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LATERAL HYPOTHALAMIC NEURONES: OSMOSENSITIVITY AND THE INFLUENCE OF ACTIVATING MAGNOCELLULAR NEUROSECRETORY NEURONES

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SUMMARY

1. The activity of single cells in the supraoptic nucleus and the lateral hypothalamus of urethane-anaesthetized rats was studied during electrical stimulation of the neural stalk.

2. Neurones activated antidromically from the neural stalk were either unaffected or, rarely, slightly affected by single shocks or by brief trains of shocks presented at an intensity just below that necessary for antidromic invasion. Thus there does not appear to be a strong synaptic coupling between rat supraoptic neurones.

3. In contrast, many lateral hypothalamic neurones, and particularly those in the perinuclear zone, were strongly affected by neural stalk stimulation, being either orthodromically excited or inhibited. Thus, rat magnocellular neurosecretory neurones appear to project, directly or indirectly, to the lateral hypothalamus.

4. Thirty-one lateral hypothalamic neurones were studied following an I.P. injection of 1 ml. 1·5 M-NaCl. Eighteen neurones responded to this osmotic stimulus with a change of at least 1 spike/sec in mean firing rate.

5. Fifteen of the thirty-one neurones were strongly affected by neural stalk stimulation (eight inhibited, seven excited). Ten of these fifteen neurones were affected in the same way by osmotic stimulation. The remaining sixteen out of thirty-one neurones were unresponsive to neural stalk stimulation, and of these, ten were also unresponsive to osmotic stimulation. Thus most lateral hypothalamic neurones responded in a similar way to neural stalk stimulation and to systemic osmotic stimulation.

6. Thus the osmosensitivity of some neurones in the lateral hypothalamus may be mediated by synaptic input arising from the magnocellular neurosecretory neurones. However, the osmosensitivity of some lateral hypothalamic neurones cannot be explained in this way, and probably derives from osmoreceptors other than the magnocellular neurones.

INTRODUCTION

The magnocellular neurosecretory cells of the supraoptic and paraventricular nuclei have in the past been regarded primarily as 'output' neurones. However, these cells may be interconnected (Léránth, Zaborsky, Marton & Palkovits, 1975) and they

project extensively to neural targets within the C.N.S. as well as to the neurohypophysis (Sofroniew, 1980). The function of these central projections is not established.

In recent experiments with an *in vitro* brain slice preparation, Mason (1980) demonstrated that rat supraoptic neurones were depolarized by small increases in the osmolarity of the extracellular medium. These results support the suggestion that supraoptic neurones are osmoreceptors (Jewell & Verney, 1957; Leng, 1980). In addition to a direct effect upon the cell resting potential, Mason (1980) observed that an increase in extracellular osmolarity also produced an increase in the excitatory synaptic input to the supraoptic neurones, and he suggested that this reflected a synergistic osmoreceptive mechanism as a consequence of synaptic coupling between supraoptic neurones. There is morphological evidence of interconnection between supraoptic neurones (Léránth *et al.* 1975) and electrophysiological evidence of some interaction (Leng, 1981*a*) possibly mediated by vasopressin (Krisch, 1979; Leng & Wiersma, 1981) but there is no direct evidence of excitatory interaction. The present study sought such evidence by recording the discharge of antidromically identified supraoptic neurones during electrical stimulation of the neural stalk.

The lateral hypothalamic region adjacent to the supraoptic nucleus is known to contain osmosensitive neurones (Hayward & Vincent, 1970; Vincent, Arnauld & Nicolescu-Cartagi, 1972; Weiss & Almli, 1975). The origin of these osmotic responses is not established. It is possible that some neurones in the perinuclear zone adjacent to the supraoptic nucleus are osmoreceptors (Hayward & Vincent, 1970). Alternatively, the osmosensitivity of lateral hypothalamic neurones may derive from synaptic input from magnocellular osmoreceptors. The present study examined the extent and nature of the synaptic communication from magnocellular neurones to lateral hypothalamic neurones, by recording the discharge of lateral hypothalamic neurones during electrical stimulation of the neural stalk. These responses were compared with the responses of the same neurones to systemic osmotic stimulation.

METHODS

Female Wistar rats of about 300 g were anaesthetized with urethane (ethyl carbamate; 1·25 g/kg body weight, I.P.). The hypothalamus was approached from the ventral surface, and the neural stalk and the supraoptic region were exposed separately (Leng, 1981*b*). The activity of single neurones was recorded extracellularly with glass micropipettes (tip diameter ~ 1 μm; impedance 15–30 MΩ) filled with 2% pontamine sky blue in 0·5% sodium acetate (Hellon, 1971). Supraoptic neurones fulfilled the constant latency and collision tests for antidromic invasion from the neural stalk. Lateral hypothalamic neurones were not activated antidromically by currents of 2 mA peak-to-peak. At the end of each experiment dye was expelled from the micropipette by ionophoresis (2–5 μA tip negative, for 10–15 min) and the brain was perfused with 10% formal saline. The final recording position was established histologically in 30 μm frozen sections stained with neutral red.

