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SPONTANEOUS ACTIVITY OF SINGLE NEURONES IN THE HYPOTHALAMUS OF RABBITS DURING SLEEP AND WAKING

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SUMMARY

1. A method is described for recording from single cells in the hypothalamus of unanaesthetized freely moving rabbits. Behaviour, bodily movement, skin and brain temperatures and e.e.g. were monitored.

2. Patterns of unit firing during slow sleep, paradoxical sleep and waking were studied in several regions of the hypothalamus, thalamus and in the septum.

3. Of the 144 cells analysed from waking to slow sleep, fifty-six (39 %) decreased mean firing rates, thirty (21 %) increased spike discharges and fifty-eight (40 %) showed no marked change. Dorsal hypothalamic and massa intermedia thalamic cells fired in brief high frequency clusters during slow sleep with a characteristic 'bimodal' interspike interval histogram. Waking and paradoxical sleep abolished these cluster discharges with a concomitant change to an 'asymmetric' histogram.

4. Of the thirty-two cells observed during the three states of waking, slow sleep and paradoxical sleep, a majority (twenty-five or 78 %) showed their highest rates of spontaneous discharge during paradoxical sleep. Discharge rates of cells sometimes changed in the course of paradoxical sleep according to the presence or absence of phasic events such as myoclonic motor activity. Two hypothalmic cells were almost totally arrested during paradoxical sleep.

5. Analysis of unit firing rates during spontaneous rises in brain temperature during waking and paradoxical sleep revealed that a majority of the neurones (22/24) changed their discharge rates in relation to behaviour rather than to brain temperature. Two cells did appear to respond specifically to the central thermal stimulus.

6. Hypothalamic cells do not behave as a homogeneous population in

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relation to changes in the state of arousal of the rabbit. Spontaneous changes in cell discharge related to sleep-waking behaviour must be considered in any interpretation of hypothalamic unit activity as related to neuroendocrine or autonomic mechanisms.

INTRODUCTION

The cyclic nature of hypothalamic functions in mammals is closely related to environmental stimuli (light, sound, etc.) and to patterns of behaviour. Despite the physiological importance of sleep and waking behaviour for hypothalamic function, no systematic attempts as far as we are aware have been made to study the spontaneous activity of the single hypothalamic neurone during natural sleep and wakefulness in the freely moving animal.

Studies of extra-hypothalamic unit activity in the lateral geniculate body (Hubel, 1960), mid-brain (Huttenlocher, 1961), pons and medulla (Bizzi, Pompeiano & Somogyi, 1964) and visual and motor cortex (Evarts, 1962, 1964) of unanaesthetized animals show a high correlation between cell firing rates and the behavioural state. Temporal patterns of discharge of hypothalamic units have been studied in the anaesthetized animal in response to osmotic, thermal, humoral and sensory stimuli (Cross & Silver, 1966 review). Experiments in the urethanized rat have displayed an important relationship between shifts in e.e.g. activity and hypothalamic unit discharges (64 % responsive, Komisaruk, McDonald, Whitmoyer & Sawyer, 1967). However, a study of thermosensitive neurones in the hypothalamus of the unanaesthetized rabbit indicated that only a small proportion (7 %) of the hypothalamic cells examined were responsive to shifts in behaviour (Hellon, 1967).

It was our expectation, based on the known anatomy and physiology of the hypothalamus, that hypothalamic cells in the unanaesthetized animal would actually be as responsive to changes in behaviour as cells in the medulla, pons, mid-brain, thalamus or cortex are. Recent studies in this laboratory on brain temperature (Baker & Hayward, 1967*a*, *b*, 1968; Hayward & Baker, 1968*a*, *b*; Hayward, 1968) and those of other workers (von Euler & Söderberg, 1957) have indicated a close relationship between changes in e.e.g., behaviour and temperature regulation. In the present experiments we examine the effects of sleep and wakefulness on the spontaneous activity of single cells in the hypothalamus and adjacent regions of the freely moving rabbit. A preliminary account of some of this work has been published (Findlay & Hayward, 1968).

METHODS

Sixteen adult female New Zealand white rabbits weighing 2·5–4·5 kg. were used. Under pentobarbitone anaesthesia (30 mg/kg) the calvarium was aseptically opened without excision of the underlying dura mater and a rigid cylinder (stainless-steel bone-fixed adapter) 12 mm outside diameter at the skull, see Fig. 1) was stereotaxically placed on the dura with its centre at APO (Sawyer, Everett & Green, 1954). The cylinder was cemented to an elevated lucite platform (Baker, Burrell, Penkhus & Hayward, 1968) which was itself cemented to three stainless-steel epidural skull screws. The margins of the opening around the cylinder were sealed with dental cement, and the cylinder, which subsequently supported the micro-electrode carrier, was filled with bone wax and capped (Fig. 1). Biparietal insulated silver-ball electrodes with a small surface bared were implanted epidurally and the lead wires soldered to a five pin receptacle (Winchester Electronics Co.) which was cemented to the platform. A copper-constantan arc-welded thermocouple in glass tubing (o.d. 0·7 mm) was stereotaxically implanted in the mid-line at P6 and pushed through the brain to the basal subarachnoid space so that the thermojunction at the tip of the tube lay at the basilar artery. We have previously established that the temperature measured in the basal subarachnoid space near the cerebral arteries is a sensitive index of temperature of cerebral arterial blood, central arterial blood and shifts in hypothalamic temperature (Baker & Hayward, 1967*a*; Hayward & Baker, 1968*b*). Thermocouple wires were attached to miniature copper-constantan connectors (Thermoelectric Co., No. MX-JTX) which were cemented to the platform. Two five-pin connectors (Amphenol Co., No. 233–1105) were mounted on the front of the platform for attachment of a field effect transistor amplifier, power input, and unit output connexions.

Figure 1 illustrates the calibrated hydraulic microdrive arrangement (Trent H. Wells, Jr., Mechanical Developments Co.) used in these experiments. The remote 'slave' cylinder of the microdrive (h.m.) is attached to the rigid cylinder (b.f.a.) on the skull by an aluminium bracket (c.a.) and a Starr guide (S.g.). By use of a series of Starr guides with holes eccentrically placed, a sequence of tracks could be explored around the circumference of circles with radii varying from 0·25 to 2·5 mm. During recording, the stainless-steel guide tube (24 gauge) (g.t.), which is attached to the guide tube clamp of the cylinder adapter, was passed stereotaxically through the Starr guide, bone wax (b.w.), dura mater (d) and into the brain to lie 10 mm below the surface just above the hypothalamus. Set screws hold these components together (Fig. 1). The micro-electrode (e) was then lowered out of its protected position in the guide tube to pass through the brain to the hypothalamus. Bone wax was applied to all metal surfaces and bone wax filled the rigid cylinder to provide a tight seal thus lessening cerebral pulsations due to a change in position and fluctuations of venous and arterial pressure.

Micro-electrodes were made from 100 μ platinum iridium (70–30 %) wire electrolytically etched and polished to a tip diameter of 1 μ or less with a 22 degree taper. Electrode tips were plated with platinum black to reduce electrical resistance. The 50 mm length of sharpened wire was insulated to within 10–20 μ of the tip with 2–4 coats of hi-bake primer paint (Dupont, No. 828–014) by the method of Kinnard & MacLean (1967). Detection of the length of the uninsulated tip and possible breaks in the insulation was made with a capacitance meter (Tektronix, Type 130 L-C Meter). Electrodes with a total tip capacitance of 10–30 pF were used.

Extracellular unit potentials recorded between the micro-electrode tip and the indifferent cylinder in the skull were initially amplified by a field effect transistor amplifier (Motorola, MPF-105) mounted on the platform. The signal was led via a high impedance probe into a high gain a.c. coupled preamplifier of band pass 80–10,000 c/s and input resistance 10 MΩ (Grass P511). Unit activity was continuously monitored with a dual beam oscilloscope (Tektronix 565) and the signal from the Y-plates of the oscilloscope led to an audio-amplifier

(Grass), to one channel of a FM/Direct magnetic tape recorder (Ampex CP 100) and to a pulse height discriminator ('Peak amplitude selector', Martin, 1969). Four outputs of this discriminator were displayed on the four trace amplifier (Type 3A74) of the second beam of the oscilloscope: 2 d.c. levels showing upper and lower thresholds of the discriminator, the raw unit data, and short pulses (0·5 msec) triggered by action potentials of the spike in the window. Three outputs of this pulse height discriminator were displayed on an ink-writing oscillograph (Offner Type-R Dynograph): a pulse out for every impulse in the window, a pulse out for every 10 impulses in the window, and an analog output proportional to the rate of arrival of impulses in the window. Electroencephalogram potentials, skin and brain thermocouple e.m.f.s (ice water reference junction, 0° C) and accelerometer outputs were amplified and recorded simultaneously with the unit data on the ink writer.

