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OSMOSENSITIVE SINGLE NEURONES IN THE HYPOTHALAMUS OF UNANAESTHETIZED MONKEYS

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SUMMARY

1. We recorded with tungsten micro-electrodes the activity of single neurones in the supraoptic nucleus (NSO) and adjacent regions of the hypothalamus while repeatedly injecting solutions of varying tonicity into the common carotid artery of trained, unanaesthetized monkeys who accepted the experimental restraints without anxiety.

2. Intracarotid injections of mildly hypertonic solutions of sodium chloride produced a characteristic behavioural response during and immediately after injection: e.e.g. 'arousal,' lip and tongue smacking, chewing, irregular sniffing respiration and associated mildly increased movement of face, eyes and body.

3. Of the 130 cells analysed during hypertonic intracarotid injections, 105 (81 %) were osmosensitive. Twenty-five (19 %) of the cells studied during similar injections were non-osmosensitive. On the basis of the anatomical location of the cells, the pattern of discharge to intracarotid osmotic stimuli and the response to arousing sensory stimuli, we divided the osmosensitive cells into two major groups, 'specific' and 'non-specific' osmosensitive cells.

4. Fifty-two (50 %) of the osmosensitive cells we labelled 'specific' because they responded to an intracarotid injection of hypertonic sodium chloride, generally did not respond to non-noxious arousing sensory stimuli and were located in or near the supraoptic nucleus. We found two subtypes of these 'specific' osmosensitive cells: (*a*) twenty-one (20 %) NSO cells with 'biphasic' responses, that is, acceleration followed by inhibition; (*b*) thirty-one (30 %) cells in the immediate perinuclear zone of the NSO with 'monophasic' responses, subdivided into twenty-one (20 %) cells that accelerated and ten (10 %) that were inhibited.

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(The work referred to in this paper was carried out in the U.S.A.)

5. Fifty-three osmosensitive cells (50 %), located diffusely in the anterolateral hypothalamus, were 'non-specific', responding both to intracarotid injections of hypertonic sodium chloride and also to sensory stimuli that were mildly arousing. Two groups of 'non-specific' osmosensitive cells showed monophasic responses; thirty-five (34 %) cells accelerated and seventeen (16 %) of them were inhibited.

6. The 'monophasic' specific osmosensitive neurones lying in the immediate perinuclear zone of the supraoptic nucleus in the primate could conceivably be the 'osmoreceptors' of Verney. The 'biphasic' specific osmosensitive neurones in the NSO may well represent the secretory cells of this system. From our data, the 'non-specific' osmosensitive neurones, scattered diffusely in the anterolateral hypothalamus, have little to do with osmoregulation. Some of these cells located in the perinuclear zone of the NSO could act as interneurones, however, conveying afferent input to the osmoreceptor-secretory complex of the supraoptic nucleus.

INTRODUCTION

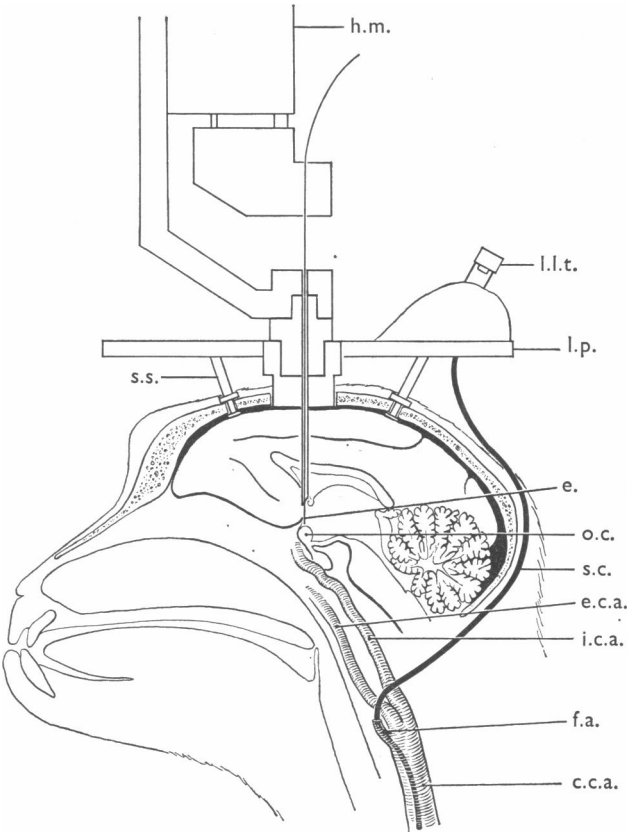
On the basis of his studies in conscious dogs, Verney (1947) concluded that the 'osmoreceptors' lay in the central nervous system in the supply area of the internal carotid arteries. Later, by selectively perfusing different parts of the dog's brain, Jewell & Verney (1957) concluded that the osmoreceptors could be further localized within the anterior hypothalamus-preoptic area. Others (Sundsten & Sawyer, 1961; Woods, Bard & Bleir, 1966), using the method of hypothalamic deafferentation, narrowed the minimal osmoreceptor zone to the region immediately around the supraoptic nucleus (NSO). Whether the neurones of the NSO themselves contain osmoreceptor elements, whether the receptors lie in the perinuclear zone of the NSO, whether a diffuse system of osmoreceptors exists in the hypothalamus and whether there is a coupling at the cellular level between osmoregulatory and arousal mechanisms are some of the problems remaining for a more precise identification of the osmoreceptors of Verney. Our stylized techniques of chronic single unit recording (Findlay & Hayward, 1968, 1969; Text-fig. 1), intracarotid injections (Hayward & Baker, 1968*a*; Baker, Burrell, Penkhus & Hayward, 1968; Text-fig. 1) and polygraphic recording of behavioural parameters (Hayward & Baker, 1968*a*, *b*; 1969; Text-fig. 2) seemed to provide the means for a possible breakthrough in the field of osmoreceptor research.

The spontaneous activity of single neurones in the hypothalamus of unanaesthetized rabbits (Findlay & Hayward, 1968, 1969; Vincent, Benoit, Scherrer & Faure, 1967) and monkeys (Hayward, 1969*a*, *b*) correlates closely with changes in sleep–waking behaviour. When we recorded

from single units in the supraoptic nucleus and the adjacent hypothalamus in the waking monkey, we were faced with the problem of determining whether the responses of these cells were triggered by the 'osmotic' or by the 'arousal' component (or both) of the intracarotid hypertonic injection (Text-figs. 2, 3). Verney (1947) had found antidiuretic responses in the conscious dog to both osmotic stimuli and to pain and emotional stress. We found antidiuretic responses in the conscious monkey to electrical stimulation of the amygdala (Hayward & Smith, 1963) and mid-brain reticular formation (Hayward & Smith, 1964), areas associated with arousing and emotional behaviour and with known hypothalamus and cortical connexions (Hayward, Fairchild & Stuart, 1966). The supraoptic neurones should, therefore, be responsive to both osmotic and nociceptive or emotionally stressful sensory stimuli. Brooks and his co-workers (Brooks, Ushiyama & Lange, 1962; Brooks, Ishikawa, Koizumi & Lu, 1966; Ishikawa, Koizumi & Brooks, 1966; Koizumi, Ishikawa & Brooks, 1964; Suda, Koizumi & Brooks, 1963) found the majority of their osmosensitive cells responsive to both osmotic and sensory stimuli. On the other hand, von Euler (1953) proposed that an 'osmoreceptor' cell should respond 'specifically' to an osmotic stimulus and not act additionally as an interneurone or secretory cell. In line with von Euler's dictum, Cross & Green (1959) and Joynt (1964) described many osmosensitive cells that seemed to qualify as osmoreceptor neurones. These neurones were located in the perinuclear zone of the NSO and responded to osmotic but not to natural sensory stimuli. None of these workers monitored behaviour (e.e.g.) in their anaesthetized animals. In view of the arousing effects of intracarotid hypertonic solutions (Verney, 1947; Hayward & Vincent, 1970*a*, *b*; Sawyer & Gernandt, 1956; Text-fig. 2) and the effects of a change in behaviour on hypothalamic unit activity in both anaesthetized (Komisaruk, McDonald, Whitmoyer & Sawyer, 1967) and unanaesthetized animals (Findlay & Hayward, 1968, 1969; Hayward, 1969*a*, *b*; Vincent *et al.* 1967), it would seem that the key to positive identification of 'osmoreceptors' is careful monitoring of behaviour during osmotic and sensory stimulation.

When we assessed behaviour by measuring several physiological parameters simultaneous to single unit recording in relaxed conscious monkeys, we found 'specific' osmosensitive neurones in and around the NSO (Text-fig. 4) responsive almost exclusively to intracarotid osmotic stimuli and poorly responsive to non-noxious arousing sensory stimuli. We, therefore, identify the 'osmoreceptors' of Verney as our 'specific' osmosensitive neurones, having a monophasic discharge and lying in the perinuclear zone of the NSO (Text-fig. 4). The secretory cells we identify as being our 'specific' osmosensitive neurones, those that discharge biphasically and lie in the NSO (Text-figs. 3, 4). Other 'non-specific' osmosensitive cells,

responsive to both osmotic and arousing stimuli, are distributed throughout the antero-lateral hypothalamus (Text-fig. 4) and appear less directly related to osmoregulation. Preliminary accounts of part of this work have been published (Hayward & Vincent, 1970*a*, *b*).