Stimulation techniques

A bipolar stainless steel stimulating electrode (Rhodes Medical Instruments, SNE 200) was placed upon the neural stalk. Stimuli were matched biphasic pulses of 1 msec duration programmed via a Devices 'Digitimer'. Post-and peristimulus time histograms and inter-spike interval histograms (300 × 1 msec bins for 8 min) of neural activity were constructed on-line with a PDP8/a computer.

Single shock studies. Post-stimulus time histograms were constructed of the activity of supraoptic neurones following 200 shocks to the neural stalk. Shocks were presented (*a*) regularly at 1 Hz, at an intensity either well above or just below the threshold for antidromic invasion or (*b*) at a

suprathreshold intensity, but programmed to follow 1 msec after a spontaneous action potential, so that the stimuli failed, through collision, to invade the recorded neurone antidromically.

Control histograms were also constructed of the activity following spontaneous action potentials in the absence of neural stalk stimulation. In some experiments stimuli were presented at 4 Hz for 300 sec.

Trains of shocks. Repeated trains of shocks were applied to the neural stalk at an intensity just below that which resulted in antidromic invasion of the recorded neurone (15 Hz or 40 Hz trains; 400 msec duration; 200 presentations at 2 sec intervals). Non-antidromically activated neurones in the lateral hypothalamus were tested with longer trains (15 Hz trains; 5 sec duration; fifteen presentations at 20 sec intervals; 0·25–1 mA peak-to-peak).

Systemic osmotic stimulation

In each experiment one cell was selected for study during osmotic stimulation. After characterizing the cell's response to neural stalk stimulation, and after at least 15 min without further stimulation, 1 ml of 1·5 M-NaCl was injected I.P. over 1 min. The discharge activity from 10 min before the injection to 25 min after the injection was recorded with a timer/counter-printer. The inter-spike interval histogram obtained before the hypertonic injection was compared with a histogram obtained 15–25 min after the injection. In some experiments a second neurone was tested with an injection of 1 ml. of 0·15 M-NaCl.

Plasma osmolarity at the end of experiments was determined by the depression of freezing point method (Knauer semi-micro osmometer) from 1 ml. cardiac blood samples.

RESULTS

From forty-eight rats, 287 cells were recorded, including 123 supraoptic neurones. The histology confirmed that the remaining cells lay within the hypothalamus, 1·6–2·4 mm lateral to the mid line, between stereotaxic co-ordinates A4·6 and A6·0 (König & Klippel, 1963), and within 1·5 mm of the ventral surface of the brain. Thresholds for antidromic invasion of supraoptic neurones lay between 0·1 and 2 mA: more than 75% (100 out of 123) of supraoptic neurones were antidromically invaded at current intensities of 1 mA or less.

Single-shock studies. For each of forty-three supraoptic neurones, including eight 'phasic' neurones (Wakerley & Lincoln, 1971), antidromic invasion was followed by a period of inactivity whose mean duration varied between 20 and 100 msec. However, for each cell a comparable period of inactivity was found to follow spontaneous action potentials, whether or not the action potentials were followed immediately by a shock to the neural stalk (Fig. 1). This period of inactivity reflected the spontaneous pattern of inter-spike activity as revealed by the relevant inter-spike interval histogram. In no supraoptic neurone did stimulation at a maximal subthreshold intensity evoke any response that was detected in the averaged response to 200 shocks. Thus these experiments produced no evidence for interaction between supraoptic neurones.

Trains of shocks. Forty supraoptic neurones, including fifteen phasic neurones, were tested with trains of shocks at 15 Hz and at a maximal subthreshold intensity. The firing rate during stimulation was altered by at least 20% in only four cells, two of which were inhibited and two excited. The remainder (thirty-six out of forty cells: 90%) were less affected or were unaffected. Stimulation at 40 Hz was slightly more effective in eliciting orthodromic responses. Of twenty-five supraoptic neurones, two were inhibited by 20% or more and five excited by 20% or more during 40 Hz stimulation. The repeated application of trains of shocks had no progressive effect