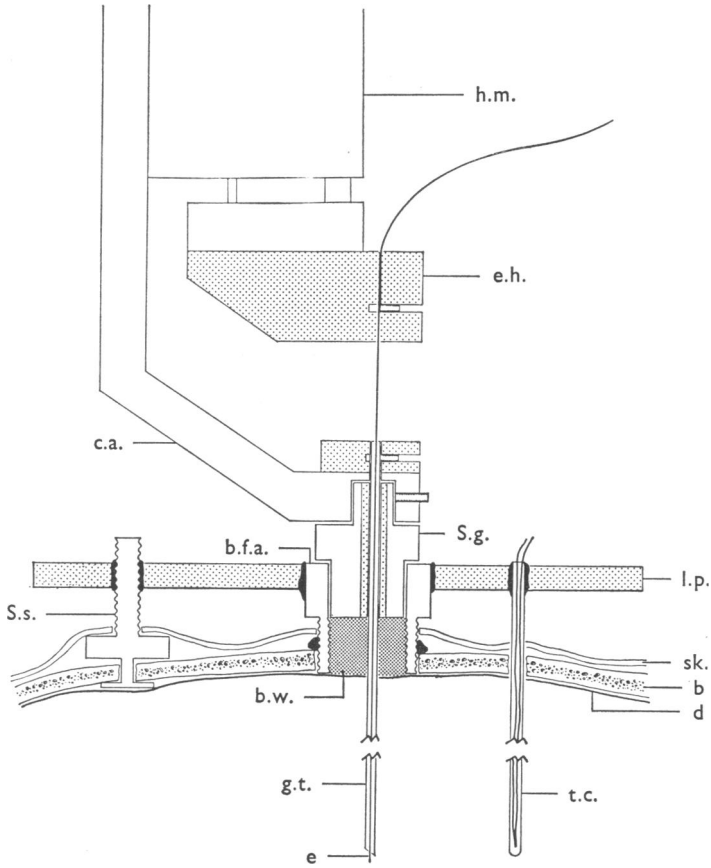

Fig. 1. Diagrammatic view of the hydraulic microdrive on the head of a rabbit (not to scale). A cranial platform holding the stereotaxic bone fixed adapter cylinder and brain thermocouple is chronically fixed above the scalp on three screws cemented in the skull (Baker *et al.* 1968). Dental cement shown in black. Note set screws holding e, g.t. and S.g. Labels: h.m. hydraulic microdrive-slave cylinder; e.h., electrode holder; c.a., cylinder adapter and guide tube clamp; S.g., Starr guide; b.f.a., stereotaxic bone fixed adapter, 12 mm diam. at skull; l.p., lucite platform; S.s., Sheatz screws; sk., skin of the scalp; b, bone of the skull; d, dura mater; b.w., bone wax; g.t., guide tube; e, micro-electrode; t.c., thermocouple.

After each animal had recovered from surgery, as indicated by its eating and drinking at preoperative levels (within 3–7 days), it was placed in a well ventilated, lucite panelled observation box (25 × 50 × 32 cm) that was suspended from strong springs in a wooden frame. A vibration pickup accelerometer (MB Electronics, Type 124) was attached to the bottom of the box to allow detection of gross body movements. A bared thermocouple (copper-constantan) was taped to the hairless dorsal surface of the ear. The animal and apparatus were housed within a sound attenuated thermoregulated chamber with a one-way glass observation window and a low level of overhead illumination. The rabbits initially explored their surroundings for some minutes and then lay in a relaxed posture with alternating periods of slow sleep, quiet waking and, if undisturbed, infrequent episodes of paradoxical sleep. Coprophagia and grooming occurred intermittently. Most animals were allowed unrestrained movement. Certain active rabbits were tethered to the bottom of the box by a lucite neck collar to prevent standing and violent body movements. Over one hundred experiments, from 4 to 8 hr in duration, were conducted 3 to 4 times each week at ambient temperatures of 20–25° C. If the recording session lasted longer than 4 hr the animals were given food and water within the recording cage. A total of eight electrode penetrations was performed in each rabbit. During these recording sessions the temporal relationships between spontaneous changes in behaviour, hypothalamic unit activity, e.e.g. activity, body movement and brain and skin temperatures were observed. The presence of sleep or waking during each period of observation was judged on the basis of the e.e.g., body movement record and observed behaviour.

Three clearly distinct states were chosen for analysis. (A) *SS: slow sleep with e.e.g. high voltage slow waves.* This showed the recumbent rabbit with eyes closed and head and ears elevated, no gross body movement, high amplitude slow waves in the electrocorticogram. (B) *PS: paradoxical sleep with e.e.g. low voltage fast waves.* This showed the recumbent rabbit with eyes closed and head and ears down, an e.e.g. indistinguishable from the waking record, initially without body movements, later twitching of vibrissae, ears, jaws, facial muscles, limbs and rapid eye movements, intense vasoconstriction of the skin of the ear with elevation of brain temperature, and abrupt termination of episode signalled by elevation of ears and head, cutaneous vasodilatation with resumption of SS or W. (*C*) *W: waking with e.e.g. low voltage fast waves.* This showed the rabbit lying, sitting or standing with eyes open and head and ears up and varying amount of body movement (Baker & Hayward, 1967*a*; Jouvet, 1967).

At the end of the studies the chronically prepared animal was anaesthetized and iron deposited electrolytically at several known levels along the electrode tracks using a stereotaxically positioned epoxy-insulated steel dental broach (Cross & Green, 1959). The brain was then perfused through the carotid artery with isotonic saline solution containing formalin (10 %) and sodium ferrocyanide (2 %). Serial frozen sections were cut at 80 μ in the stereotaxic coronal plane and stained with thionin. The marked micro-electrode tracks were easily identified by the Prussian blue spots and the Horsley–Clarke co-ordinates for each cell determined. Electrode tracks were reconstructed and unit locations placed on outline drawings of the rabbit diencephalon (Sawyer *et al.* 1954).

Analysis of data began with identification of the various states described above (SS, PS, W) which follow the criteria of others (Jouvet, 1967). Because there were often long periods of transition between W and SS not all of the recording time was classified. Following identification of the periods of recording which were to be subjected to further analysis, the corresponding sections of the magnetic tape record were replayed and the discharges monitored on an oscilloscope so as to establish the stability of the wave form of the unit. Single cell spike trains, clearly separable from base line activity and neighbouring units, were led into the pulse height discriminator and short (0·5 msec) pulses triggered by the unit action potentials were recorded on magnetic tape. An SDS 930 computer, using a sampling rate of 2000 samples/sec, was programmed to identify the times of occurrence of the spikes, and to

record these times on a digital tape. The statistical analysis was then performed by the IBM 360/75 computer with a programme which calculated the mean firing rate, interspike interval mean, standard deviation, coefficient of variation, and histogram of any desired order, and computed the spike train autocorrelogram (Perkel, Gerstein & Moore, 1967*a*, *b*). This programme allowed accumulated statistics to be performed on a unit during identical behavioural states which were temporally dispersed. Thus, firing patterns of a neurone during several periods of a particular behavioural state, for example slow sleep, could be analysed separately and the accumulated statistics and histogram presented.

RESULTS

We recorded more than 1000 cells in the diencephalon of the unanaesthetized, moving rabbit; 234 of these were selected for detailed statistical analysis. One hundred and forty-eight were recorded during both waking (W) and slow sleep (SS). Thirty-two cells were examined during the three states of W, SS and paradoxical sleep (PS). Recording time for each cell varied from 3 to 60 min with an average duration of about 15–20 min. Sustained unit discharges varied from mean rates of less than 0·1 spikes/sec to a maximum of 90 spikes/sec. Brief, high frequency cluster discharges of up to 500 spikes/sec or more were also observed.

