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# ELECTRICAL PROPERTIES OF AXONS AND NEUROHYPOPHYSIAL NERVE TERMINALS AND THEIR RELATIONSHIP TO SECRETION IN THE RAT

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SUMMARY

1. Isolated rat pituitary stalk–neurohypophysial complexes were electrically stimulated and the evoked compound action potentials were recorded at the level of both axons and nerve terminals.

2. The latency of the nerve terminal response increased during continuous stimulation of the stalk at frequencies as low as 1 Hz.

3. At similar frequencies continuous stimulation of the stalk produced an increase in the latency of the response of the nerve fibres and a decrease in the amplitude of the compound action potential.

4. The increase in the latency of the response of both axons and nerve terminals was related to the frequency and number of stimuli.

5. The time necessary for full recovery of the response of the axons and the nerve endings, following stimulation at frequencies above 5 Hz, was not linearly related to the frequency of stimulation.

6. Stimulation of the stalk with a pulse pattern (bursts) imitating the electrical activity of vasopressin-containing magnocellular neurones showed that the latency of the compound action potential had increased by the end of the first burst.

7. The latency of the response of axons and nerve endings was inversely proportional to the time interval between bursts.

8. Prolonged stimulation of the isolated neural lobe with 'vasopressin'-like bursts induced the release of vasopressin. Twelve bursts, separated by 3 min intervals, released more hormone than fifty bursts given during the same period of time, but separated by a 21 s interval.

9. Leu-enkephalin ($10^{-5}$ M) did not modify the latency or the amplitude of the action potentials evoked with low frequency of stimulation (0·5 Hz) or with 'vasopressin'-like bursts.

10. In conclusion, it is suggested that the electrical properties of the nerve fibres and the nerve endings goes some way to explain the pattern of hormone release observed during sustained stimulation.

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## INTRODUCTION

The mechanism of stimulation–secretion coupling, as proposed two decades ago (Douglas & Rubin, 1963; Douglas & Poisner, 1964), implies that, following the arrival at the nerve terminal of action potentials, there is an influx of calcium which gives rise to release of the neurosecretory granule content (for review see Nordmann, 1983). Although the frequency of electrical stimulation of the neural lobe *in vitro* has long been known to be an important parameter for the amount of vasopressin (AVP) and oxytocin (OT) released (Dreifuss, Kalnins, Kelly & Ruf, 1971; Nordmann & Dreifuss, 1972), only recently has the importance of the pattern of stimulation for inducing neurohypophysial hormone release *in vitro* been recognized (Dutton & Dyball, 1979; Bicknell, Flint, Leng & Sheldrick, 1982; Shaw, Bicknell & Dyball, 1984; Cazalis, Dayanithi & Nordmann, 1985). Extensive electrophysiological studies of the magnocellular neurones in the hypothalamus (review by Poulain & Wakerley, 1982) have shown that upon demand for AVP or OT the cells increase their firing rate. Haemorrhage (Poulain, Wakerley & Dyball, 1977), intraperitoneal injection of hypertonic saline (Brimble & Dyball, 1977), occlusion of the carotid (Dreifuss, Harris & Tribollet, 1976) or dehydration (Arnauld, Dufy & Vincent, 1975) give rise to an increase in the frequency of firing of vasopressin-containing cells. From slow tonic firing the cell develops a characteristic phasic pattern. OT-containing cells, recorded from anaesthetized lactating rats, likewise show a specific bursting discharge of action potentials for a period of 0·5–4 s. The frequency of discharge, however, is much greater than for the AVP-containing cells and can be as high as 80 spikes/s (Wakerley & Lincoln, 1973). This high frequency discharge triggers the release of OT which promotes an increase of the intramammary pressure leading to milk ejection.

Increasing evidence obtained from experiments performed on the isolated neural lobe have demonstrated that, within a certain range, the same number of pulses given at a high frequency (50–100 Hz) induces the release of a larger amount of hormone than when delivered at lower frequencies (Dreifuss *et al.* 1971; Nordmann & Dreifuss, 1972). This potentiation of hormone secretion under maintained high frequency stimulation only continues for periods up to approximately 10 s. Recent experiments in which neural lobes were stimulated with bursts of pulses to mimic the firing pattern of the magnocellular neurones have shown that the intraburst firing rate and the interburst intervals are two crucial parameters for promoting hormone release (Cazalis *et al.* 1985). Prolonged stimulation of the neural lobe with a regular (Bicknell, Brown, Chapman, Hancock & Leng, 1984; Cazalis *et al.* 1985) or a phasic pulse pattern (Cazalis *et al.* 1985) gives rise to an increased rate of release which wanes with time even though the stimulus is maintained. Among plausible mechanisms, this decrease could result from a change in the electrical properties of the neurone. In the present study, using an isolated preparation, we have analysed *in vitro* the electrophysiological properties of the axons and nerve endings of the rat hypothalamic magnocellular neurones. Our results strongly suggest that the changes in the rate of neurohypophysial hormone release induced by prolonged electrical stimulation can be correlated with the capacity of the axons and the terminals to conduct action potentials.

## METHODS

The experiments were performed on single isolated pituitary stalk–neurointermediate preparations from decapitated male Wistar rats of 250–280 g body weight. The stalk was sectioned at the level of the median eminence and the stalk–neurointermediate complex was transferred immediately to saline. After removal of the anterior pituitary the preparation was transferred to a chamber formed by a depression in a Sylgard (Dow Corning) surface. Temperature was maintained at $36 \pm 1$ °C (except as indicated) and the isolated system perfused (180 $\mu$l/min) continuously with oxygenated (95 % $O_2$, 5 % $CO_2$) saline. Rapid exchange of the perfused saline was facilitated by maintenance of a small chamber volume (300 $\mu$l). The physiological saline was of the following composition (mM): NaCl, 150; KCl, 5; $CaCl_2$, 2·2; $MgCl_2$, 1·0; glucose, 10; HEPES, 10 adjusted to pH 7·2 with NaOH. Tetrodotoxin (TTX; Calbiochem) was added to saline just prior to use from a more concentrated stock solution.

Electrical activity was recorded simultaneously from nerve (hypophysial stalk) and terminal (neural lobe) regions by separate extracellular bipolar suction electrodes formed from glass pipettes. Application of slight suction following placement of the electrodes facilitated electrical recording from regions under the electrode tip. Electrode tip diameters measured 7–10 $\mu$m and 20–30 $\mu$m for terminal and nerve recordings respectively. The signal was amplified conventionally (Grass P15B), filtered (7200 Hz) and monitored on a storage oscilloscope (Tektronix 5111) from which pictures were taken. Electrical stimulation of the hypophysial stalk was obtained by isolating a portion of the stalk within a bipolar suction electrode and applying 0·4 ms duration current pulses at particular frequencies (see Results). The magnitude of the applied current pulse was set 50 % above the value at which a maximum nerve response was recorded (supra-threshold). The stimulus artifact was reduced at the recording electrodes by placement of a ground electrode formed from flattened silver wire between the stimulating and recording electrodes.