Text-fig. 1. Diagrammatic view of the hydraulic microdrive and the intracarotid cannula on the head of a monkey (not to scale). A cranial platform holding the stereotaxic bone fixed adapter cylinder and capped arterial cannula is chronically fixed above the scalp on four screws cemented in the skull (Baker *et al.* 1968). Note that the silicon rubber cannula is placed into the common carotid artery through the cut end of the common facial artery and fixed without interruption of flow in the common, external or internal carotid vessels. The silicon cannula is threaded under the skin of the neck to the cranial platform. Labels: h.m., hydraulic microdrive-slave cylinder; l.l.t., luer-lok tip with rubber diaphragm capping the arterial cannula; l.p., lucite platform; s.s., stainless-steel screws; e., tungsten micro-electrode; o.c., optic chiasma; s.c., silicon rubber cannula; e.c.a., external carotid artery; i.c.a., internal carotid artery; f.a., common facial artery; c.c.a., common carotid artery.

METHODS

Text-fig. 1 illustrates the following technical procedures. With pentobarbitone (30 mg/kg) we anaesthetized eight adult female Rhesus monkeys (*Macaca mulatta*, 4·5–5·5 kg) that had previously been conditioned to primate restraining chairs for several weeks. We fixed to the calvarium the four stainless-steel epidural bolts that would, by protruding 1/2 in. through the lateral scalp, help to steady the head during later recordings (Evarts, 1968). Without excising the dura mater, we then opened the calvarium of the vertex aseptically in order to place a rigid cylinder (stainless-steel bone-fixed adapter, 12 mm o.d. at the skull) stereotaxically on the dura with its centre at Fr. 17, L 2·5 (Snider & Lee, 1961). We cemented this cylinder to an elevated lucite platform (Baker *et al.* 1968; Hayward & Baker, 1969) which was in turn cemented to four additional stainless-steel epidural skull screws. We sealed the margins of the opening around the cylinder with dental cement, and filled the cylinder, which subsequently supported the micro-electrode carrier, with bone wax and capped it. We implanted biparietal insulated silver-ball electrodes with a small surface bared epidurally for electrocorticographic recording (e.e.g.), and fixed insulated stainless-steel wires with bared tips in the periorbital tissues for recording extraocular movements. Lead wires for extra-ocular movements and e.e.g. we soldered to a five-pin receptacle (Winchester Electronics Co.) previously cemented to the platform. We implanted a copper–constantan arc-welded thermocouple in glass tubing (o.d. 0·7 mm) stereotaxically, 1 mm lateral to the mid line at Fr. 7, penetrating the brain to the basal subarachnoid space. The thermojunction at the tip of the tube lay at the bifurcation of the basilar artery. Temperature measured in the basal subarachnoid space near the cerebral arteries is, as previously established (Hayward & Baker, 1968*b*; 1969), a sensitive index of cerebral and central arterial blood temperature, as well as of shift in hypothalamic temperature. Miniature copper–constantan connectors (Thermoelectric Co., No. MX-JTX), cemented to the platform, held the thermocouple wires. Two five-pin connectors (Amphenol Co., No. 233–1105) screwed to the platform front held a field effect transistor amplifier (Motorola, MPF-105), a power input and unit output connexions. Silicon rubber cannulae (Beckton–Dickinson Co., o.d. 1–2 mm), implanted 4 cm into the left common carotid artery through the common facial artery and into the right atrium (o.d. 1·6 mm) through the left internal jugular vein, continued under the skin of the neck and head, terminating in capped hypodermic needles (Baker *et al.* 1968; Hayward & Baker, 1968*a*, 1969).

We mounted a calibrated hydraulic microdrive arrangement (Trent H. Wells, Jr., Mechanical Developments Co.) on the cranial platform. For recording purposes, the stainless-steel guide tube (22 gauge), which was attached to the cylinder adapter, passed stereotaxically through the Starr guide, bone wax, dura mater and into the brain, lying 25 mm below the cortical surface and 5 mm above the optic tract or chiasm (Findlay & Hayward, 1969). We then lowered the micro-electrode out of its protective position in the guide tube, passing it through the hypothalamus to the supraoptic nucleus. Bone wax covered all metal surfaces and sealed the cylinder tightly, lessening cerebral pulsations resulting from positional changes and other venous and arterial fluctuations.

Following a modified Hubel method (1957), we made electrodes of 0·008 in., 80 mm length tungsten wire, electrolytically etched and polished to a tip diameter of 1 μ or less and insulated to within 10–20 μ of the tip with two to four coats of Isonel-31 varnish (Schnectary Chemicals, Inc.). A capacitance meter (Tektronix, Type 130 L–C Meter) monitored the uninsulated tip length and any possible leaks in the insulation (Bak, 1967). Our electrodes had a total tip capacitance of 50–80 pF.

The field effect transistor mounted on the platform amplified extracellular unit potentials recorded between the micro-electrode tip and the indifferent cylinder on the skull. Led through a high impedance probe, the signal went into a high gain a.c.-coupled preamplifier of band pass 80–10,000 Hz and input resistance of 10 MΩ (Grass P 511). A dual beam oscilloscope (Tektronix 565) continuously monitored the signal which ran from the Y-plates of the oscilloscope to an audio-amplifier (Grass), to one channel of a direct magnetic tape recorder (Tanberg 64X) and to a pulse height discriminator (Martin, 1969), two outputs of which appeared on an ink-writing oscillograph (Offner Type-R Dynograph). There was one pulse out for every impulse in the window. The ink-writer amplified and recorded simultaneously (the e.e.g., and eye movement potentials, skin, nasal and brain thermocouple e.m.f.s (ice water reference junction, 0° C) and accelerometer output with unit data.

After each animal had recovered from surgery, as indicated by its eating at pre-operative levels (within 3–7 days), we placed it in its special thermoregulated chamber (Hayward & Baker, 1968*a*, *b*; 1969). A vibration pick-up accelerometer (MB Electronics, Type 124) attached to the chair bottom allowed us to detect gross body movements. Rods attached at one end to the four protruding cranial bolts via ball-joint couplings and to the frame of the primate chair at the other end steadied the head painlessly, allowing the animal to doze intermittently during unit recording and to behave normally otherwise (Evarts, 1968). We taped bared thermocouples (copper–constantan) to the hairless dorsal surface of the ear and into the external nasal meatus. Arterial and venous cannulae connected to extension tubes allowed us to inject solutions outside the chamber. We ran forty-nine experiments at ambient temperatures of 25° C from 4–8 hr in duration, 3 to 4 times a week, performing a total of about eight electrode penetrations per monkey. During these recording sessions we observed the temporal relationships between intracarotid injections or arousing stimuli, and changes in behaviour, hypothalamic single unit activity, e.e.g. activity, body movement, eye movement, respiration and brain and skin temperatures. On the basis of the e.e.g., eye and body movement record, respiration and observed behaviour, we were able to judge whether the animal was sleeping, in quiet waking or aroused.

When we detected a stable neurone, we first tested its sensitivity to intracarotid injections of hypertonic, isotonic and hypotonic solutions, using sodium chloride solutions 1·4–3·6 % (0·22–0·60 M) or dextrose solutions 25 % (1·38 M), sodium chloride solutions 0·9 % (0·15 M) or dextrose solutions 5 % (0·27 M) and distilled water, respectively. We use either a slow injector (Harvard Instrument Co.) or a manually operated 1 ml. syringe, graduated in 0·025 ml. for intracarotid injections given at rates ranging between 0·003 and 0·25 ml./sec. A test of the cell's sensitivity to sound stimuli, light flashes and touches on various parts of the body concluded our tests.

Terminally, we anaesthetized the chronically prepared animal and deposited iron electrolytically at several known levels along the electrode tracks with a stereotaxically positioned epoxy-insulated steel dental brooch (Cross & Green, 1959). We then perfused the brain through the carotid artery with isotonic saline solution containing formalin (10 %) and sodium ferrocyanide (2 %). We cut serial frozen sections from the brain at 80 μ in the sterotaxic plane, then stained them with thionin. Prussian blue spots helped us to identify the micro-electrode tracts (Pl. 1) which we then reconstructed, placing the unit locations on outline drawings of the monkey diencephalon (Text-fig. 4; Snider & Lee, 1961).

Analysis of data began with identification of those cells responsive to either osmotic or sensory stimuli or both. After we had noted the periods of recordings which were to be analysed further, we replayed the sections of the magnetic tape

record corresponding to the discharges monitored on an oscilloscope in order to establish the stability of the wave form of the unit. We photographed single cell spike trains that were clearly separable from base line activity and neighbouring units, led them into the pulse height discriminator (Martin, 1969) while recording short (0·5 msec) pulses triggered by the unit action potentials on magnetic tape.

