Analysis of bursting responses of oxytocin neurones in the rat in late pregnancy, lactation and after weaning

Q. B. Jiang and J. B. Wakerley*

Department of Anatomy, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD, UK

1. Electrophysiological recordings were undertaken to compare bursting characteristics of oxytocin (OT) neurones at four reproductive stages: day 20 of pregnancy, day 22 of pregnancy (expected day of parturition), day 7–11 of lactation, and day 5–6 after weaning.

2. Each OT neurone was recorded for 1 h of suckling, combined with cervico–vaginal probing at 5 min intervals as an additional stimulus for bursting. Intracerebroventricular (I.C.V.) oxytocin (2·2 ng) was given after 30 min to facilitate bursting responses. Bursts observed during suckling were classified as 'spontaneous' or 'probe-evoked'.

3. The percentage of cells displaying spontaneous and/or probe-evoked bursts during the recording was low in day 20 pregnant animals, high in lactators and intermediate in day 20 pregnant and weaner groups. These differences may relate to variation in the proportion of animals with a responsive milk-ejection reflex, as well as the relative size of the population of bursting OT neurones.

4. In the period before I.C.V. OT, overall burst frequency (including both spontaneous and probe-evoked bursts) was similar across groups. After I.C.V. OT, overall burst frequency was much higher in lactators compared with other groups. Similar results were obtained when spontaneous bursts were analysed separately.

5. Burst amplitude (action potentials per burst, including both spontaneous and probe-evoked bursts) prior to I.C.V. OT was similar between the day 20 pregnant, day 22 pregnant and lactating groups, but was lower in weaners. All groups showed an increase in burst amplitude after I.C.V. OT, but values in weaners remained lower than in other groups. In a separate analysis of spontaneous bursts, burst amplitude after I.C.V. OT was higher in lactators, and lower in weaners, than in pregnant animals.

6. Background firing rates of OT cells were higher in the day 20 and day 22 pregnant groups compared with lactators, and lower in weaners. Only OT cells in lactators showed a significant increase in background firing rates following I.C.V. OT.

7. It is concluded that the bursting characteristics of OT cells change markedly between late pregnancy, mid-lactation and weaning. The factors underlying these changes, which are only loosely correlated with the sequence of morphological adaptations in OT cells surrounding lactation, remain to be established.

Magnocellular oxytocin (OT) neurones within the paraventricular and supraoptic (SO) nuclei of the suckled lactating rat have an unusual ability to fire in intermittent high frequency bursts, which are highly synchronized within the whole population of neurones (see reviews by Poulain & Wakerley, 1982; Richard, Moos & Freund-Mercier, 1988; Wakerley & Ingram, 1993). Each burst consists of a brief 2–4 s period of intense excitation, followed by an after-inhibition lasting about 15 s. This results in the pulsatile release of neurohypophysial OT (Higuchi, Honda, Fukuoka, Negoro & Wakabayashi, 1985) which acts on the myoepithelium of the mammary gland to evoke a milk-ejection response. Bursting activity is also observed in oxytocin neurones during birth (Summerlee, 1981), where OT has an essential role in the expulsion of fetuses from the uterus (Luckman *et al.* 1993).

* To whom correspondence should be addressed.

From the evidence to date, it would seem that only stimuli specific to the parturient and lactating female can induce bursting activity in OT neurones. Thus stimuli which are not specifically associated with motherhood, such as haemorrhage (Poulain, Wakerley & Dyball, 1977), osmotic challenge (Brimble, Dyball & Forsling, 1978) or cholecystokinin (CCK) administration (Hamamura, Leng, Emson & Kiyama, 1993), only cause a sustained increase in the firing of OT neurones, without the occurrence of intermittent bursting responses. In view of this association between OT cell bursting activity and the state of motherhood in the rat, it is particularly interesting that between late pregnancy and lactation, the oxytocinergic systems of the hypothalamus undergo a profound morphological (Theodosis, Poulain & Vincent, 1981; Hatton & Tweedle, 1982; Hatton, Modney & Salm, 1992; Theodosis & Poulain, 1992) and biochemical (Jirikowski, Caldwell, Pilgrim, Stumpf & Pederson, 1989; Brooks, Lund, Stumpf & Pederson, 1990; Insel, 1990) reorganization. It has been proposed (see Theodosis & Poulain, 1992) that these changes in the hypothalamic oxytocinergic systems, which are reversed following weaning, may somehow be concerned with enabling synchronous bursting activity during birth and suckling. However, the precise relationship between these adaptations and the firing patterns which the OT neurones are capable of generating remains to be investigated. Thus the present study was undertaken to compare the bursting characteristics of OT neurones in late pregnant, lactating and weaned rats.

Most previous studies of OT cell bursting activity (e.g. Belin & Moos, 1986; Dyball & Leng, 1986; Coles & Poulain, 1991) have solely involved suckling as the means of evoking bursting responses. However, in the present study, in which we wished to analyse the behaviour of OT cells in different reproductive states, it seemed important to use an experimental protocol which would maximize the number of bursts we were likely to record from each OT neurone. Preliminary studies showed that cervico–vaginal (CV) probing briefly applied to the anaesthetized suckled rat operated rather like vaginal distension (Negoro, Uchide, Tadokoro, Honda & Higuchi, 1987) to trigger an immediate burst additional to the on-going sequence of 'spontaneous' bursts triggered by suckling alone. Thus, in the present investigation we decided to use a test procedure which included regular (5 min intervals) CV probing applied during suckling to evoke additional responses. Furthermore, each OT neurone in this study was recorded both before and after giving an intracerebroventricular (I.C.V.) injection of OT, so that we could compare the facilitatory effect on bursting (Freund-Mercier & Richard, 1984) between the different reproductive states.

METHODS

Animals

Experiments were performed on Wistar rats which were purchased as young adults (Bantin & Kingman, Hull, UK) and mated in our own animal house colony. Animals were fed on a standard rat diet (Bantin & Kingman) and housed in a controlled environment (19–23 °C, 14 h light, 10 h dark). Four groups of animals were used at the following stages: day 20 of pregnancy, day 22 of pregnancy, day 7–11 of lactation and day 5–6 after weaning. Date of mating of pregnant rats was confirmed by detection of a vaginal plug on the morning following mating, which was counted as day 1 of pregnancy. Our animals would be expected to give birth on the afternoon or evening of day 22. In order to ensure that their nipples were well developed, so making it easier to attach the pups, all pregnant animals used in the study had been through a previous lactation. All rats were left for a minimum of 2 weeks before re-mating, to allow time for reversal of the adaptations in OT neurones associated with lactation. Animals studied during lactation had litters of 10–12 pups. On the evening before the experiment, lactating rats were separated from all but one of their pups. Animals to be used for the weaned group also had litters of 10–12 pups, which were weaned on day 20 of lactation.