Latencies of the compound action potentials were measured by taking the stimulation artifact as the reference. Most of our study involves the analysis of the change of the latency of response during a train of pulses. Thus, in order to compare these variations among different experiments we defined the following equation:

$$K(t) = 1 - \frac{L_t - L_0}{L_t}, \tag{1}$$

where $L_t$ = latency at time $t$,
$L_0$ = latency at the onset of the stimulus.

Therefore $K(t)$ is inversely proportional to the latency of the compound action potential (c.a.p.).

Hormone release from electrically stimulated isolated neurohypophyses was studied as described in Cazalis *et al.* (1985).

## RESULTS

### *Effects of repetitive stimulation on c.a.p.s*

Fig. 1 illustrates a typical and representative extracellular recording from the neural lobe of a c.a.p. induced by stimulation of the pituitary stalk. In the present work several lines of evidence suggest the c.a.p.s, recorded in this manner, arise from a relatively small number of nerve endings. The evidence includes: (1) presence of a well-defined threshold, (2) short duration ( $< 2$ ms), and (3) lack of an increase in the c.a.p. with increasing stimulus intensity. The c.a.p.s also exhibited, for each recording, a well-defined latency ($7{\cdot}5 \pm 0{\cdot}2$ ms; mean $\pm$ S.E. of mean; $n = 74$). The range of latencies was between 5 and 12 ms. Experiments in which the temperature of the incubating medium was varied showed that the latency of the response increased linearly with a decrease in temperature in the range of 27–37 °C. From these experiments it was calculated that the latency of the response had a $Q_{10}$ of 1·36. The c.a.p.s were abolished in the presence of $3 \times 10^{-7}$ M-TTX.

The latency of the nerve terminal c.a.p. was not altered by maintained stimulation given at frequencies below 1 Hz (see below), but a marked increase of the latency or disappearance of the response was observed at higher frequencies. Fig. 2 shows the effect of increasing the stimulus frequency on the latency of the recorded c.a.p. In this particular case, the terminal(s) responded to stimuli given at 0·5–1·0 Hz throughout the 15 s stimulation period. At frequencies of 5 Hz or above, however,

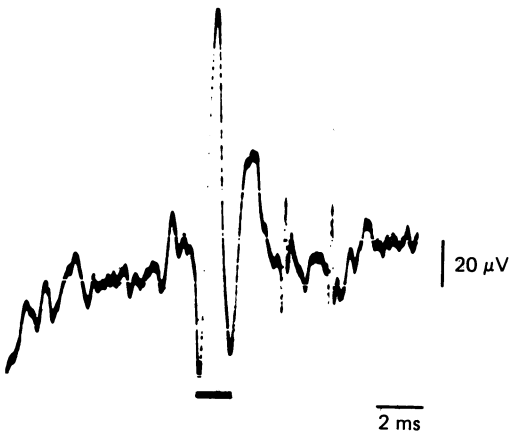


Fig. 1. Extracellularly recorded c.a.p. from neurosecretory nerve endings of the rat neurohypophysis. The pituitary stalk was stimulated with a current pulse of 0·4 ms duration at a magnitude set 50% above the value at which a maximum nerve c.a.p., as indicated by the heavy bar, was recorded.

the latency of the c.a.p.s increased and the terminals failed to respond after very few stimuli (see Figs. 5 and 6 for the mean of the results). Furthermore, the c.a.p. could not be observed even when the stimulus intensity was increased. Note that in these particular recordings, a single pulse given after the offset of the trains of stimuli, shows that recovery of the response is nearly complete 15 s after the cessation of the stimulus. The mean time for complete recovery in all our experiments was, however, longer (see Figs. 7 and 8).

In order to analyse the relationship between nerve conduction and the recorded terminal c.a.p., an additional recording electrode was placed on the pituitary stalk between the stimulating electrode and the electrode focused on the neurohypophysis. Fig. 3 illustrates both axon and terminal c.a.p.s during a 2 min train of pulses given at 13 Hz. Two effects should be noted. First, the latency of response of both the axons and the terminal(s) was increased with stimulus durations as short as 15 s. The latency increases further with the duration of the stimulus with the terminal c.a.p. being abolished before completion of the 2 min stimulation. Secondly, the amplitude of the axonal c.a.p. also showed a large decrease during stimulation. Both terminal and axonal responses showed recovery after the cessation of stimulation. Full recovery of the latency and of the amplitude, however, required *ca.* 5 min. Fig. 4*A* illustrates further the decrease of the amplitude and the increase of the latency of the axonal c.a.p. during a train of pulses delivered at 13 Hz for a period of 1 min.