We programmed the SDS 9300 computer, using a sampling rate of 2000 samples/sec, to identify the times of occurrence of the spikes and to record these times on a digital tape. The IBM 360/91 computer then performed the statistical analysis with a program 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). This programme allowed accumulated statistics to be performed on a unit during identical periods of response states, temporally dispersed. Thus, firing patterns of a neurone during several periods of osmotic injection could be analysed separately and the accumulated statistics and histogram presented.

RESULTS

With the centre of the bone-fixed adapter (Text-fig. 1) positioned stereotaxically over the left supraoptic nucleus (Text-fig. 4, Pl. 1; Snider & Lee, 1961) of the monkey, we gave injections into the ipsilateral common carotid artery. By stereotaxic co-ordinates, optic 'noise' during experiments and histological identification of Prussian blue spots terminally (Pl. 1), we localized the cells in and around the supraoptic nucleus. Our accuracy of cell localization above the optic chiasm or tract we estimate to be $\pm 150\,\mu$ physiologically, $\pm 500\,\mu$ with the metallic iron deposits. From the records of more than 500 single cells reached in the antero-lateral hypothalamus of eight unanaesthetized, moving, trained monkeys, we were able to analyse 130 cells statistically. One hundred and five (81 %) of these cells were osmosensitive, while twenty-five (19 %) failed to change firing rates during intracarotid hypertonic solution injections (Text-fig. 4). This ratio of 4:1, osmosensitive to non-osmosensitive, does not represent the distribution of these cells in the hypothalamus, but is a biased sample. Many cells either failed to survive the initial testing procedures or were clearly non-osmosensitive and discarded as extraneous. The recording time for each cell varies from 5 to 30 min with an average duration of about 15–20 min. Sustained unit discharges vary from mean rates of less than 0·2 spikes/sec, to a maximum of 50 spikes/sec, with a few cells showing cluster discharges of up to 500 spikes/sec. In the resting, waking monkey, diencephalic cells fired at slow rates with no significant difference between mean base line firing of cells in such diverse areas as the lateral preoptic area, lateral hypothalamus, globus pallidus, supraoptic nucleus and the perinuclear zone (Tables 1, 2). The perinuclear zone is that area immediately surrounding the NSO for 0·5–1 mm. Three parts of the monkey NSO, the pars dorsolateralis, pars dorsomedialis, pars ventromedialis (Pl. 1; Nauta & Haymaker, 1969), were identifiable.

TABLE 1. Diencephalic cells recorded in the waking monkey without stimulation

Brain site*	No. of cells	Mean firing rate (spikes/sec)	S.D. (±)	Coefficient of variation
LPO	22	2·9603	2·2057	0·7451
LH	27	2·6371	2·2119	0·8088
GP	13	2·6546	3·5860	1·3509
NSO-PNZ	45	2·0938	1·6268	0·7770
NSO	23	2·3474	1·5283	0·6511
Total	130	—	—	—

* Anatomical areas correspond to Snider & Lee (1961): LPO, lateral preoptic area; LH, lateral hypothalamus; GP, globus pallidus; NSO-PNZ, perinuclear zone of the supraoptic nucleus; NSO, supraoptic nucleus.

TABLE 2. Mean rates of discharge (spikes/sec) of fifty-two 'specific' osmosensitive cells in the hypothalamus of the unanaesthetized monkey before and during intracarotid injections of hypertonic sodium chloride

Cell no.	Brain* site	Cell† type	MFR‡ control (spikes/sec)	MFR osmotic stimulation	% Change in MFR (Δ %)
6	NSO-PDL	B	3·98	13·95	250
10	NSO-PDL	B	1·54	5·10	231
31	NSO-PDL	B	3·79	5·21	37
32	NSO-PDL	B	1·13	4·31	283
33	NSO-PDL	B	1·45	10·76	642
34	NSO-PDL	B	4·10	13·89	239
35	NSO-PDL	B	4·24	7·76	83
36	NSO-PDL	B	2·38	12·82	440
52	NSO-PDL	B	5·81	13·89	139
60	NSO-PDL	B	2·15	3·61	68
61	NSO-PDL	B	4·49	5·88	31
62	NSO-PDL	B	3·52	4·61	31
70	NSO-PDL	+	3·57	4·85	30
109	NSO-PDM	B	3·18	4·53	47
110	NSO-PDM	B	10·99	14·25	30
131	NSO-PDM	B	1·17	9·26	689
132	NSO-PDM	B	2·38	5·92	148
136	NSO-PDM	B	2·52	9·90	293
137	NSO-PDM	B	1·25	1·80	44
141	NSO-PDM	B	2·48	3·12	26
142	NSO-PDM	B	1·13	4·17	267
143	NSO-PDM	B	1·45	3·33	130
9	NSO-PNZ	+	2·37	4·54	92
27	NSO-PNZ	+	2·56	4·61	80
28	NSO-PNZ	+	2·65	3·38	27
29	NSO-PNZ	+	2·50	3·45	38

TABLE 2 (*cont.*)

Cell no.	Brain* site	Cell† type	MFR‡ control (spikes/sec)	MFR osmotic stimulation	% Change in MFR (Δ %)
30	NSO-PNZ	−	4·46	0·22	95
48	NSO-PNZ	+	5·99	14·93	149
49	NSO-PNZ	+	3·77	32·64	765
51	NSO-PNZ	−	3·52	0·00	100
54	NSO-PNZ	+	0·65	2·16	231
55	NSO-PNZ	+	1·34	2·67	99
72	NSO-PNZ	+	0·66	5·59	750
77	NSO-PNZ	+	4·12	5·84	42
80	NSO-PNZ	−	2·21	1·43	35
81	NSO-PNZ	−	1·87	0·00	100
84	NSO-PNZ	+	6·49	7·34	22
90	NSO-PNZ	−	2·00	0·00	100
91	NSO-PNZ	+	1·37	2·85	107
92	NSO-PNZ	+	0·74	1·71	131
93	NSO-PNZ	+	0·67	1·37	103
94	NSO-PNZ	+	2·17	3·12	44
95	NSO-PNZ	+	1·29	1·75	36
100	NSO-PNZ	−	3·14	0·00	100
107	NSO-PNZ	+	1·58	5·13	224
113	NSO-PNZ	+	1·47	2·44	44
124	NSO-PNZ	+	9·43	13·83	47
125	NSO-PNZ	+	4·61	5·94	30
133	NSO-PNZ	−	2·07	0·00	100
135	NSO-PNZ	−	3·17	0·00	100
50	LPO	−	10·31	3·16	69
139	LH	−	2·16	0·00	100

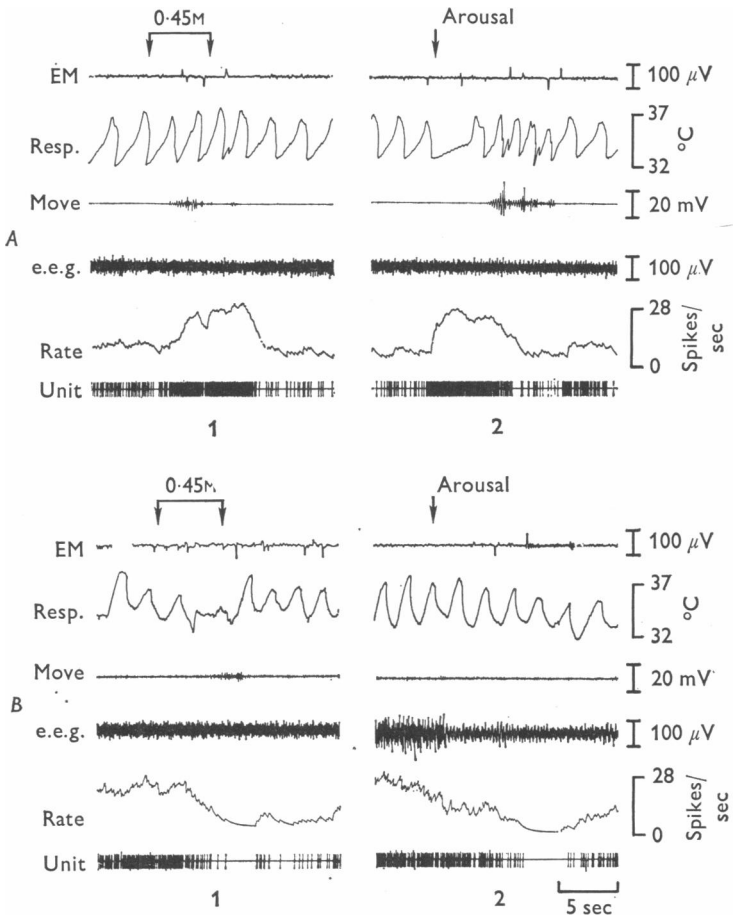
* Cells are arranged by anatomical location. Abbreviations (Snider & Lee, 1961): NSO-PDL, supraoptic nucleus, pars dorsolateralis; NSO-PDM, supraoptic nucleus, pars dorsomedialis; NSO-PNZ, supraoptic nucleus, perinuclear zone; LPO, lateral preoptic area; LH, lateral hypothalamus.