Spontaneous activity of cells during slow sleep and waking

Slow sleep and quiet waking were the two behavioural states most often exhibited by our animals. Of the 144 cells analysed during SS and W, ninety-eight were situated in the hypothalamus, eighteen in the septum, twenty-two in the massa intermedia and ten in other ventral thalamic nuclei (Table 1). Using the criteria of a greater than 50 % change in mean firing rate as indicative of 'significant' change we found that fifty-six (39 %) of these cells decreased, thirty (21 %) increased, and fifty-eight (40 %) showed no change in firing rate from waking to slow sleep (Table 1).

Figure 2 illustrates a dorsal hypothalamic cell that fires faster during

Legend to Fig. 2.

Fig. 2. Pattern of discharge of a dorsal hypothalamic unit during sleep and waking in the unanaesthetized rabbit. During slow sleep the rabbit lies quietly, e.e.g. synchronized high voltage and the unit exhibits intermittent brief high frequency bursts of up to 500 spikes/sec or more at an over-all mean rate of 2·7/sec with a 'bimodal' interspike interval histogram. Upon waking at arrow, eyes open, sniffs, sits up, e.e.g. desynchronized low voltage and the unit accelerates to a higher mean rate (6·4/sec) with a sustained train of spikes and an 'asymmetric' unimodal histogram. Time marker for upper three traces, 30 sec, for unit trace, 20 msec. Labels: Move, accelerometer measure of body movement; e.e.g., biparietal cortical electroencephalogram; mean rate, analogue output proportional to the rate of discharge; unit, spikes photographically recorded from oscilloscope; accumulated interval histograms. N = number of intervals, μ = mean interspike intervals, σ = standard deviation and CV = coefficient of variation. Note the different scale of the ordinate for SS (0·5) and W (0·1).

TABLE 1. Diencephalic cells recorded during waking and slow sleep

Brain site[1]	No. of cells	> 50 % decrease[2]	> 50 % increase	< 50 % difference
AH	21	7 (33 %)	8 (38 %)	6 (29 %)
VMH	9	4 (44 %)	2 (22 %)	3 (33 %)
LHA	15	3 (20 %)	3 (20 %)	9 (60 %)
DHA	25	14 (56 %)	1 (4 %)	10 (40 %)
DMH	16	4 (25 %)	3 (19 %)	9 (56 %)
PHA	8	1 (12 %)	3 (38 %)	4 (50 %)
M	22	13 (59 %)	4 (18 %)	5 (23 %)
SP	18	7 (39 %)	4 (22 %)	7 (39 %)
Other thalamic nuc.	10	3 (30 %)	2 (20 %)	5 (50 %)
Total	144	56 (39 %)	30 (21 %)	58 (40 %)

1. Anatomical areas correspond to Sawyer *et al.* (1954) with the exception that AH (anterior hypothalamus) comprises their AHA (anterior hypothalamic area), MPO (medial preoptic area) and LPO (lateral preoptic area); VMH, ventromedial hypothalamus; LHA, lateral hypothalamic area; DHA, dorsal hypothalamic area; DMH, dorsomedial hypothalamus; PHA, posterior hypothalamic area; M, massa intermedia of the thalamus; SP, septum pellucidum.

2. Those cells showing greater than 50 % changes in mean firing rate from waking to slow sleep are distinguished from those showing a difference in firing rate of less than 50 %.

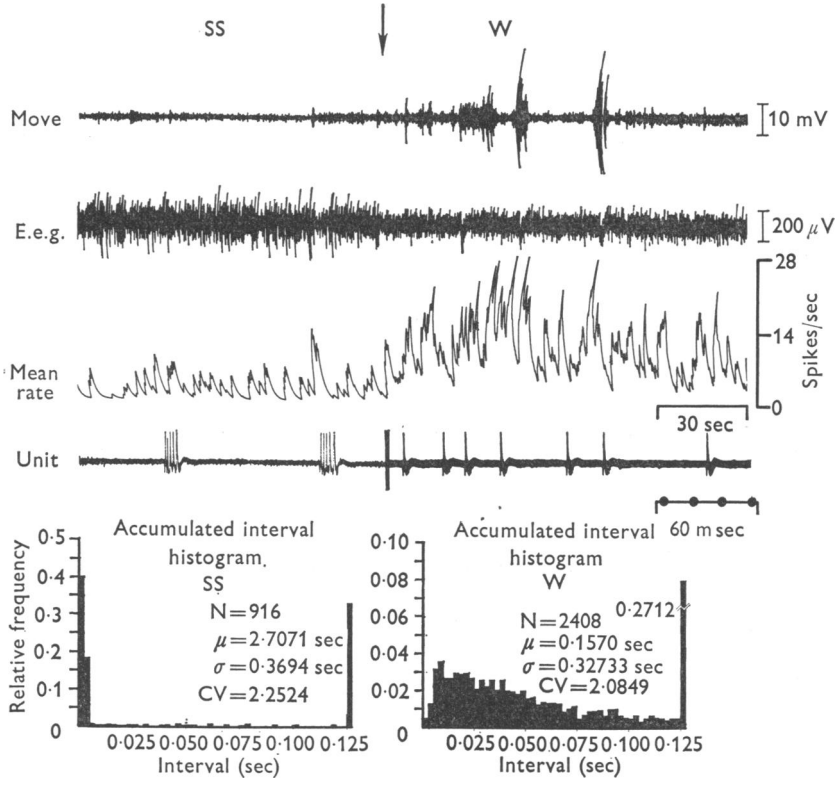

Fig. 2. For legend see opposite page.

waking (6·4/sec) than during slow sleep (2·7/sec.) During slow sleep this cell displayed brief high frequency clusters of spikes (2–6 spikes of up to 500/sec or more) in contrast to the more steady spike trains during waking. This pattern of discharge during SS is described by the 'bimodal' interspike interval histogram where the left peak indicates the short intervals of the clusters and the right peak indicates the long intercluster intervals. The absence of very short intervals and the altered distribution during waking in this cell is described by the 'asymmetric' histogram (Fig. 2). Cells of this type were common in the massa intermedia (14/22) and dorsal

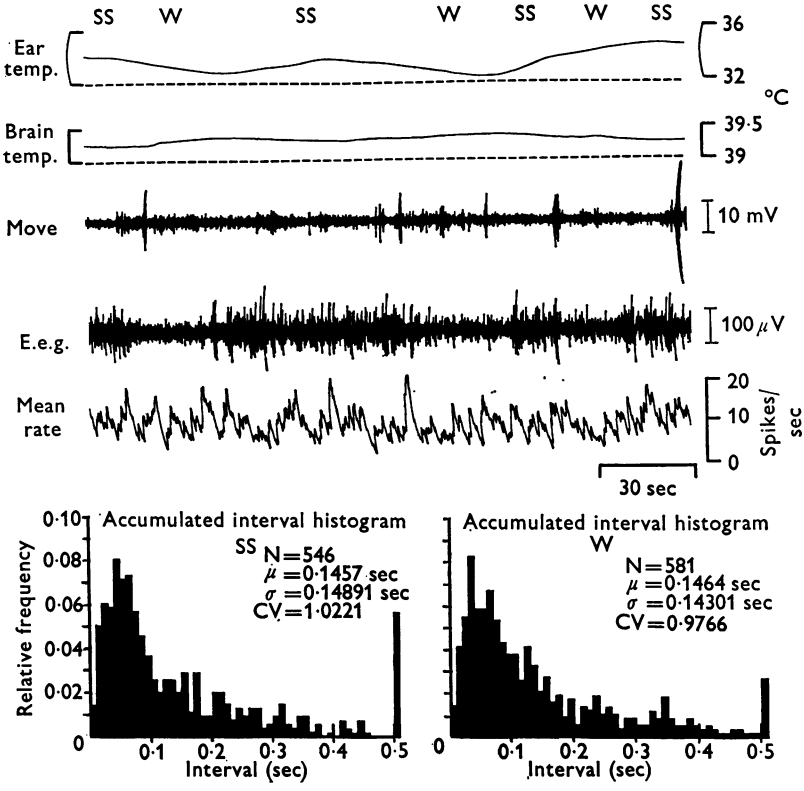

Fig. 3. Spontaneous unit activity in the lateral hypothalamus of the rabbit. The discharge pattern of this single hypothalamic neurone is not responsive to shifts in behaviour nor to change in temperature of the skin or brain. Identical mean firing rates (6·7 spikes/sec) and similar 'asymmetric' interspike interval histograms are shown during slow sleep and waking. Labels: ear temp., temperature of the skin of the ear; brain temp., temperature of the intracranial cerebral arterial blood at the basilar artery, the best measure of changing brain temperature; move, accelerometer measure of body movements, e.e.g., biparietal electrocorticogram; mean rate, analogue output proportional to the rate of unit discharge; accumulated interval histogram, N = number of intervals, μ = mean interspike intervals, σ = standard deviation and CV = coefficient of variation.

hypothalamic area (14/25); they were found less often in the posterior hypothalamic area (3/8) and rarely elsewhere in the diencephalon (1/92).