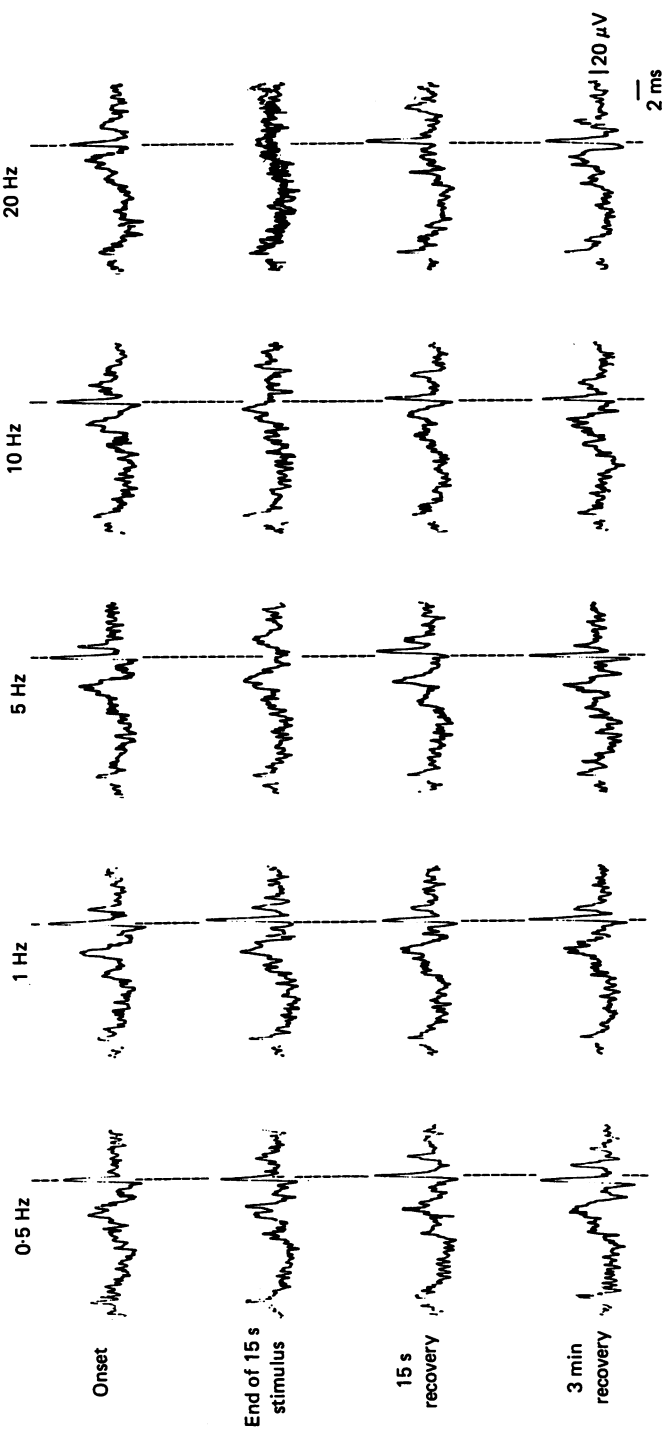


Fig. 2. Extracellularly recorded c.a.p.s from neurosecretory nerve endings stimulated, via the pituitary stalk, at different frequencies for a period of 15 s. A single stimulus, applied either 15 s or 3 min after the offset of the 15 s stimulation period provides a measure of recovery. The dashed lines provide a reference for the latency of later responses to that of the first stimulus. Note that for this particular ending an increased latency was observed after 15 s of stimulation at 1 Hz. The superimposed traces at 20 Hz (second from top) were taken 4 and 15 s after the onset of stimulation.

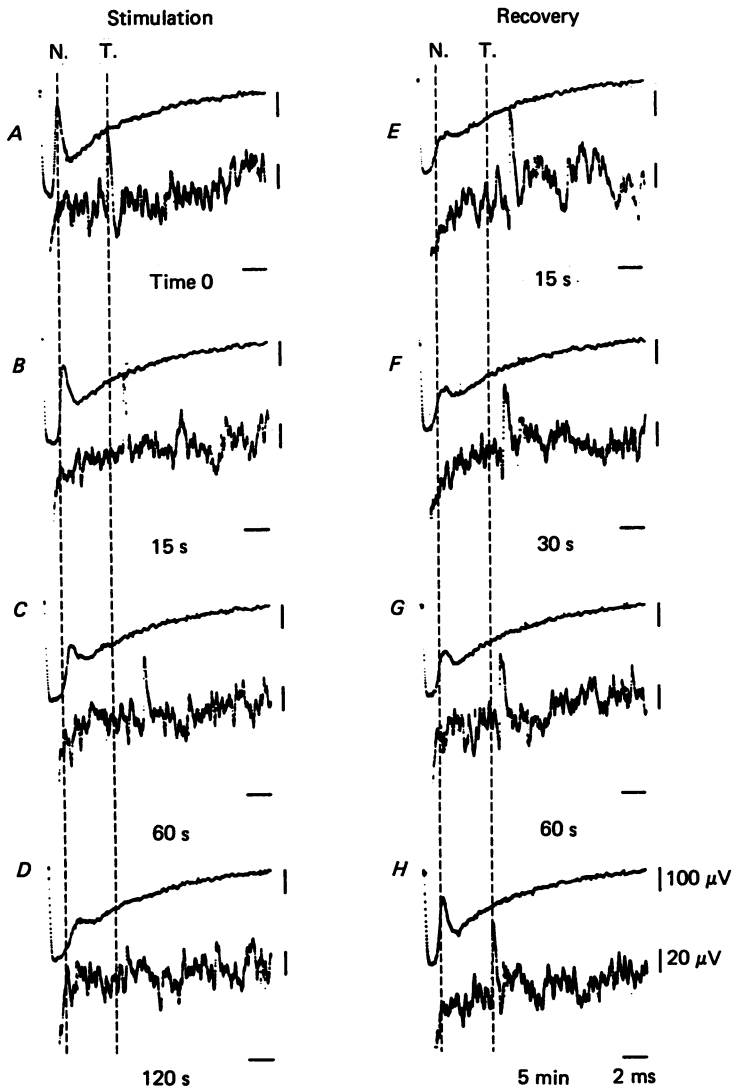


Fig. 3. Simultaneous extracellularly recorded c.a.p.s from the pituitary stalk and from neurosecretory nerve endings during and after stimulation of the axons. Stimuli were delivered at 13 Hz for a period of 120 s. Recovery was tested by applying a single pulse after 15, 30, 60 and 300 s. The dashed lines provide a reference for the latency of the responses to that of the first stimulus for both the nerve trunk (N.) and the terminals (T.). Note the increased latency of the nerve trunk (top trace) and of the terminal (bottom trace) responses during stimulation and the decrease of the amplitude of the axonal c.a.p. Full recovery occurs 5 min after the cessation of the maintained stimulation.

The time course of the recovery of the response is shown in Fig. 4*B*. Note that approximately 3 min was necessary for complete recovery.

Quantification of the changes in latency with stimulus frequency is shown in Fig. 5. The latency of the response has been converted into a function, $K(t)$, which is inversely proportional to latency (see Methods). Stimulation at 0·5 Hz resulted in no

change of $K(t)$ in either the axons (Fig. 5*A*) or terminals (Fig. 5*B*) during stimulus periods over 11 min. However, a significant decrease of $K(t)$ ($P < 0{\cdot}001$) (i.e. latency increase) occurs with stimuli delivered at frequencies as low as 1 Hz. A decrease in the amplitude of the c.a.p. occurs concomitantly with a decrease of $K(t)$ at stimulus frequencies greater than 1 Hz. Fig. 6 illustrates the time course of the decrease in

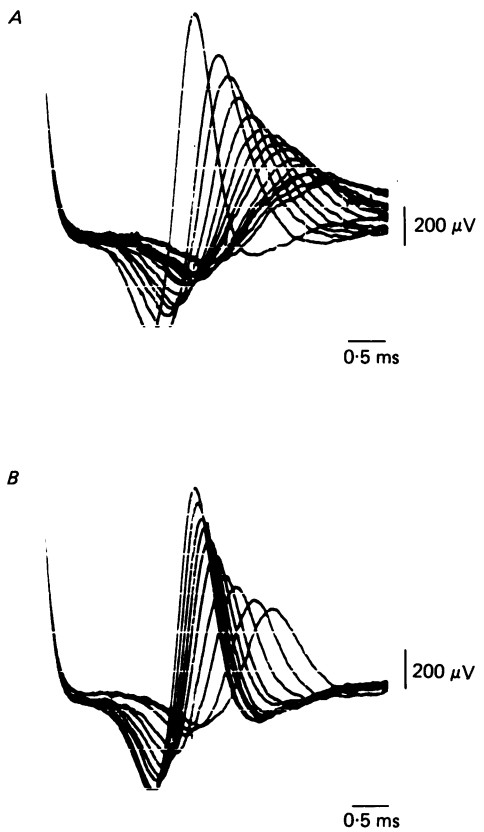


Fig. 4. Effect of maintained electrical stimulation on the pituitary stalk c.a.p. *A*, response to a stimulus given at 13 Hz for a period of 60 s. The traces were taken at the onset of the stimulus (left) and then every 4 s. *B*, recovery of the axonal c.a.p. A single pulse was given (from right to left) every 15 s for a period of 120 s and then 180 and 300 s after the cessation of stimulation.

amplitude of the c.a.p. recorded from the pituitary stalk for stimulation at various frequencies. The values have been normalized: the amplitude of the action potential induced by the first of a train of pulses was taken as unity. The observed decrease of the amplitude during a train of stimuli does not result from a spread of the compound potential since the area of the response was also decreased (Fig. 3; see Baertschi & Dreifuss, 1979).