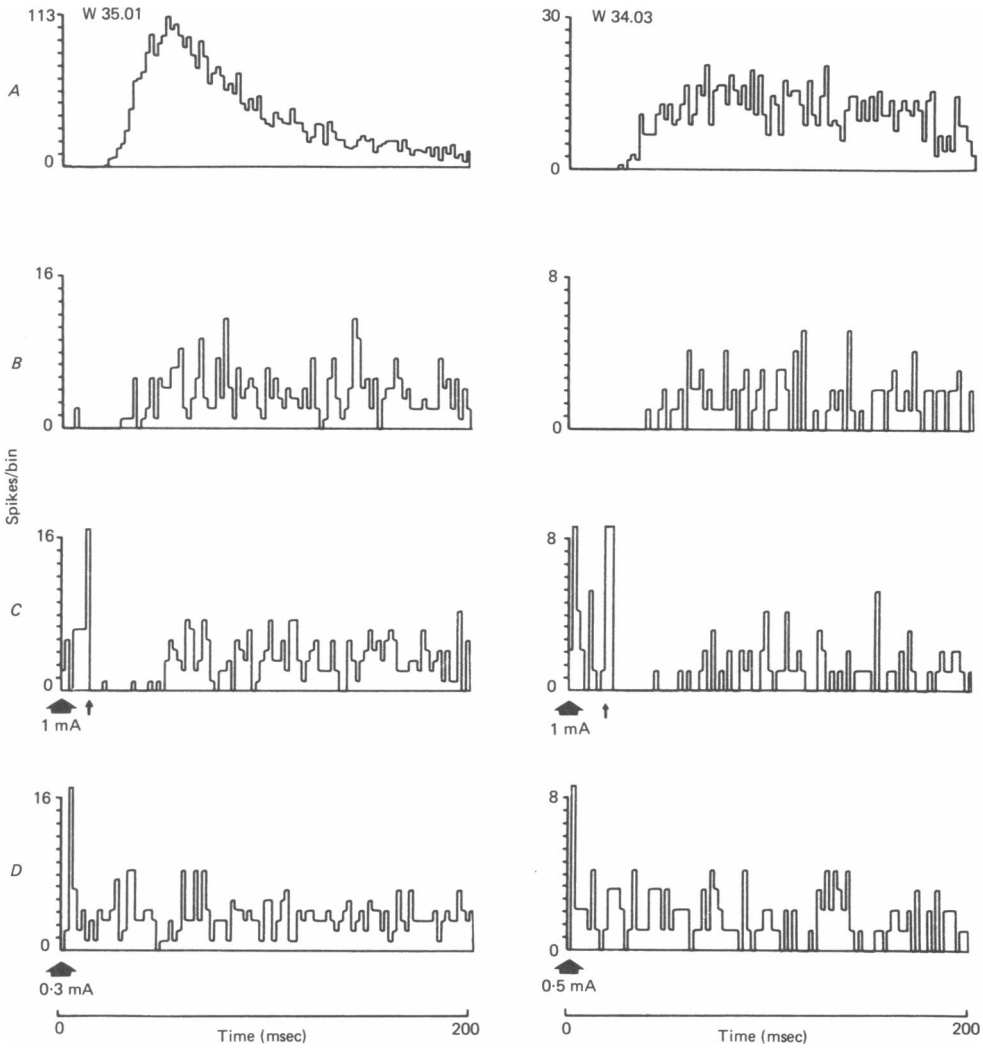

Fig. 1. Effects of single shocks to the neural stalk on single unit activity of two supraoptic neurones. *A*, inter-spike interval histograms of the spontaneous activity of the two neurones. Note the relatively long modes. *B–D*, post-stimulus time histograms: 200 sweeps triggered in *B* by spontaneous orthodromic action potentials, in *C* by 1 mA stimulus pulses applied to the neural stalk which invaded each neurone antidromically (arrow) and in *D* by subthreshold stimulation of the neural stalk. Antidromic action potentials are followed by an apparent inhibition of about 50 msec duration (*C*). However, a similar inhibition follows spontaneous orthodromic action potentials (*B*) and reflects the characteristic shape of the inter-spike interval histogram. Subthreshold stimuli (*D*) have little or no effect.

upon the discharge of any supraoptic neurone during the 6 min test period. Four supraoptic neurones that responded orthodromically to trains of shocks were re-tested using 1200 shocks presented at 4 Hz. The post-stimulus time histograms revealed slight orthodromic responses at a latency of 30–60 msec, and of the same polarity as the responses to trains of shocks (Fig. 2).