Figure 3 shows a typical lateral hypothalamic cell which failed to change its mean rate (6·7 spikes/sec) or its temporal pattern of discharge, as described by identical 'asymmetric' interval histograms, from waking to slow sleep.

The medial preoptic cell in Fig. 4 accelerated its firing rate from 23 spikes/sec during SS to 32 spikes/sec during W. The low variability of interval length of the spike trains of this cell is described by the sharp

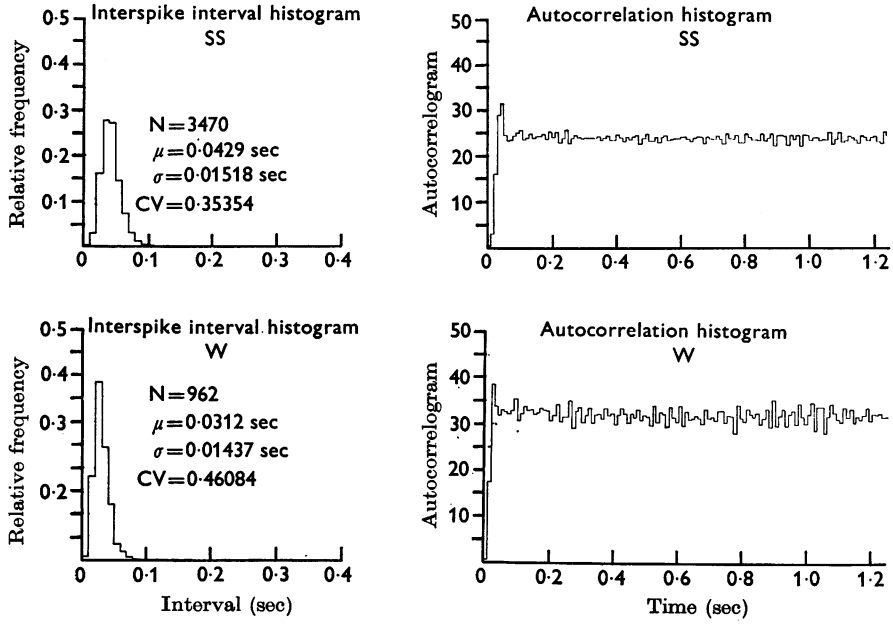

Fig. 4. Interspike interval histograms (left) and corresponding autocorrelograms (right) of a medial preoptic neurone during slow sleep and waking in a rabbit. Upper row: neuronal activity (23 spikes/sec) described by an 'asymmetric' histogram and an 'early mode' autocorrelogram during slow sleep. Lower row: acceleration of unit firing (32 spikes/sec) with 'asymmetric' histogram and 'early mode' autocorrelogram during waking. Labels: N = number of intervals; μ = mean interspike intervals; σ = standard deviation; CV = coefficient of variation.

unimodal peak of the 'asymmetric' interspike interval histogram as well as the small standard deviation (0·015) and coefficient of variation (standard deviation/mean interval, 0·35–0·46). The narrow ranges of interspike interval variation of this MPO cell contrast markedly with the pattern of discharge of the DHA and LHA cells shown in Figs. 2 and 3, respectively. The autocorrelation histograms (Perkel *et al.* 1967*a*) in Fig. 4 show an early high density peak or 'early mode' autocorrelogram, indicating that

each spike tends to be followed by another spike at a relatively constant interval. The subsequent flat part indicates that the influence of the spike at time zero is short lived (Fujita, Rosenberg & Segundo, 1968). Most of our autocorrelation histograms were 'flat' when performed on cells with high variability of interspike intervals and with 'asymmetric' interval histograms.

The over-all distribution of mean firing rates for the hypothalamic cells during W and SS is shown in Fig. 5. Figure 6 illustrates the mean firing

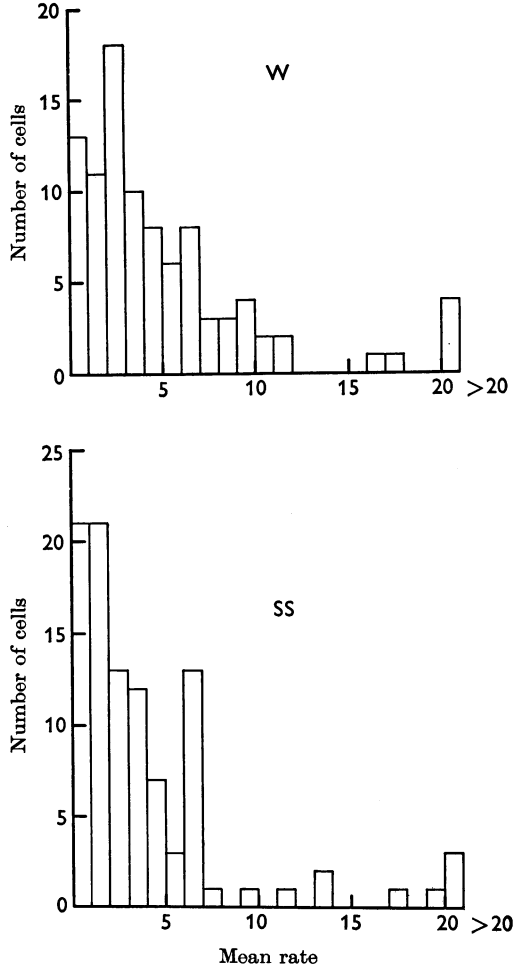

Fig. 5. Bar graph shows mean spontaneous activity (mean rate = spikes/sec) of ninety-eight units in the hypothalamus of sixteen unanaesthetized rabbits. Upper diagram: waking. Lower diagram: slow sleep. Note the tendency for less dispersion and slower mean firing rates during slow sleep in contrast to the greater spread and shift to the right (faster) discharge rates during waking.

rates for individual hypothalamic and extra-hypothalamic cells from W to SS. The mean firing rates for the anatomical groups of cells are also shown in Fig. 6. Cells of the massa intermedia and dorsal hypothalamus showed the most consistent and pronounced drop in mean firing rates (spikes/sec) between W and SS (Table 1, Figs. 2 and 6). Lateral hypothalamic cells showed the lowest mean firing rates and the least alteration of firing of any hypothalamic area with a change in behaviour (Table 1, Figs. 3 and 6).

The regions with the greatest percentage of 'significant' change in discharge rates from W to SS were the massa intermedia (77 %), anterior hypothalamus (71 %) and ventromedial hypothalamus (67 %) (Table 1, Fig. 6).

Spontaneous activity of units during paradoxical sleep

Thirty-two units were observed during the three states of waking, slow sleep and paradoxical sleep. A majority of these cells (25/32) showed their highest rates of spontaneous discharge during paradoxical sleep compared with lower rates during waking or slow sleep (Table 2). Figure 7 illustrates the temporal pattern of discharge of a massa intermedia cell showing accelerated firing during PS, lower discharge rates during W and the slowest firing during SS. The 'bimodal' interval histogram during SS (brief high frequency cluster discharges, see Fig. 2) becomes 'asymmetric' during the faster firing of this M cell during PS and W. This pattern of response is characteristic of cells in the massa intermedia and dorsal hypothalamus (Fig. 2 and Table 2).

Some of the episodes of paradoxical sleep in our rabbits were non-uniform, in that the initial 'atonic' phase (without movement) lasting from 20 to 75 sec was followed by a 'myoclonic' phase associated with intermittent jerking of the ears, face and limbs as well as with rapid eye movements. Unit discharge rates often remained low during the quiet period of PS; with the onset of twitching, the increased firing rate ensued. One septal cell, for example, fired at 0·82 spikes/sec during the first 75 sec of PS (immobile), and at 5·34 spikes/sec during the last 50 sec of PS (twitching). An exception to this general pattern is shown in Fig. 7 where accelerated firing of this massa intermedia cell occurred during the initial quiet phase of PS while the discharge rate fell during the final twitching phase of paradoxical sleep.