Surgical preparation

Animals were anaesthetized with urethane (1·2 g kg^{-1}) and were given an I.V. injection (10 μg kg^{-1}) of the short-acting opioid analgesic, fentanyl (Sublimaze; Janssen Pharmaceutical, Oxford, UK) prior to surgery. An abdominal or inguinal mammary gland was cannulated, together with a maxillary branch of the internal jugular vein. The animals were then placed in a stereotaxic frame and the brain exposed. Following ligation of the superior sagittal sinus, a bipolar stimulating electrode (SNEX 100; Rhodes Medical Instruments, supplied by Clarke Electromedical Instruments, Reading, UK) was positioned near the neural stalk to antidromically activate neurosecretory cells within the supraoptic nuclei. Under microscopic control, this electrode was carefully lowered to the basosphenoid bone and then withdrawn by 0·2 mm. After connecting the mammary cannula to a pressure transducer (Type EM750; Elcomatic, Glasgow, UK), correct positioning of this electrode was confirmed by recording an intramammary pressure response following a brief stimulus train (2 ms biphasic pulses of 1 mA peak to peak, applied at 50 Hz for 4 s). In pregnant and weaned animals, an intramammary injection of cows milk (0·1–0·3 ml) was often necessary to achieve a mammary response to OT (see Sutherland, Aizlewood & Wakerley, 1986). A Hamilton microsyringe (10 μl) was positioned with its needle in the third ventricle (checked by withdrawing cerebrospinal fluid) for the subsequent I.C.V. injection of OT. Electrophysiological recording was started 2–3 h after surgery and all animals were first given 1 mg kg^{-1} I.V. propanolol (Inderal; Zeneca, Macclesfield, UK) to eliminate central inhibition of the milk-ejection reflex mediated by β-adrenoceptors (Tribollet, Clarke, Dreifuss & Lincoln, 1978).

Electrophysiological recording procedure and protocol for testing bursting responses in OT neurones

Extracellular recordings were obtained with glass micropipettes (5–10 MΩ) filled with 0·5 M NaCl solution. The recording microelectrodes were stereotaxically positioned to track through

the supraoptic nucleus, using co-ordinates based on Paxinos & Watson (1986): 1·8–2·2 mm lateral to mid-line, 6·8–7·2 mm anterior to inter-aural line, 8·8–9·2 mm from brain surface. As the electrode was lowered, single shocks (1–2 mA) were delivered to the posterior pituitary. Location of the recording microelectrode within the supraoptic nucleus was indicated by the presence of a field potential, and the sudden appearance of several antidromic spikes over a short tracking distance. Once the electrode was located in the supraoptic nucleus, a litter of eight to nine pups was applied to the nipples. In experiments on pregnant and weaned animals, a foster litter (5–10 days of age, previously separated overnight) was employed.

With the exception of phasically active neurones, which were rapidly discarded on the grounds that they were likely to be vasopressin cells (Poulain & Wakerley, 1982), each supraoptic neurone encountered was recorded for 1 h of suckling. During suckling, brief episodes of CV probing were undertaken at intervals of approximately 5 min in order to evoke additional burst responses, except in a few experiments in which the effects of suckling alone were being investigated. CV probing was performed using a small metal rod (diameter 3 mm) inserted into the vagina and pushed against the cervix 4 times over a 3–4 s period, using sufficient pressure to evoke reflex contractions of the abdominal muscles. When a spontaneous suckling burst occurred just before a probing episode was about to be given, probing was delayed for 30 s. After the first 30 min of the recording, an I.C.V. injection of 2·2 ng OT (Syntocinon; Sandoz, Frimley, Camberley, UK) in 2 μl isotonic saline was administered into the third ventricle to facilitate the milk-ejection reflex (Freund-Mercier & Richard, 1984). If the cell had shown no bursts during the 1 h recording, an injection of 20 μg kg^{-1} cholecystokinin I.V. (CCK 26–33 sulphated amide; Cambridge Research Biochemicals, Northwich, UK) was administered to further characterize the cell as an OT-secreting neurone (Leng, Way & Dyball, 1991). To avoid biasing the data, no more than two cells were studied in any one animal. At the end of the experiment, the rats were killed by an overdose of sodium pentobarbitone I.V. (Sagatal; May and Baker, Dagenham, UK).

In the early experiments an attempt was made to obtain an intramammary pressure record alongside each neuronal recording, as in previous studies (Wakerley & Lincoln, 1973; Poulain *et al.* 1977). However, in the pregnant and weaned animals it proved very difficult to maintain a meaningful intramammary pressure record, which necessitated frequent attention to the cannulated mammary gland, whilst simultaneously undertaking electrophysiological recording. Thus no intramammary pressure data are presented in this study.

Data analysis

An on-line permanent record of neuronal activity was obtained by passing the recorded signal through a laboratory-built window discriminator which delivered pulses to a computer-based rate analyser (BBC Electron, with purpose-built interface), the analog output of which was connected to a pen-recorder (Watanabe, Graphtek, Nantwich, UK). The extracellular signal was also stored on a tape-recorder (Racal, Southampton, UK) for later off-line computer analysis (Modulog System; Grafitek, Bristol, UK). For each neuronal recording, an analysis was made of the background firing rate, and the occurrence and number of spikes in each high frequency burst of firing. Bursts which occurred during suckling but were unrelated to a probing episode were classified as *spontaneous bursts*, whereas bursts which were related to a probing episode were classified as *probe-evoked bursts*. A separate analysis was made of the 30 min period before and after administering I.C.V. OT. If a cell was lost towards the end of the second period of analysis, the available data were still included.

Because of the rather small number of bursting responses displayed by some cells, it was decided to base the initial calculation of means for the various bursting parameters on all bursts, without distinction between spontaneous and probe-evoked responses. Mean parameters for spontaneous and probe-evoked bursts were then calculated separately. In this separate analysis, the incidence of probe-evoked responses was expressed in terms of the number of bursts per probe episode, rather than bursts per ten minutes, thereby eliminating possible variation relating to the number of probe episodes given during each test period.

Comparison of mean bursting parameters between groups was undertaken using the non-parametric Mann–Whitney U test. Differences in numbers of responsive cells between groups were compared by χ^2 test. Changes in background firing rate were evaluated using Student's paired t test.

RESULTS

Experiments were initially performed on fifty-three rats, in which a total of seventy-five OT neurones were recorded during suckling. These neurones were all antidromically activated by stimulation of the posterior pituitary (response latencies, 9–15 ms; threshold current, 0·2–2·4 mA), and were identified as putative OT neurones according to one or more of the following criteria: (a) occurrence of high frequency bursts, (b) excitation following I.V. CCK injection (Leng *et al.* 1991), or (c) presence of fast (> 3 spikes s^{-1}) continuous background activity (Poulain & Wakerley, 1982).

General features of bursting responses recorded from OT neurones in pregnant, lactating and weaned rats

Sixty percent (45/75) of all OT neurones displayed one or, more usually, repetitive high frequency bursts during suckling. Approximately half (25/45) of these neurones showed both spontaneous bursts and probe-evoked bursts (Fig. 1*A* and *B*). Spontaneous bursts only were shown by seven out of forty-five cells and thirteen out of forty-five cells showed only probe-evoked bursts (Fig. 1*C*).

Bursts evoked by cervical probing were identical in character to spontaneous bursts, and consisted of a brief (2–3 s) period of intense (> 20 spikes s^{-1}) firing, usually starting coincidentally with the first probe (Fig. 1*A*). Probe-evoked bursts, like the spontaneous bursts, showed both an increase in incidence and amplitude following I.C.V. injection of OT (Fig. 1*C* and *E*). Where probing failed to evoke a full burst of firing, there was sometimes a transient increase in background activity (see example in Fig. 1*B*, periods 1–3), but this occurred in too few cells for comparison between groups.

