cells had similar burst patterns in four cases after haloperidol and in three cases after d$(CH_2)_5$OVT injection. After haloperidol injection the mean background activity decreased rapidly, from $3{\cdot}4 \pm 1{\cdot}0$ spikes/s (5 min before injection) to $2{\cdot}0 \pm 0{\cdot}7$ spikes/s (5 min after injection) for the p.v. cells, and from $2{\cdot}6 \pm 0{\cdot}6$ to $0{\cdot}5 \pm 0{\cdot}3$ spikes/s for the s.o. cells. Though the decrease in firing rate was less pronounced for the p.v. cells than for their s.o. counterparts, the timing of the variations was parallel (Fig. 6*B*1). The evolution of the burst frequency of the p.v. and s.o. cells was similar: each of the paired cells always displayed bursts simultaneously and at greater intervals than during the control period (Figs. 5 and 6*B*2). The decreases in burst amplitude were of the same order for both the p.v. and the s.o. cells. The mean amplitude of the first bursts after haloperidol injection was low, about 42 % of the p.v. or the s.o. control values (Fig. 6*B*2). Consequently, not enough oxytocin was released to induce any increase in intramammary pressure (Fig. 5). The next bursts had progressively greater amplitudes and the return to control values occurred in a similar way for both the p.v. and the s.o. cells about 30 min after haloperidol injection (Fig. 6*B*2). The synchronization parameters of successive corresponding bursts were in the same range both before and after haloperidol injection (Fig. 6*B*3). After d$(CH_2)_5$OVT injection, the background activity decreased in two cases out of three, but in all cases bursts became smaller (10–50 % of control values), less frequent than during the control period and were not always followed by milk ejections. The synchronization parameters for successive corresponding bursts were in the same range before and after d$(CH_2)_5$OVT injection.

Failure to display bursts during inhibition occurred in four cases out of eight after haloperidol injection and in one case out of three after d$(CH_2)_5$OVT injection: one of the paired p.v.–s.o. cells did not display any burst (a p.v. cell three times, a s.o. cell twice) for a time varying according to the cell whereas its counterpart displayed smaller and delayed bursts (see Fig. 7). For all cells, background activity decreased after drug injections except once after haloperidol when the background activity of the non-responsive cell was little affected. In most cases (four out of five) it was the cells with the lowest bursts (amplitudes of 13–40 spikes) under control conditions which failed to display bursts after drug injection. After the non-responsive period, the bursts progressively reappeared as the inhibitory effects of the drugs wore off, the first burst being always smaller than the subsequent ones. In two cases, it took 1 ng of oxytocin to facilitate the occurrence of bursts in the non-responsive cells (see Fig. 7). After the non-responsive period, the synchronization parameters of the successive corresponding bursts were in the same range as the control ones.

After injection of each drug, the decreases in background activity and burst amplitude varied with the cells and did not depend on whether they were in the p.v. or the s.o. nucleus. Further evidence for this was provided by twice recording a pair

