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INFLUENCE OF MEDIAL PREOPTIC–ANTERIOR HYPOTHALAMIC AREA STIMULATION ON THE EXCITABILITY OF MEDIOBASAL HYPOTHALAMIC NEURONES IN THE RAT

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SUMMARY

1. Extracellular action potentials recorded from 798 neurones in the mediobasal hypothalamus (MBH) of pentobarbitone anaesthetized male rats were analysed for a change in excitability following stimulation in the medial preoptic and anterior hypothalamic areas.

2. An increase in excitability characteristic of orthodromic excitation was observed from 11·5% (n = 92) of MBH neurones. Latencies for excitation were shorter for cells tested with anterior hypothalamic area stimulation (n = 42; mean 5·4 ± 2·6 msec S.D.) than for cells tested with medial preoptic stimulation (n = 50; mean 15·2 ± 7·2 msec S.D.). With spontaneously active neurones, excitation was followed by a decrease in excitability lasting 150–250 msec. An initial decrease in excitability, suggestive of post-synaptic inhibition, over a wide latency range (4–30 msec) and with durations of 100–400 msec was observed from 3·6% of MBH neurones.

3. Features of antidromic invasion were observed from 149 MBH neurones. From the medial preoptic area, the latency range was 0·5–38 msec (mean 7·8 ± 5·5); from the anterior hypothalamic area the latency range was 0·4–9·5 msec (mean 3·1 ± 2·3). Occasionally an abrupt decrease in latency followed an increase in stimulus intensity. Most cells followed paired stimuli at frequencies up to 500 Hz. Axon conduction velocities were estimated to be under 2·0 m/sec. Antidromic invasion was usually followed by a decrease in excitability lasting approximately 100–150 msec.

4. Twenty MBH neurones displayed antidromic invasion from *both* the medial preoptic or anterior hypothalamic areas *and* one other stimulation site: the median eminence (five cells); the amygdala (six cells); the region of thalamic nucleus medialis dorsalis (three cells) and the midbrain

periaqueductal gray (six cells). Interaction studies indicated that the axons of these cells branched close to the origin of the axon itself.

5. Antidromic invasion from the surface of the median eminence identified thirty-nine tuberoinfundibular neurones. Stimulation in the medial preoptic and anterior hypothalamic area produced orthodromic excitatory ($n = 5$) and inhibitory ($n = 4$) actions on HVM neurones, but was without an action on most other neurones ($n = 30$). Tuberoinfundibular neurones in the ventromedial nucleus also responded to stimulation in the amygdala, but usually at latencies greater than that for medial preoptic area evoked responses.

6. These observations indicate a close relationship between MBH neurones and cells located in both the amygdala and the medial preoptic-anterior hypothalamic area. The data for tuberoinfundibular neurones indicates that several extrahypothalamic areas may send fibres to these cells. These pathways may be important for the adaptive neuroendocrine responses reported in the literature.

INTRODUCTION

The hypothalamic ventromedial nucleus is a neuronal complex important in activities related to aggressive and emotional expression (Clemente & Chase, 1973; Glusman, 1974), control of feeding and appetitive behaviour (Hoebel, 1971) and regulation of adenohypophyseal secretion (Blackwell & Guillemin, 1973). Since this neuronal structure is so intimately involved in behaviour and homoeostasis, it probably has substantial extrahypothalamic connexions. Recent anatomical and electrophysiological studies have attempted to identify and characterize the organization of afferent and efferent connexions of ventromedial nucleus neurones (Szentágothai, Flerkó, Mess & Halász, 1968; Millhouse, 1973*a*, *b*; Renaud & Martin, 1975; Saper, Swanson & Cowan, 1975; Renaud & Hopkins, 1976). This communication reports on the electrophysiology of certain ascending and descending connexions between mediobasal hypothalamic (MBH) neurones and the medial preoptic (MPOA) and anterior hypothalamic (AHA) areas.

The medial preoptic area is concerned functionally with thermoregulation (Hammel, 1968; Hayward & Baker, 1968), water balance (Hayward & Baker, 1968; Fitzsimons, 1972; van Gemert, Miller, Carey & Moses, 1975), sexual behaviour (Numan, 1974; Pfaff, Daikow, Zigmond & Kow, 1974) and endocrine function, in particular the regulation of pituitary gonadotropin secretion (Halász, 1969; Cramer & Barraclough, 1973). Until recently there was relatively little data on neuronal connexions between MPOA–AHA and MBH. However, anatomical evidence

(Szentágothai *et al.* 1968; Millhouse, 1973*a*, *b*; Conrad & Pfaff, 1975, 1976*a*, *b*; Saper *et al.* 1975; Swanson, 1976) is now available for both ascending and descending connexions between these two regions. Recent electrophysiological investigations have also demonstrated evidence for direct connexions between MBH and MPOA–AHA (Dyer, 1973; Harris & Sanghera, 1974; Makara & Hodacs, 1975; Renaud & Martin, 1975).

The present investigation provides additional details on connexions between these two areas, and demonstrates the heterogeneity in afferent and efferent connexions of MBH neurones that project to MPOA–AHA. A portion of these results has been briefly reported (Renaud & Martin, 1975; Renaud, 1976*a*).

METHODS

Preparation. Experiments were performed on male Sprague-Dawley rats anaesthetized with intraperitoneal pentobarbitone (50 mg/kg initially, with supplemental intravenous doses of 3·0 mg every 2–3 hr). The heart rate was monitored continuously and body temperature maintained at 38·5° C throughout each experiment. The hypothalamus was exposed via a transpharyngeal approach; insertion of a non-toxic mixture of plasticine in Vaseline into the diploic spaces occupied by venous sinuses provided adequate haemostasis during and after surgery.

Stimulation. Electrical stimulation of the medial preoptic-anterior hypothalamic area (Fig. 1) was effected by means of bipolar electrodes (nichrome wire o.d. 230 μm, insulated except for the terminal 0·5 mm with tip separations of 0·5 mm) implanted stereotaxically. Similar electrodes were stereotaxically positioned within the basolateral, basomedial or cortical nuclei of the amygdala, i.e. sites defined on the basis of earlier studies (Renaud, 1976*b*) to project to the hypothalamic ventromedial nucleus. In some experiments additional electrodes were stereotaxically inserted into the periaqueductal gray and the mid line thalamic nucleus medialis dorsalis. Single 1 Hz current pulses 0·05 msec in duration and at intensities of 150–550 μA were applied to the electrodes from isolated stimulation units controlled by a programmable clock (Digitimer).

In order to identify tuberoinfundibular neurones by antidromic invasion, a concentric 28 ga bipolar electrode whose tip had been polished so as to protrude not more than 150 μm from the outer ring was positioned gently by micromanipulation on the surface of the median eminence pituitary stalk junction just posterior to the origin of the portal capilliary plexus. Current pulses of 0·05 msec duration and at intensities of 70–350 μA were applied by isolated stimulation units.

The criteria for antidromic invasion include the ability to display constant latency responses following stimulation at or just above threshold, and at frequency rates greater than 150 Hz. For spontaneously active neurones, the presence of collisions between an orthodromic (spontaneously occurring) action potential and an antidromic evoked spike discharge constituted a third criterion. In the event that one cell displayed these criteria from more than one stimulation site, appropriately timed suprathreshold stimulation at each site yielded evidence of cancellation of antidromic impulses.