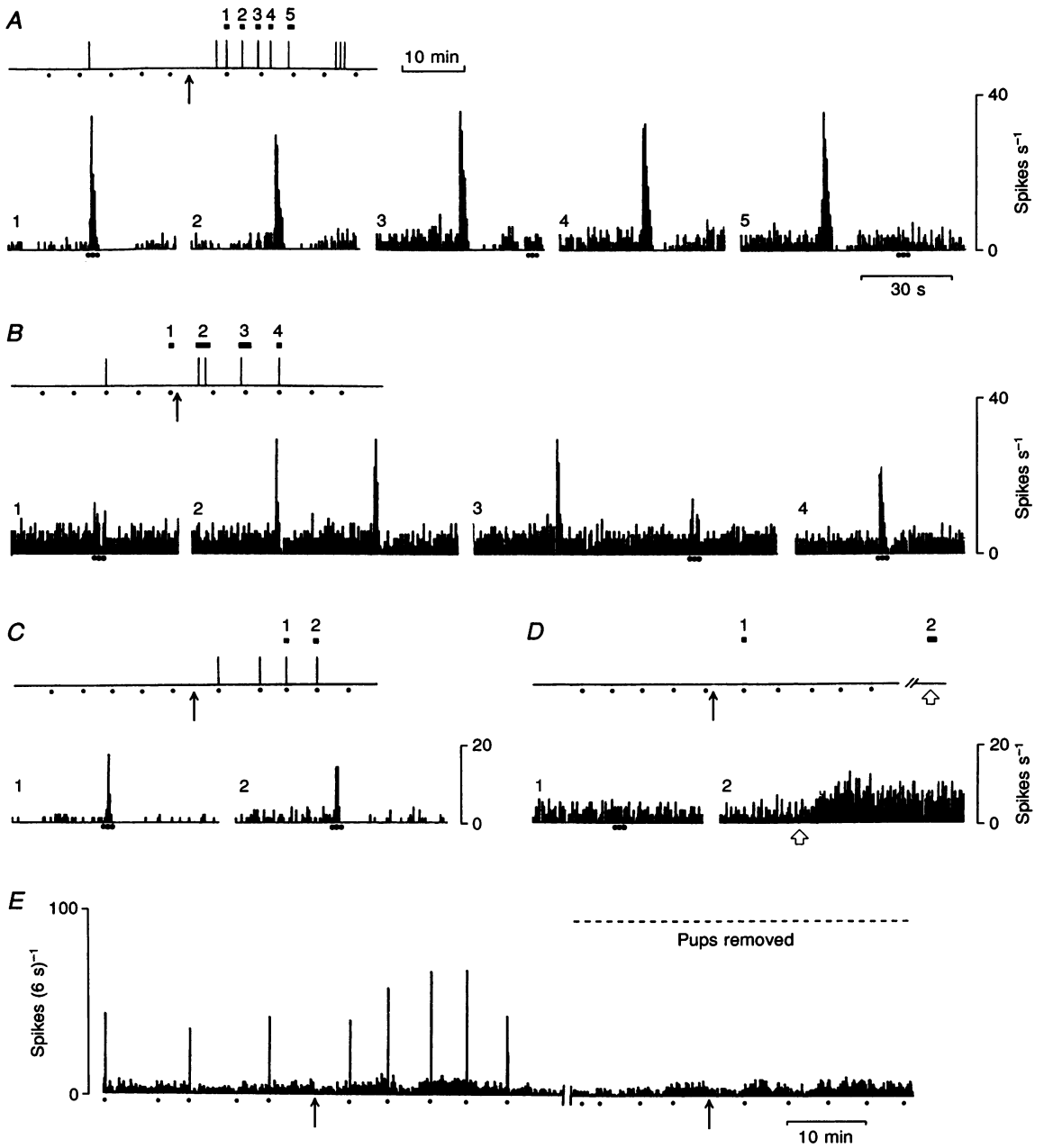

Figure 1. Response characteristics of OT neurones during suckling combined with intermittent CV probing

A–E show edited periods of activity of individual OT cells in the form of sequential firing rate histograms (1 s bin width). Insets above the histograms (included in *A–D*) illustrate the sequence of bursts (indicated by vertical bars) that were recorded 30 min before and after I.C.V. OT administration (filled arrows). The numbered horizontal bars above these insets correspond to the edited periods of activity that are shown on a much expanded time scale in the sequential histograms beneath. Single dots represent an episode of CV probing, merged dots represent individual probes in an episode. *A* shows a cell displaying both spontaneous bursts (i.e. unrelated to CV probing; see periods 2–5), as well as probe-evoked bursts (see period 1). *B* shows a cell also displaying both spontaneous (period 2) and probe-evoked bursts (period 4) but, additionally, this cell sometimes displayed small increases in background firing when probing failed to elicit a full burst (periods 1 and 3). *C* shows a cell which displayed bursts only in response to CV probing. *D* shows a cell which displayed neither spontaneous nor probe-evoked bursts but was excited by CCK (open arrow). *E* (note the contracted time scale of the histogram; bin width 6 s) shows that removal of the pups caused disappearance of probe-evoked bursts. These examples were recorded at the following stages: *A*, day 10 lactating rat; *B*, day 22 pregnant rat; *C* and *E*, 5 day weaned rat; *D*, day 20 pregnant rat.

The remaining 40% (30/75) OT cells showed neither spontaneous nor probe-evoked bursts, although all of the unresponsive cells which were later tested with CCK showed excitation (Fig. 1*D*).

Probe-evoked bursts were dependent upon the suckling stimulus and ceased if the pups were removed from the nipples (Fig. 1*E*). A brief footpinch applied during suckling was ineffective at evoking bursts, suggesting a degree of specificity in the response to CV probing.

Comparison of bursting characteristics of OT neurones between the different experimental groups

Out of the seventy-five OT neurones that were studied, eighteen were from the day 20 pregnant group, seventeen from the day 22 pregnant group, twenty-seven from the lactating group and thirteen from the weaned group. The higher number of cells recorded in the lactating group reflected the fact that the pups found the nipples and became attached more quickly to lactating animals than to pregnant and weaned animals, resulting in fewer cells being lost during the initial phase of the recording. Two animals in the day 22 pregnant group had a vaginal discharge indicating the onset of labour, but the responses obtained in these animals were similar to those in the remainder of the group.

Percentage of OT neurones showing bursting responses in pregnant, lactating and weaned rats

The percentage of OT neurones showing bursting responses (including both spontaneous and probe-evoked bursts) is shown in Fig. 2*A*. For the period before (□) I.C.V. OT, the highest percentage of bursting cells was found in the lactation group. The percentage of bursting OT cells was increased (but not significantly) after (▩) I.C.V. OT in all groups, except the day 20 pregnant animals. For the period following I.C.V. OT, there was a significant difference between the day 20 pregnant and lactating rats ($P < 0{\cdot}05$). In the separate analysis of spontaneous bursts (Fig. 2*B*), it was notable that in the period prior to I.C.V. oxytocin, the day 20 pregnant and, more especially, the weaner groups showed fewer responsive cells compared with lactators ($P < 0{\cdot}05$ for lactators *versus* weaners). For probe-evoked bursts (Fig. 2*C*), there was a highly significant ($P < 0{\cdot}01$) difference between the day 20 pregnant group and lactators in the period following I.C.V. OT. Since only one to two cells were recorded in each experiment, it was not possible to undertake further analysis to determine whether these differences in the percentage of responsive cells reflected differences in the extent of neuronal recruitment within individual animals, or the proportion of responsive animals within the group as a whole.