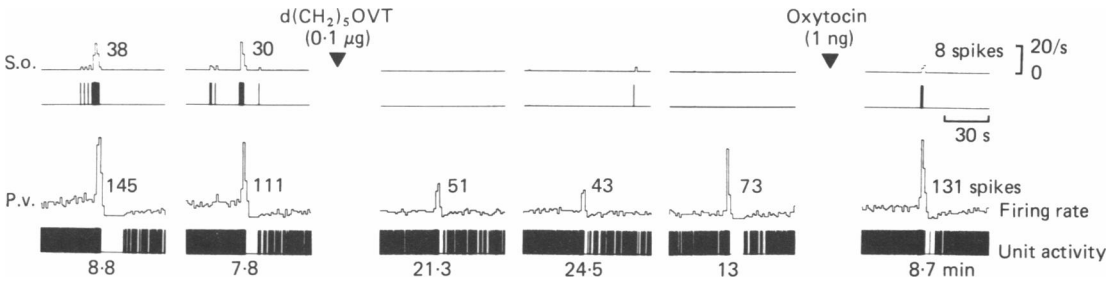

Fig. 7. Responses of a pair of p.v. and s.o. oxytocin cells after $d(CH_2)_5OVT$ and oxytocin injections. Same representation as in Fig. 2*A*. In this experiment, 0·1 μg of $d(CH_2)_5OVT$ was injected I.C.V. 30 min after 1 ng oxytocin (not shown) when the two cells were displaying bursts every 8 min. The oxytocin antagonist strongly inhibited the milk ejection reflex without significantly modifying the background activities of either cell. During the inhibitory period, three successive bursts with lower amplitudes (about half the control values) occurred at very long intervals (about every 20 min) on the p.v. cell, whereas, in each case, the s.o. cell failed to display any such burst. I.C.V. injection of 1 ng oxytocin 65 min after injection of $d(CH_2)_5OVT$ had a facilitatory effect and permitted the occurrence of a burst on the s.o. cell simultaneously with that of the p.v. cell. The amplitude of the s.o. cell burst was very low (8 spikes only) whereas that of the p.v. cell burst reverted to control values.

of s.o.–s.o. cells, once after haloperidol injection and once after $d(CH_2)_5OVT$ injection. In the first case, the background activities of the cells decreased slightly but their burst pattern was strongly affected: one cell displayed small bursts after a complete 30 min blockage whereas the other was not yet recruited. In the second case, the firing rate decreased in both cells and the bursts were less frequent and smaller (50 %) than the control bursts.

DISCUSSION

Paired recordings of p.v. and s.o. cell activities have recently provided evidence for the simultaneous activation of these cells at milk ejection (Belin *et al.* 1984). This technique, applied in the present study, not only confirmed the synchronization of oxytocin cells whatever the experimental conditions but also permitted the comparison of p.v. and s.o. cell firing characteristics, which showed: (1) the absence of a correlation between background activity and burst amplitude; (2) the similarity and simultaneity of the responses of p.v. and s.o. cells to the I.C.V. injection of drugs; and (3) the existence of a recruitment phenomenon which might be favoured by intrahypothalamic oxytocin release.

Comparison of p.v.–s.o. cell firing characteristics

Under our control conditions, the background activity of p.v. cells was significantly higher than that of s.o. cells recorded simultaneously, but the mean amplitude of p.v. cell bursts did not differ significantly from that of s.o. cells. Recording from p.v. and s.o. cells separately, Poulain *et al.* (1977) also observed that p.v. cells have higher background activities than s.o. ones but they found p.v. cell bursts to be higher than

s.o. ones. Like Wakerley & Lincoln (1973), we did not find any correlation between background activity and burst amplitude for either type of cell at a given time during the milk ejection reflex. We recorded both p.v. and s.o. cells with low firing rates displaying high neurosecretory bursts and, conversely, cells with high firing rates displaying low bursts, all combinations in between being also possible.

However, for a given cell recorded throughout the milk ejection reflex, Lincoln & Wakerley (1975) observed that spontaneous variations in background activity were accompanied by parallel variations in burst amplitude, whatever the initial level of background activity. In our experiments, this correlation was observed after injection of oxytocin (increase in both parameters) and in the majority of cases after injection of haloperidol and $d(CH_2)_5OVT$ (decrease in both), but was not so evident when the reflex was normally triggered at the beginning of suckling: the progressive increase in burst amplitude was not detectable against the background activity.

Thus, for each cell, the ability to produce bursts and the burst amplitude cannot be assessed from the background activity level alone. Individual differences were evident and did not depend on whether the cells were in the p.v. or the s.o. nuclei. They accounted for differences in the recruitment of the oxytocin cells at the beginning of the reflex and during its inhibition by drugs. The cells recruited long after the reflex had begun always displayed mini-bursts (few spikes and short-lived) whatever their background activity levels. During inhibition of the reflex, cells failing to display bursts were not those with the lowest background activities during the control period but, in most cases, cells already having low burst amplitudes.

