

Electrical Properties of Hypothalamic Neuroendocrine Cells

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ABSTRACT Goldfish hypothalamic neuroendocrine cells have been investigated with intracellular recordings. The cells showed resting potentials of 50 mv and action potentials up to 117 mv followed by a long lasting and prominent diphasic hyperpolarizing afterpotential. The action potential occurred in two steps indicating sequential invasion. "Total" neuron (input) resistance was measured to be $3.3 \times 10^7 \Omega$ and total neuron time constant was 42 msec. Orthodromic volleys, produced by olfactory tract stimulation, generated graded excitatory postsynaptic potentials. These neuroendocrine cells seem, therefore, to have electrical membrane properties that are similar to those of other central neurons. Antidromic volleys (pituitary stimulation) produced inhibitory postsynaptic potentials whose latency was only slightly longer than that of the antidromic spike indicating the presence of recurrent collaterals. This finding suggests that the concept of the neuroendocrine cell as a neuron whose axon forms contacts only on blood vessels and not on other neurons or effector cells is too restrictive. Perfusion of the gills with dilute (0.3 per cent) sea water produced an inhibition of spontaneous activity. This inhibition is discussed in relation to recent work which demonstrates that goldfish hypothalamic hormones facilitate Na^+ influx across the gill membrane.

Since their initial description by Ernst Scharer (44), neurons having the secretory morphology characteristic of gland cells have been identified in the hypothalamus of all classes of vertebrates (34, 45). It is now well established that these neurosecretory cells form an elongated endocrine gland in which their axons serve as a transport channel for hormone that is produced in the soma and released into the blood stream by specialized presynaptic terminals (2, 34, 35, 39). This endocrine function has given rise to the notion that, in their neural properties, hypothalamic as well as all other neuroendocrine cells differ from central neurons in that they end only on capillaries and do not form synaptic junctions with other nerve or effector cells (6, 7, 10).

The purpose of the present study, on the preoptic neuroendocrine cells of the goldfish, is to investigate how these morphological and functional speciali-

zations manifest themselves in the neurons' electrical behavior. In particular, this investigation will focus on whether the electrical functioning of the neuroendocrine cell resembles that of non-endocrine neurons or of non-nervous glandular cells (12, 21, 28).¹

The preoptic nucleus of lower vertebrates, which differentiates into the supraoptic and paraventricular nuclei in higher forms, produces the hormones of the neural lobe of the pituitary (40). This nucleus and its outflow tract, the hypothalamic pituitary pathway, constitute the most clearly established neuroendocrine system of vertebrates and consequently have been the object of extensive morphological and endocrine-physiological investigations (1, 19, 34, 37, 38, 43, 45). In addition, several studies have recently explored certain electrophysiological characteristics of this system. Potter and Loewenstein investigated the unusually long pituitary stalk of *Lophius* and demonstrated that the stalk axons conduct propagating action potentials which can be fired orthodromically by hypothalamic stimulation (41). This direct demonstration of electrical activity in a neuroendocrine axonal tract has been confirmed by Carlisle (9) and by Bennett (3). Cross and Green (11) and more recently, Brooks *et al.* (8) have recorded extracellularly in the anterior hypothalamus the unit activity of unidentified elements, some of which were probably neuroendocrine in nature, and have shown that these cells may respond to sensory and/or to osmotic stimuli. The use of intracellular recordings and direct identification in the present study permits a further specification of several types of membrane responses of hypothalamic neuroendocrine cells. Related data on the spinal neurosecretory cells of fish have recently been obtained by Morita *et al.* (32) and Bennett and Fox (5).

The goldfish preoptic nucleus offers several specific advantages in the electrophysiological investigation of neuroendocrine cells. In goldfish the anterior hypothalamus is relatively large and the magnocellular portion of the preoptic nucleus, which is made up entirely of neuroendocrine cells, is directly visible following removal of the choroid plexus. The cells are moderate in size and the brain is pulseless; this makes it possible to obtain stable intracellular recordings from these neurons. In addition, electrodes can be placed into the sella turcica for stimulation of the pituitary and for antidromic activation of the preoptic hypophysial tract. This provides an additional and electrophysiological method for identifying neuroendocrine cells.

Although there is great morphological similarity between the teleost and mammalian neuroendocrine systems the two are not identical in function. The posterior pituitary hormone which differs somewhat in its structure in

¹ Some of the findings were described in a preliminary abstract (24) and presented at the 1962 Spring Meetings of the American Physiological Society.

the two classes (43) does not affect renal tubular absorption of water in fish as it does in higher vertebrates (40). Instead, teleost hormone facilitates Na^+ influx across the gill membrane of the fresh water fish (29–31).