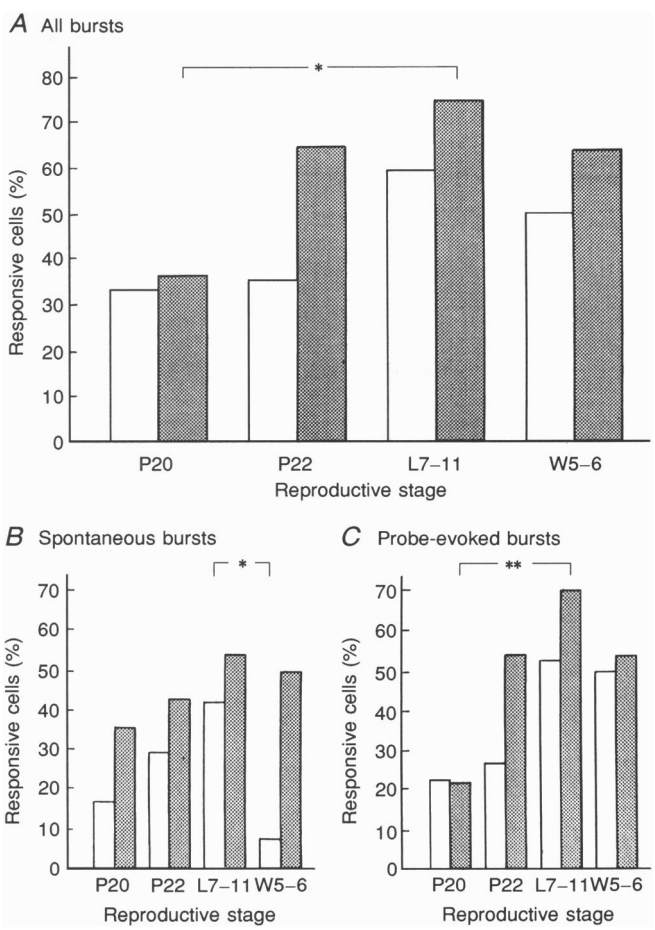

Figure 2. Composite data showing the percentage of responsive OT neurones in the different experimental groups

The percentage of responsive cells was calculated by inclusion of: *A*, all bursts (i.e. responsive cells showed at least 1 spontaneous and/or probe-evoked burst); *B*, spontaneous bursts (i.e. responsive cells showed at least 1 spontaneous burst); or *C*, probe-evoked bursts (i.e. responsive cells showed at least 1 probe-evoked burst). □, data for the 30 min period prior to giving I.C.V. OT; ▩, data for the 30 min period after I.C.V. OT. Asterisks indicate level of significance (* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$). Values are based on analysis of 13–27 OT cells from 8–19 rats. P20, day 20 pregnant group; P22, day 22 pregnant group; L7–11, day 7–11 lactation group; W5–6, day 5–6 weaned group.

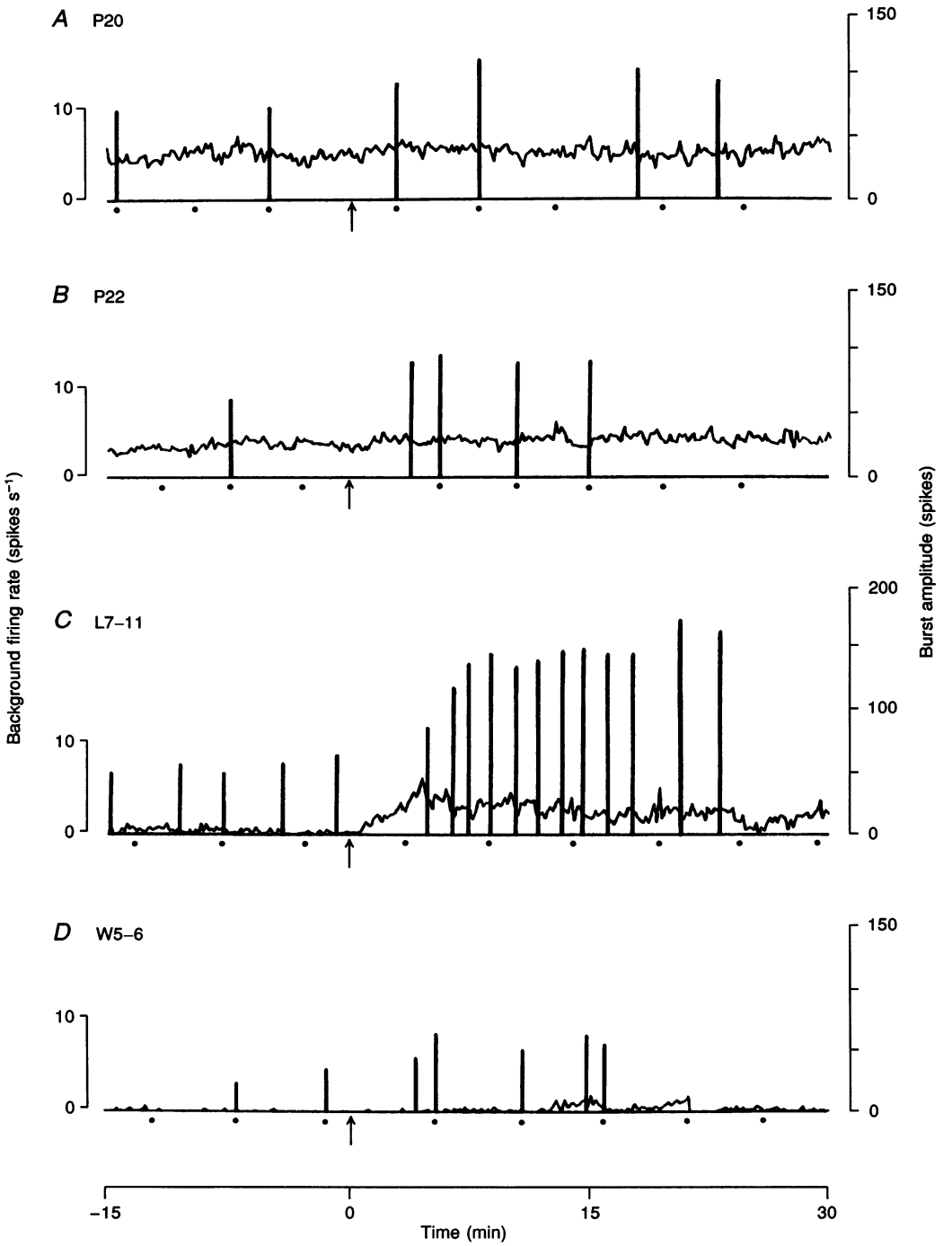

Figure 3. Examples of the different patterns of OT neurone activity in the four groups

The 4 different examples are from the day 20 pregnant group (P20, *A*), the day 22 pregnant group (P22, *B*), the day 7–11 lactation group (L7–11, *C*), and the day 5–6 weaned group (W5–6, *D*). In each case, background activity (left scale) is indicated by the continuous line and burst amplitude is indicated by the vertical bars (right scale); dots indicate episodes of CV probing and arrows indicate where OT (2·2 ng I.C.V.) was given. Note the differences in background firing and bursting activity between these different examples, and that only the cell recorded during lactation displayed a clear increase in background firing rate following I.C.V. OT (for further explanation see text).

Frequency of bursting responses in OT neurones in pregnant, lactating and weaned rats

Individual examples showing the frequency of bursting responses are given in Fig. 3 and composite data are given in Fig. 4. Analysis of all bursts revealed no significant differences before I.C.V. OT (Fig. 4*A*). Burst frequency was increased in all groups by I.C.V. OT, but this was only significant ($P < 0{\cdot}01$) in the lactators, which showed by far the biggest effect. Burst frequency after I.C.V. OT was significantly higher ($P < 0{\cdot}05$) in the lactators than in both the day 22 pregnant and the weaned groups. Separate analysis of spontaneous bursts (Fig. 4*B*) showed a similar pattern, with the most frequent bursts after I.C.V. OT again occurring in the lactators ($P < 0{\cdot}05$ compared with both day 22 pregnant and weaned animals). Interestingly, the lowest frequency of spontaneous bursts before I.C.V. OT was observed in the weaners, whereas this group showed the highest incidence of probe-evoked bursts over the same period (Fig. 4*C*). Only lactators showed a significant ($P < 0{\cdot}05$) increase in the incidence of probe-evoked bursts after I.C.V. OT.