Recording. Glass micropipettes with tip impedance of 5–7 MΩ, tip diameters of approximately 1 μm and filled with 3 M-NaCl were used to record unit activity. Micropipettes were connected through a chlorided silver wire lead to a preamplifier and conventional system amplifier with variable band pass. Suitable action potentials

were recorded on photographic film from an oscilloscope. A variable voltage gate was utilized to select action potentials for probability studies using a PDP 11/40 computer programmed for single spike train analysis (time interval analysis, post-stimulus-latency discharge probability).

Localization. Methods for localization of the tip of the recording micropipette within the mediobasal hypothalamus have been outlined in a previous publication (Renaud, 1976*b*). The most useful localizing feature was the amygdala evoked field potential response within the ventromedial nucleus (Renaud, 1976*b*). Additional localizing features included the depth location of all recorded neurones, and precise measurements of distances from mid line. The locations of electrode tips broken and left in place during perfusion with 10% formalin, and locations of all stimulating electrodes, were verified from histological sections stained with thionin.

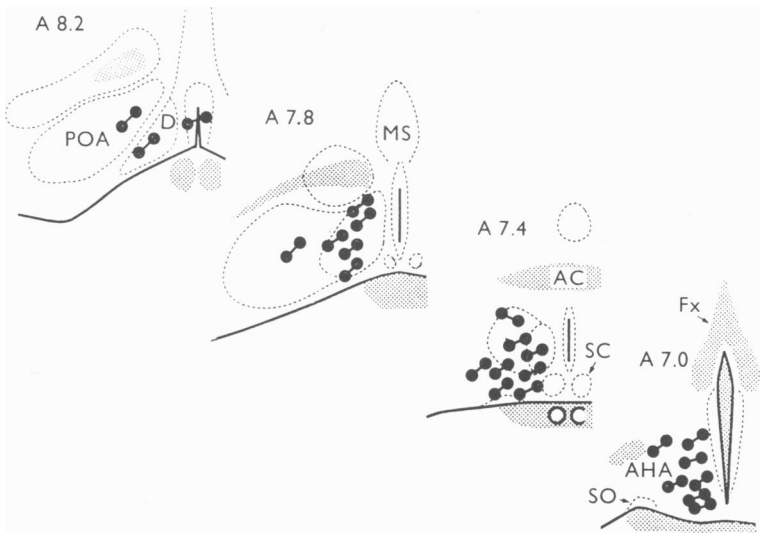

Fig. 1. The pairs of dots, superimposed on schematic coronal sections through four levels (A 7.0 to A 8.2 in stereotaxic co-ordinants) of the preoptic-anterior hypothalamic area, summarize the locations of tips of bipolar stimulation electrodes in this series of experiments. AC, anterior commissure; AHA, anterior hypothalamic area; D, diagonal band; Fx, fornix; MS, medial septum; OC, optic chiasm; POA, preoptic area; SC, suprachiasmatic nucleus; SO, supraoptic nucleus.

RESULTS

The observations reported in this communication were derived from an analysis of the responses of 798 MBH neurones tested with stimulation at MPOA–AHA sites illustrated in Fig. 1. Of the neurones tested, 33·8% displayed a response interpreted as orthodromic excitation (ninety-two cells), inhibition (twenty-nine cells) or antidromic activation (149 cells). These neurones were located in the ventromedial (584 cells), dorsomedial (twenty-eight cells) and arcuate (ninety-two cells) nuclei, and the peri-

ventricular region (ninety-four cells). In animals under pentobarbitone anaesthesia, hypothalamic neurones usually displayed little spontaneous activity; in the present studies, 42 % of the cells were silent except for their evoked action potentials. Cells were characterized according to evoked orthodromic or antidromic responses from amygdala and MPOA–AHA stimulation. Further characterization of neurones was obtained from evidence of antidromic invasion after midbrain (periaqueductal gray), median eminence and thalamic medialis dorsalis stimulation.

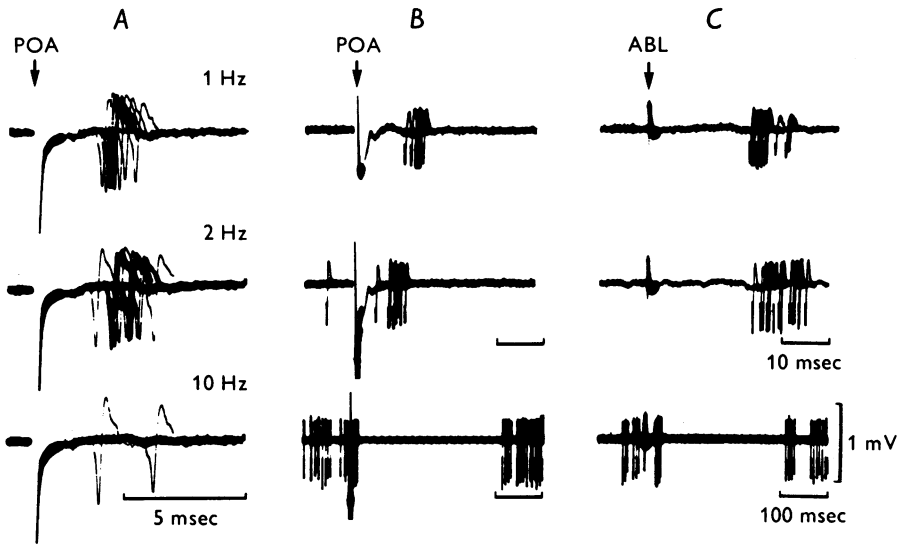

Fig. 2. Superimposed oscilloscope traces to illustrate the usual excitatory responses from MBH neurones after medial preoptic area (POA) stimulation. In *A* the series of traces from one cell show failure to follow orthodromic activation at frequencies beyond 10 Hz. The upper row in *B* and *C* illustrates similar excitatory patterns from one cell after 1 Hz preoptic and basolateral amygdala (ABL) stimulation. The lower traces in *B* and *C* illustrate excitation (middle row) and a subsequent silent interval (bottom row) obtained from an adjacent spontaneously active neurone tested with the same stimulation parameters. Note that the latencies for MPOA evoked excitations (cf. Fig. 5) were generally briefer than for amygdala evoked excitations (cf. Fig. 5 in Renaud, 1976*b*).

Orthodromic responses

Excitation. An increase in excitability over a latency range of 1·8–29·5 msec (Fig. 5) following MPOA or AHA stimulation characterized the responses of ninety-two MBH neurones (11·5 %). These neurones were located as follows: ventromedial nucleus (sixty-six cells), arcuate nucleus (fourteen cells), periventricular region (nine cells) and the ventral portion of the dorsomedial nucleus (three cells). The majority of neurones

responded with single action potentials whose latencies varied by less than 10 msec from sweep to sweep (Fig. 2*A*, *B*); only three neurones displayed a more prolonged change in excitability with a duration of 40–50 msec (Fig. 3*A*). With responsive neurones that displayed spontaneous activity, excitation was followed by a silent interval of 150–250 msec (Fig. 2*B*, lowest trace; Fig. 3*B*). The presence of this prominent post-excitatory depression from neurones that were both spontaneously active and from otherwise silent neurones whose activity was maintained post-synaptically by iontophoretic application of L-glutamate (12 neurones) suggested the presence of a post-synaptic inhibitory process. This opinion was further supported by the presence of an initial silent interval in the spontaneous or glutamate evoked discharges of other MBH neurones tested in the same experiment (Fig. 3*C*, *D*). Excitatory responses seldom followed stimulation rates in excess of 5–10 Hz (Fig. 2*A*), possibly owing to cumulative post-synaptic inhibitory effects.