In two units spontaneous activity was nearly totally arrested during PS (Fig. 8), and in four other cells a reduction of activity occurred. Figure 9 illustrates the mean firing rates for individual hypothalamic and extra-hypothalamic cells from waking, slow sleep to paradoxical sleep. The overall mean firing rates for these thirty-two cells indicate a progressive in-

crease in discharge frequency when going from SS to W to PS. Cells in the hypothalamus fired at lower average rates than cells in extra-hypothalamic sites under similar behavioural conditions.

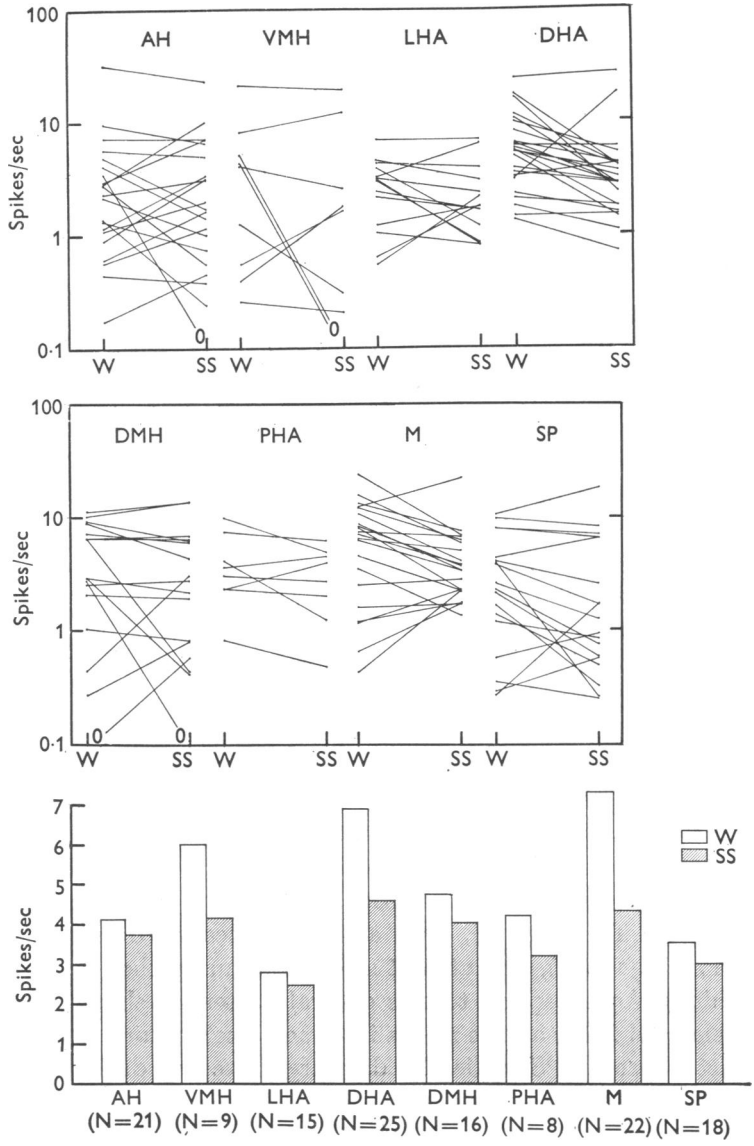

Fig. 6. For legend see opposite page.

Spontaneous changes in brain temperature and diencephalic unit activity

Twenty-four units were studied during spontaneous elevation in brain temperature related to movement during waking (increased heat production) or to peripheral cutaneous vasoconstriction (decreased heat loss) during paradoxical sleep.

We attempted to answer three questions about brain temperature and hypothalamic units. (1) Does the rise in brain temperature during paradoxical sleep in the rabbit result from vasoconstriction of the skin of the ear, retention of body heat with elevation of central arterial blood, cerebral arterial blood and brain temperatures (Baker & Hayward, 1967*a*)? (2) Are the spontaneous and physiological shifts in brain temperature (non-thermode heating and cooling) during behaviour adequate stimuli for 'thermosensitive' or other hypothalamic neurones and can the responses be separated from behavioural ones? (3) Are there hypothalamic neurones which change their firing rates in relation to cutaneous vasomotor activity in the behaving rabbit?

During seventeen episodes of paradoxical sleep in the rabbit we measured a 0·2° C (0·1–0·5° C, mean and range) rise in brain temperature due to ear cooling (vasoconstriction) of 3·5° C (2·0–10·0° C). Measurement of brain temperature at the basilar artery gives not only the most sensitive index of changes in brain temperature but also a direct measure of central arterial blood and cerebral arterial blood temperature (Hayward & Baker,

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Legend to Fig. 6.

Fig. 6. Rates of spontaneous discharge (spikes/sec) of 138 diencephalic and septal units during waking and slow sleep in the rabbit. In upper and middle rows cells from specific anatomical regions are grouped with spontaneous discharge rates on the ordinate (log. scale) and plots obtained from the same unit during each behavioural state connected along the abscissa. Lower row: mean firing rates (spikes/sec) of the groups of cells in each anatomical region with white columns waking and hatched columns slow sleep. Note the tight grouping, the minimal shift of discharges with behaviour and the lower mean rate of LHA cells, in contrast to the wider spread of firing rates and the higher mean rates of the AH, DMH and SP units. Units in DHA and M both showed the highest firing rates during waking, the most consistent decline of firing during slow sleep and a greater uniformity of firing pattern than elsewhere in the diencephalon. Labels: these anatomical areas correspond to those of Sawyer *et al.* (1954) except that AH (anterior hypothalamus) comprises their AHA (anterior hypothalamic area), MPO (medial preoptic) and LPO (lateral preoptic); VMH, ventromedial hypothalamus; LHA, lateral hypothalamic area; DHA, dorsal hypothalamic area; DMH, dorsomedial hypothalamus; PHA, posterior hypothalamic area; M, massa intermedia of the thalamus; SP, septum pellucidum.

1968*b*). Seventeen units examined during this spontaneous elevation of brain temperature during PS showed acceleration of eleven cells, slowing or arrest of four cells and no change in firing rate of two cells (see Figs. 7 and 8). The temporal pattern of discharge of 15/17 of these units was clearly related to the behavioural state of the rabbit and not to the changes

TABLE 2. Mean rates of discharge (in spikes/sec) of thirty-two cells in the diencephalon during the three states of waking, slow sleep and paradoxical sleep

Brain site*	Waking	Slow sleep	Paradoxical sleep
AHA	2·82	9·92	15·59
AHA	0·45	0·38	1·52
MPO	5·62	4·90	7·20
MPO	0·61	1·60	0·70
LPO	1·10	1·97	8·68
VMH	0·55	1·66	5·86
VMH	8·10	11·98	15·39
ARC	20·74	25·16	29·45
LHA	0·64	1·74	4·36
LHA	3·12	2·34	5·84
LHA	3·90	1·20	4·04
LHA	2·14	1·64	0·00
DHA	17·43	3·94 (B)†	15·49
DHA	3·83	2·80 (B)	4·14
DHA	5·32	3·33 (B)	3·67
DHA	6·33	2·71 (B)	7·17
DHA	3·21	4·06	4·35
DHA	3·50	2·76	9·54
DHA	2·09	1·77 (B)	2·28
DMH	6·56	0·44	4·53
DMH	0·27	0·80	0·33
DMH	2·56	2·75	2·57
DMH	1·04	0·81	1·21
DMH	7·19	6·00	0·00
PHA	2·34	3·93	8·12
M	12·63	22·93	34·55
M	6·29	3·42 (B)	7·71
M	8·56	3·81 (B)	6·24
M	24·03	7·09 (B)	15·15
M	1·19	2·30 (B)	8·69
SP	0·27	1·78	2·58
AM	1·03(B)	2·75 (B)	0·80

* Cells are arranged by anatomical location. Abbreviations (Sawyer *et al.* 1954): ARC, arcuate nucleus; AM, nucleus anteromedialis; remainder of abbreviations same as in Table 1.