† 'Specific' osmosensitive cells classified as to firing pattern: B, biphasic firing-acceleration followed by inhibition; +, monophasic acceleration; −, monophasic inhibition.

‡ MFR, mean firing rate in spikes/sec.

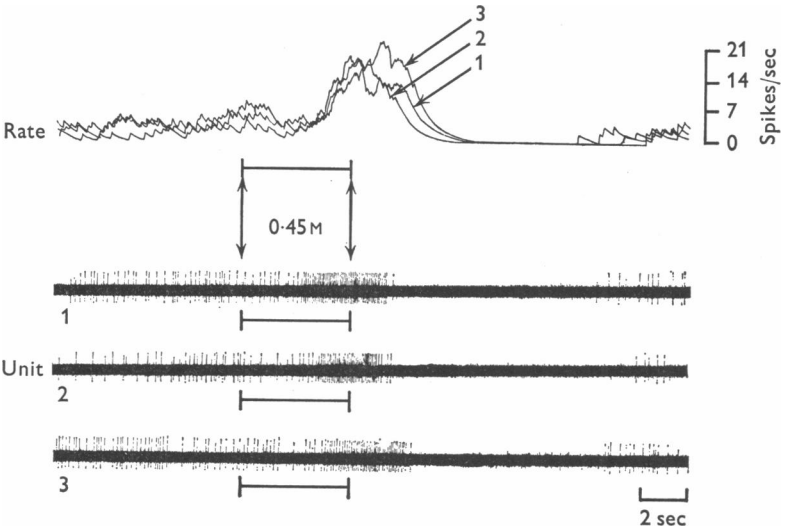
The osmosensitive cell

After the hypothalamic neurone had established a steady base line rate of firing, we injected 0·5–1·0 ml. 0·45 M (0·22–0·60 M) sodium chloride solution into the ipsilateral common carotid artery. The standard injection time was 4–5 sec (0·10–0·25 ml./sec). If there was a greater than 20% change in firing rate during the period of injection, with a return to base line firing within 30–60 sec after the end of the injection (Text-figs. 2, 3),



Text-fig. 2. Firing patterns of two 'non-specific' osmosensitive neurones in the lateral hypothalamus of the unanaesthetized monkey. In *A* the cell shows a monophasic acceleration of firing during intracarotid infusion of hypertonic sodium chloride (1) and a similar increase in discharge rate with opening the door of the environmental chamber (2). A second lateral hypothalamic cell (*B*) responded to intracarotid hyperosmotic injection (1) and to an auditory stimulus (2) with inhibition of cell discharge. Note the e.e.g. arousal (*B*, 2) and varying degrees of change in respiration, eye and body movement with these osmotic and arousing stimuli. Labels: EM, eye movements; Resp., nasal respiration measured by a thermocouple; Move, accelerometer measure of body movements; e.e.g. biparietal electrocorticogram; Rate, analogue output proportional to the rate of unit discharge; Unit, pulse output from pulse height discriminator triggered by action potentials of the spike in the window.

the cell was judged to be osmosensitive. Intracarotid isotonic saline (0·15 M) or distilled water produced no response in our osmosensitive cells (Text-fig. 5). All of our osmosensitive cells conformed to these criteria. There were no cells in our waking monkeys with a delayed initial response to an osmotic stimulus, and none with a response that continued for 2–4 min.

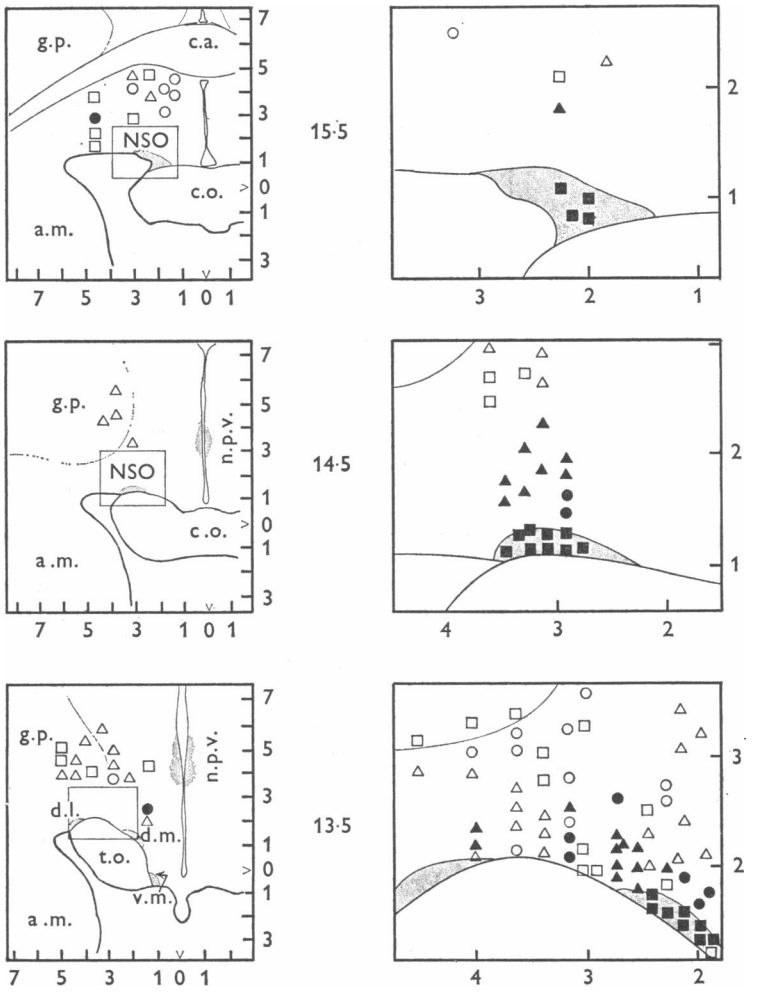


Text-fig. 3. Three 'biphasic' excitatory–inhibitory responses produced by repeated intracarotid injections of hypertonic sodium chloride while recording from a 'specific' osmosensitive supraoptic neurone in the waking monkey. Superimposed polygraph tracings (above) of the mean rate of cell firing (spikes/sec) for three consecutive osmotic stimuli (1, 2, 3) of 0·45 M sodium chloride at 0·25 ml./sec injected at 2-min intervals. Unit (below), spikes photographically recorded from the oscilloscope, during the same three (1, 2, 3) osmotic stimuli. Cell did not respond to arousing stimuli. Note the highly reproducible phase of unit acceleration and period of cell silence following each stimulus.

Osmotic stimulation produced a variety of monophasic (acceleration or inhibition) and biphasic (acceleration followed by inhibition) responses (Text-figs. 2, 3, 4). Osmotic stimuli applied at 2 min intervals or greater during the testing of a cell furnished us with highly reproducible results (Text-fig. 3), but intracarotid injections at intervals of less than 1 min caused the cell firing to decrease, the cell presumably becoming refractory (Text-fig. 5). A stepwise increase in osmolality of the solution injected into the carotid, volume and rate of injection held constant, produced a graded increase in firing frequency roughly proportional, within limits, to the osmotic stimulus (Text-fig. 5). Adjustment of the osmolality, volume and

rate of injection to produce an equivalent osmotic stimulus led to roughly similar cell responses (Text-fig. 8).

Intracarotid injections of hypertonic saline may have a variety of behavioural and vegetative effects. Holland, Sundsten & Sawyer (1959) described a biphasic arterial blood pressure response (hypotension followed by hypertension) in the acutely prepared, post-ether, locally anaesthetized,



Text-fig. 4. For legend see facing page.

immobilized rabbit. Repeated injections of hypertonic sodium chloride into the common carotid artery of the unanaesthetized, sitting monkey produced no significant change in systemic arterial blood pressure (Statham

P 23 pressure transducer). There was a 15 % increase in carotid blood flow (100 ml./min to 112 ml./min) in two lightly anaesthetized monkeys during intracarotid injections measured by a non-cannulating flow probe (2·3–3 mm diam.) of a pulsed-field electromagnetic flowmeter (Statham Instruments Co., Model 0–5000). With an injection of 0·45 M sodium chloride at 0·20 ml./sec, we calculated a rise in blood osmolality from a control level of 300 m-osmole/kg (Hayward & Baker, 1968*a*) to a theoretical peak of 385 m-osmole/kg, a peak 20 % increase in plasma osmolality during our intracarotid injections. Apparently the injected solutions equilibrated rapidly. Depending upon the tonicity and rate of injection, the blood in the internal carotid artery probably shows a 10–20 % increase in osmolality on reaching the hypothalamus.