Latencies for excitation were shorter for cells tested with AHA stimulation (n = 42 cells; mean $5{\cdot}4 \pm 2{\cdot}6$ msec S.D.) than with MPOA stimulation (n = 50 cells; mean $13{\cdot}2 \pm 7{\cdot}2$ msec S.D.). It is possible that AHA stimulation sites activated some caudally directed fibres originating in MPOA or more rostral regions, since there is anatomical evidence for substantial projections to MBH from MPOA (Conrad & Pfaff, 1975, 1976*a*; Swanson, 1976). Since AHA neurones also project posteriorly to MBH (Conrad & Pfaff, 1976*b*) the mean latency differences observed between AHA and MPOA stimulation sites may indicate separate caudal projections from neurones in both areas. However, there was no significant difference in the response latencies of arcuate nucleus neurones compared with ventromedial nucleus neurones in these two groups.

Most of the ventromedial nucleus neurones that displayed excitation after MPOA or AHA stimulation also displayed excitation after stimulation in the basolateral or basomedial amygdala (n = 44 cells) or after stimulation of amygdalofugal fibres in the stria terminalis (n = 10 cells). Response latencies from the amygdala were generally 7–12 msec longer than for those from MPOA–AHA. Two examples are illustrated in Fig. 2*B*, *C*. In comparison with neurones located in the ventromedial nucleus, other MBH neurones that displayed excitation from MPOA–AHA were generally unresponsive to amygdala stimulation.

Three spontaneously active ventromedial nucleus neurones excited by MPOA–AHA stimulation displayed an initial inhibition of 100–150 msec after amygdala stimulation, thus indicating that excitatory responses observed from one area were not necessarily associated with similar responses observed from other regions.

Inhibition. An initial silent period in either spontaneous or glutamate

evoked spike discharges was observed from twenty-nine MBH neurones in response to MPOA–AHA stimulation. These responses occurred over a wide latency range (4–30 msec; Fig. 5) with no significant difference between MPOA and AHA stimulation sites. For MPOA stimulation sites, latencies for neurones in the arcuate nucleus (eight cells; mean $15{\cdot}7 \pm 6{\cdot}2$ msec S.D.) were marginally longer than for those in ventromedial nucleus (twenty-one cells; mean $11{\cdot}2 \pm 6{\cdot}1$ msec S.D.). The duration of the silent period was usually 100–150 msec (Fig. 3*C*); spike discharges of a small

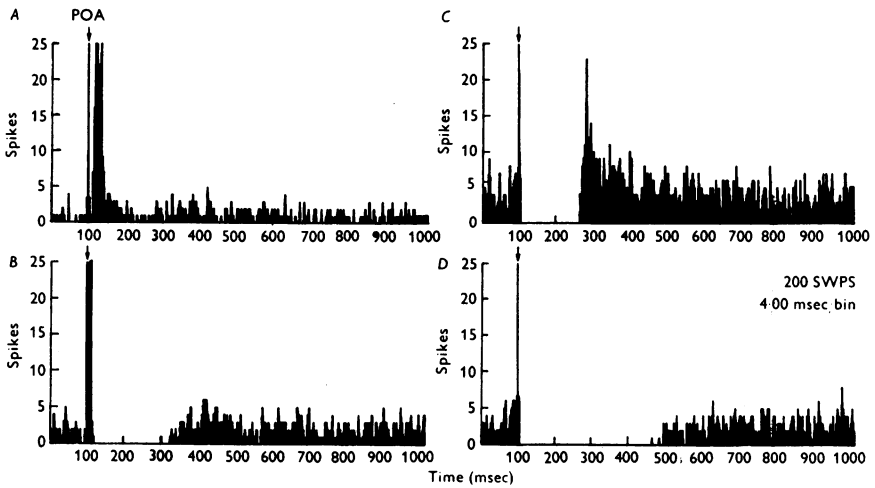

Fig. 3. Post-stimulus latency histograms derived from four different MBH neurones to indicate the four excitability patterns observed after medial preoptic (POA) stimulation (depicted by the arrows). The arcuate nucleus neurone in *A* displayed a long duration facilitation. The ventromedial nucleus neurone in *B* showed short latency excitation followed by a silent interval. An initial silent period, with different durations, characterized the responses of two other ventromedial nucleus neurones illustrated in *C* and *D*. Each histogram contains 200 sweeps, with 4 msec bin widths.

number of active neurones did not return until 300–400 msec after the stimulus (Fig. 3*D*). Post-inhibition enhancement of excitability (Fig. 3*C*) was occasionally observed, and not necessarily associated with the duration of the inhibitory period.

Of fourteen ventromedial neurones that displayed inhibitory responses from MPOA, four cells displayed a similar response pattern from amygdala stimulation (for example, Fig. 10*D*, *E*) while five cells showed amygdala evoked excitation, and five other neurones were unresponsive to amygdala stimulation. Of four arcuate nucleus neurones tested, two were inhibited and two were unresponsive to amygdala stimulation.

Unresponsive neurones. Of the population of MBH neurones tested in

this series of experiments, comparatively few cells displayed evidence of orthodromic activation (11·5 %) or inhibition (3·6 %). This low percentage of responses may therefore indicate a paucity of MPOA–AHA projections to MBH. It is also possible that a substantial number of responses were not observed because neurones were not spontaneously active, partly due to the presence of pentobarbital anaesthesia that may enhance the effect of endogenous inhibitory influences (cf. Nicoll, 1975).

Antidromic responses

Latencies. 149 MBH neurones exhibited features of antidromic invasion (Fig. 4*A*) from either MPOA or AHA. Twenty cells were located within the arcuate nucleus or periventricular region, while the remainder were in or near the ventromedial nucleus (Fig. 9). The histograms in Fig. 5

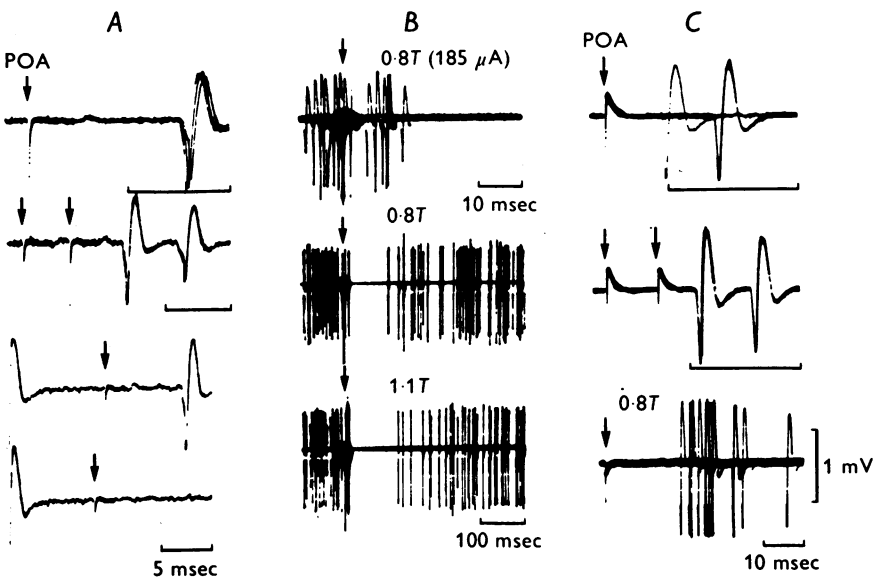

Fig. 4. *A*, features characteristic of antidromic invasion after medial preoptic stimulation (POA) include constant latency responses at or above threshold (upper trace) at high frequencies of stimulation (second trace) and collisions between spontaneous action potentials (shown at the onset of the lower traces) and the antidromic action potential at appropriate intervals (approximately twice that of the antidromic latency). *B*, superimposed sweeps from the same spontaneously active neurone shown in *A* illustrate that a silent interval with a latency of 15 msec (upper trace) and a duration of approximately 100 msec (lowest traces) follows preoptic stimulation even at stimulus intensities subthreshold (0·8*T*, 185 μA) for antidromic invasion. *C*, data from another ventromedial nucleus neurone illustrates antidromic invasion from medial preoptic stimulation (upper traces) and facilitation of spike discharges at subthreshold stimulus intensities (lowest trace).