The time necessary for the complete recovery of the $K(t)$, the inverse latency function, of the axonal and terminal responses following stimulation for different lengths of time at different frequencies is shown in Figs. 7 and 8. The results show

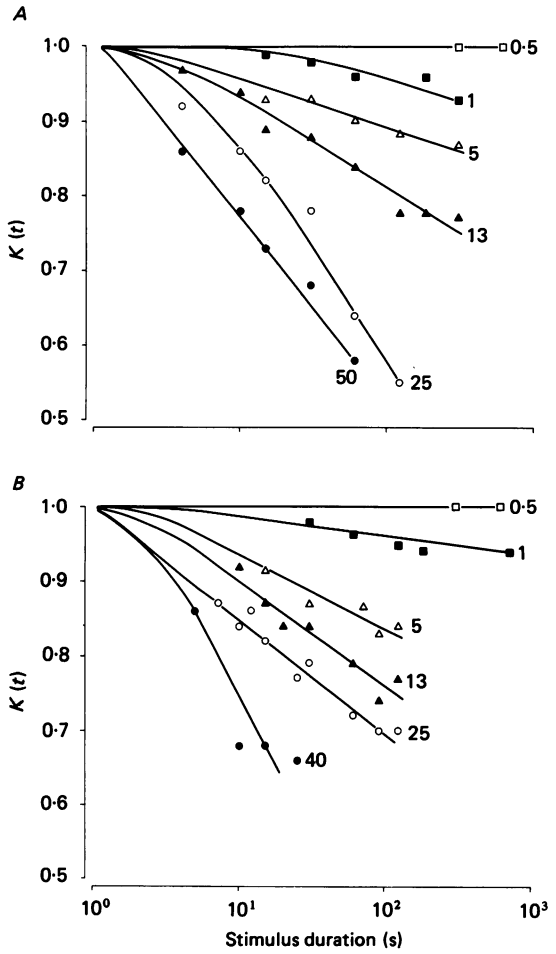


Fig. 5. Effect of stimulus trains on the latency of the response of the pituitary stalk and of the nerve endings. The latency has been transformed and normalized to give the inverse latency function $K(t)$ (see Methods). *A*, axons; *B*, nerve endings. The pituitary stalk was stimulated for different periods at the indicated frequencies (in Hz). Each value corresponds to the mean of seven to twelve experiments. The standard error of the mean varied between 0 and 11 % of the values given on the Figure.

that the time necessary for full recovery was not related to the duration of the stimulation period. This was observed when the pituitary stalk was stimulated over the frequency range of 1–50 Hz for periods of 15–120 s. It is worth pointing out that for frequencies equal to or below 1 Hz, the time necessary for full recovery, even after prolonged stimulation, was much shorter (Figs. 6 and 7*D*).

In another series of experiments, the stalk–neural lobe complex was stimulated with a constant number of pulses (350, 700, or 2100) applied at different frequencies. The $K(t)$ values of the response of the nerve trunk and the terminals are compared in Fig. 9. Stimulation at frequencies as low as 1 Hz for a total of 350 pulses or more (see Fig. 9) gave rise to a significant decrease of $K(t)$, the inverse latency function,

for both the nerve and the terminals. Interestingly, the value of $K(t)$ observed for 350 s of stimulation at 1 Hz did not decrease further with a longer period of stimulation. This was not the case for higher frequencies. Stimulation at 13 Hz to give a total of 350 pulses (Fig. 9*A*) reduced $K(t)$ of the response recorded from the pituitary stalk much less than did 2100 pulses (Fig. 9*E*). This is consistent with the

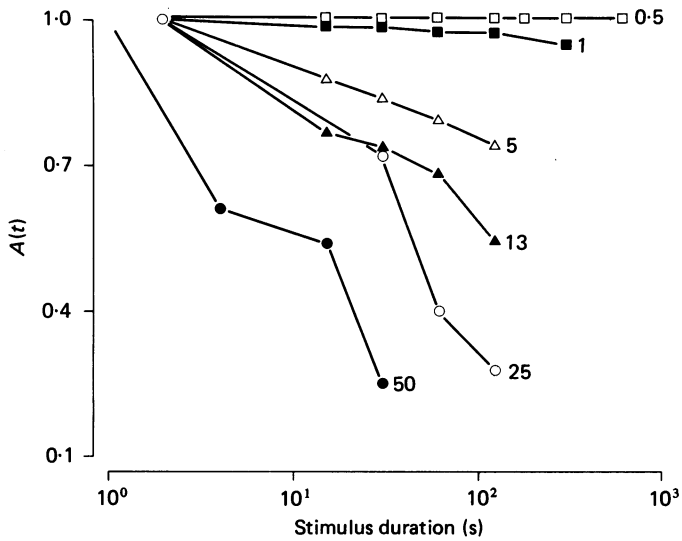


Fig. 6. Effect of stimulus duration on the c.a.p. amplitude ($A(t)$) recorded from the pituitary stalk. The axons were stimulated at different frequencies for different lengths of time. The amplitude of the response has been normalized by taking as a unit the amplitude of the c.a.p. from the first stimulus. The stimuli were delivered at the indicated frequencies. The results represent the mean of six to twelve experiments. The standard error of the mean varied between 0 and 2% of the values given in the Figure.

results of Fig. 5 in which the changes of $K(t)$ of the axons and the terminals were analysed during stimulation at different frequencies for different periods of time. Note that in this experiment recovery following a train of 2100 pulses given at 13 Hz (Fig. 9*E*, *F*) was faster for the nerve trunk than for the terminals.

In the majority of our experiments utilizing maintained stimulation, we observed first the disappearance of the terminal response, followed by that of the pituitary stalk. In a few instances, however, the terminal response was maintained during a train of pulses in which a marked decrease of the amplitude of the nerve stalk c.a.p. had occurred (see Fig. 11). Comparison of the $K(t)$ values for both the nerve and the terminal responses of each individual experiment gave a correlation coefficient of 0·80 ($n = 350$; $P < 0{\cdot}001$). This suggests that failure to respond to a stimulus at the level of the axon can be correlated with a failure in the terminal response.