† Those cells with spike trains characterized by 'bimodal' interspike interval histograms are indicated by the letter B in parentheses after the appropriate behavioural state.

in brain temperature (Figs. 7 and 8). Two units appeared to change discharge rates in relation to changes in brain temperature rather than to shifts from SS to PS to SS or W. The arcuate cell shown in Fig. 10 accelerates only several seconds after the onset of paradoxical sleep (e.e.g. and observed behaviour) during the rising phase of brain temperature and slows only after cessation of PS when brain temperature is falling toward control level of 39·3° C. These same relationships between rise and fall of brain temperature and this arcuate cell firing are shown during the

second episode of PS (Fig. 10). A second cell in the lateral preoptic area was partially inhibited during elevation of brain temperature during PS, only resuming control levels of spike discharges when brain temperature had returned to base line, long after PS had ended.

During seven episodes of waking behaviour in our rabbits brain temperature increased by 0·2° C (0·1–0·5° C). Four neurones increased firing rates, one neurone decreased firing and two cells showed no change during

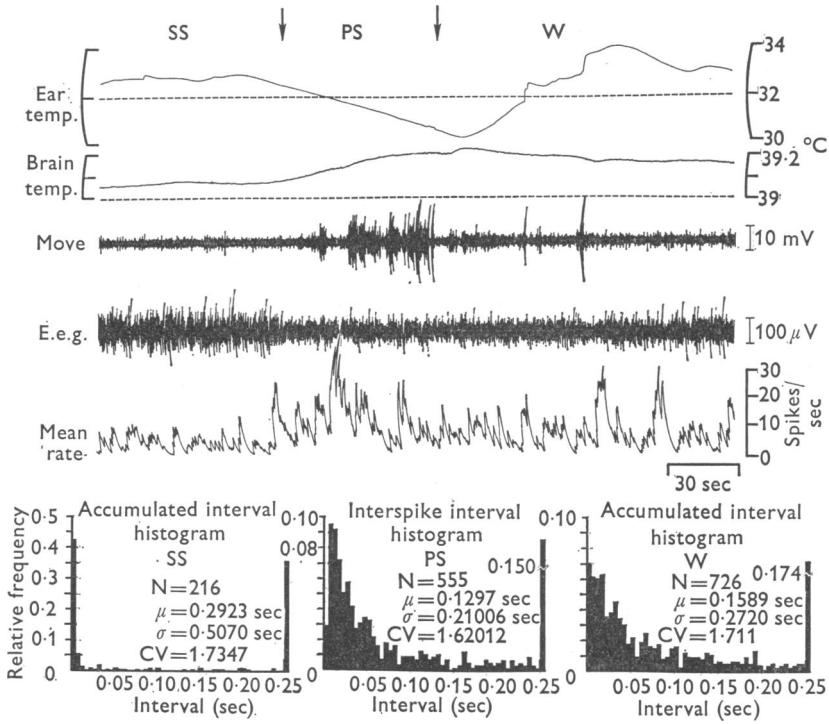

Fig. 7. Temporal relationships between single thalamic unit activity recorded from the massa intermedia and other manifestations of sleep and wakefulness in the rabbit. Head and ears down at first arrow and up at second arrow. During slow sleep the cell is firing at a low mean rate (3·4 spikes/sec) with intermittent bursts as described by the 'bimodal' histogram. During paradoxical sleep and waking the unit accelerates (7·7 spikes/sec and 6·3 spikes/sec respectively) with more sustained spike trains and a different distribution of intervals as described by the 'asymmetric' histograms. The thalamic unit fires maximally during the early atonic phase of PS and slows somewhat during the later twitching phase. Note the intense cutaneous vasoconstriction during PS with heat retention and elevation of brain temperature. Labels: ear temp., temperature on the skin of the ear; brain temp., temperature of the cerebral arterial blood at the basilar artery; move, accelerometer measured body movements; e.e.g., biparietal electrocorticogram; mean rate, analogue output proportional to the rate of unit discharge; accumulated interspike interval histogram, N = number of intervals, μ = mean interspike intervals, σ = standard deviation, and CV = coefficient of variation.

this rise in brain temperature (Fig. 2). The firing pattern of all these cells paralleled changes in behavioural state more closely than changes in brain temperature. In several instances lengthy periods of cyclic waking and slow sleep were interrupted by sustained motor activity, a rise in brain temperature and a subsequent prolonged period of slow sleep. The unit

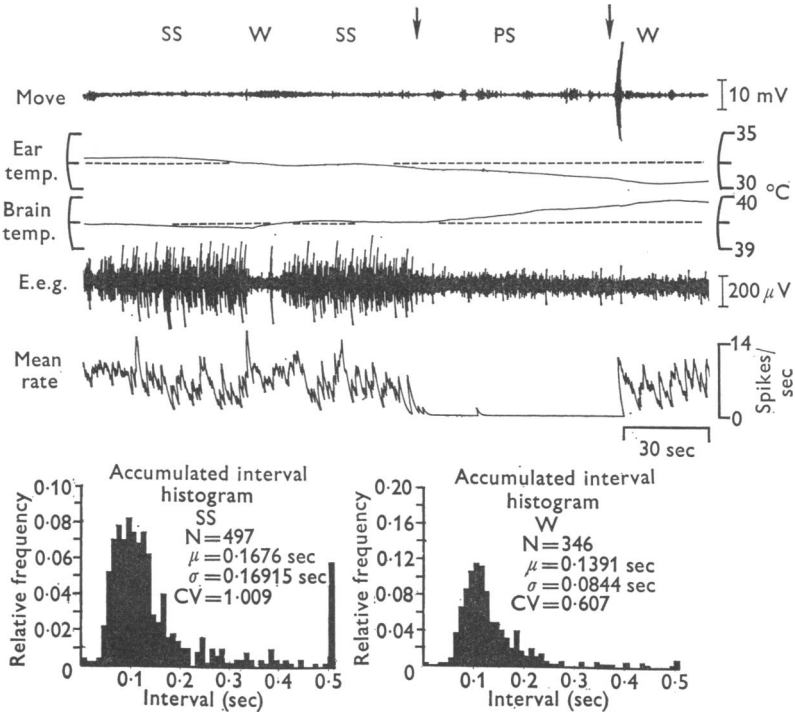

Fig. 8. Inhibition of firing of a hypothalamic unit during paradoxical sleep in the unanaesthetized rabbit. Pattern of firing as described by 'asymmetric' interval histograms and mean rates (6·0 spikes/sec) in SS and 7·2 spikes/sec in W) is similar during slow sleep and waking in this dorsomedial hypothalamic neuron. First arrow indicates head and ears down. Second arrow indicates head and ears raised. Three spikes were recorded during the 70 sec of paradoxical sleep. Labels: move, accelerometer measured body movements; ear temp., temperature on the skin of the ear; brain temp., temperature of the cerebral arterial blood at the basilar artery; e.e.g., biparietal electrocorticogram; mean rate, analogue output proportional to the rate of unit discharge; accumulated interval histogram, N = number of intervals, μ = mean interspike intervals, σ = standard deviation and CV = coefficient of variation.

in these instances reflected the behavioural state; the behaviour was, however, influenced by brain temperature in that the slow sleep was clearly induced by the rise in brain temperature (von Euler & Soderberg, 1957). We found no neurones which appeared to discharge in relation to cutaneous

vasomotor activity in the behaving rabbit. Detection of such neurones should be possible during PS because the cutaneous vasoconstriction occurs 20–60 sec before the behavioural and e.e.g. signs of this behavioural state (see Figs. 7 and 8 and Baker & Hayward, 1967*a*).