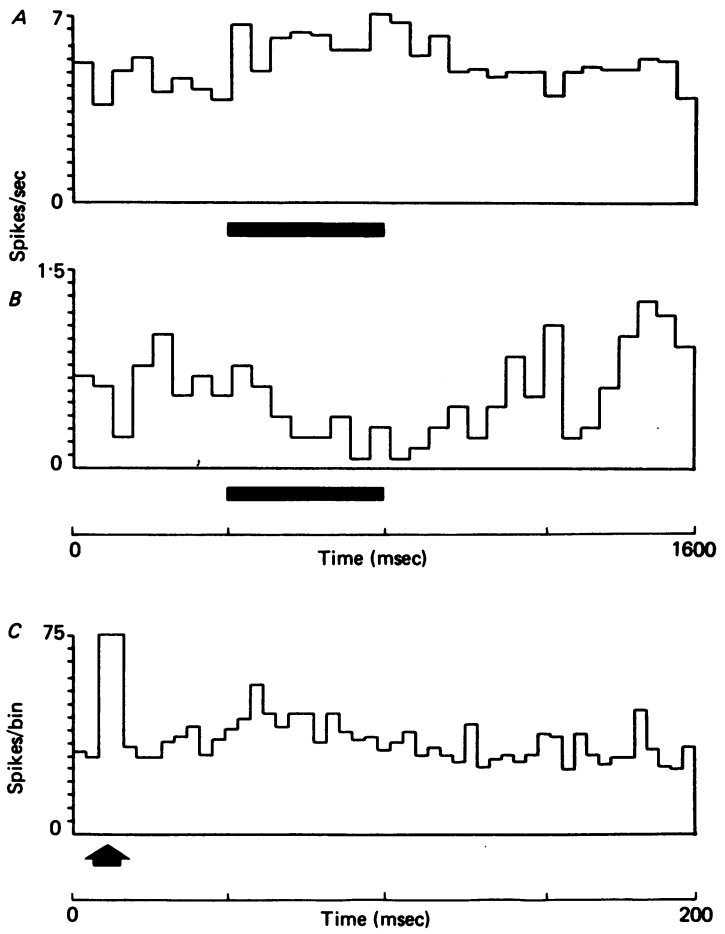

Fig. 2. *A*, *B*, peristimulus time histograms of the activity of an antidromically identified supraoptic neurone during trains of stimuli (40 Hz, 400 msec repeated 200 times at 2 sec intervals). The stimuli were presented for each cell at an intensity below that which produced antidromic action potentials in that cell. The cell in record *A* is excited by the trains; the cell in record *B* is inhibited. Most supraoptic neurones were scarcely affected by such stimulation. *C*, post-stimulus time histogram of the activity of the supraoptic neurone illustrated in *A*, produced by 1200 stimuli presented to the neural stalk at 4 Hz at a maximal subthreshold intensity (0·5 mA). There is a slight orthodromic excitation observable with a latency of about 60 msec. The arrow indicates the stimulus artifact.

Lateral hypothalamic neurones

Recordings were obtained from 164 lateral hypothalamic neurones. These neurones were spontaneously active at between 0·3 and 24 spikes/sec (4·6 $\pm$ 0·5 spikes/sec: means $\pm$ S.E.M.). Each of 125 neurones was studied during 5 sec of 1 mA neural stalk stimulation at 15 Hz, repeated at 20 sec intervals in a 5 min test period. Eleven of the 125 neurones showed a progressive change in mean firing rate during the test period, and these were omitted from further study because of the ambiguities in characterizing their responses to the stimulus trains. In forty-four of the remaining

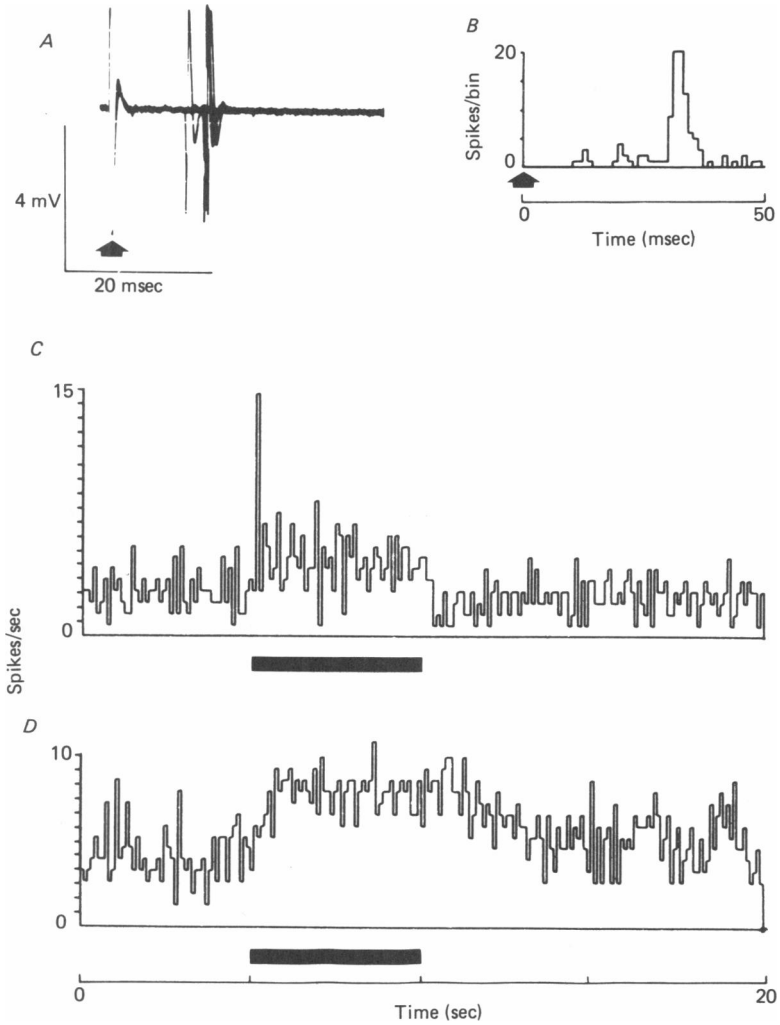

Fig. 3. Orthodromic excitation of lateral hypothalamic neurones following stimulation of the neural stalk. *A*, action potentials photographed from the screen of a storage oscilloscope. The spikes are triggered orthodromically following stimuli (0·5 mA) applied at 0·5 Hz to the neural stalk (arrow). Ten superimposed oscilloscope sweeps. *B*, post-stimulus time histogram of the activity of a neurone in the perinuclear zone following 0·4 mA stimuli applied to the neural stalk (200 shocks at 2 sec intervals; 2 msec bins; arrow). *C*, *D*, peristimulus time histograms of the activity of two lateral hypothalamic neurones. Stimulus trains (15 trains, 15 Hz at 1 mA; black bars) produce sustained excitation of each cell. The cell illustrated in *C* is the same cell shown in *B*.