In his study of the waking dog's antidiuretic response to intracarotid sodium chloride, Verney (1947) described lip smacking as a simultaneous behavioural response incidental to the osmotic stimuli. He also emphasized the importance of emotional stress for the release of antidiuretic hormone from the neurohypophysis. Because we knew, from our previous single unit recordings in the hypothalamus of the unanaesthetized monkey (Hayward, 1969*a*, *b*) and rabbit (Findlay & Hayward, 1968, 1969; Vincent *et al.* 1967) that there was a high correlation between sleep–waking behaviour and single cell activity, we were alerted to the animal's behaviour in the current study. Others (Brooks *et al.* 1962, 1966; Cross & Green, 1959; Joynt, 1964; Ishikawa *et al.* 1966; Koizumi *et al.* 1964; Suda *et al.* 1963) who recorded from single cells in the hypothalamus during intracarotid hypertonic saline

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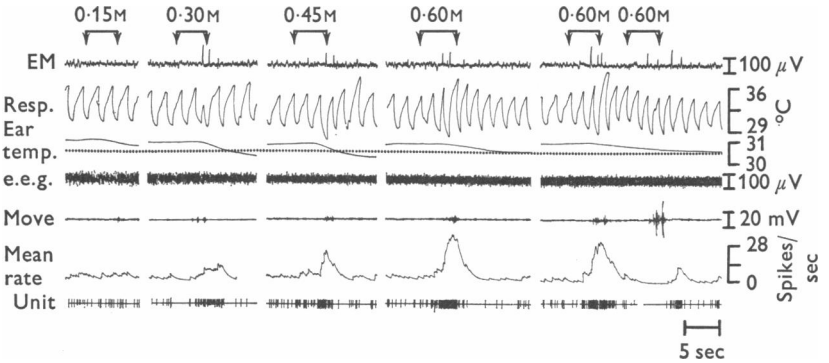
Text-fig. 4. Diencephalic localization of the one hundred and thirty cells recorded in eight monkeys. Frontal sections are Fr. 15·5 (above), Fr. 14·5 (middle) and 13·5 (bottom) after the atlas of Snider & Lee (1961), with the full field on the left and an enlarged box-insert shown on the right. Symbols: ■, 'specific' biphasic osmosensitive cells; ▲, 'specific' monophasic inhibitory osmosensitive cells; △, 'non-specific' monophasic acceleration osmosensitive cells; ○, 'non-specific' monophasic inhibitory osmosensitive cells; □, 'non-osmosensitive' cells. Note the uniform grouping of the 'specific' biphasic osmosensitive cells in the dorsomedial and dorsolateral parts of the supraoptic nucleus and the localization of most of the 'specific' monophasic neurones in the perinuclear zone of the supraoptic nucleus. The 'non-specific' osmosensitive cells and the 'non-osmosensitive' cells lie diffusely throughout the antero-lateral hypothalamus, a few in the perinuclear zone but most elsewhere. We find dorsolateral, dorsomedial and ventromedial parts of the NSO in the monkey. For further histological details see Pl. 1. Labels: g.p., globus pallidus; c.a., anterior commissure; NSO, supraoptic nucleus; c.o., optic chiasma; a.m., amygdala; n.p.v., paraventricular nucleus; d.l., pars dorsolateralis of the supraoptic nucleus; d.m., pars dorsomedialis of the supraoptic nucleus; v.m., pars ventromedialis of the supraoptic nucleus; t.o., optic tract.

injection, had no such opportunity to observe behaviour in their anaesthetized animals.

In our conscious, moving monkeys, intracarotid hypertonic saline often produced a characteristic behavioural response. Some or all of the following occurred: e.e.g. 'arousal,' lip and tongue smacking, chewing, irregular sniffing respiration, turning toward the side of the injection and other associated mildly increasing facial, ocular and bodily movement (Text-figs. 2, 5, 6). Depending upon the type of intracarotid osmotic injection, some or all of this complex response occurred after each injection with little habituation (Text-fig. 5). It should be noted that while these behavioural

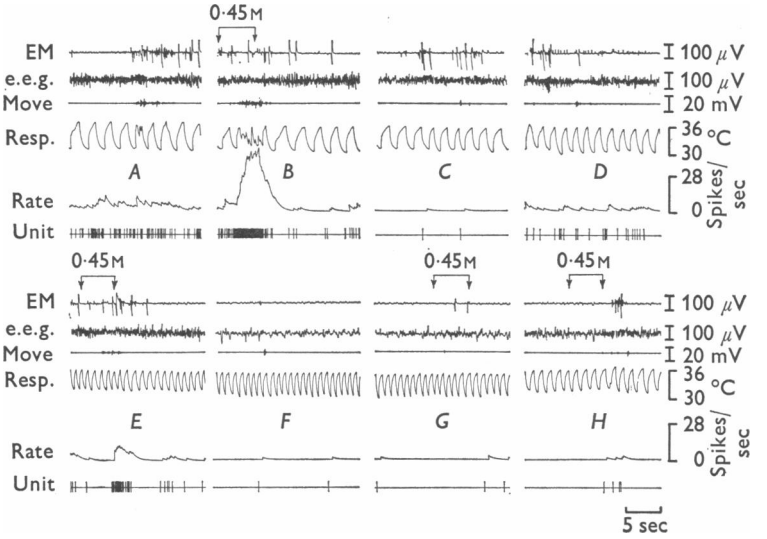


Text-fig. 5. A 'specific' osmosensitive neurone in the supraoptic nucleus responds with a 'biphasic' discharge pattern to intracarotid injections of sodium chloride repeated at about 2-min intervals. Injection of isotonic sodium chloride (0·15 M) causes no change in cell firing, while 2× molality (0·30 M) increases cell discharge during injection with some following inhibition. Intracarotid injection of 3× (0·45 M) and 4× (0·60 M) concentration of sodium chloride causes proportionally greater increase of cell acceleration during injection and more prolonged and total cell inhibition after injection. When the interval between injections is shortened from 2 min to 5 sec (0·60 M and 0·60 M), this supraoptic neurone shows refractoriness with less response to the second rapid osmotic stimulus. Note the cooling of the skin of the ear and changes in respiration, eye and body movement during these repeated injections. We recorded no cooling of the cerebral arterial blood and brain. Labels: EM, eye movement; Resp., nasal respiration measured by a thermocouple; Ear temp., temperature of skin of the ear; e.e.g. biparietal electrocorticogram; Move, accelerometer measure of body movements; Mean rate, analogue output proportional to the rate of unit discharge; Unit, pulse output from pulse height discriminator triggered by action potentials of the spike in the window.

responses were clear they were also rather subtle. We observed that none of these responses occurred, however, when we gave intracarotid injections of distilled water, isotonic saline (0·25 M) or isotonic glucose solutions

(0·27 M, Text-fig. 5). Injections given at 25° C into the common carotid produced mild cooling of the ear (Text-fig. 5), but no cooling at the level of the cerebral arterial blood (Hayward & Baker, 1968*b*, 1969).

As we studied the responsiveness of cells in the hypothalamus to intracarotid injections of hypertonic and isotonic saline, separating our osmo-



Text-fig. 6. Effects of urethane anaesthesia on the spontaneous firing rate and the response to osmotic stimulation of a 'specific' osmosensitive neurone of the 'biphasic' type in the supraoptic nucleus of the chronic monkey. In *A*, spontaneous firing of cell did not change, with a period of spontaneous arousal with increased eye and body movements and a brief sniff, while in *B*, intracarotid injection of 0·45 M sodium chloride produced cell acceleration, sniffing irregular respiration, increased body and eye movements. After acceleration of the cell there followed 10 sec of marked inhibition followed by resumption of base line discharge rates. After an initial intravenous injection of urethane (1·3 g), spontaneous cell firing was depressed at 30 sec (*C*), 9 min (*D*) and 11 min (*E*) with decreased responsiveness to intracarotid hypertonic sodium chloride (*E*). Further depression of spontaneous firing occurs following a second (additional) injection of urethane (1·3 g) at 1 min (*F*), 3 min (*G*) and 11 min (*H*). This level of urethane anaesthesia completely blocks the response of this 'specific' 'biphasic' osmosensitive cell to intracarotid hypertonic sodium chloride at 3 min (*G*) and 11 min (*H*). In this 5 kg monkey a full I.V. anaesthetizing dose of urethane (ethyl carbamate) would be 5–6 g. Depression occurred at 2·6 g or about one half of an anaesthetizing dose. Labels: EM, eye movements; e.e.g. biparietal electrocorticogram; Move, accelerometer measure of body movements; Resp., nasal respiration measured by a thermocouple; Rate, analogue output proportional to a rate of unit discharge; Unit, pulse output from pulse height discriminator triggered by action potentials of the spike in the window.

sensitive from non-osmosensitive cells by the criteria already listed, we wondered about the specificity, of the osmosensitive cell. Was it responding to the 'osmotic' or to the 'arousal' effects of the intracarotid hypertonic injection? Accordingly, we tested cells not only to the osmotic stimulus but also to non-noxious arousing sensory stimuli such as light, sound and touch. We found that arousal would occur 'spontaneously' or could be produced by our tapping on the environmental chamber, shining a light into the monkey's eyes, making a noise in the room opening the door of the chamber and touching various parts of the monkey's body (Text-figs. 2, 6). All of these procedures aided us in further separating the osmosensitive cells into two groups; the 'specific' osmosensitive cells exclusively responsive to osmotic input; the 'non-specific' osmosensitive cells responsive to osmotic and to arousing sensory stimuli.