illustrate the antidromic invasion latency range for 112 neurones tested with MPOA stimulation (range 0·5–38 msec; mean 7·8 ± 5·5) and 37 cells tested with AHA stimulation (range 0·4–9·5 msec; mean 3·1 ± 2·3). The majority of neurones displayed only one latency for antidromic invasion when tested with stimulation at different intensities. However, seven cells tested with MPOA and two cells tested with AHA stimulation demonstrated a decrease of 0·5–1·5 msec in antidromic invasion latencies at higher stimulus intensities. Fig. 6*A*, *B* illustrates one exceptional example where two distinctly different latencies at differences of 3 msec were recorded at higher stimulus intensities.

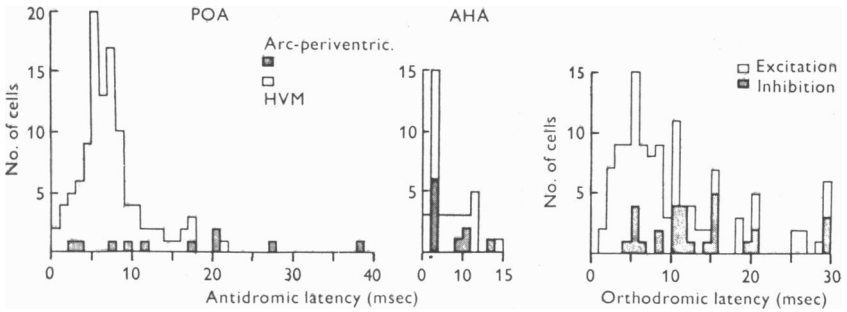

Fig. 5. The two histograms on the *left* plot the frequency distribution for antidromic invasion of 149 MBH neurones from medial preoptic (POA) or anterior hypothalamic area (AHA) stimulation sites. The hatched area depicts the cells located in the arcuate nucleus or periventricular area, the open sites indicate ventromedial nucleus neurones. The histogram on the *right* is a frequency distribution of the latencies for excitatory (open areas) or inhibitory (hatched areas) responses observed from 121 MBH cells after medial preoptic stimulation.

Response to paired stimuli. In most instances the antidromic responses followed paired stimuli at frequencies up to 500 Hz. At interstimulus intervals under 30 msec the shape of the second antidromic response changed; there was a diminution in amplitude and an accentuation of the inflexion of the rising phase of the action potential. These observations presumably reflected the presence of the relative refractory period of the neurone. At interstimulus intervals under 3 msec the latency of the second antidromic action potential increased beyond that of the first response (Fig. 6*B*) in association with further diminution in amplitude and apparent prolongation in the duration of the action potential. Despite rather slow conduction velocities estimated for the axons of these neurones (under 2·0 m/sec), the vast majority of neurones followed paired stimuli at intervals as brief as 2 msec.

Excitability patterns after MPOA–AHA stimulation. Twenty-four

spontaneously active ventromedial neurones were examined for changes in excitability at different intensities of MPOA–AHA stimulation. At suprathreshold intensities for antidromic invasion, 19 cells (80%) displayed a *decrease* in spike discharge probability that lasted approximately 100 msec; for 7 of these neurones this change in activity was evident even with

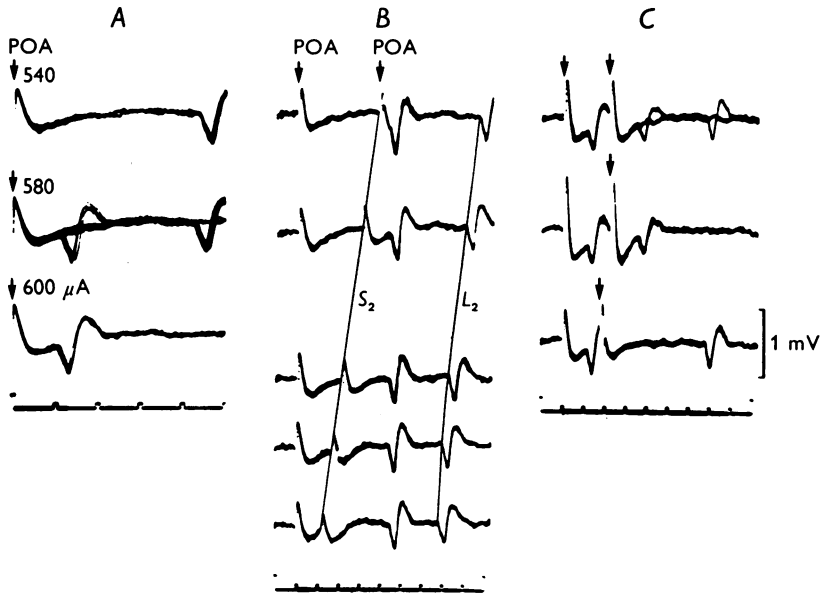

Fig. 6. Two latencies for antidromic invasion from the medial preoptic area (POA). This figure illustrates data from a periventricular neurone. *A*, two latencies for antidromic invasion, dependent upon stimulation intensities (measured in μA). The traces in *B* were obtained during paired preoptic stimuli at lower stimulus intensities. Two lines were drawn through the onset of the second stimulus (S_2) and the onset of the second antidromic response (L_2); their divergence at the shortest interstimulus intervals (under 3 msec) indicates an increase in the latency of the second antidromic spike. In *C*, at higher stimulus intensities (600, 610 and 620 μA from above downwards) the first of two stimuli evoked shorter latency antidromic invasion. The latency of the antidromic spike following the second stimulus varied with stimulation intensities, indicative of an increased threshold for antidromic invasion to the second of two stimuli at short interstimulus intervals. Time calibration: 1 msec.

stimulus intensities subthreshold for antidromic invasion (Fig. 4*B*) suggesting that the silent interval did not arise from post-activation depression but was most likely due to a post-synaptic inhibitory process activated through descending fibres or recurrent collaterals of other neurones antidromically activated by the same stimulus (cf. Fig. 12). Four cells, including one neurone that displayed antidromic invasion from both MPOA and the

amygdala (Fig. 8) showed *no change* in excitability after MPOA stimulation at intensities up to 1·5 times threshold for antidromic invasion. Two cells displayed a short latency enhanced activity at stimulus intensities subthreshold for antidromic invasion (Fig. 4*C*).