Amplitude of bursting responses of OT neurones in pregnant, lactating and weaned rats

Burst amplitude (based on combined spontaneous and probe-evoked bursts) was similar in the two pregnant groups and slightly higher (not significantly) in the lactators, both before and after I.C.V. OT (see examples in Fig. 3 and composite data in Fig. 5*A*). The weaners showed much smaller bursts, both before ($P < 0{\cdot}05$ *versus* lactators and $P < 0{\cdot}01$ *versus* day 22 pregnant animals) and after I.C.V. oxytocin ($P < 0{\cdot}01$ for weaners *versus* lactators and day 22 pregnant groups, and $P < 0{\cdot}05$ *versus* day 20 pregnant groups). Separate analysis of the amplitude of spontaneous bursts (Fig. 5*B*) revealed a broadly similar pattern, except that during the period after I.C.V. OT there was a more pronounced difference between the pregnant and lactating animals. Probe-evoked bursts (Fig. 5*C*) were of similar amplitude in the day 20 pregnant, day 22 pregnant and lactating groups, but again much smaller in the weaned group.

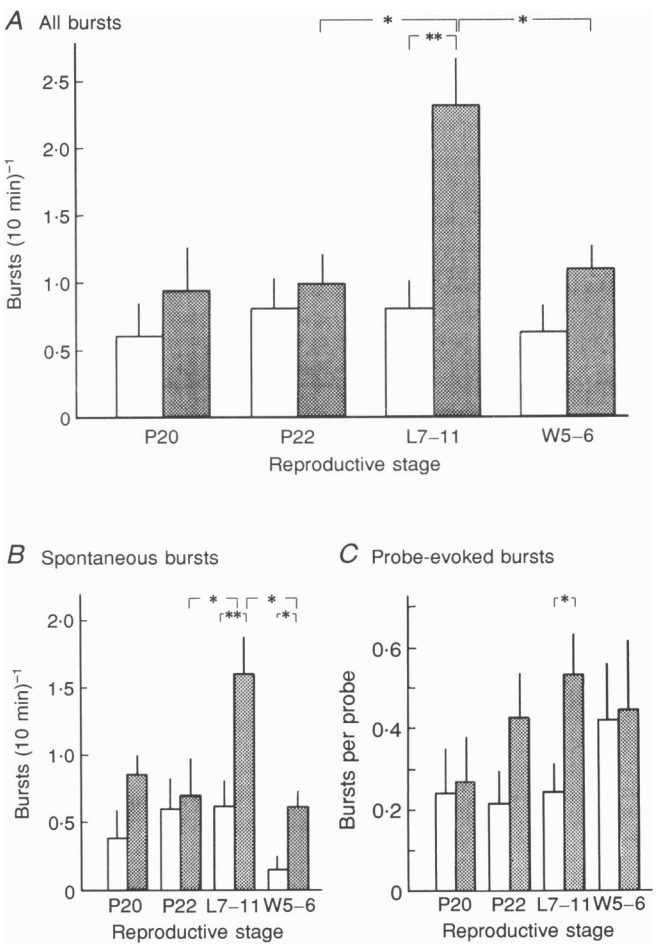

Figure 4. Analysis of the frequency of bursting responses in OT neurones in the different groups

Burst frequency (mean ± S.E.M.) was calculated on the basis of: *A*, inclusion of all bursts (i.e. spontaneous as well as probe-evoked bursts); *B*, inclusion of only spontaneous bursts; or *C*, inclusion of only probe-evoked bursts. □, data for the 30 min period prior to giving I.C.V. OT; ▨, data for the 30 min period after I.C.V. OT. In responsive OT cells showing no bursts during the particular 30 min analysis period, frequency was ascribed a value of zero. Means are based on 5–19 cells from 4–15 rats. Asterisks indicate level of significance (* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$). See Fig. 2 for explanation of group abbreviations.

Examination of bursting responses in the absence of CV probing

Since probe-evoked bursts were dependent upon suckling (Fig. 1*E*), the response to CV probing probably involved central pathways which overlapped with those transmitting the suckling stimulus. This raised the possibility of inhibitory interactions, whereby CV probing during suckling might actively inhibit spontaneous bursting. Such inhibitory interactions seemed most likely in the weaned group in particular, in which there was a high incidence of probe-evoked bursts, whereas the frequency of spontaneous bursts was low (Fig. 4). Thus, it was decided to undertake a further series of experiments (Fig. 6) in weaned rats to re-examine spontaneous bursting during suckling in the absence of CV probing. In eight cells from six rats, parameters for spontaneous bursts were similar to those previously obtained in the presence of CV probing (Figs 4*B* and 5*B*).

Background activity of OT neurones in pregnant, lactating and weaned rats

Background firing rates of the OT neurones varied in the different groups (see Figs 3 and 7). Mean firing rates of OT cells in day 20 and day 22 pregnant animals were $3{\cdot}3 \pm 1{\cdot}5$ and $4{\cdot}4 \pm 0{\cdot}8$ spikes s^{-1}, respectively. Values for day 22 pregnant animals were significantly higher ($P < 0{\cdot}01$) than means for both lactating ($1{\cdot}6 \pm 0{\cdot}4$) and weaned ($0{\cdot}6 \pm 0{\cdot}3$ spikes s^{-1}) rats.

The effect of I.C.V. OT on the background firing rates of the OT neurones also varied between groups. In the lactators, I.C.V. OT caused a significant ($P < 0{\cdot}01$) increase in background firing (see Figs 3 and 7) which persisted for 10–15 min, as previously reported by Freund-Mercier & Richard (1984), whereas in the other groups there was no significant change.

DISCUSSION

The present electrophysiological results, which extend previous indirect evidence based on intramammary pressure recordings (Sutherland *et al.* 1986), show that the various neuronal mechanisms producing high frequency bursts in OT neurones can undergo dynamic changes in their functional characteristics according to reproductive state. However, it is clear that these changes do not correlate in any simple way with the well-established morphological adaptations of OT cells associated with lactation (Theodosis & Poulain, 1987; Hatton *et al.* 1992; Theodosis & Poulain, 1992). In particular, there were no significant changes in bursting parameters between the two pregnant groups, yet morphological reorganization of the OT neurones probably

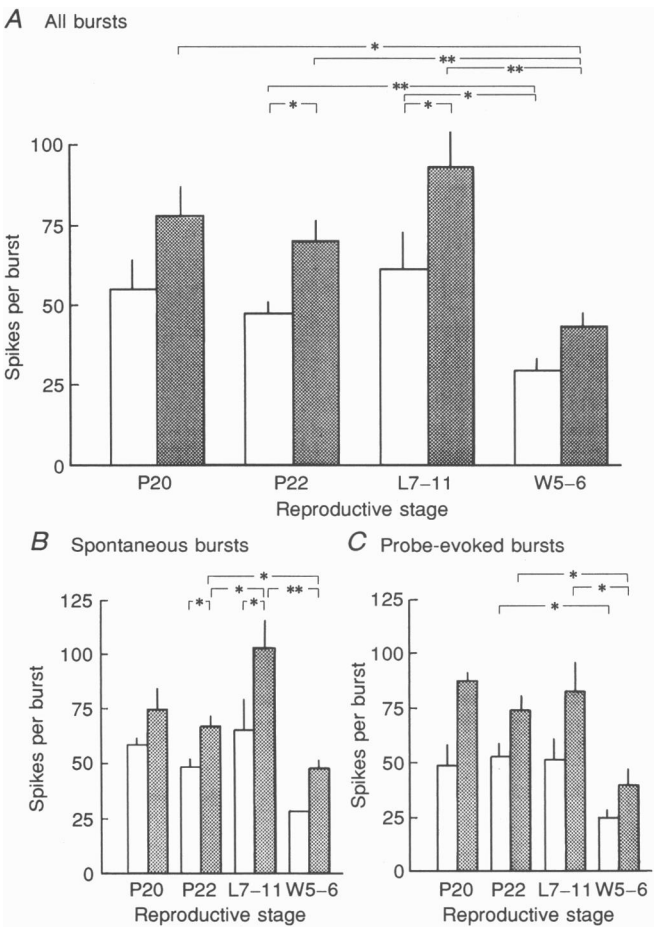

Figure 5. Analysis of the amplitude of bursting responses in OT neurones in the different groups