The 'specific' osmosensitive cell

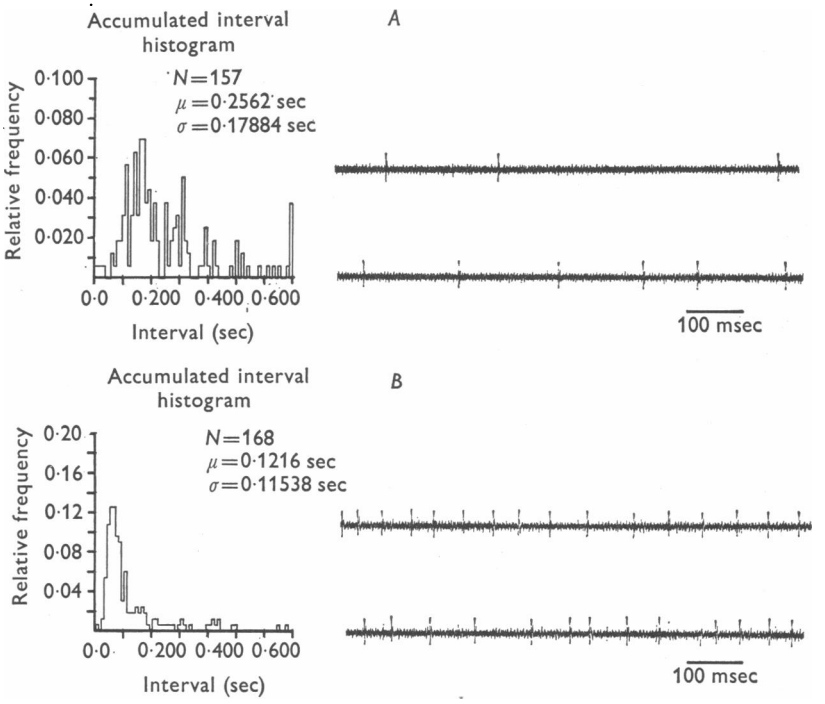
Fifty-two osmosensitive cells (50%) responded to an intracarotid hypertonic saline injection with a greater than 20% change in firing rate during injection, returned to base line within 30–60 sec and failed to respond to a variety of mild arousing stimuli (Table 2, Text-figs. 3–9). These cells also failed to change their firing rates to intracarotid injections of isotonic saline or to distilled water. We define such cells as 'specific' osmosensitive cells, partly because of their selective functional response, partly because of their grouping anatomically in the supraoptic nucleus and the immediate perinuclear zone of the NSO (Table 1, Pl. 1, Text-fig. 4).

All of our 'specific' osmosensitive cells are not the same. Depending upon the pattern of response to osmotic stimulus and anatomical location, we define two sub-types of 'specific' osmosensitive cells: 'biphasic' specific cells in the NSO; 'monophasic' specific cells, showing acceleration or inhibition in the perinuclear zone of the NSO.

'Biphasic' specific osmosensitive supraoptic neurones. We recorded from twenty-one cells (20%) located in the NSO (Text-fig. 4, Pl. 1, Table 2) which showed a distinct 'biphasic' pattern of firing to an intracarotid osmotic stimulus (Text-fig. 3). This pattern consists of an initial burst of activity (2–4 sec), followed by a period of silence (5–12 sec) before a return to a base line firing (Text-figs. 3, 5, 6). All of the cells that we recorded from in the supraoptic nucleus showed this 'biphasic' pattern of response, with two exceptions: one cell was non-osmosensitive (Text-fig. 4), the other a 'monophasic' specific osmosensitive cell (Text-fig. 4, Table 2). We located all the cells with a 'biphasic' pattern of response in the NSO and nowhere else in the hypothalamus (Text-fig. 4, Table 2).

These cells do not respond to intracarotid isotonic saline (0·15 M, Text-fig. 5), nor to mild arousing stimuli (Text-fig. 6). Repeated identical

osmotic stimuli delivered at 2-min intervals produce reproducible excitatory and inhibitory phases of these supraoptic neurones (Text-fig. 3) while repeated injections at 5-sec intervals decrease both phases (Text-fig. 5). When volume and rate of injections are constant and concentration of osmotic stimulus increased, repeated injections produce a graded enhancement of both the acceleratory and inhibitory phases (Text-fig. 5). The per-



Text-fig. 7. Accumulated interspike interval histogram (left) and corresponding spike trains (right) of a 'specific' osmosensitive neurone of the 'biphasic' type in the supraoptic nucleus of a waking monkey. In *A*, neuronal activity (3·9 spikes/sec) described by an 'assymetric' histogram accumulated during three 5-sec control periods prior to stimulation and a slow spike train. In *B* accumulated 'assymetric' histogram during three 5-sec periods of injection of intracarotid 0·45 M sodium chloride solution with acceleration of unit firing (8·3 spikes/sec) and a faster spike train. Not shown is the absence of cell firing during the post-injection period (0·00 spikes/sec). Cell showed no change in firing rate to isotonic sodium chloride nor to mild arousing sensory stimuli. Labels: N = the number of intervals; μ = mean interspike intervals; σ = standard deviation.

centage increase in mean firing rate from control state to the period during hypertonic injection ranges from 25 to 689% (distribution, Table 2) for 'biphasic' specific osmosensitive neurones. These cells are highly sensitive

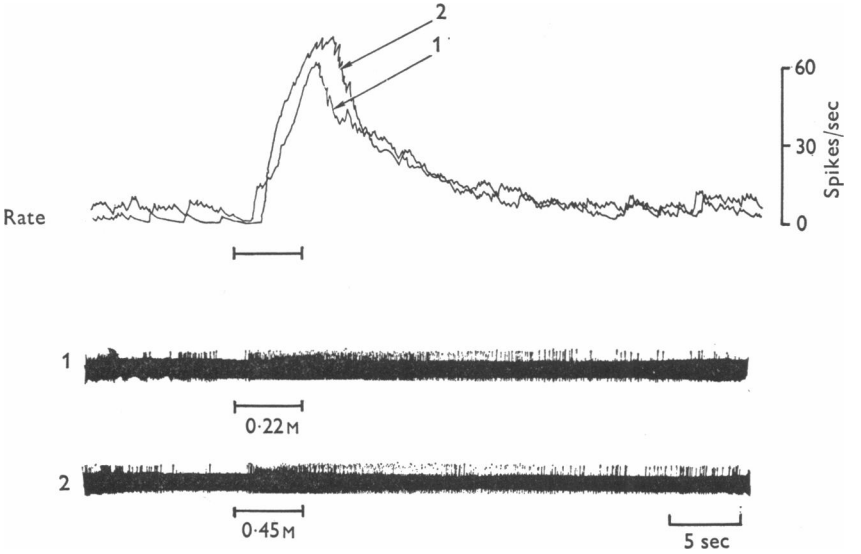
to anaesthesia. Low doses of urethane suppress spontaneous firing and osmosensitivity (Text-fig. 6).

We find 'biphasic' specific osmosensitive cells in the pars dorsolateralis (twelve cells) and in the pars dorsomedialis (nine cells), but none in the pars ventromedialis of the NSO (Text-fig. 4, Pl. 1, Table 2; Nauta & Haymaker, 1969). Cells of the supraoptic nucleus in the monkey discharge at slow rates in the resting, waking animal (Tables 1, 2), showing no special pattern of spontaneous or stimulated discharge as indicated in the 'asymmetrical' histogram (Text-fig. 7). When the osmotic stimulus (0·45 M sodium chloride) was applied as a chronic infusion (0·1 ml./sec) over 100–200 sec, the mean firing rates of biphasic specific osmosensitive cells increased by 100% and a slow oscillatory excitatory–inhibitory pattern of firing having a period of 10–20 sec developed. No shorter term pattern of rhythmicity was evident in the interval histograms or the autocorrelograms. We find no cells in the NSO firing with brief, high frequency clusters as seen in the dorsolateral hypothalamus (Findlay & Hayward, 1969). On the basis of cell location and the 'specific' nature of response to osmotic stimulus with a unique 'biphasic' discharge, we identify these cells as the secretory cells of the supraoptic nucleus.