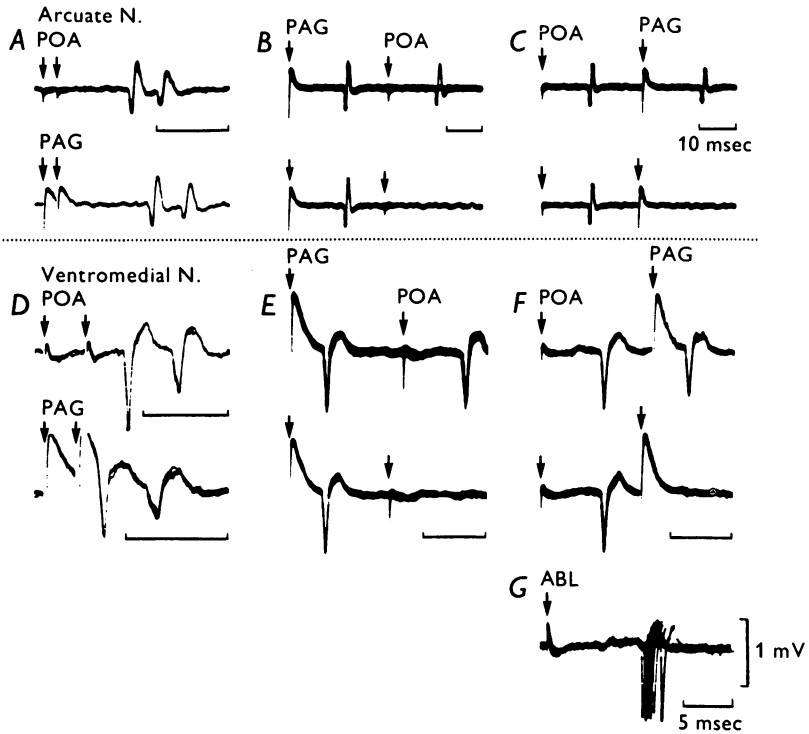

Fig. 7. Axon branching in two different neurones, one in the arcuate nucleus (*A–C*) and one in the ventromedial nucleus (*D–F*) encountered within the same micro-electrode penetration. In this instance both neurones displayed antidromic invasion from medial preoptic area (POA) and periaqueductal gray (PAG) stimulation sites. The superimposed traces in *B*, *C*, *E*, *F* obtained during interaction experiments illustrate the critical intervals for cancellation of the second antidromic response. The ventromedial nucleus neurone also displayed orthodromic activation (*G*) from basolateral amygdala (ABL) stimulation.

Axon branching. Twenty neurones displayed antidromic invasion from *both* MPOA or AHA *and* from one other stimulation site; the details of each neurone are listed in Table 1. Three cells were located within the arcuate nucleus, the remainder within the ventromedial nucleus. Fig. 7 illustrates two examples of neurones that appear to have axons that bifurcate in rostral and caudal directions. In other instances axon branches were directed dorsally to the mid line thalamic area (medialis dorsalis)

TABLE 1. Summary of the data obtained from hypothalamic neurones that demonstrate antidromic invasion from either the medial preoptic area (MPOA) or anterior hypothalamic area (AHA) *and* one other stimulation site.

The column on the left denotes the location of neurones within the arcuate (ARC) or ventromedial (HVM) nucleus. The centre columns list the antidromic invasion latencies from MPOA, AHA, median eminence (ME), basolateral-basomedial amygdala (AMG), medialis dorsalis nucleus of the thalamus (MDT) and midbrain periaqueductal gray (PAG). The column on the right (*) refers to data (where available) from interaction experiments (cf. Fig. 7*B*, *C*, *E*, *F*; Fig. 8*C*, *D*; Fig. 11*C*, *D*) and lists the critical interval (in msec) between suprathreshold stimulation at each site that resulted in cancellation of the antidromic response to the second stimulus. Note that the cancellation interval approximates the sum of the individual antidromic latencies, suggesting axon bifurcation close to the cell soma

	Antidromic latencies (msec)						Cancellation interval
Location	MPOA	AHA	ME	AMG	MDT	PAG	(msec)*
ARC	20·0	—	6·0	—	—	—	—
ARC	28·0	—	1·5	—	—	—	30
ARC	12·0	—	—	—	—	14·5	26
HVM	8·2	—	4·2	—	—	—	13
HVM	12·3	—	—	31·5	—	—	41
HVM	0·5	—	—	3·8	—	—	5
HVM	13·0	—	—	14·5	—	—	—
HVM	6·2	—	—	18·5	—	—	25
HVM	8·2	—	—	8·0	—	—	16
HVM	—	0·5	—	3·5	—	—	5
HVM	—	1·9	—	—	10·0	—	—
HVM	—	2·5	—	—	13·0	—	—
HVM	—	3·0	—	—	15·6	—	19
HVM	—	4·3	1·5	—	—	—	6
HVM	—	5·0	1·3	—	—	—	7
HVM	12·8	—	—	—	—	21·8	32
HVM	6·0	—	—	—	—	8·5	—
HVM	6·0	—	—	—	—	22·0	—
HVM	4·7	—	—	—	—	3·0	7
HVM	5·5	—	—	—	—	2·5	8

laterally to the amygdala (Fig. 8) or ventrally to the median eminence (Fig. 11). In these instances a correlation between the antidromic latency from each region and the distance to the stimulation site indicated that conduction velocities were approximately similar in each axon branch, and seldom exceeded 1 m/sec. In the interaction experiments (Figs. 7*B*, *C*, *E*, *F*, 8*C*, *D*, 11*C*, *D*) the antidromic spike cancellation interval (Table 1) approached the sum of the two distinct antidromic latencies suggesting that the axon bifurcation occurred close to the origin of the axon (cf. Horrobin, 1966).

Response from amygdala. Neurones located in the ventromedial nucleus

usually display excitation or inhibition after amygdala stimulation (Renuad, 1976*b*). Many MBH neurones that projected to MPOA were located in the ventromedial nucleus and usually responded to amygdala stimulation with either excitatory or inhibitory responses (Fig. 9). Since the amygdala is also known to receive fibres from ventromedial nucleus neurones (Renaud & Hopkins, 1976) it was not surprising to observe occasional examples of antidromic invasion after amygdala stimulation (Fig. 8; Table 1).