Burst amplitude was calculated on the basis of: *A*, inclusion of all bursts (i.e. spontaneous as well as probe-evoked bursts); *B*, inclusion of only spontaneous bursts; or *C*, inclusion of only probe-evoked bursts. □, data for the 30 min period prior to giving I.C.V. OT; ▨, data for the 30 min period after I.C.V. OT. Values are means ± S.E.M. Asterisks indicate level of significance (* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$). Means in *A* are based on 5–19 cells recorded from 4–15 rats, except for P20 before I.C.V. OT (5 cells from 3 rats); means in *B* are based on 5–13 cells from 4–10 rats, except for the 2 P20 values (3–5 cells from 3 rats) and for W5–6 before I.C.V. OT (1 cell only); means in *C* are based on 4–14 cells from 3–11 rats, except for P20 after I.C.V. OT (3 cells from 2 rats). See Fig. 2 for explanation of group abbreviations.

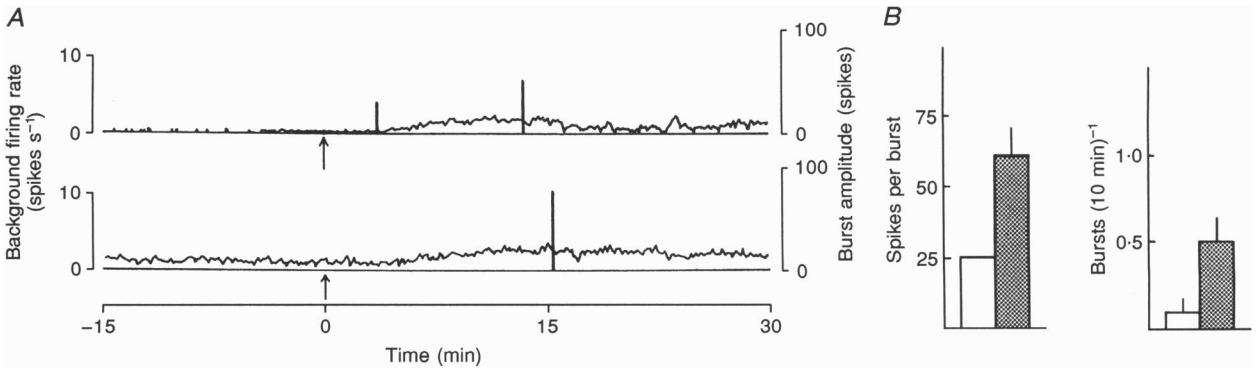

Figure 6. Occurrence of spontaneous bursts during suckling in the absence of CV probing

A further group of weaned animals was investigated to determine whether the low incidence of spontaneous bursts was related to an inhibitory effect of CV probing. In recordings obtained from 8 cells in 6 rats, numbers of neurones showing spontaneous bursts before and after I.C.V. OT were 1 (13%) and 6 (75%), respectively. *A* illustrates two examples; background activity (left scale) is indicated by the continuous line and burst amplitude (right scale) is indicated by the vertical bars. Arrows indicate I.C.V. injection of 2·2 ng OT. *B* shows group means which were similar to those for spontaneous bursts in weaned animals given the additional stimulus of CV probing (see Figs 4*B* and 5*B*). □, before I.C.V. OT; ▨, after I.C.V. OT.

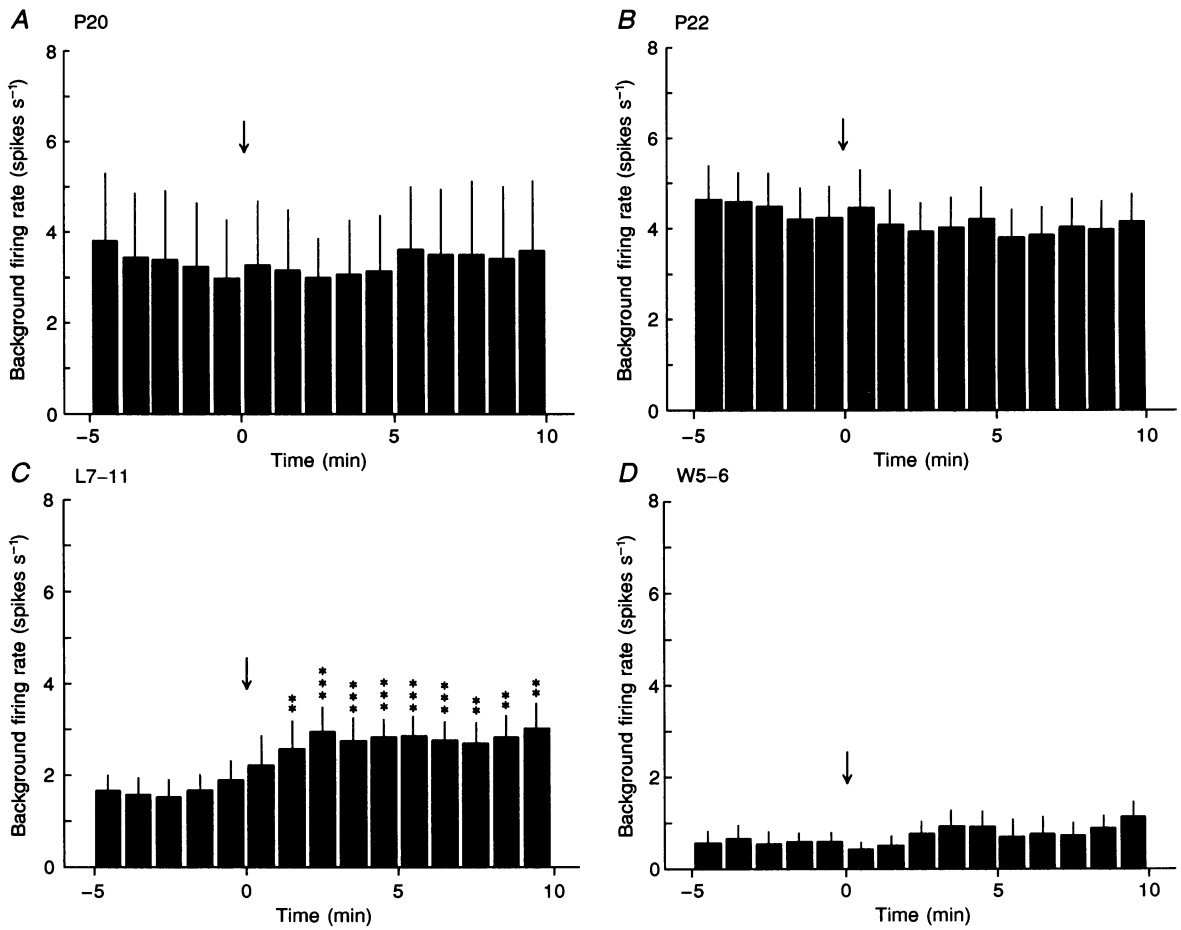

Figure 7. Histograms showing the effect of I.C.V. OT on the background firing rate of OT neurones during suckling

Each bar shows the mean (± S.E.M.) firing rate for 5 min before and 10 min after giving I.C.V. OT (2·2 ng). For this analysis, bursts were removed and replaced with mean activity of an equivalent period before the burst. Asterisks above the bars indicate a significant difference from the mean for the 5 min period before I.C.V. OT (** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$). See Fig. 2 for explanation of group abbreviations.

progresses rapidly over the last 24–48 h of pregnancy (Theodosis & Poulain, 1987, 1992). Similarly, the rapid decline in bursting observed following 5 days of weaning might seem to occur too quickly for it to be ascribed solely to reversal of the morphological adaptations, which probably take somewhat longer to regress. Thus, whilst morphological adaptations of the OT cells may have played some part in the present results, other important factors must have also contributed to the observed changes in bursting activity.