'Monophasic' specific osmosensitive cells in the perinuclear zone of NSO. Thirty-one cells (30%) was the total number of 'monophasic' osmosensitive cells recorded. Of these, twenty-eight were located in the perinuclear zone of the NSO, one was in the supraoptic nucleus, one in the lateral hypothalamus and one in the lateral preoptic area (Text-fig. 4, Table 2). All displayed a 'monophasic' response to intracarotid hypertonic sodium chloride (Text-figs. 8, 9), with no response to intracarotid distilled water, isotonic saline or non-noxious arousing sensory stimuli. Twenty-one of these 'specific' osmosensitive cells (20%) show monophasic accelerated responses (Text-figs. 8, 9) and (10%) show monophasic inhibitory responses (Table 2). Adjustment of rate and concentration of injection to yield a roughly equivalent osmotic stimulus produces nearly identical acceleration responses (Text-fig. 8). The percentage increase in mean firing rate between control state and the period during osmotic injection ranges from 22 to 765% (distribution, Table 2) for monophasic specific perinuclear zone neurones. All the osmosensitive cells that we recorded from in the perinuclear zone of the NSO showed these monophasic patterns of response. Some were specific and others non-specific cells (Text-fig. 4).

Specific cells with a 'monophasic' pattern to intracarotid hypertonic saline were mainly distributed in the perinuclear zone of NSO with only an occasional cell located elsewhere in the antero-lateral hypothalamus (Text-fig. 4). Cells of the perinuclear zone in the monkey discharge at slow rates in the resting, waking animal (Tables 1, 2). The monophasic specific cells

show no particular rhythmicity of spontaneous or stimulated firing activity on the computer-produced histogram ('asymmetric') and autocorrelogram ('flat', Text-fig. 9). On the basis of cell location and the specific nature of response to the osmotic stimulus, we identify these cells in the peri-nuclear zone of the NSO as the 'osmoreceptors' of Verney.

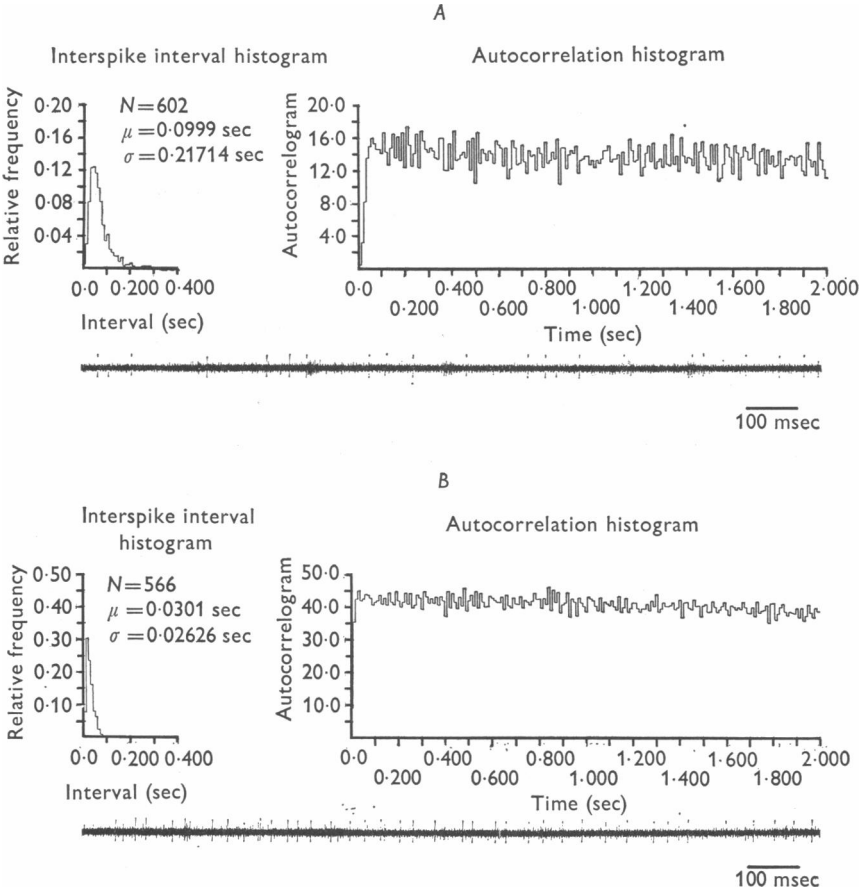


Text-fig. 8. Responses of a 'monophasic' specific osmosensitive neurone in the perinuclear zone of the supraoptic nucleus to osmotically different solutions. After a 5-sec intracarotid injection of 1 ml. 0·22 M (1) and 0·5 ml. 0·45 M (2) sodium chloride solution to give roughly equal osmotic stimuli this cell shows nearly equivalent acceleration of firing. Superimposed polygraph tracings (above) of the mean rate of cell firing (spikes/sec) and unit spikes photographically reproduced (below) during the same two osmotic stimuli. Note the absence of post-stimulus inhibition. Cell did not respond to mild arousing sensory stimuli nor to isotonic sodium chloride intracarotid.

The 'non-specific' osmosensitive cell

We recorded from fifty-three cells (50 %) located diffusely in the antero-lateral hypothalamus which showed 'monophasic' responses to both intra-carotid hypertonic sodium chloride and to mild arousing sensory stimuli (Text-fig. 2). These cells are non-specific. They are widely distributed throughout the antero-lateral hypothalamus, although a few are in the perinuclear zone (Text-fig. 4), and they all lack a specific response to hypertonic intracarotid stimulus (Text-fig. 2). These cells respond neither to intracarotid isotonic saline nor to distilled water. Thirty-five of these cells (34 %) show monophasic accelerated responses to both osmotic and sensory stimuli (Text-fig. 2*A*). Seventeen of these cells (16 %) show mono-

phasic inhibitory responses to both osmotic and sensory stimuli (Text-fig. 2*B*). The presence of a behavioural response to the osmotic stimulus, the response of these cells to non-noxious arousing sensory stimuli, and the wide distribution throughout the hypothalamus suggest to us that these cells are not directly related to osmoregulation. Perhaps some of those cells



Text-fig. 9. Interspike interval histogram (left) and corresponding autocorrelogram (right) of a 'specific' osmosensitive cell of the monophasic type in the perinuclear zone of the supraoptic nucleus of an unanaesthetized monkey. In *A*, neuronal activity (10·1 spikes/sec) described by an 'asymmetric' histogram, a 'flat' autocorrelogram and a slower spike train (below). In *B*, during a 5-sec injection of intracarotid 0·45 M sodium chloride solution, acceleration of unit firing (33·3 spikes/sec) with 'asymmetric' histogram, a 'flat' autocorrelogram and a faster spike train (below). Cell showed no change in rate to non-noxious sensory stimuli nor to isotonic carotid solutions. Labels: N = the number of intervals; μ = mean interspike intervals; σ = standard deviation.

located in the immediate perinuclear zone (Text-fig. 4) act as an interneuronal convergence mechanism for funnelling sensory input into the osmoreceptor-secretory complex of the supraoptic nucleus.

DISCUSSION

In our attempt to identify the 'osmoreceptors' of Verney we first noted that an intracarotid injection of hypertonic sodium chloride in the waking monkey produced a behavioural response consisting of e.e.g. 'arousal', irregular sniffing respiration, lip and tongue smacking, chewing and a mild increase in movement of face, eyes and body. These observations confirm the observations of lip-smacking (Verney, 1947) in the dog and suggest that there is an 'osmotic' and an 'arousal' component to intracarotid hypertonic injections. Such a finding is pertinent to the present analysis of osmosensitive neurones and 'osmoreceptors' with single unit recording techniques. Previously we had found many hypothalamic cells (60–70%) sensitive to shifts in sleep–waking behaviour (Findlay & Hayward, 1968, 1969; Hayward, 1969*a*, *b*; Vincent *et al.* 1967). By testing hypothalamic single neurones with both osmotic and mild sensory stimuli (auditory, visual, tactile), we now find two populations of responsive cells: 'specific' osmosensitive cells excited by osmotic but not by pure arousal stimuli; 'non-specific' osmosensitive cells responsive to both osmotic and arousing sensory stimuli.

We classify the cells of the supraoptic nuclei and the perinuclear zone as 'specific' osmosensitive cells. They are sensitive to small injections of hypertonic sodium chloride into the common carotid artery, but show little or no response to the arousing sensory stimuli of sound, light or touch. The characteristic and reproducible changes in firing frequency of these 'specific' osmosensitive cells undoubtedly result from the 10–20% increase in osmolality of the circulating cerebral arterial blood. These specific cells do not respond to isotonic saline or distilled water, thus eliminating the remote injection procedure itself or the 10–15% increase in carotid blood flow or associated pressure changes as the primary causes of the cell response. The absence of systemic blood pressure changes during osmotic stimulus eliminates this as a possible non-specific cause of unit response.

Assuming then, that the electrical activity of these 'specific' osmosensitive neurones is related to neurohypophysial activity, is there a distinction between 'osmoreceptors' and 'secretory' cells? Cells in the perinuclear zone of the supraoptic nucleus respond to osmotic stimuli with 'monophasic' acceleration or inhibition. We propose, therefore, on the basis of location, pattern of response and specificity of response that these

cells are the 'osmoreceptors' of Verney. Our data supports the suggestion by Cross & Green (1959) in the anaesthetized rabbit and Joynt (1964) in the locally anaesthetized, immobilized cat that 'osmoreceptors' lie in the perinuclear zone of the NSO and are generally unresponsive to sensory stimuli. These findings also fulfil the main criteria of von Euler (1953) for identification of a true receptor in the central nervous system. We can be more dogmatic in our identification of the osmoreceptors of Verney than earlier investigators (Cross & Green, 1959; Joynt, 1964) because we can clearly separate functionally and anatomically the 'osmoreceptors' from the closely related 'secretory' cells of the supraoptic nucleus.