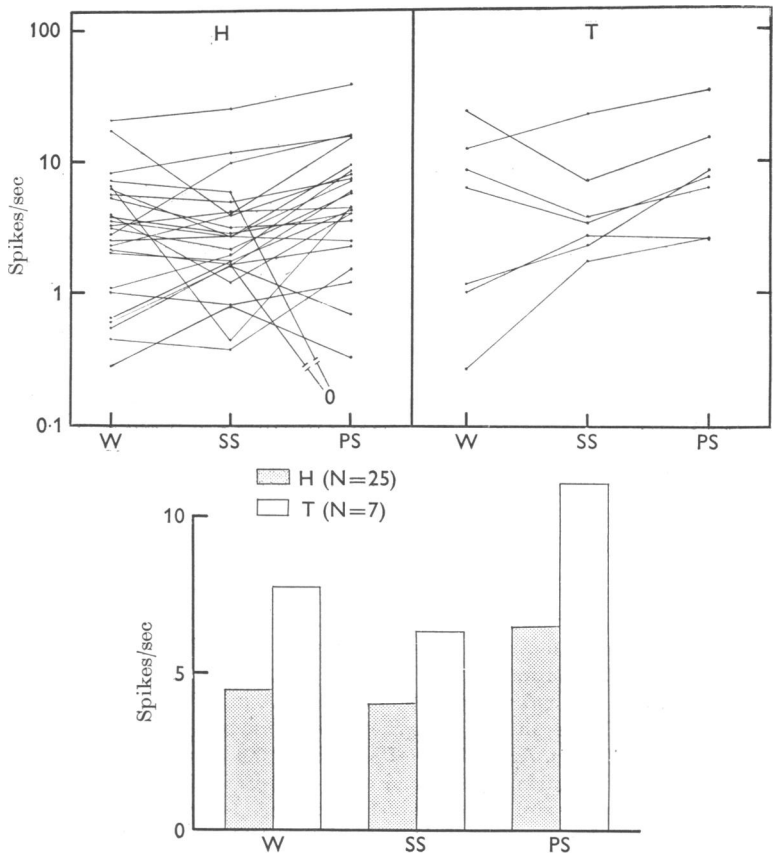

Fig. 9. Spontaneous discharge rates (spikes/sec) of twenty-five hypothalamic and seven extra-hypothalamic cells during waking, slow sleep and paradoxical sleep in rabbit. Upper row: spontaneous discharge rates on the ordinate (log. scale) and plots obtained from the same cell during each behavioural state (W, SS, PS) connected along the abscissa. Lower row: mean firing rates (spikes/sec) of the groups of cells, hypothalamic (H, shaded) and extra-hypothalamic (T, unshaded), during the three states of behaviour.

DISCUSSION

One of the first reports of the recording of single unit activity in relation to the state of arousal in an unrestrained animal was by Hubel (1960). He found that brief high frequency clusters of spikes occur in cells of the cat lateral geniculate body during slow sleep and that this pattern is not seen

during waking. The pattern and rate of cell activity during slow sleep as described by Hubel closely resembles the single unit pattern and rate which we found in the massa intermedia, dorsal hypothalamic area and posterior hypothalamic area.

An over-all decrease in cell activity from waking to slow sleep has been found in monkey pyramidal tract neurones by Evarts (1964) and in cat vestibular neurones by Bizzi *et al.* (1964). On the other hand, Huttenlocher

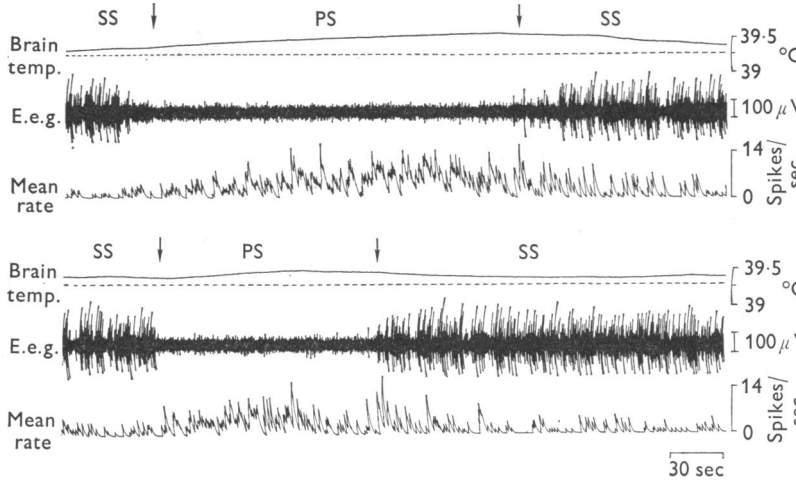

Fig. 10. Elevation of brain temperature and temporal patterns of discharge of a hypothalamic neurone during paradoxical sleep in a rabbit. Upper and lower sections are part of a continuous record. Single cell in the arcuate area discharges at a low mean rate (0·4 spikes/sec) with intermittent bursts during slow sleep and discharges at a higher mean rate (4·0 spikes/sec) with more sustained spike trains during paradoxical sleep. Arrow before PS indicates fall of head and ears, arrow after PS indicates elevation of head and ears. Changes in firing pattern of this hypothalamic neurone parallel the rise and fall of brain temperature more closely than the e.e.g. or behavioural manifestations of PS or SS. Label: e.e.g., biparietal electrocorticogram; mean rate, analogue output proportional to the rate of unit discharge; brain temp., temperature of the intracranial cerebral arterial blood at the basilar artery.

(1961) found that most units in the medial brain stem of the cat showed more spontaneous activity during slow sleep, although a group of cells in the ventral part of the mid-brain reticular formation showed more rapid and continuous discharges during waking than during a slow sleep. Over-all results in the hypothalamus of the rabbit (Figs. 5, 6 and 9) in our study show a decrease in cell activity from waking to slow sleep with a wide variety of change in individual cell response (Figs. 6 and 9).

There is general agreement that units in the cortex, thalamus and mid-brain show an increase in activity during paradoxical sleep. We found in

the hypothalamus of the rabbit that the highest spontaneous discharge rates of most cells occur during PS (Fig. 9). Interspike interval histograms of diencephalic spike trains recorded in paradoxical sleep bore more resemblance to those produced by the same cell during waking than during slow sleep. This appears to be in contrast to pyramidal tract cells in which firing patterns in paradoxical sleep are more similar to those in slow sleep than to those in waking (Evarts, 1964). The relationship between hypothalamic cell firing (abrupt acceleration or slowing) and the sporadic twitching of PS may be related to ascending reticular (vestibular) influences. Bizzi *et al.* (1964) found bursts of activity from vestibular nuclei associated with rapid eye movements of PS in the cat.

The complexity of the alterations in cell activity which we have found to occur between the different states of sleep and waking indicates that the hypothalamus possesses a population of less homogeneity than that of most other parts of the brain studied. But then the anatomy of the hypothalamus is a complex meshwork of fibres and interneurones with few well defined cell layers, nuclei or fibre-tracts where direct and indirect afferent connexions are received from numerous other brain areas. This diversity of behaviour of adjacent hypothalamic neurones clearly places severe limitations on the value of 'mass activity' techniques (e.e.g., evoked potentials, d.c. potentials and multiple unit activity) in elucidating the details of the complex integrating processes occurring in this area of the brain (Hayward, Fairchild & Stuart, 1966; Malliani, Rudomin & Zanchetti, 1965; Komisaruk *et al.* 1967). Our unit studies in the hypothalamus suffer from the same limitations recognized by others, namely, the neuronal population sample is biased in favour of larger units; the number of cells that could be recorded and analysed in each hypothalamic region during each state of behaviour is too small to give reliable information of the over-all activity of the region; interpretation of the changes in hypothalamic single unit firing patterns is made difficult by the complete lack of information about simultaneous activity of other cells in the brain.

Early brain lesioning and stimulating studies (eg. Ranson, 1939; Hess, 1947) linked the hypothalamus with sleep control. The intensive studies on sleep over the last ten years have served to emphasize the difficulties entailed in assigning crucial roles to specific areas of the brain. Despite these problems, however, there now seems good reason to conclude that the areas primarily involved in the initiation of slow sleep and paradoxical sleep are situated more caudally in the brain stem (Jouvet, 1967). Thus it seems probable to us that changes in hypothalamic cell activity during different behavioural states are the result, rather than the cause, of the change in the sleep–waking state of the animal.

An important regulatory function of the hypothalamus acting in part via the autonomic nervous system is the control of body temperature. Experiments on hypothalamic neurone activity in anaesthetized (Cross & Silver, 1966 review) and unanaesthetized (Hellon, 1967) animals in relation to local changes in brain temperature have led to the suggestion that there exists a relatively discrete population of cells in the anteromedial region of the hypothalamus and preoptic area which shows a high degree of sensitivity to local temperature. These cells have accordingly been labelled as 'warm sensitive' and 'cold sensitive' thermoreceptors involved in temperature regulation. In few, if any, of these unit experiments has cortical e.e.g. been recorded, and the behavioural effects of the hypothalamic heating and cooling have seldom been systematically observed. We found that two thirds of the anterior hypothalamic cells from which we recorded were sensitive to the state of behaviour of the animal. In the light of the findings of von Euler & Söderberg (1957) that moderate hypothalamic heating or cooling synchronized or desynchronized, respectively, the e.e.g. in rabbits and cats, we would suggest that some, at least, of 'temperature sensitive' units described by previous authors may have been primarily affected by sleep–waking changes which were themselves induced by changes in hypothalamic temperature.