METHODS

Preparation Large goldfish weighing 200 to 300 gm and measuring 10 to 12 inches were anesthetized and paralyzed with an intramuscular injection (1 cc/100

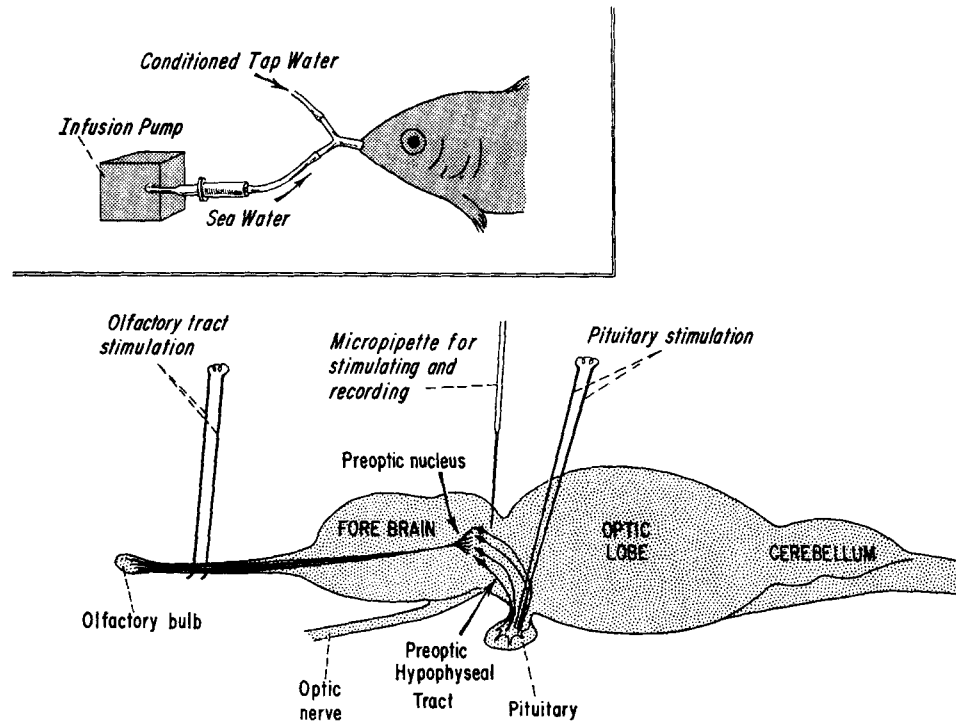


FIGURE 1. Schematic diagram of the goldfish brain indicating the location of the preoptic nucleus and the position of the electrodes for olfactory tract (orthodromic) and pituitary (antidromic) stimulation. Arrangement for perfusing with tap and with dilute sea water is indicated in the insert.

gm) of a fresh water teleost physiological solution (52) containing nembital (300 mg per cent) and either *d*-tubocurarine (15 mg per cent) or metabine (30 mg per cent). In view of the actions of anesthetics on the neuroendocrine system (22, 51) several additional experiments were carried out on animals maintained only under paralyzing agents in order to insure that the effects studied were not due, in part, to the anesthetic agent used.

The animals were fixed semirigidly in a lucite chamber and the gills perfused with circulating conditioned tap water using a simple system designed by Furshpan and Furukawa (personal communication). A Harvard Instrument Co. infusion pump was

inserted in parallel with this system and permitted a regulated flow of sea water to enter the main inflow tube (see Fig. 1). A balanced solution of dilute sea water was used because of the reported toxicity of sodium chloride solutions for fresh water fish (15). The ratio of tap water to injected sea water was greater than 100:1 so that the introduction of sea water produced practically no change in total flow rate. The actual amount of Na^+ perfusing the gills, at various settings of the infusion pump, was determined by spectrophotometric analysis. The range used in these experiments was 0.8 to 1.6 mEq Na^+ /liter. This represents a three- to sixfold increase of the 0.3 mEq Na^+ /liter found in the tap water used for these experiments.

The brain was exposed from the olfactory bulb to the cerebellum, and the choroid plexus was carefully removed with fine dissecting forceps, care being taken to control bleeding. The cerebral hemispheres were then gently spread further apart; the third ventricle was filled with mineral oil at room temperature. This permitted good access to and visibility of the floor and the walls of the ventricle.

Recording and Stimulation K^+ citrate-filled glass micropipettes were used for intracellular recording. These were led through a Bak unity gain "negative capacitance" preamplifier to two high gain DC differential amplifiers of a double beam oscilloscope. A Wheatstone bridge was used for intracellular stimulation through the recording microelectrode. These techniques, and the criteria used for obtaining bridge balance, were similar to those employed in an earlier investigation on hippocampal neurons and are described there in greater detail (26, 48). The fish was grounded through one of two stainless steel bars used for fixation.

Bipolar stimulating electrodes were placed on the olfactory tract and a fine pair of Teflon insulated sharpened tungsten microwires (23) with exposed tip diameters of about 50μ were inserted through the optic lobe into the pituitary gland (Fig. 1) Passage through the diaphragma sellae could be felt as a definite resistance to electrode movement and was helpful in locating the sella turcica. The large size of the teleost pituitary made it a relatively easy target. At the end of each experiment the brain was removed by suction and the location of the electrodes in the sella turcica was verified by visual inspection.