In the monkey the neurones of the NSO exhibit a 'biphasic' response to osmotic stimuli, that is, acceleration followed by inhibition, without any response to arousing sensory stimuli. We identify these biphasic specific osmosensitive cells as the secretory neurones of the NSO. As far as we are aware, no previous description of this biphasic pattern of response in the NSO has been published. It is possible that these responses are unique to the primate. We think it more likely, since these cells are easily depressed by small doses of anaesthesia, that they may be the 'silent' cells of earlier workers (Cross & Green, 1959). The inhibitory phase of this biphasic response might be accounted for by a slow inhibitory post-synaptic potential (Libet & Tosaka, 1969), it might be related to recurrent collaterals of neuronendocrine neurones (Kandel, 1964; Koizumi & Yamashita, 1969) acting through an interneurone, or it might be due to post-tetanic hyperpolarization. Future studies in unanaesthetized animals using pharmacological agents may allow us to distinguish between a slow inhibitory post-synaptic potential or post-tetanic hyperpolarization mechanisms of inhibition. At present we have no information about the nature of the synaptic coupling between the osmoreceptors and the secretory cells of the NSO.

We classify cells lying diffusely throughout the anterolateral hypothalamus as 'non-specific' osmosensitive cells. They are sensitive to small injections of hypertonic sodium chloride into the common carotid artery and react with identical responses to mild arousing sensory stimuli. These cells show no responses to isotonic saline and hypotonic solutions. We consider that most of these cells are responding to the behavioural component of the osmotic stimulus. We have not studied the exact mechanism of arousal but likely trigger zones include receptors in the tongue and mouth (taste), osmoreceptors in the olfactory bulb (sniffing respiration; Sundsten & Sawyer, 1959), hypothalamic osmoreceptors for drinking (Verney, 1947) and brain stem osmoreceptors for vasomotor or respiratory control (Clemente, Sutin & Silverstone, 1957; Holland *et al.* 1959), limbic and reticular osmoreceptors (Sawyer & Gernandt, 1956).

There is a basic conflict in the literature regarding the criteria for defining a cell as 'osmosensitive'. We use the same criteria as Cross & Green (1959) and Joynt (1964), a prompt response to intracarotid hypertonic solutions during or immediately after injection with a return to base line firing within 30–60 sec. Brooks and co-workers (Brooks *et al.* 1962, 1966; Ishikawa *et al.* 1966; Koizumi *et al.* 1964 and Suda *et al.* 1963), in studies in the anaesthetized and immobilized cat, have chosen to disregard the rapidly responding cells. They prefer the 'osmosensitive' cells to be the ones that respond during or after the intracarotid injection and continue to respond (acceleration or inhibition) for 2–4 min after the beginning of the osmotic stimulus. We have not observed these long-lasting cellular responses to osmotic stimuli in the unanaesthetized monkey nor have others reported them in the urethane anaesthetized rabbit (Cross & Green, 1959) or in the locally anaesthetized, paralysed cat (Joynt, 1964). It seems to us, on a theoretical basis alone, that osmoreceptor cells would need to have a shorter response time than 2–4 min in order to closely regulate plasma osmolality. Brooks and co-workers usually used a choloralosane anaesthetized, immobilized, hemispherectomized cat with ligated cerebral arteries on the hemispherectomized side. Intracarotid injections, given into the common carotid artery on the hemispherectomized side, must traverse the carotid rete (Baker & Hayward, 1967; Hayward & Baker, 1969) to reach the opposite circle of Willis and the supraoptic nucleus. Perhaps some aspect of their experimental design, such as disinhibition by removal of the opposite NSO, or after-discharge related to chloralosane, or delayed passage of the osmotic injection or prolonged behavioural arousal may account for their observed delayed responses.

A second basic conflict in the literature relates to the responsiveness of the 'osmosensitive' cells to sensory stimuli. Brooks and co-workers find the osmosensitive cells in and near the NSO quite responsive to electrical stimulation of peripheral nerves and central brain sites. Cross & Green (1959) in the rabbit, Joynt (1964) in the cat and we in the unanaesthetized monkey find the osmosensitive cells in the vicinity of the NSO poorly responsive to natural sensory stimuli. It seems likely that these divergent views concerning the definition of osmosensitive cells and their responsiveness to sensory stimuli is related to the fact that different groups, Brooks and co-workers on the one hand and Cross & Green, Joynt, and ourselves on the other, are talking about totally different cells. Similarly, basic differences in the experimental design could account for these major discrepancies in the neurophysiological definition of supraoptic neurones.

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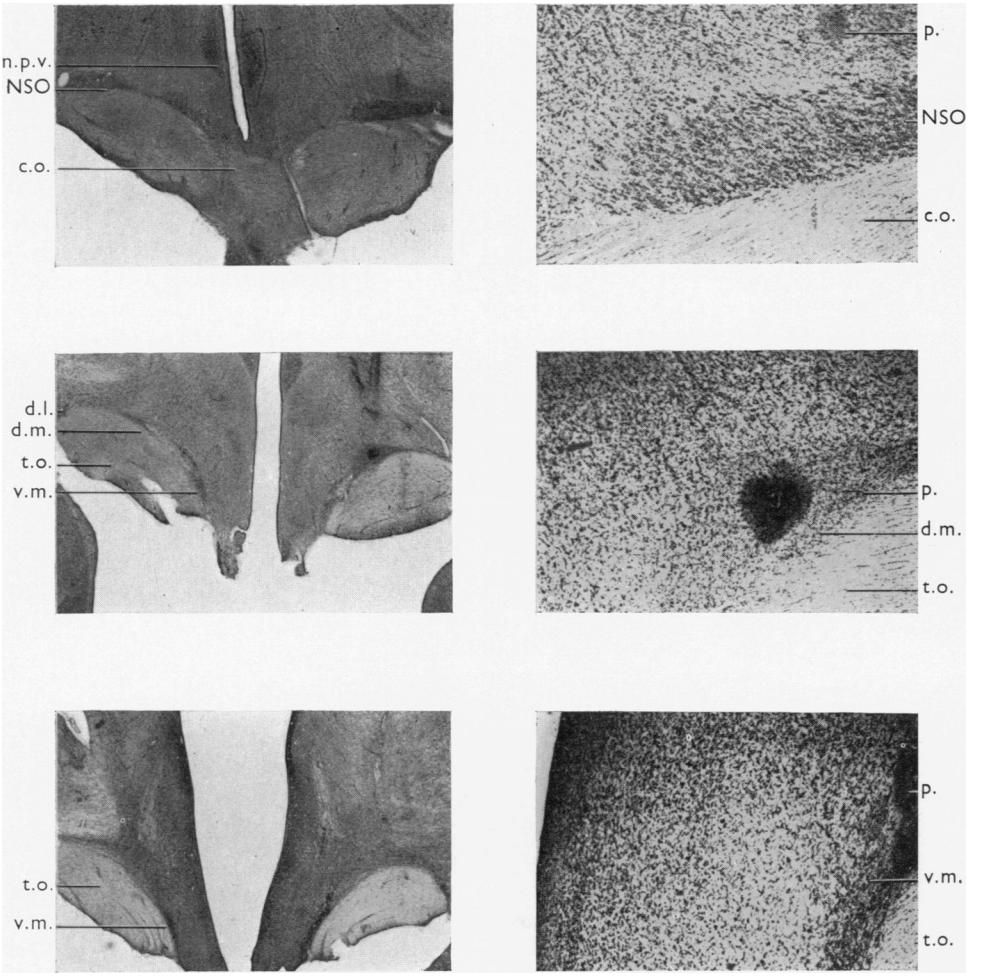
EXPLANATION OF PLATE

Photomicrographs of the hypothalamus and the supraoptic nucleus in three monkeys at approximate stereotaxic planes of Fr. 15·5 (upper), Fr. 14·5 (middle) and Fr. 13·5 (lower) after the co-ordinates of Snider & Lee (1961). The lower power views (× 15, left) show the three anatomical parts of the supraoptic nucleus, the surrounding structures in the anterior hypothalamus and the Prussian blue spots marking the sites of hypothalamic electrode tracks. On the right is the corresponding higher power view (× 48) showing in detail the Prussian blue spots and the three parts of the supraoptic nucleus. The sections were cut transversely at 80 μ and stained with thionin. Labels: n.p.v., paraventricular nucleus; NSO, supraoptic nucleus; d.l., pars dorsolateralis of the supraoptic nucleus; d.m., pars dorsomedialis of the supraoptic nucleus; t.o., optic tract; v.m., pars ventromedialis of the supraoptic nucleus; p., Prussian blue spot; c.o. optic chiasm.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