We studied the response of hypothalamic neurones to spontaneous and physiological (no thermode) shifts in brain temperature in the behaving rabbit. While our total sample was small, most hypothalamic units exposed to a rise in brain temperature altered their discharge rates only in relation to behaviour. We conclude that either the thermal stimulus (0·2° C) was inadequate or that these cells were not thermosensitive or both. The two cells responsive primarily to brain temperature (not behaviour) were located within (lateral preoptic) and without (arcuate) the usual thermosensitive region. On occasion these elevations in brain temperature did appear to induce slow sleep with unit firing following the change in behaviour (von Euler & Söderberg, 1957). The state of arousal of an animal is such a critical factor in body temperature regulation that it would seem essential for future studies to monitor e.e.g. and observe sleep–waking behaviour.

A number of changes such as cutaneous vasoconstriction (Baker & Hayward, 1967*a*, *b*), nasal vasoconstriction (Baker & Hayward, 1968), decline in blood pressure and heart rate (Gassel, Gheladucci, Marchiafava & Pompeiano, 1964), and pupil meiosis with phasic midriasis (Berlucchi, Moruzzi, Salvi & Strata, 1964) indicate that profound changes in autonomic nervous system function occur during paradoxical sleep. Our results suggest that the firing rate of cells in regions involved in such autonomic control may be markedly affected during paradoxical sleep.

A large number of unit studies have now been reported (Cross & Silver, 1966 review) demonstrating the sensitivity of hypothalamic neurones to a wide variety of humoral stimuli. Within recent years, it has become clear that caution is required in the interpretation of many of the earlier studies because even in the anaesthetized animal hypothalamic unit activity can vary with changes in the e.e.g. (Komisaruk *et al.* 1967). It is clear from our results that the cells of the hypothalamus are closely attuned to the state of behaviour of the animals by neural mechanisms yet obscure. If the unanaesthetized animal is to be used in future studies of humoral effects on hypothalamic single neurones, considerable care will have to be exercised to separate non-specific arousal or depressor effects of stimuli from specific effects of those stimuli on limited populations of neurones. Further developments in this field may depend on the simultaneous analysis of spike trains from two or more single hypothalamic cells (Perkel *et al.* 1967*b*). Hormonal changes may dramatically affect the interneuronal transmission of information and such changes could be overlooked if the activity of only one cell is studied.

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REFERENCES

Baker, M. A., Burrell, E., Penkhus, J. & Hayward, J. N. (1968). Capping and stabilizing chronic intravascular cannulae. *J. appl. Physiol.* **24**, 577–579.

Baker, M. A. & Hayward, J. N. (1967*a*). Autonomic basis for the rise in brain temperature during paradoxical sleep. *Science, N.Y.* **157**, 1586–1588.

Baker, M. A. & Hayward, J. N. (1967*b*). Carotid rete and brain temperature of cat. *Nature, Lond.* **216**, 139–141.

Baker, M. A. & Hayward, J. N. (1968). The influence of the nasal mucosa and the carotid rete upon hypothalamic temperature in sheep. *J. Physiol.* **198**, 561–579.

Berlucchi, G., Moruzzi, G., Salvi, G. & Strata, P. (1964). Pupil behaviour and ocular movements during synchronized and desynchronized sleep. *Archs ital. Biol.* **102**, 230–244.

Bizzi, E., Pompeiano, O. & Somogyi, I. (1964). Spontaneous activity of single vestibular neurons of unrestrained cats during sleep and wakefulness. *Archs ital. Biol.* **102**, 308–330.

Cross, B. A. & Green, J. D. (1959). Activity of single neurones in the hypothalamus: effect of osmotic and other stimuli. *J. Physiol.* **148**, 554–569.

Cross, B. A. & Silver, I. A. (1966). Electrophysiological studies on the hypothalamus. *Br. med. Bull.* **22**, 254–260.

Evarts, E. V. (1962). Activity of neurones in visual cortex of the cat during sleep with low voltage fast EEG activity. *J. Neurophysiol.* **25**, 812–816.

Evarts, E. V. (1964). Temporal patterns of discharge of pyramidal tract neurones during sleep and waking in the monkey. *J. Neurophysiol.* **27**, 152–171.

FINDLAY, A. L. R. & HAYWARD, J. N. (1968). Hypothalamic single unit activity during sleep and arousal in the rabbit. *Proc. XXIV Intern. Congr. Physiol. Sci., Washington, D.C.* **7**, 134.

FUJITA, Y., ROSENBERG, J. & SEGUNDO, J. P. (1968). Activity of cells in the lateral vestibular nucleus as a function of head position. *J. Physiol.* **196**, 1–18.

GASSEL, M. M., GHELARDUCCI, B., MARCHIAFAVA, P. L. & POMPEIANO, O. (1964). Phasic changes in blood pressure and heart rate during the rapid eye movement episodes of desynchronized sleep in unrestrained cats. *Archs ital. Biol.* **102**, 530–544.

HAYWARD, J. N. (1968). Brain temperature regulation during sleep and arousal in the dog. *Expl Neurol.* **21**, 201–212.

HAYWARD, J. N. & BAKER, M. A. (1968*a*). Diuretic and thermoregulatory responses to preoptic cooling in the monkey. *Am. J. Physiol.* **214**, 843–850.

HAYWARD, J. N. & BAKER, M. A. (1968*b*). The role of the cerebral arterial blood in the regulation of brain temperature in the monkey. *Am. J. Physiol.* **215**, 389–403.

HAYWARD, J. N., FAIRCHILD, M. D. & STUART, D. G. (1966). Hypothalamic and cortical D-C potential changes induced by stimulation of the midbrain reticular formation. *Expl. Brain Res.* **1**, 205–219.

HELLON, R. F. (1967). Thermal stimulation of hypothalamic neurones in unanaesthetized rabbits. *J. Physiol.* **193**, 381–395.

HESS, W. R. (1947). Vegetative Funktionen und Zwischenhirn. *Helv. physiol. pharmac. Acta*, Suppl. IV, 65 pp.

HUBEL, D. H. (1960). Single unit activity in lateral geniculate body and optic tract of unrestrained cats. *J. Physiol.* **150**, 91–104.

HUTTENLOCHER, P. R. (1961). Evoked and spontaneous activity in single units of medial brain stem during natural sleep and waking. *J. Neurophysiol.* **24**, 451–468.

JOUVET, M. (1967) Neurophysiology of the states of sleep. *Physiol. Rev.* **47**, 117–177.

KINNARD, M. A. & MACLEAN, P. D. (1967). A platinum micro-electrode for intracerebral exploration with a chronically fixed stereotaxic device. *Electroenceph. clin. Neurophysiol.* **22**, 183–186.

KOMISARUK, B. R., MCDONALD, P. G., WHITMOYER, D. I. & SAWYER, C. H. (1967). Effects of progesterone and sensory stimulation on EEG and neuronal activity in the rat. *Expl Neurol.* **19**, 494–507.

MALLIANI, A., RUDOMIN, P. & ZANCHETTI, A. (1965). Contribution of local activity and electric spread to somatically evoked potentials in different areas of the hypothalamus. *Archs ital. Biol.* **103**, 119–135.

MARTIN, A. R. (1969). A peak amplitude selector for electrophysiological data analysis. *IEEE Trans. bio-med. Engng.* (In the Press.)

PERKEL, D. H., GERSTEIN, G. L. & MOORE, G. P. (1967*a*). Neuronal spike trains and stochastic point processes. I. The single spike train. *Biophys. J.* **7**, 391–418.

PERKEL, D. H., GERSTEIN, G. L. & MOORE, G. P. (1967*b*). Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys. J.* **7**, 419–440.

RANSON, S. W. (1939). Somnolence caused by hypothalamic lesions in the monkey. *Archs Neurol. Psychiat., Chicago* **41**, 1–23.

SAWYER, C. H., EVERETT, J. W. & GREEN, J. D. (1954). The rabbit diencephalon in stereotaxic coordinates. *J. comp. Neurol.* **101**, 801–824.

VON EULER, C. & SÖDERBERG, U. (1957). The influence of hypothalamic thermoceptive structures on the electroencephalogram and gamma motor activity. *Electroenceph. clin. Neurophysiol.* **9**, 391–408.