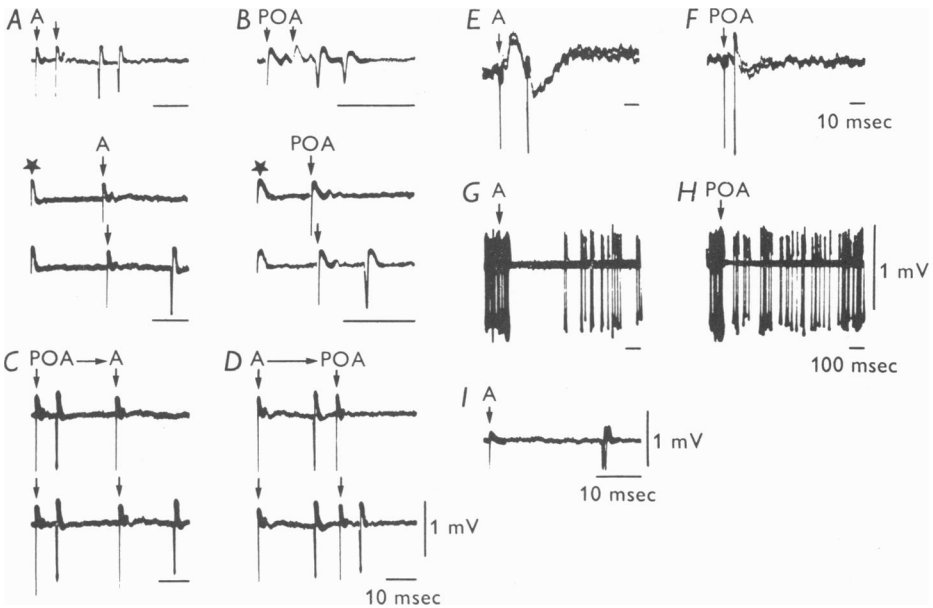

Fig. 8. *A–D*, oscilloscope traces obtained from a ventromedial nucleus neurone that demonstrated features of antidromic invasion from both the basolateral amygdala (A) and the medial preoptic area (POA). *E–F*, records obtained with a wide band pass (10 Hz to 10 kHz) to illustrate the antidromic unit responses superimposed on the local field potentials. *G–H*, a marked depression of activity for 500 msec was only observed after amygdala (but not preoptic) stimulation at intensities suprathreshold for antidromic invasion. *I*, amygdala stimulation at intensities subthreshold for antidromic invasion evoked orthodromic responses at a latency of 25–27 msec (band pass 500 Hz–10 kHz).

Tuberoinfundibular neurones

A total of 39 MBH tuberoinfundibular neurones identified by antidromic invasion after stimulation of the surface of the median eminence (Makara, Harris & Spyer, 1972; Sawaki & Yagi, 1973; Renaud, 1976*a*, *c*) were tested with MPOA (thirty cells) or AHA (nine cells) stimulation. These neurones were located in the ventromedial nucleus (eighteen cells),

the arcuate nucleus (fifteen cells) and the periventricular region (six cells). During the period of observation only seven tuberoinfundibular cells displayed any spontaneous activity.

Excitation. The majority of tuberoinfundibular neurones were unresponsive to MPOA–AHA stimulation (twenty-two cells from MPOA; five cells from AHA). Of the responsive neurones, five cells displayed short latency

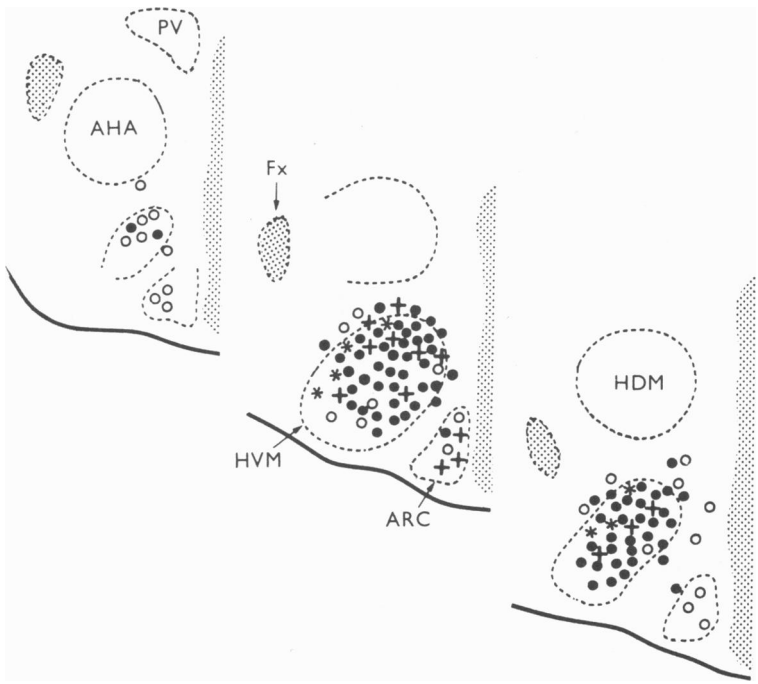

Fig. 9. The various symbols indicate the approximate locations of neurones that displayed antidromic invasion from MPOA–AHA superimposed on a schematic diagram through three levels of the mediobasal hypothalamus. The various symbols depict the nature of their response to amygdala stimulation: short latency excitation (●), inhibition (*) and unresponsive neurones (○). Note that most of the responsive neurones were located within the ventromedial nucleus. The Figure also indicates the location of fourteen neurones with axons that branch (+) to other sites in addition to the medial preoptic area (see Table 1). ARC, arcuate nucleus; Fx, fornix; HDM, hypothalamic dorsomedial nucleus; HVM, hypothalamic ventromedial nucleus; PV, paraventricular nucleus.

orthodromic activation (latency range 5·5–30·0 msec). Three of these cells were located within the ventromedial nucleus, and all exhibited short latency excitation from amygdala stimulation (cf. Fig. 2 in Renaud, 1976*d*).

Inhibition. The discharges of four of seven spontaneously active tuberoinfundibular neurones were depressed for 100–150 msec after MPOA-AHA stimulation (latency range 6·0–20·0 msec). All four cells were located within the ventromedial nucleus. Three cells displayed similar responses from amygdala stimulation (Fig. 10*D*, *E*); the activity of the other tuberoinfundibular cell in this group was increased following amygdala stimulation.

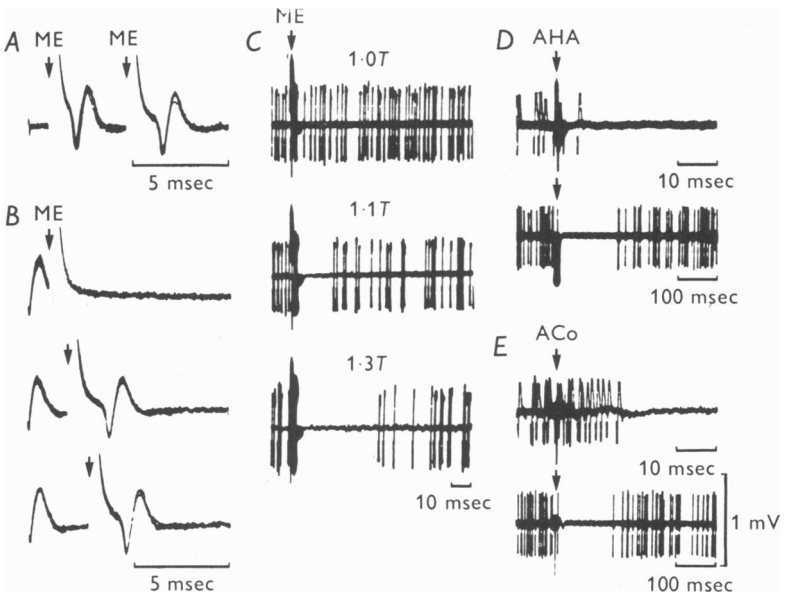

Fig. 10. Data from a ventromedial nucleus tuberoinfundibular neurone. *A*, constant latency responses observed after median eminence (ME) stimulation; note the slightly longer latency for the second antidromic response. *B*, the upper trace illustrates cancellation of the antidromic spike by a spontaneous spike (shown at the onset of each trace). The lower traces illustrate that the antidromic latency is slightly prolonged as the stimulus is delivered closer to this critical interval. *C*, this spontaneously active cell displayed a progressively longer silent period at increasing suprathreshold intensities for antidromic invasion from median eminence stimulation, suggesting a recurrent post-synaptic inhibitory process. *D*, *E*, stimulation in the anterior hypothalamic area (AHA) and cortical nucleus of amygdala (ACo) also evoked short latency silent intervals with a duration of 100 msec.