There thus seem to be two different controls for each cell, one which determines its background activity and the other its ability to produce bursts. These results raise the question of the meaning of background activity. If, like Lincoln & Wakerley (1974) we consider that 'the background firing of a neurosecretory cell reflects the ongoing levels of depolarizing and hyperpolarizing synaptic inputs', does the difference observed in the p.v. and s.o. cell firing rates reflect differences in the nature of the inputs to the p.v. and s.o. nuclei and, consequently, differences in the importance of their functional role during the milk ejection reflex? Poulain *et al.* (1977) suggested that the s.o. nuclei might 'receive fewer excitatory connexions from the afferent components of the milk ejection reflex'. Our present data do not support this hypothesis since: (1) the mean amplitudes of the s.o. and p.v. cell bursts were similar; (2) when compared, two by two, during a given milk ejection reflex, the s.o. cells more frequently displayed higher neurosecretory bursts than the p.v. cells; (3) whatever the experimental conditions, the characteristics of the p.v. and s.o. cells waxed and waned in unison (the variations in the amplitude of neurosecretory bursts being parallel and of the same order); (4) the recruitment phenomenon occurred in both the p.v. and the s.o. nuclei (see below). As regards the milk ejection reflex, and, more precisely, the firing acceleration linked with the reflex release of oxytocin, p.v. and s.o. cells seem to be similarly involved and to play functional roles of similar importance. The differences observed between p.v. and s.o. cell background activities have not yet been satisfactorily explained.

Recruitment and synchronization of p.v. and s.o. oxytocin cells. The putative role of oxytocin

Both failure to burst and progressive recruitment were observed at the beginning of the milk ejection reflex and during its inhibition by drugs but never during a well-established reflex. Unlike O'Byrne & Summerlee (1983), we never observed, during a regular pattern of milk ejection, any oxytocin cell failing to produce one burst unexpectedly whereas the other cells did. However, these authors recorded only one cell in non-anaesthetized rats over extended periods (> 12 h), and only the behavioural response of the suckling young at milk ejection was taken as an index of oxytocin release. Moreover, in spite of the variability of the synchronization parameters (onset and maximum firing time-lags) no desynchronization, *sensu stricto*, was ever observed.

Recruitment phenomenon

When the reflex began, the whole pool of oxytocin cells was not recruited right away at the first neurosecretory burst: some cells in both nuclei failed to display the first few bursts when the others did so. Whatever the time of its recruitment, as soon as the non-responsive cell began to display bursts, they were simultaneous with those of the other oxytocin cells. The first burst occurring on all oxytocin cells was always of lower amplitude than the following ones, as was also observed by Lincoln & Wakerley (1975). Moreover, for the late-recruited cells, the first burst was often a mini-burst, indicating a delayed potential ability to burst, i.e. perhaps low excitability. Progressive recruitment was not observed when I.C.V. oxytocin injection enhanced milk ejections at the beginning of suckling; here all the cells recorded displayed bursts from the beginning because of the facilitatory role of oxytocin in triggering bursts (see below).

During maximum inhibition of the reflex by haloperidol or $d(CH_2)_5OVT$, some oxytocin cells failed to produce neurosecretory bursts while, at the same time, the bursts of other oxytocin cells were smaller and delayed but still detectable. These differences in cell responsiveness also reflected differences in potential ability to burst.

Under both experimental conditions, the changes in the amplitude of the milk ejections (due to changes in the amount of oxytocin released) reflected not only variations in the burst amplitude, as suggested by Lincoln & Wakerley (1975), but also variations in the number of oxytocin cells recruited. These two variations explained particular results (V. Belin & F. Moos, unpublished observations and Freund-Mercier & Richard, 1984) previously obtained with single oxytocin cell recordings when the reflex began or was inhibited: (1) the occurrence of neurosecretory bursts not followed by an increase in intramammary pressure; (2) the occurrence of milk ejections not preceded by neurosecretory bursts.