It would be wrong at this stage to conclude that the morphological adaptations of OT neurones during lactation play no role in burst generation. The highest frequency and amplitude of bursting were observed in the mid-lactation group, at a time when the numbers of double synapses, dendritic bundling and membrane apposition would all be maximal (Theodosis *et al.* 1981; Perlmutter, Tweedle & Hatton, 1984; Theodosis & Poulain, 1987). The increased membrane apposition occurring during lactation would be expected to enhance cell coupling, either by direct electrotonic interaction (Hatton *et al.* 1992) or indirectly through transient spike-related increases in local potassium concentration (Coles & Poulain, 1991). Increased coupling of OT neurones during synchronous excitatory synaptic input would increase the probability of burst initiation and augment burst amplitude. From the present results though, it must be assumed that the functional expression of these adaptations is subordinate to other variable determinants of bursting activity.

It is evident that the most striking differences between lactating and other groups, especially in spontaneous bursts, occurred during the period following I.C.V. OT, rather than during the initial control period (Figs 3*C* and 4*B*). This suggests that one important change occurring in lactation is an increase in the efficiency of the neuronal circuitry mediating the facilitatory effect of I.C.V. OT. A recent electrophysiological study of neurones within the bed nucleus of the stria terminalis, a structure strongly implicated in OT-induced facilitation of the milk-ejection reflex (Moos, Ingram, Wakerley, Guerne, Freund-Mercier & Richard, 1991), showed that sensitivity to OT was low in late pregnancy, high in lactation, and low again at weaning (Ingram & Wakerley, 1993). These changes correspond well with the currently observed differences in burst frequency between groups following I.C.V. OT. Furthermore, it is notable that the usual increase in background firing rate of the OT neurones following I.C.V. OT, which is known to be linked to the facilitatory response (Freund-Mercier & Richard, 1984), was only seen in lactating animals, further supporting the idea of enhanced OT sensitivity in this group. This enhanced OT sensitivity might relate to differences in the density of OT binding sites in the bed nucleus, although it has been reported that OT binding in established lactation is similar to that during late pregnancy (Insel, 1990; Kremarik, Freund-Mercier & Stockel, 1991). Ingram & Wakerley (1993) have suggested that modification of the postreceptor transduction mechanism may provide a possible explanation between OT binding and the potency of the central actions of the peptide.

Another variable which may have been important in determining differences in bursting activity between the groups, both during the basal period and following I.C.V. OT, was the efficiency of transmission of the sensory input between the nipples and hypothalamus, required both for spontaneous and probe-evoked bursts. It might be argued, for example, that the greater frequency and amplitude of bursting in lactating animals was related to the fact that mammary glands of these animals were distended with milk, and this somehow altered the afferent input, or suckling behaviour of the pups. However, previous work has shown that the degree of mammary distension has little effect on the characteristics of the milk-ejection reflex (Riggs, Sutherland & Wakerley, 1985). Nevertheless there still remains the possibility of changes occurring centrally in the afferent circuitry of the milk-ejection reflex, for example in the ascending noradrenergic projections implicated in oxytocin release (Crowley, Parker, Armstrong, Spinolo & Grosvenor, 1992), which might be modified in lactation.

An important methodological consideration in the current investigation was the novel use of CV probing during suckling as an additional stimulus for evoking bursting responses. The response to CV probing resembled the effect of vaginal distension, reported by Negoro *et al.* (1987). They concluded that vaginal distension acted to trigger a burst through excitatory interaction with pathways concerned with the milk-ejection reflex, since this stimulus was ineffective in the absence of suckling. It is likely that the response to CV probing involved a similar interaction, and this is supported by the observation that probe-evoked bursts were dependent upon suckling and, like spontaneous bursts, were facilitated by OT. Comparison of results obtained by analysis of all (i.e. both spontaneous and probe-evoked) bursts (Figs 2*A*, 4*A* and 5*A*) with results which included only spontaneous bursts (Figs 2*B*, 4*B* and 5*B*), would suggest that inclusion of the probe-induced responses had relatively little impact on the overall pattern of group differences. A notable exception was the case of the weaned animals in which, in the period before I.C.V. OT was given, very few spontaneous bursts occurred (Figs 2*B* and 4*B*), whereas CV probing was highly effective compared with other groups (Figs 2*C* and 4*C*). However, weaners were subsequently found to display a low frequency of spontaneous bursts, regardless of intermittent CV probing. In some instances the triggering of an additional probe-evoked burst may have delayed the next spontaneous response, but this is unlikely to have been significant

except in those cases (mostly lactators) in which spontaneous bursts were occurring relatively frequently.

The differences found in the percentage of OT neurones showing bursting responses, with overall values (based on all bursts) being lowest for the day 20 pregnant group (Fig. 2*A*), must be treated with caution. It is tempting to interpret these differences in terms of changes in the numbers of OT neurones recruited into the pool of cells contributing to the generation of OT pulses, especially since neuronal recruitment has been previously demonstrated in the oxytocinergic magnocellular system (Belin & Moos, 1986). However, it is notable that, even in lactating animals, the maximum percentage of responsive cells was only in the order of 75%, whereas previous data from lactating rats under similar conditions suggest that virtually all OT neurones are correlated with the milk-ejection reflex (Poulain *et al.* 1977; Poulain & Wakerley, 1982). This would indicate that the absence of bursting, in some cells at least, may have been related to failure of milk-ejection responses to occur during the period of the recording. Further work using the technique of simultaneous double recordings of OT neurones (Belin & Moos, 1986) in the different groups is required to resolve this particular problem of neuronal recruitment.

The background activity of the OT neurones recorded in the lactating animals was similar to that reported in previous studies (Poulain *et al.* 1977; Freund-Mercier & Richard, 1984; Belin & Moos, 1986). Background activity was significantly elevated on day 22 of pregnancy, and this is compatible with an elevation in basal levels of plasma OT reported by Douglas, Dye, Leng, Russell & Bicknell (1993) over the period equivalent to days 19 and 22 of pregnancy in the present study. Summerlee (1981) also reported an increase in background activity of OT neurones in late pregnant rats, although in his experiments the increase in firing was not observed until close to the onset of parturition. Presumably, the increase in basal activity towards the end of pregnancy could contribute to the onset of parturition. The stimulus for the increased firing is unclear but one possibility is the ovarian hormone, relaxin, which is known to excite OT neurones (see Way & Leng, 1992), and will be elevated at this stage of pregnancy. The pre-partum rise of oestradiol may also contribute, since this steroid is known to excite OT neurones (Akaishi & Sakuma, 1985).

In conclusion, the present study provides electrophysiological evidence that bursting characteristics of OT neurones change dramatically between pregnancy, lactation and weaning. The neural basis for these changes, particularly the relative importance of local adaptations of the OT cells *versus* other factors such as modification of the afferent pathways controlling bursting, remains to be established.

Akaishi, T. & Sakuma, Y. (1985). Oestrogen excites oxytocinergic but not vasopressinergic cells in the paraventricular nucleus of the female rat hypothalamus. *Brain Research* **353**, 302–305.