Antidromic invasion. Five inactive neurones (two in the arcuate nucleus, three in the ventromedial nucleus) displayed antidromic invasion from both the median eminence and from MPOA–AHA stimulation (Fig. 11). Details of their location, latencies of activation and critical intervals for cancellation of the antidromic spike from each stimulation site are included in Table 1. These observations provide further support for

extrahypothalamic axon collaterals in the tuberoinfundibular pathway (Renaud, 1976*a*, *c*).

From the population of MBH neurones studied, those with a termination on the median eminence, i.e. tuberoinfundibular cells, are the only cells with a role that can be partially defined since they are presumably involved in the regulation of adenohypophyseal secretion (Harris, 1975; Szentágothai *et al.* 1968; Makara *et al.* 1972; Blackwell & Guillemin, 1973;

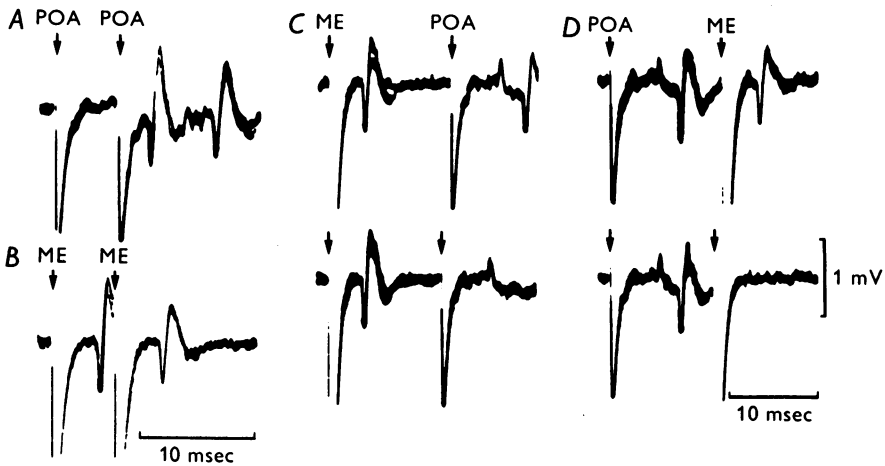

Fig. 11. Axon branching in tuberoinfundibular neurones. *A*, *B*, constant latency unit responses at high stimulation frequencies were observed after both medial preoptic area (POA) and median eminence (ME) stimulation. *C*, *D*, the upper and lower traces in each set illustrate the critical interstimulus intervals that result in cancellation of the second antidromic response.

Sawaki & Yagi, 1974; Renaud, 1976*c*). Therefore, the responses recorded from tuberoinfundibular neurones were utilized to construct a schema of possible neural connexions between MBH tuberoinfundibular neurones and both MPOA–AHA and the amygdala. The afferent and efferent neuronal connexions of the different tuberoinfundibular cells depicted in Fig. 12 are proposed on the basis of electrophysiological observations, and indicate the apparent heterogeneity in the connexions of some tuberoinfundibular neurones, particularly those located within the ventromedial nucleus.

DISCUSSION

The present results indicate that MPOA–AHA stimulation is associated with orthodromic responses from at least 11·5 % of MBH neurones. While MPOA–AHA stimulation probably excites both fibres of passage and perikarya of local neurones, there is anatomical evidence of a substantial

neuronal projection from MPOA–AHA to MBH (Conrad & Pfaff, 1975, 1976*a*, *b*; Swanson, 1976). Dyer (1973) has also reported electrophysiological evidence that 41% of MPOA–AHA neurones project directly to the ventromedial-arcuate region, an estimate that probably includes the preoptic tuberoinfundibular pathway (Renaud, 1976*e*). Thus there may

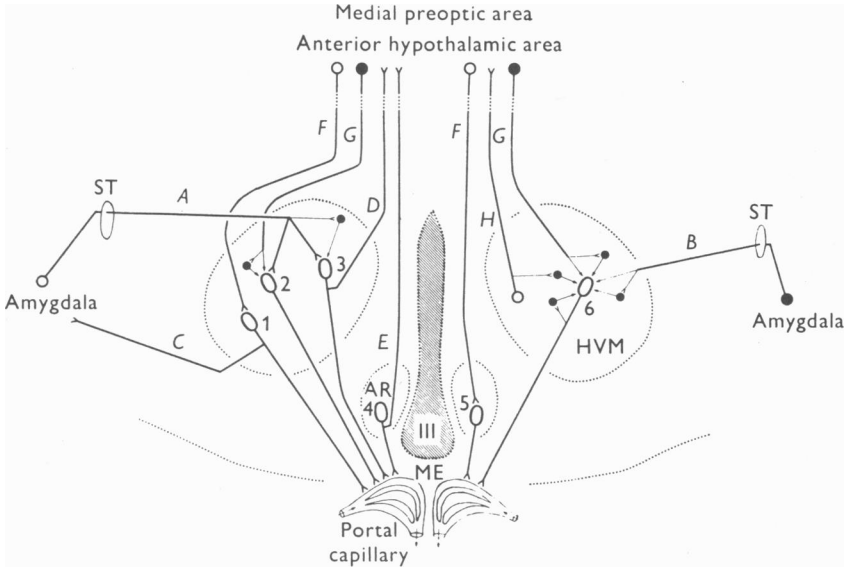

Fig. 12. This schematic coronal section through the mediobasal hypothalamus outlines the relative positions of the ventromedial (HVM) and arcuate (AR) nuclei. The Figure illustrates postulated connexions between tuberoinfundibular neurones (nos. 1–6, connected directly to the median eminence (ME) portal capillary plexus), the amygdala and medial preoptic and anterior hypothalamic areas. The heavy continuous lines depict principal connexions. Tuberoinfundibular neurones in HVM (nos. 1–3, 6) receive connexions from both the amygdala and more rostral regions, while arcuate tuberoinfundibular neurones (nos. 4, 5) are mostly connected only with rostral regions. Afferent extrahypothalamic pathways presumed to mediate excitatory connexions originate from open circles (*A*, *F*). Extrahypothalamic inhibitory connexions are shown to originate from filled circles (*B*, *G*) and may be mediated either directly or through afferent collateral pathways to local inhibitory interneurones (identified as small filled circles). The fine continuous lines indicate *intranuclear* recurrent or afferent collateral inhibitory pathways. Note that some tuberoinfundibular neurones (e.g. no. 6; cf. Fig. 10) may receive inhibitory connexions from several sources: through direct or afferent collateral pathways from amygdala (*B*) and medial preoptic–anterior hypothalamic area (*G*); through recurrent collateral pathways from ascending fibres of adjacent HVM neurones (*H*) or axon collaterals in the tuberoinfundibular tract. Axons of some tuberoinfundibular neurones (nos. 1, 3, 4) bifurcate and send fibres to the amygdala (*C*) and medial preoptic area (*D*, *E*).

be an even greater number of MPOA–AHA afferents to MBH than is indicated by the responsive cells reported here. Furthermore, synaptic excitation and inhibition patterns observed from MBH neurones in this investigation can be presumed to indicate activity in these descending fibres or their axon collaterals, in many instances through monosynaptic connexions (Fig. 12). The generally longer latencies for inhibition than for excitation suggests that the former may be mediated indirectly through di- or polysynaptic connexions through afferent collateral fibres that terminate locally on MBH inhibitory interneurones (Fig. 12).