*Effects of interburst interval on c.a.p.s in response to patterned stimulation*

A series of experiments were performed in which the stalk was stimulated with bursts of pulses of a pattern similar to that recorded from vasopressinergic cells of

rats following activation by dehydration or haemorrhaging (an 'AVP' burst, see Cazalis *et al.* 1985). Recordings from terminals during six successive bursts, separated by 21 s intervals, show that, by the end of the first burst, there had been a significant decrease of $K(t)$, the inverse latency function (Fig. 10). Although recovery occurred, it was not complete within the 21 s interburst interval. By the end of the sixth burst,

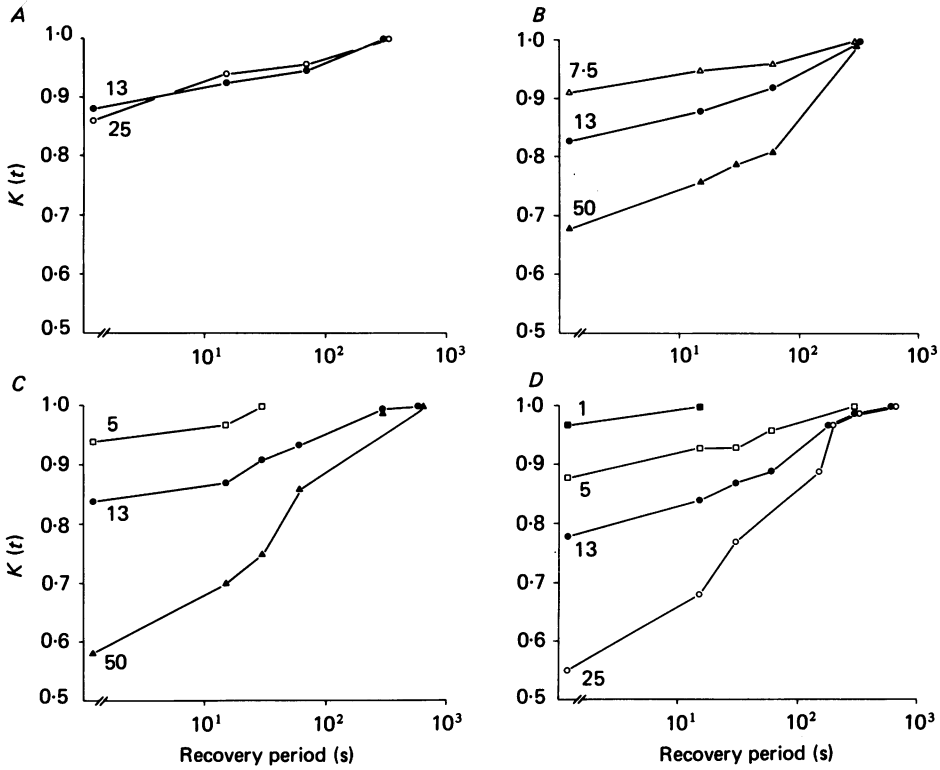


Fig. 7. Time course of the recovery of the inverse latency function $K(t)$ of the pituitary stalk following maintained stimulation. The axons were stimulated (in Hz) for (s): 15 (*A*), 30 (*B*), 60 (*C*), and 120 (*D*) at indicated frequencies. Recovery following maintained stimulation was tested by stimulating the nerve trunk with a single pulse delivered at different times. The latency of the c.a.p. has been transformed into the function $K(t)$ as described in Methods. Each value corresponds to the mean of five to twelve experiments.

$K(t)$ had decreased to 75% of its original level. Table 1 shows that the silent period between the bursts (i.e. interburst interval) is a key event for the recovery of the axon and terminal electrical responses. At the onset of the sixth burst the mean value of $K(t)$ of the nerves was 0·69 when the bursts were delivered without intervals; this value rose to 0·98 when the bursts were separated by 40 s intervals. Similar results were obtained for the nerve terminals. At the end of the sixth burst, $K(t)$ had a value of 0·69 when the bursts were given without silent intervals whereas this value was 0·87 when 40 s intervals separated the bursts of pulses.

In the majority of the preparations stimulated with the 'AVP' burst pattern (21 s interburst interval), a failure of the terminal c.a.p. occurred after only a few bursts.

However, Fig. 11 illustrates an experiment in which after twenty bursts separated by 21 s silent periods most of the axons failed to respond but the terminal(s) still showed a well-defined c.a.p. This strengthens the above-mentioned data showing that there is a variability in the capacity of the nerve fibres and the terminals to respond to stimuli given at different frequencies for different periods of time.

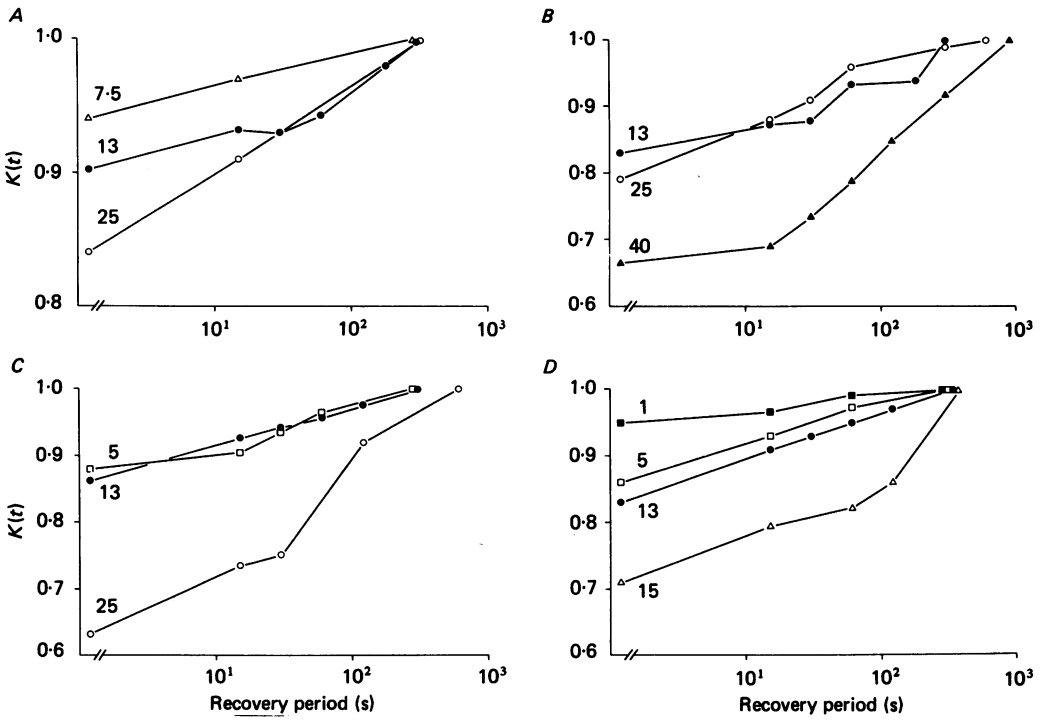


Fig. 8. Time course of the recovery of the inverse latency function $K(t)$ of the neurohypophysial nerve endings following stimulation of the pituitary stalk. The axons were stimulated for (s): 15 (*A*), 30 (*B*), 60 (*C*), and 120 (*D*) at the indicated frequencies (Hz). Recovery was measured as described in the legend of Fig. 7. The latency was transformed according to eqn. (1) (Methods). Each value corresponds to the mean value of five to twelve experiments.