Synchronization of oxytocin cells

The great variability in the onset time-lags between p.v. and s.o. cell bursts showed that bursts did not begin preferentially in one or the other nucleus. Furthermore, for a given pair of cells, the order and duration of the onset time-lag varied randomly from one pair of bursts to another. The regular prior activation of one cell *versus*

another was only seen for cells displaying the highest bursts whether in the p.v. or the s.o. nucleus. The synchronization parameters of two neighbouring cells had the same characteristics. Thus, the synchronization of oxytocin cells between and inside the nuclei appeared more irregular, and the parameters longer than in other systems: about 40 ms between the onset of the paroxysmal potentials of two neocortical cells (Gutnik, Connors & Prince, 1982) and up to 7 ms between the onset of simultaneous bursts of two hippocampal cells (Miles & Wong, 1983). For the oxytocin cells, the onset time-lag related to burst duration (about 5%) was in fact sufficient to ensure the massive and pulsatile oxytocin release required to produce milk ejection. The maximum firing time-lag, being half the onset time-lag, indicates that there is a better synchronization of the periods of highest spike frequency which are the most efficient for oxytocin release.

Hypotheses for the synchronization of oxytocin cells inside each nucleus. Several possible mechanisms for the synchronization of oxytocin cells clustered inside each magnocellular nucleus might be envisaged on the basis of morphological and electrophysiological data.

The structural reorganization of neurosecretory cells in the magnocellular nuclei during lactation could permit interactions between the cells. Thus, the presence of 'double synapses' (Theodosis, Poulain & Vincent, 1981; Hatton & Tweedle, 1982; Theodosis & Poulain, 1984*a*), especially including the oxytocin cells (Theodosis & Poulain, 1984*b*), could contribute to neural synchronization by transmitting the same information to two cells simultaneously. The cross-correlograms of spikes of paired neurosecretory cells (Thomson, 1984) partly substantiate this hypothesis. The numerous membrane appositions between neurosecretory cells (Theodosis *et al.* 1981; Hatton & Tweedle, 1982), and especially between oxytocin cells (Theodosis & Poulain, 1984*b*) might favour cell synchronization and recruitment. Several mechanisms, including fluctuations in extracellular ions (accumulation of K^+) and electrical field effects could intervene, as was suggested for hippocampal CA_1 neurones (Yarom & Spira, 1982; Yaari, Konnerth & Heinemann, 1983; Richardson, Turner & Miller, 1984; Taylor & Dudek, 1984). The anatomical description of gap junctions (Andrew, McVicar, Dudek & Hatton, 1981; Mason, 1982, 1983) and the electrophysiological results on s.o. slices (Mason, 1982; Thomson, 1984) suggested that electrotonic coupling might synchronize the activities of cells. However, in the present study, the onset time-lags of two neighbouring cells (a few hundred milliseconds) did not corroborate this mechanism. Synchronization mechanisms via excitatory chemical synapses, such as those described for hippocampal cells (Traub & Wong, 1982; Miles, Wong & Traub, 1984) might also intervene. In the magnocellular nuclei, oxytocin-immunoreactive neurones were shown to make synaptic contacts with one another (Theodosis, 1985) but, according to Leng (1981), electrical interactions between neurosecretory cells were weak. The possibility of indirect connexions is supported by both morphological and electrophysiological data. Most of the chemical synapses in the s.o. nucleus have been shown to derive from cell bodies in, or very close to, the nucleus (Léránth, Záborsky, Marton & Palkovits, 1975). In the s.o. nucleus, neurosecretory cells can project onto some interneurones inside the nucleus and onto some cells outside the nucleus (Dyball & Leng, 1982). Bruni & Perumal (1984) described an extensive network of small-diameter fibres that pervade the s.o. nucleus

and make contacts with several neurones and their processes. An excitatory synaptic interaction in the s.o. nucleus has been demonstrated between a neurosecretory cell and a putative interneurone behaving as a follower cell (Dyball & Leng, 1982; Leng & Dyball, 1983). So, if two oxytocin cells were connected via an interneurone, one of the cells might display smaller bursts than the other and these bursts might start later. This hypothesis is sustained by our data showing that, for two simultaneous bursts, the highest amplitude one started first. However, such synaptic interactions between oxytocin cells imply that one of the neurotransmitters ensuring chemical transmission is oxytocin itself; this is discussed below.