Antidromic activation of 20 % of MBH neurones indicates that a significant population of these cells project rostrally at least as far as MPOA–AHA. Both the localization (i.e. arcuate and ventromedial nuclei and periventricular region) and the percentage of MBH neurones with MPOA–AHA projections are in agreement with the results of other electrophysiological studies (Harris & Sanghera, 1974; Makara & Hodacs, 1975). Since Dyer (1973) has recorded orthodromic responses in 32 % of preoptic and anterior hypothalamic neurones after stimulation in the ventromedial-arcuate region, one must assume that some of these ascending axons do have terminals in MPOA–AHA, even through their final destiny may be elsewhere.

One interesting observation recorded from certain hypothalamic neurones that exhibit all of the criteria considered to characterize antidromic invasion (see Methods) either from intra- or extrahypothalamic sites (cf. Renaud, 1976*c*, *d*; Renaud & Hopkins, 1976) has been their rather wide range of antidromic invasion latencies. The occasional instance of neurones with very long antidromic invasion latencies (up to 38 msec over distances of 3–4 mm) would imply very slow axon conduction velocities. These findings probably reflect the diversity of axon diameters observed in morphological studies of the mediobasal hypothalamus where some of the smaller unmyelinated fibres demonstrate axon diameters under 1·0 μm (Brawer, 1972; Brawer & Sonnenschein, 1975). Despite their small size, these axons appear to conduct impulses antidromically at frequencies greater than 200 Hz.

This study has utilized stimulation in other areas to add further details to the neural organization of MBH neurones. For example, amygdala stimulation has been shown to evoke synaptic excitation and inhibition from many ventromedial neurones that project to MPOA–AHA. This may provide an indirect disynaptic pathway through which the amygdala can influence the excitability of MPOA–AHA neurones. These connexions would therefore supplement the direct amygdalo-preoptic fibres reported anatomically (Heimer & Nauta, 1969; Field, 1972). The amygdala itself receives afferent connexions from mediobasal hypothalamus (Renaud &

Hopkins, 1976) and occasionally the same neurone projects to both MPOA and to the amygdala (cf. Fig. 8; Table 1). These observations suggest that there must be a close functional relationship between MBH neurones on the one hand, and neurones in the amygdala and MPOA on the other hand.

The electrophysiological data presented here indicate a rather extensive axon collateral organization for MBH neurones (Table 1). While the electrophysiological results do not provide an indication of the magnitude of axonal arborization within the hypothalamus, golgi studies (e.g. Szentágothai *et al.* 1968; Millhouse, 1973*a*, *b*) suggest that axon collaterals are frequently observed in the mediobasal hypothalamus. Is there evidence of a role for these axon collaterals? Presumably many of the *intranuclear* collaterals are engaged in local inhibitory networks since post-synaptic-inhibition appears to be prominent within the hypothalamic region (Murphy & Renaud, 1969; Renaud, 1976*b*). The diverging *extrahypothalamic* axon branching patterns reported here may be necessary for the complex behavioural and homoeostatic functions of the hypothalamus, but our limited knowledge of the neurophysiology of emotionality, aggression and feeding behaviour precludes adequate descriptions of their function. Only in the area of neuroendocrine control can one elaborate on the possible significance of extrahypothalamic axon collaterals of identified tuberoinfundibular neurones, since these cells are presumed to represent the neurosecretory neurones responsible for adenohypophyseal regulation (Szentágothai *et al.* 1968). Thus, according to Dale's hypothesis (Dale, 1935) activity in the tuberoinfundibular system may be associated with peptide release not only at median eminence nerve terminals, but also at central nervous system termination sites of axon collaterals of tuberoinfundibular neurones (Renaud & Martin, 1975; Renaud, 1976*c*; Renaud, Martin & Brazeau, 1976). This postulate may require revision since more than one substance appears to be secreted at sites of synaptic contact (cf. Silinsky, 1975; Schubert, Lee, West, Deadwyler & Lynch, 1976). Nevertheless, the hypothesis is supported by observations that certain hypothalamic peptides are localized in specific extrahypothalamic regions (Brownstein, Arimura, Sato, Schally & Kizer, 1975; Wilbur, Montoya, Plotnikoff, White, Gendrich, Renaud & Martin, 1976) primarily within nerve terminals and synaptosomes (Zimmerman, Hsu, Ferin & Kozlowski, 1974; Barnea, Ben-Jonathan, Colston, Johnston & Porter, 1975; Sétáló, Vigh, Schally, Arimura & Flerko, 1975; Tsang, Tan, Brazeau, Lal, Renaud & Martin, 1975; Ramirez, Gautron, Epelbaum, Pattou, Zomoro & Kordon, 1975). Axoplasmic transport in axon collaterals in peptidergic pathways may represent a mechanism for extrahypothalamic distribution of certain hypothalamic peptides (Martin, Renaud & Brazeau,

1975; Renaud *et al.* 1976). Evidence that these peptides can influence neuronal excitability (Dyer & Dyball, 1974; Kawakami & Sakuma, 1976; Renaud, Martin & Brazeau, 1975) and animal behaviour (Prange, Nemeroff, Lipton, Breese & Wilson, 1977) further supports a role for some of these peptides in neuronal function, possibly as modulators of neuronal activity or as neural transmitter agents. However, there are still very important questions to be answered. For example, there is as yet no information on the association between activity in the tuberoinfundibular system and neural secretion of releasing factors. Nor is it certain whether the tuberoinfundibular system contains mechanisms that can restrict impulse traffic at points of axon bifurcation (cf. Grossman, Spira & Parnas, 1973; Waxman, 1976).

The medial preoptic and anterior hypothalamic areas are important in the regulation of several adenohypophyseal hormones. Stimulation in these areas results in a rise in plasma TSH (Martin & Reichlin, 1972) and a decrease in plasma growth hormone (Martin, Tannenbaum, Willoughby, Renaud & Brazeau, 1975). This area also appears responsible for the preovulatory surge of luteinizing hormone (Halász, 1969; Cramer & Barraclough, 1973; Wuttke, 1974). The same region contains neuronal perikarya immunoreactive for somatostatin (Alpert, Brawer, Patel & Reichlin, 1976) and luteinizing hormone releasing hormone (Barry, Dubois & Carrette, 1974). The presence of tuberoinfundibular neurones in the medial preoptic area (Makara, Harris & Spyer, 1972; Harris & Sanghera, 1974; Renaud, 1976*e*) further supports the role of this region in the regulation of adenohypophyseal secretion.

Surgical deafferentation of mediobasal hypothalamus does not alter basal levels of adenohypophyseal secretion or certain pulsatile secretory patterns (Halász, 1969; Blake & Sawyer, 1974; Willoughby, Terry, Brazeau & Martin, 1976) but does result in the loss of an adaptive flexibility normally present in the neuroendocrine axis. For example, cyclic ovulation in the female rat ceases (Hálasz, 1969) and diurnal or inductive variations in corticosterone are absent (Krey, Lu, Butler, Hotchkiss, Piva & Knobil, 1975; Wilson & Critchlow, 1975). According to the schema in Fig. 12, two extrahypothalamic regions, i.e. the medial preoptic area and the amygdala can influence the excitability of mediobasal tuberoinfundibular neurones, and these areas are presumably part of the neuronal connexions important for this adaptive modification in the neuroendocrine axis. The neural pathways that connect the tuberoinfundibular neurones depicted in the schema in Fig. 12 may be oversimplified, but are presumed to represent the network through which these effects are manifested, and provide a useful preliminary framework for further studies on the neural organization within the mediobasal hypothalamus.

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