114 neurones, the mean firing rate changed by 1 spike/sec or more during neural stalk stimulation. Of these, twenty-six neurones (23 %) were excited and eighteen inhibited (Figs. 3 and 4). The inhibited neurones comprised 36 % (eighteen out of fifty) of cells spontaneously active at greater than 1·5 spikes/sec.

Twenty-two neurones which responded strongly to 1 mA trains were also tested at lower stimulus intensities. Orthodromic responses were demonstrated in these neurones using currents of 0·25–0·75 mA. Fifteen strongly responsive neurones were

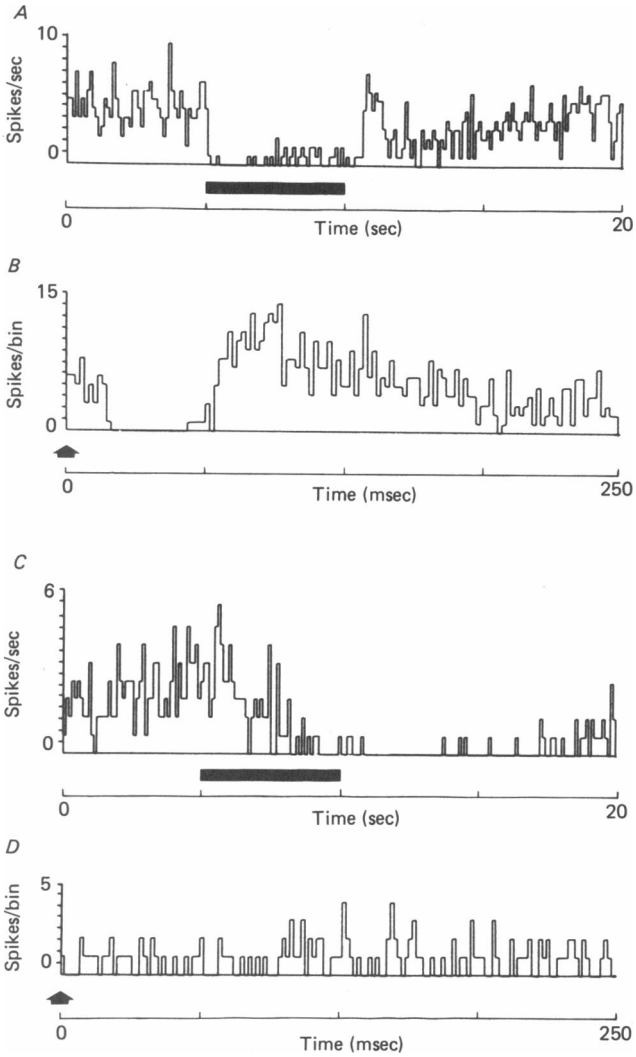

Fig. 4. Orthodromic inhibition of lateral hypothalamic neurones following stimulation of the neural stalk. *A*, *C*, peristimulus time histograms of the activity of two lateral hypothalamic neurones. Stimulus trains (fifteen trains, 15 Hz at 1 mA; black bars) inhibit each neurone. *B*, *D*, post-stimulus time histograms of the cells illustrated in *A* and *C* respectively, following 1 mA stimuli applied to the neural stalk (200 shocks at 2 sec intervals; 2 msec bins; arrows). *B* shows orthodromic inhibition between 30 and 70 msec after each shock. The neurone illustrated in *C* shows a late onset inhibition during trains of stimuli, but no short latency inhibition following single shocks.

tested using repeated single shocks (0·5–1 mA; 1 Hz for 200 sec) and in ten of these cells a marked excitation or inhibition followed the shocks with a latency of 15–40 msec (Fig. 4).

In one experiment, an electrode at the rostral limit of the supraoptic nucleus recorded the simultaneous activity of two cells, one of which was antidromically identified as projecting to the neural stalk. The second cell was activated orthodromically but not antidromically, and the spontaneous activity of this cell was strongly