Belin, V. & Moos, F. (1986). Paired recordings from supraoptic and paraventricular oxytocin cells in suckled rats: recruitment and sychronization. *Journal of Physiology* **377**, 369–390.

Brimble, M. J., Dyball, R. E. J. & Forsling, M. L. (1978). Oxytocin release following osmotic activation of oxytocin neurones in the paraventricular and supraoptic nuclei. *Journal of Physiology* **278**, 69–78.

Brooks, P. J., Lund, P. K., Stumpf, W. E. & Pedersen, C. A. (1990). Oxytocin mRNA levels in the medial preoptic area are increased during lactation. *Journal of Neuroendocrinology* **2**, 621–626.

Coles, J. A. & Poulain, D. A. (1991). Extracellular K^+ in the supraoptic nucleus of the rat during reflex bursting activity by oxytocin neurones. *Journal of Physiology* **439**, 383–409.

Crowley, W. R., Parker, S. L., Armstrong, W. E., Spinolo, L. H. & Grosvenor, C. E. (1992). Neurotransmitter and neurohormonal regulation of oxytocin secretion in lactation. *Annals of the New York Academy of Sciences* **652**, 301–325.

Douglas, A. J., Dye, S., Leng, G., Russell, J. A. & Bicknell, R. J. (1993). Endogenous opioid regulation of oxytocin secretion through pregnancy in the rat. *Journal of Neuroendocrinology* **5**, 307–314.

Dyball, R. E. J. & Leng, G. (1986). Regulation of the milk ejection reflex in the rat. *Journal of Physiology* **380**, 239–256.

Freund-Mercier, M.-J. & Richard, P. (1984). Electrophysiological evidence for facilitatory control of oxytocin cells by oxytocin during suckling in the rat. *Journal of Physiology* **352**, 447–466.

Hamamura, M., Leng, G., Emson, P. C. & Kiyama, H. (1993). Electrical stimulation and c-fos mRNA expression in rat neurosecretory neurones after systemic administration of cholecystokinin. *Journal of Physiology* **444**, 51–63.

Hatton, G. I., Modney, B. K. & Salm, A. K. (1992). Increases in dendritic bundling and dye coupling of supraoptic neurones after the induction of maternal behaviour. *Annals of the New York Academy of Sciences* **652**, 142–155.

Hatton, G. I. & Tweedle, C. D. (1982). Magnocellular neuropeptidergic neurones in hypothalamus: increases in membrane apposition and number of specialised synapses from pregnancy to lactation. *Brain Research Bulletin* **8**, 197–204.

Higuchi, T., Honda, K., Fukuoka, T., Negoro, H. & Wakabayashi, K. (1985). Release of oxytocin during suckling and parturition in the rat. *Journal of Endocrinology* **105**, 339–346.

Ingram, C. D. & Wakerley, J. B. (1993). Post-partum increase in oxytocin-induced excitation of neurones in the bed nuclei of the stria terminalis *in vitro*. *Brain Research* **602**, 325–330.

Insel, T. R. (1990). Regional changes in brain oxytocin receptors post-partum: time course and relationship to maternal behaviour. *Journal of Neuroendocrinology* **2**, 539–545.

Jirikowski, G. F., Caldwell, J. E., Pilgrim, Ch., Stumpf, W. E. & Pedersen, C. A. (1989). Changes in immunostaining for oxytocin in the forebrain of the female rat during late pregnancy, parturition and early lactation. *Cell and Tissue Research* **256**, 411–417.

Kremarik, P., Freund-Mercier, M. J. & Stoeckel, M. R. (1991). Autoradiographic detection of oxytocin- and vasopressin-binding sites in the various subnuclei of the bed nucleus of the stria terminalis in the rat: effects of functional and experimental sexual steroid variations. *Journal of Neuroendocrinology* **3**, 689–698.

Leng, G., Way, S. & Dyball, R. E. J. (1991). Identification of oxytocin cells in the rat supraoptic nucleus by their response to cholecystokinin injection. *Neuroscience Letters* **122**, 159–162.

Luckman, S. M., Antonijevic, I., Leng, G., Dye, S., Douglas, A. J., Russell, J. A. & Bicknell, R. J. (1993). The maintenance of normal parturition in the rat requires neurophypophysial oxytocin. *Journal of Neuroendocrinology* **15**, 7–12.

Moos, F., Ingram, C. D., Wakerley, J. B., Guerne, Y., Freund-Mercier, M. J. & Richard, P. (1991). Oxytocin in the stria terminalis and lateral septum facilitates bursting of hypothalamic oxytocin neurones in suckled rats. *Journal of Neuroendocrinology* **3**, 163–171.

Negoro, H., Uchide, K., Tadokoro, Y., Honda, K. & Higuchi, T. (1987). Vaginal distension induces milk-ejection related burst of oxytocin neurones interacting with suckling stimuli in lactating rats. *Brain Research* **404**, 331–374.

Paxinos, G. & Watson, C. (1986). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, London.

Perlmutter, L. A., Tweedle, C. D. & Hatton, G. I. (1984). Neuronal/glial plasticity in the supraoptic dendritic zone: dendritic bundling and double synapse formation at parturition. *Neuroscience* **13**, 769–779.

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

Poulain, D. A., Wakerley, J. B. & Dyball, R. E. (1977). Electrophysiological differentiation of oxytocin- and vasopressin-secreting neurones. *Proceedings of the Royal Society* B **196**, 367–384.

Richard, P., Moos, F. & Freund-Mercier, M. J. (1988). Bursting activity in oxytocin cells. In *Pulsatility in Neuroendocrine Systems*, ed. Leng, G., pp. 75–97. CRC Press Inc, FL, USA.

Riggs, C. M., Sutherland, R. C. & Wakerley, J. B. (1985). Re-appraisal of the influence of mammary distension on the frequency of milk ejection in the rat. *Journal of Endocrinology* **105**, 127–132.

Summerlee, A. J. S. (1981). Extracellular recordings from oxytocin neurones during the expulsive phase of birth in unanaesthetized rats. *Journal of Physiology* **321**, 1–9.

Sutherland, R. C., Aizlewood, E. S. & Wakerley, J. B. (1986). Changing characteristics of the milk-ejection reflex during pregnancy, lactation and after weaning in the rat. *Journal of Reproduction and Fertility* **76**, 123–130.

Theodosis, D. T. & Poulain, D. A. (1987). Oxytocin secreting neurones: a physiological model for structural plasticity in the adult mammalian brain. *Trends in Neurosciences* **10**, 426–430.

Theodosis, D. T. & Poulain, D. A. (1992). Neuronal-glial and synaptic plasticity of the adult oxytocinergic system: factors and consequences. *Annals of the New York Academy of Sciences* **652**, 301–325.

Theodosis, D. T., Poulain, D. A. & Vincent, J.-D. (1981). Possible morphological basis for synchronisation of neuronal firing in the rat supraoptic nucleus during lactation. *Neuroscience* **6**, 919–929.

Tribollet, E., Clark, G., Dreifuss, J. J. & Lincoln, D. W. (1978). The role of central adrenergic receptors in the reflex release of oxytocin. *Brain Research* **142**, 69–84.

Wakerley, J. B. & Ingram, C. D. (1993). Synchronisation of bursting in hypothalamic oxytocin neurones: possible co-ordinating mechanisms. *News in Physiological Sciences* **8**, 129–133.

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

Way, S. A. & Leng, G. (1992). Relaxin increases the firing rate of supraoptic neurones and increases oxytocin secretion in the rat. *Journal of Endocrinology* **132**, 149–158.

Acknowledgements

This work was supported by a project grant from the AFRC.

Received 24 May 1994; accepted 10 January 1995.