*Effects of interburst interval on AVP release in response to patterned stimulation*

In view of the results described above, concerning the recovery of the axonal and terminal responses as a function of the duration of the silent period between bursts (Table 1), we looked at hormone release under similar conditions. Isolated rat neural lobes were stimulated electrically with 'AVP' bursts for a total period of 40 min. The bursts were separated by intervals of different duration. Fig. 12*A* shows the total evoked AVP release after 40 min of stimulation for each treatment. While with an interburst interval of 180 s the total number of bursts given was much smaller (twelve) than the number delivered with 21 s intervals (fifty), they induced more hormone release. This is best illustrated in Fig. 12*B* in which the mean amount of AVP released per burst is plotted as a function of the duration of the silent period.

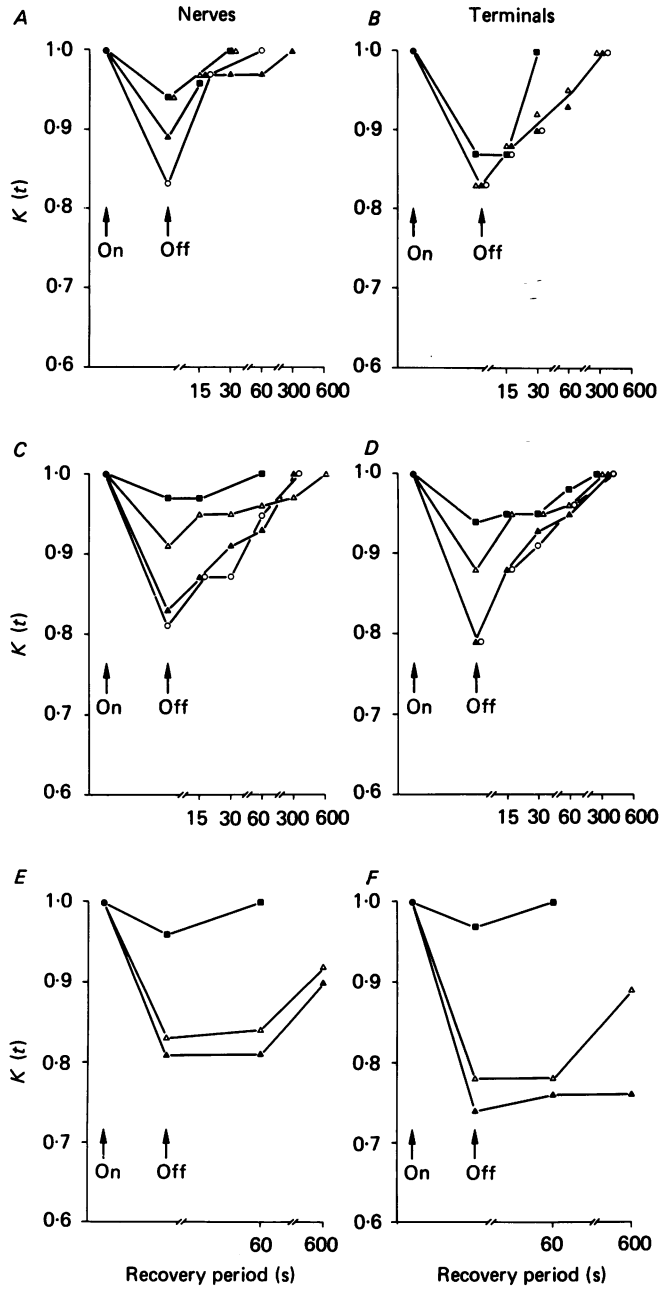


Fig. 9. Comparison of $K(t)$ of c.a.p.s of the nerve trunk and the neurohypophysial nerve endings. The pituitary stalk was stimulated with 350 (*A* and *B*), 700 (*C* and *D*) and 2100 (*E* and *F*) pulses delivered at frequencies of (Hz): 1 (■), 5 (△), 13 (▲), and 25 (○). The arrows indicate the onset and end of the stimulus. The latency of response of the nerve trunk (*A*, *C* and *E*) and of the nerve endings (*B*, *D* and *F*), has been transformed according to eqn. (1). Recovery of the response was tested by giving a single pulse at different times after the end of the stimulus.

The results demonstrate that for hormone release the length of the interburst interval is of extreme importance for inducing optimum hormone secretion.

*Failure of Leu-enkephalin to modify electrical responses*

Finally, controversial results (for review see Table 2 in Nordmann, Dayanithi & Cazalis, 1986) have been published recently concerning the role of opiates on the release of neurohypophysial hormones. In five experiments we tested if Leu-

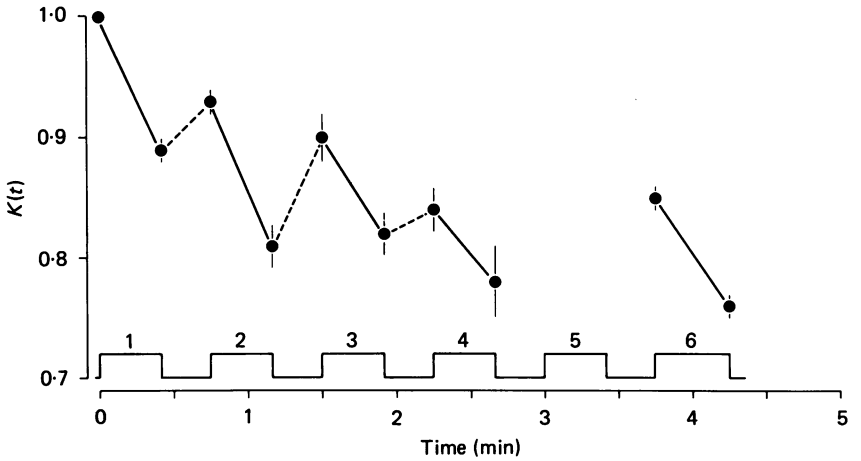


Fig. 10. Changes in the inverse latency function $K(t)$ of a neurosecretory nerve terminal (c.a.p.) following stimulation of the pituitary stalk with 'AVP' bursts separated by 21 s interburst intervals. The response latency was transformed according to eqn. (1). Note that $K(t)$ was always smaller at the end than at the onset of a burst. The results represent the mean of six to sixteen experiments (±S.E. of mean). The bursts were delivered as indicated in the lower part of the Figure.

enkephalin modified the terminal c.a.p. resulting from electrical stimulation of the pituitary stalk. The preparations were stimulated for 45 min at 0·5 Hz and perfused continuously with $10^{-5}$ M-Leu-enkephalin during the last 25 min of stimulation. In another series of experiments ($n = 4$) the stalk was stimulated with a series of ten 'AVP' bursts separated by 21 s intervals. After a resting period of 10 min the preparation was perfused with $10^{-5}$ M-Leu-enkephalin and stimulated with a series of 'AVP' bursts. In neither experiment did Leu-enkephalin modify either the amplitude or the latency of the c.a.p. as compared to controls.