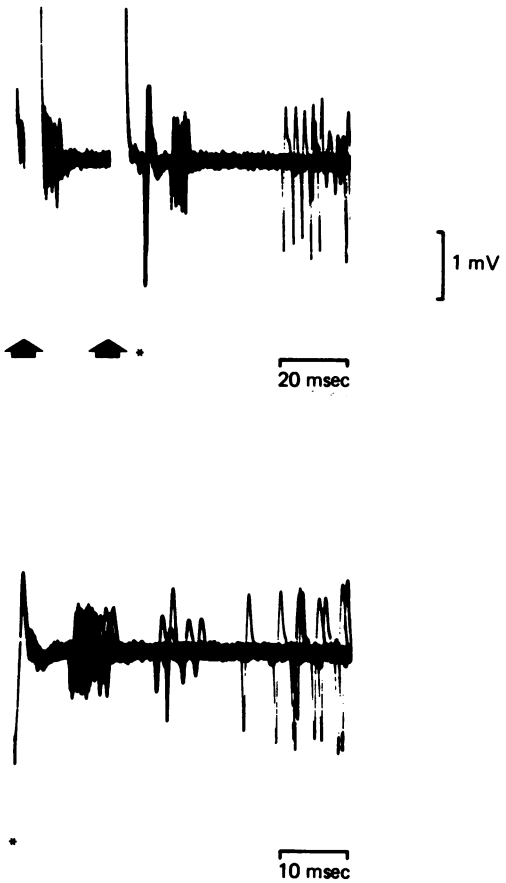

Fig. 5. Photographs taken from the face of a storage oscilloscope during an experiment. The electrode was simultaneously recording from two neurones at the rostral limit of the supraoptic nucleus. The larger cell was antidromically identified as projecting to the neural stalk, the smaller cell was not antidromically identified. The top photograph illustrates the collision test for antidromic identification of the larger cell. The photograph consists of fifteen superimposed oscilloscope sweeps: each sweep is synchronized with a pair of 1 mA stimulus pulses (arrows) applied to the neural stalk. Each pair of pulses has been triggered by a spontaneous action potential from the larger cell. The antidromic spike evoked by the first of these pulses has been extinguished by collision. The antidromic spike from the larger cell is seen to follow the second pulse with a constant latency. Note that discharges from the smaller cell occur with a variable latency of about 20 msec following the second pulse, but not the first pulse. This smaller cell could not be activated antidromically by stimuli applied to the neural stalk. The lower photograph consists of twenty-five superimposed oscilloscope sweeps each of which is synchronized with a spontaneous action potential from the larger cell. Note that the activity of the smaller cell appears to be synchronized with that of the larger cell with a variable latency of about 12 msec. This latency is similar to the latency of discharge of the same cell following the antidromic action potentials induced in the larger cell as shown in the upper photograph.

synchronized with that of the antidromically identified neurone (Fig. 5). Action potentials in the non-identified cell followed either spontaneous or antidromically evoked action potentials in the antidromically identified cell with a latency of about 10 msec. Thus this double-recording provided a direct demonstration that some supraoptic magnocellular neurones project to neighbouring neurones.

TABLE 1. Orthodromic responses to neural stalk stimulation amongst non-antidromically activated neurones of the lateral hypothalamus: distribution of response types by anatomical location (depth of pentration of recording electrode: the electrode was advanced through the supraoptic nucleus into the lateral hypothalamus at an angle of 25° to the ventral surface of the brain)

	Number of cells		
Depth of penetration (mm)	Orthodromically excited	Non-responsive	Orthodromically inhibited
< 1·5	13	13	8
1·5–2·0	6	18	3
2·0–2·8	7	39	7
Total	26	70	18

TABLE 2. Responses of lateral hypothalamic neurones to systemic osmotic stimulation (1 ml. 1·5 M-NaCl I.P.) distributed by their response to neural stalk stimulation

	Response to neural stalk stimulation		
Response to osmotic stimulation	Orthodromically excited	Non-responsive	Orthodromically inhibited
Excited	4	4	1
Unresponsive	2	10	1
Inhibited	1	2	6
Mean change in firing rate following osmotic stimulation (spikes/sec)	$+1·9 \pm 1·1$	$0·1 \pm 0·4$	$-1·1 \pm 0·8$

Cells showing strong orthodromic responses were more frequently encountered close to the supraoptic nucleus (Table 1). Twenty-one out of thirty-four cells in the perinuclear zone adjacent to the supraoptic nucleus (within 1·5 mm depth of electrode penetration) were orthodromically responsive, whereas significantly fewer (twenty-three out of eighty) of the deeper cells were responsive (χ^2 test: 1 d.f. $P < 0·025$).

Osmotic responses

As described by Brimble & Dyball (1977), a single I.P. injection of 1 ml. 1·5 M-NaCl raises plasma osmotic pressure by 10–15 m-osmole/l. over the following 15 min, and this increase is sustained for at least an hour. Thirty-one lateral hypothalamic neurones were studied for 25 min following such an injection. For eighteen neurones, the injection was followed by a change of 1 spike/sec or more in mean discharge rate: nine cells were excited and nine inhibited. The other thirteen cells were classed as unresponsive to osmotic stimulation. However, there was no significant change in the mean discharge rate of all lateral hypothalamic neurones following osmotic stimulation (mean rate $4·5 \pm 0·7$ spikes/sec; mean change $+0·2 \pm 0·5$ spikes/sec; $n = 31$).