Hypotheses for the synchronization of oxytocin cells between the four magnocellular nuclei. According to Voloschin & Tramezzani (1973), the total deafferentation of the p.v. nuclei did not prevent the milk ejection reflex. The present work gives evidence that neither nucleus prevails over the other. Thus, it is likely that the p.v. and s.o. nuclei were reached simultaneously by neural impulses from the mammary gland. The question that then arises is whether the neural impulses might already have been synchronized by some gating mechanism. This possibility has been suggested by various results: lesions of the rat ventral tegmentum probably including B_9 serotonin neurones (Juss & Wakerley, 1981) or I.C.V. injections of serotonin and serotonergic antagonists (Moos & Richard, 1983) disturbed the regularity of burst or milk ejection patterns without modifying their amplitudes. On the other hand, each nucleus might operate as an epileptic-like focus whose synchronization could be ensured by connexions between them. Some evidence has been given for connexions between a p.v. nucleus and its ipsilateral s.o. nucleus (Silverman, Hoffman & Zimmerman, 1981; Tribollet & Dreifuss, 1981; Saphier & Feldman, 1985) and between both p.v. nuclei (Silverman *et al.* 1981; Sofroniew & Glasmann, 1981). However, no connexions have been described between both s.o. nuclei (Silverman *et al.* 1981), so how can one explain that after the lesion of both p.v. nuclei paired cells in each of the s.o. nuclei display neurosecretory bursts simultaneously at each milk ejection (Belin, Moos & Richard, 1985)? The intervention of a gating mechanism thus remains likely, whatever the mechanisms involved in synchronizing oxytocin cells within each nucleus might be. The connexions between nuclei might serve to improve synchronization. Since these connexions have been assumed to stem from oxytocin cells (Silverman *et al.* 1981), oxytocin might, in this case too, play a fundamental role.

The putative role of oxytocin

Previous studies have demonstrated that intrahypothalamic oxytocin not only facilitates neurosecretory bursts during suckling but is also necessary for their occurrence (Freund-Mercier & Richard, 1981, 1984). In the present study, oxytocin injections did not improve the synchronization parameters but favoured oxytocin cell recruitment: the role of oxytocin might be to increase the excitability of all oxytocin cells until the burst threshold is reached. The oxytocin ensuring such a control could originate from the magnocellular nuclei themselves. Indeed, several results support the hypothesis of local peptide release inside the magnocellular nuclei that is somewhat independent of the neurohypophysial release: thick neurophysin-containing processes terminate freely within the neuropil (Sofroniew & Glasmann, 1981); the characteristic varicose appearance of dendrites in the s.o. nuclei suggests a large

accumulation of peptide in these swellings (Armstrong, Schöler & McNeill, 1982); and the large neurosecretory processes contain neurosecretory granules (Kiss, Palkovits, Záborsky, Tribollet, Szabó & Makara, 1983). *In vitro*, oxytocin can be released by dendrites or by axon collaterals of oxytocin cells into the extracellular spaces of the magnocellular nuclei (Chapman, Hatton, Ho, Mason & Robinson, 1983; Moos, Freund-Mercier, Guerné, Guerné, Stoeckel & Richard, 1984). The action potentials of s.o. and p.v. magnocellular cells have calcium dendritic spike components that argue in favour of local hormone release (Mason & Leng, 1984). Thus, local oxytocin release might occur during the milk ejection reflex and play a facilitatory role in the recruitment of oxytocin cells, in increasing the amplitude and frequency of neurosecretory bursts and perhaps in synchronization. However, for this last parameter, since the onset time-lags were of the same range whatever the experimental conditions (whether potential ability to burst was increased or decreased), it is likely that burst synchronization depends mainly on the local connexions of cells in and between the nuclei.

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