## DISCUSSION

The experiments presented here establish the ability of the axons and nerve terminals of the magnocellular neurones to repetitively conduct action potentials at the frequencies and durations observed in somata recordings under normal conditions. Under conditions of physiological demand for OT and AVP the somata show an increase in firing frequency accompanied by a patterning of spikes into bursts (review by Poulain & Wakerley, 1982). The present work demonstrates that both the axons

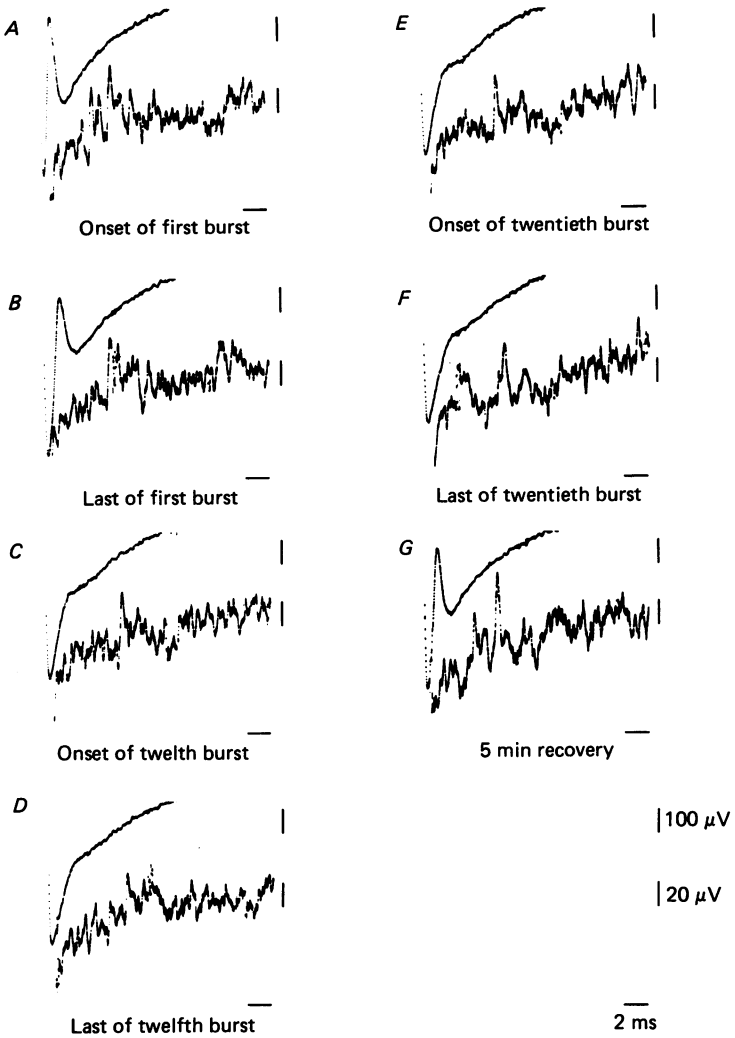


Fig. 11. Effect of stimulation with 'AVP' bursts on the pituitary stalk and neurosecretory nerve terminal c.a.p.s. The axons were stimulated with a series of twenty bursts delivered without an interburst interval. Note that in this experiment, at the end of the stimulation the response of the axons (top) has almost disappeared whereas the response of the nerve endings could still be detected. Full recovery (*G*) occurred 5 min after the end of burst stimulation.

and the neurohypophyseal nerve terminals fail to conduct action potentials for long periods of time at mean frequencies similar to those found within the AVP bursts or within the OT discharge. Further, a reduction in amplitude of both nerve and terminal c.a.p.s was found with maintained stimulation as low as 1 Hz. Dreifuss *et al.* (1971) and Baertschi & Dreifuss (1979) described similar results on *in vivo* preparations, where the amplitude of the antidromically driven nerve c.a.p. was reduced with low frequency stimulation. Similarly, Pittman (1983) observed an

TABLE 1. $K(t)$ of terminal c.a.p.s after six bursts not separated or separated by interburst intervals of different duration

| | Interburst interval (s) | $K(t)$* | |
|---|---|---|---|
| | | Nerve | Terminal |
| First burst on | | 1·0 | 1·0 |
| Sixth burst on | 0 | 0·69 | 0·73 |
| | 21 | 0·88 | 0·87 |
| | 40 | 0·98 | 0·92 |
| Sixth burst off | 0 | 0·69 | 0·65 |
| | 21 | 0·75 | 0·74 |
| | 40 | 0·87 | 0·85 |

* See Methods.

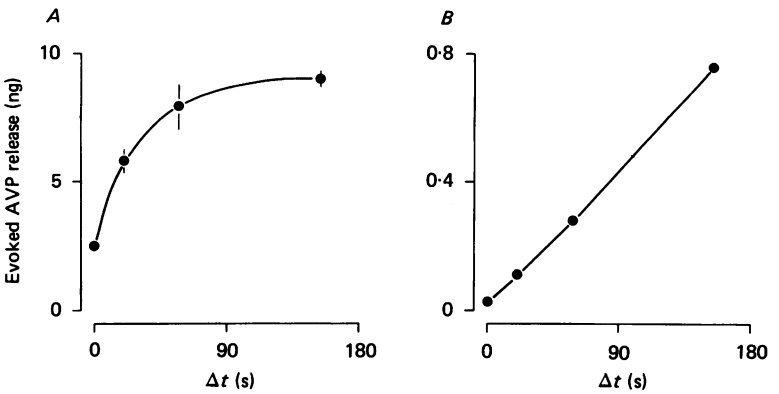


Fig. 12. The effect of silent intervals between bursts on the amount of AVP release. Neural lobes were stimulated with 'AVP' bursts for a total period of 40 min. The bursts were not separated or separated by intervals of $\Delta t$ duration. *A*, evoked AVP release; *B*, average amount of AVP released by one burst as a function of the interburst silent intervals. Evoked release was calculated by subtracting the mean basal release determined in the first fractions preceding the onset of the stimulus from the amount of horn one found in each fraction. Results are given as mean ±S.E. of mean.

increased antidromic latency of the c.a.p. recorded from rat magnocellular neurones following stimulation of the pituitary stalk at different frequencies. It is noteworthy that the magnocellular neurones, under normal conditions, exhibit a mean frequency of firing of 0·5–2 Hz (review by Poulain & Wakerley, 1982). The present data suggest that, at these frequencies, there could be a maintained release of hormone, and hence a maintained plasma hormone concentration.