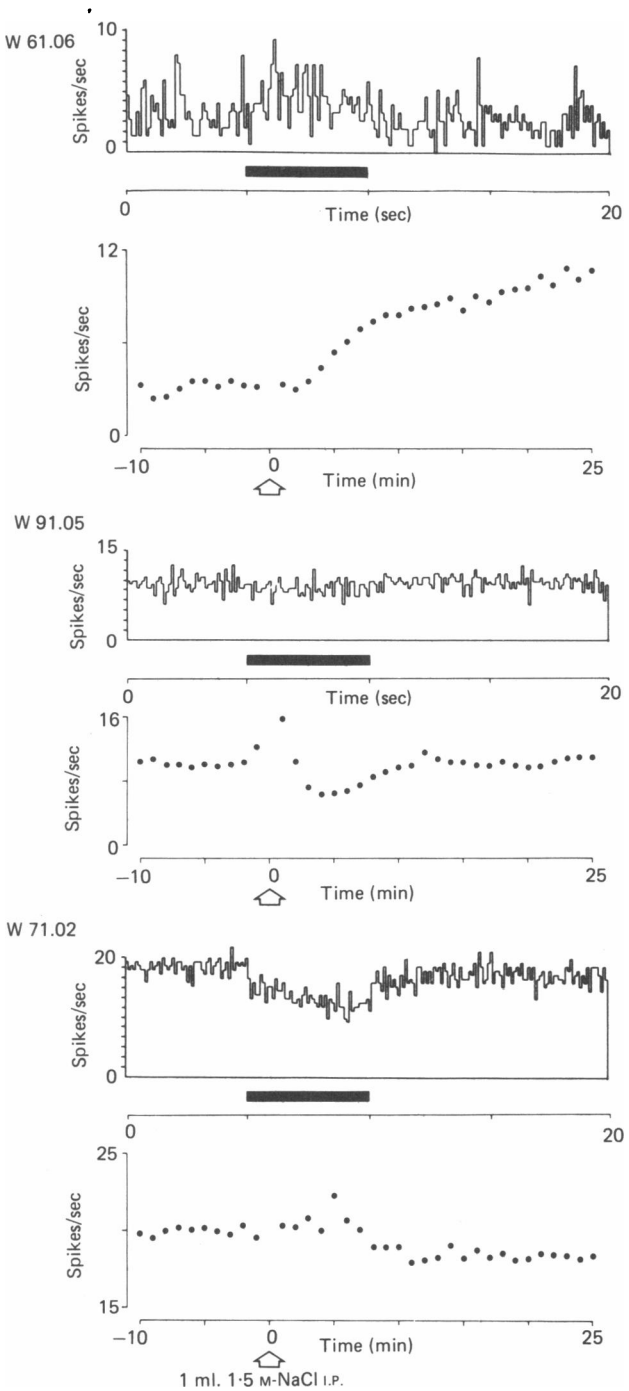

Fig. 6. Responses of three lateral hypothalamic neurones to repeated trains of stimuli applied to the neural stalk (5 sec, 15 Hz, fifteen repetitions, 1 mA) and to osmotic stimulation (1 ml. 1·5 M-NaCl I.P.). For each cell the top record is a peristimulus time histogram; the bottom record shows the mean discharge rate in successive minutes from 10 min before the injection to 25 min after the injection. Cell W61.06 (top pair of records)

Seven lateral hypothalamic neurones were studied following a control I.P. injection of 1 ml. 0·15 M-NaCl. None of these cells altered its discharge rate by more than 1 spike/sec following the injection, nor was there a significant change in the mean discharge rate of the group.

Osmotically responsive cells were apparently not confined to any particular zone of the lateral hypothalamus. However, such cells were more common in the perinuclear zone (ten cells responsive out of thirteen) than elsewhere in the lateral hypothalamus (eight cells responsive out of eighteen).

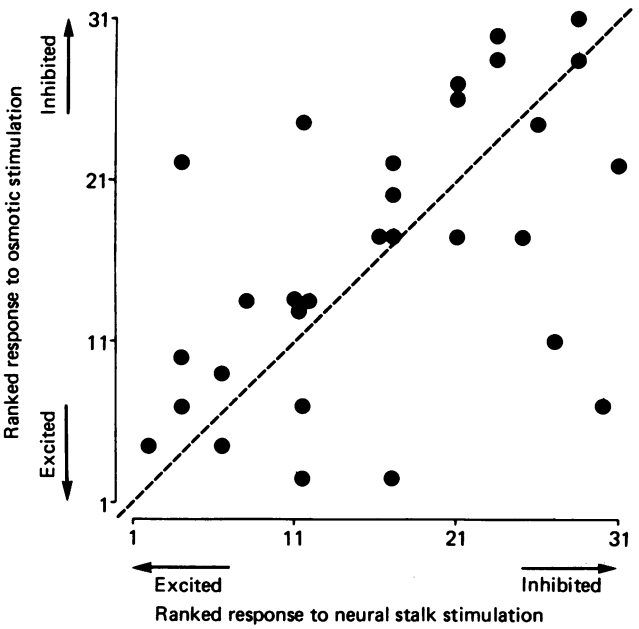

Fig. 7. Rank comparison of effects of osmotic stimulation and neural stalk stimulation on ranked firing rates of thirty-one lateral hypothalamic neurones.

Comparison of the effects of osmotic stimulation and neural stalk stimulation

Twenty of the thirty-one cells tested with an osmotic stimulus responded in a similar way to this stimulus as to neural stalk stimulation (Table 2). Four cells were excited by both types of stimulation, six were inhibited by both, and ten were unresponsive to both (Fig. 6). To assess the statistical significance of the correlation between effects, the osmotic and orthodromic responsiveness of lateral hypothalamic neurones were compared in a non-parametric analysis. The firing rates of the thirty-one

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was excited by neural stalk stimulation and by osmotic stimulation. Cell W91.05 (middle pair) was unaffected by neural stalk stimulation and only transiently affected by osmotic stimulation. Cell W71.02 (bottom pair) was inhibited by both osmotic stimulation and neural stalk stimulation.

cells before osmotic stimulation were ranked. The changes in rank firing rate accompanying osmotic stimulation and neural stalk stimulation respectively were calculated, and these rank changes were themselves ranked (Fig. 7). There was a significant correlation between the rank shift produced by osmotic stimulation and that produced by neural stalk stimulation ($P < 0{\cdot}001$; t test 29 d.f. on Spearman's Rank Correlation Coefficient).

DISCUSSION

The results of the present study suggest that there may be no strong synaptic coupling between magnocellular supraoptic neurones in the rat. However, some cells in the perinuclear zone do receive synaptic input from the magnocellular neurones, and indeed, activation of magnocellular neurosecretory neurones influences the activity of a substantial minority of neurones throughout the lateral hypothalamus. A comparison of the effects of neural stalk stimulation upon lateral hypothalamic neurones with the responses of the same neurones to osmotic stimulation suggests that the synaptic input to the lateral hypothalamus arising from magnocellular neurones may contribute to the osmosensitivity of cells in the lateral hypothalamus.

The conclusion that there is no strong synaptic coupling between supraoptic neurones in the rat appears at odds both with the morphological evidence of extensive intercommunication within the nucleus (Léránth *et al.* 1965) and with previous electrophysiological evidence. While the reports of recurrent inhibition in the rat supraoptic nucleus (Dreifuss & Kelly, 1972; Dyball, 1974) appear to have been based on a misinterpretation (Buckley & Leng, 1981), Leng (1980) has reported circumstances in which stimulation of the neural stalk modifies the discharge activity of supraoptic neurones. In the latter experiments, specific and slight modifications of firing pattern were produced by sustained trains of stimuli applied to the neural stalk, and these changes occurred with a latency of between seconds and minutes. Thus interaction between supraoptic neurones may involve a neurohormonal modulating factor rather than a classical neurotransmitter with an immediate and direct effect upon neuronal excitability (Leng & Wiersma, 1981).

However, it appears that supraoptic neurones influence neurones in the perinuclear zone in a manner explicable by a conventional neurotransmitter action. It has been reported previously that some cells in the perinuclear zone are osmosensitive (Hayward & Vincent, 1970), and indeed it has been postulated that these cells are the osmoreceptors responsible for the osmotic activation of supraoptic neurones (Hayward, 1977). The present results suggest another explanation, that the cells in the perinuclear zone are osmosensitive as a result of afferent input from the supraoptic nucleus.

Many cells throughout the lateral hypothalamus appeared to respond to systemic osmotic stimulation, though fewer than the 90% suggested by previous workers (Weiss & Almli, 1975), This discrepancy may arise from a difference in the method of osmotic stimulation. Intraperitoneal injection of hypertonic saline produces a gradual and sustained increase in plasma osmolarity (Brimble & Dyball, 1977): previous experiments used intracarotid injections, which provide a rapid and transient osmotic stimulus which cannot be separated from cardiovascular effects.

In the present experiments, many lateral hypothalamic neurones were influenced, directly or indirectly, by the activation of magnocellular neurosecretory neurones. This influence may contribute to the osmosensitivity of neurones in the lateral hypothalamus, since many of these neurones responded in the same way to neural stalk stimulation as to osmotic stimulation. However, some osmotic responses observed in the lateral hypothalamus could not be explained by an input arising from magnocellular neurones. Thus it remains likely that there is a population of osmoreceptive neurones functionally and anatomically separate from the magnocellular neurosecretory neurones. It has been suggested that the lateral preoptic area, which is known to project to the lateral hypothalamus, may contain osmoreceptors associated with thirst rather than with the control of vasopressin secretion (Blank & Wayner, 1975; Blass & Epstein, 1971; Peck & Novin, 1971; Weiss & Almli, 1975).

Finally, it is possible that such a 'second' population of osmoreceptors interacts with the supraoptic osmoreceptors. Present results suggest that there is little or no excitatory synaptic coupling between rat supraoptic neurones. Therefore the increase in e.p.s.p.'s observed by Mason (1980) in these neurones during osmotic stimulation must derive from other osmosensitive neural elements. Cells of the lateral preoptic area are a possible source of these e.p.s.p.s since this region is within the slice preparation from which the recordings of supraoptic neurones were obtained. Thus osmotic regulation of thirst and antidiuresis appear not to be served by distinct and concise neural substrates. Rather, there appears to be extensive interaction between multiple systems of osmosensitive neurones.

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