*Characterization of axonal and terminal c.a.p.s*

Stimulation of the pituitary stalk at low frequencies gives rise to a well-defined nerve c.a.p., which, as observed by others (Dreifuss *et al.* 1971; Nordmann & Dreifuss, 1972), was blocked by exposure of the preparation to TTX. Whereas previous attempts have recorded electrical activity from very large areas of the neurohypo-

physis (Dreifuss *et al.* 1971; Nordmann & Dreifuss, 1972), we have used small-sized suction electrodes in order to record from a discrete area of the neural lobe. It is, however, difficult to know if the recorded c.a.p. represented the activity of one or several units. By empirical reasoning it is unlikely that an electrode with a tip diameter of 7–10 $\mu$m would record extracellularly the activity of a single nerve ending which has a mean diameter of 1·6 $\mu$m (Nordmann, 1977). However, in the majority of our experiments, each terminal c.a.p. had a defined threshold and an amplitude which could not be increased further by higher intensities of stimulation. The 'all or none' response suggests, but does not prove, that it occurs from a small group of endings, all belonging to the same neurone. It is worth mentioning that one single magnocellular neurone gives rise, in the neural lobe, to an average of 1840 endings. Thus the above explanation seems reasonable.

The mean latency, from the stimulus artifact to the development of the nerve c.a.p., of 7·2 ms is in agreement with the work of others who recorded *in vivo* the compound field potential of the supraoptic nucleus (Dreifuss & Kelly, 1972) or of the pituitary stalk (Baertschi & Dreifuss, 1979) evoked by stimulation of the pituitary stalk or the neurohypophysis respectively. Assuming a mean distance of 3 mm between the stimulating and recording electrodes, the conduction velocity for the hypothalamo-neurohypophysial tract is about 0·4 m/s. Such a velocity is in agreement with that found by others on peripheral mammalian fibres (Brown & Holmes, 1956; Ritchie & Straub, 1956).

*Effects of physiological pattern of stimulation*

In the present study, simultaneous recordings of c.a.p.s from axon and terminal were elicited with a pattern of stimulation similar to that encountered *in vivo*. The pattern used mimicked the bursting activity of vasopressinergic neurones which occurs during haemorrhage (Poulain *et al.* 1977) or dehydration (Wakerley, Poulain & Brown, 1978). The major findings are: (1) There is a progressive decrease of $K(t)$ (increase of latency) throughout the period of stimulation by repeated bursts; the decrease of $K(t)$ is apparent at the end of a single burst. (2) The duration of the interburst silent period is crucial for the recovery of the axonal and terminal electrical responses. These results are in parallel with those found for hormone release from neural lobes stimulated *in vitro* (Cazalis *et al.* 1985). The latter experiments found that hormone output induced with four AVP bursts was much larger when the bursts were separated with silent periods than when they were delivered without silent periods. Here we report that prolonged stimulation of the neural lobe with 'AVP' bursts separated by 60 or 180 s interburst intervals induces more hormone release than does a greater number of bursts delivered during the same period of time but with 21 s interburst intervals. These results and those of others (Dutton & Dyball, 1979; Bicknell *et al.* 1984; Cazalis *et al.* 1985) can be explained by our electrophysiological data which show that the recovery of $K(t)$, the inverse latency function, is more pronounced when bursts are delivered with silent periods of 60 s duration. Surprisingly, the results for both the release experiments and the *in vitro* electrophysiological recordings suggest that the average duration of the *in vivo* intervals (20–25 s) between 'AVP' bursts (for review see Poulain & Wakerley, 1982) is not optimum because prolonged stimulation with AVP bursts separated with 60

or 180 s interval periods (which are rarely observed *in vivo*) released more AVP and OT per pulse than did stimulation with silent periods of 21 s (Fig. 12).

In the present study we found that few axons and nerve terminals rapidly failed to respond to stimuli given at frequencies as low as 5 Hz (Figs. 5 and 6). The number of axons and terminals which failed to respond to the stimulation was correlated with the increase of the frequency of stimulation. In their electrophysiological study Leng & Wiersma (1981) applied the 'constant collision stimulation' to the neural stalk for periods of 10–15 min. Their results show that under such circumstances most of the cells in the supraoptic nucleus were antidromically activated. These results are, however, not in contradiction with those reported here for a magnocellular neurone is likely to have a mean firing rate smaller than 5 Hz (Poulain & Wakerley, 1982). An attractive but purely hypothetical idea would be to know if in the hypothalamo-neurohypophysial system, the proximal part of the axons can, compared to its more distal part, conduct action potentials for a longer period of time at a rather high frequency.

The decrease in the c.a.p. of the axons and the change in the latency of response of the nerve fibres and the terminals with stimulation at frequencies of 40–50 Hz also deserve comment. Unit recordings of hypothalamic cells in anaesthetized, lactating rats have shown that periodically (every 5–10 min) the OT cells discharge action potentials for a period of 0·5–4 s at frequencies which can reach 80 spikes/s (Wakerley & Lincoln, 1973). Our results show that at high frequencies, a large number of fibres failed to respond after only a few seconds of stimulation. If the electrical membrane properties of the cell bodies are similar to those of the axons and the terminals, then our results may explain the short duration of the discharge of action potentials recorded from oxytocinergic fibres.

Finally we found that the opioid peptide Leu-enkephalin had no effect on the latency and the amplitude of the evoked c.a.p. of the stalk and the terminals. The effect of this molecule on the release mechanism at the level of the nerve endings has been and is still the subject of a large debate (see Table 2 in Nordmann *et al.* 1986). The present study and the data reported on AVP and OT release from the isolated neural lobe (Nordmann *et al.* 1986) would suggest that Leu-enkephalin acts possibly centrally on one of the mechanisms which leads to hormone release from the neurohypophysis.

In conclusion, large numbers of axons and neurohypophysial nerve terminals fail to conduct action potentials for prolonged periods at mean frequencies found to occur during AVP bursts or OT discharge. The results give insight into the mechanisms which lead to decreased hormone release upon maintained electrical stimulation. They emphasize the need for using a phasic pattern of stimulation delivered at low frequencies when neurohypophysial hormone release is studied *in vitro*.

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