Criteria for Selection of Data The data presented have been obtained from 50 cells which generated action potentials of 40 mv or greater and remained in stable condition for over 5 minutes. Several cells remained stable during impalements of 1 hour.

RESULTS

1. *Identification of Neuroendocrine Cells*

The magnocellular portion of the preoptic nucleus is made up exclusively of neuroendocrine cells. In small and medium sized goldfish these cells range in size from 12 to 30μ (38). In very large animals occasional cells up to 40μ are encountered (Roth and Kandel, unpublished observation). The nucleus lies in the wall of the preoptic recess of the third ventricle just beneath the ependymal lining (Fig. 2 and reference 38). The anterior commissure and

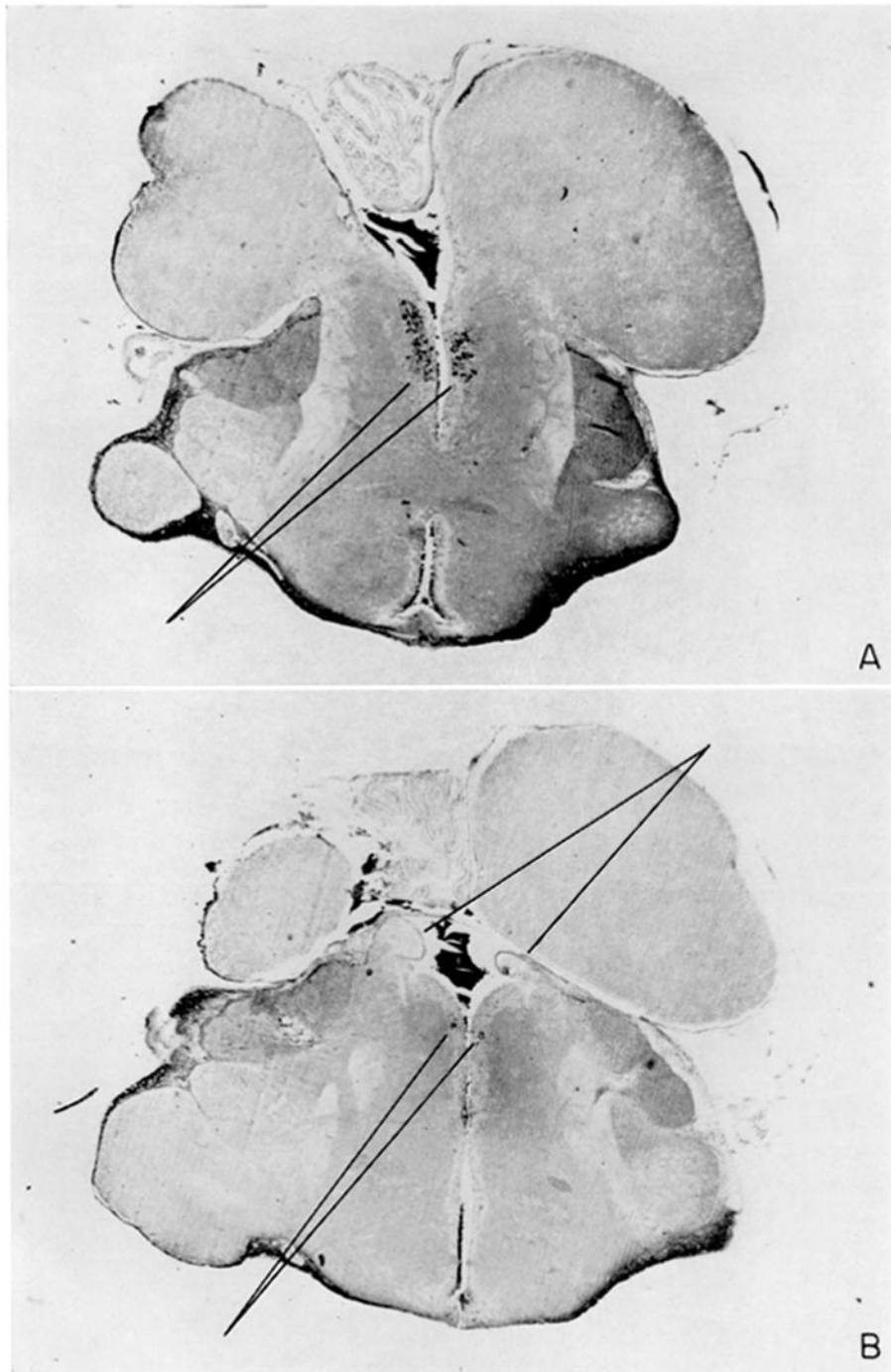


FIGURE 2. Cross-sections through a goldfish brain stained with the chrome hematoxylin method of Gomori to show the posterior portion of the preoptic nucleus. The nucleus is indicated by the lines on the left-hand side of the sections and the habenular ganglion by the lines on the right in B. The ependyma was removed in the experiments; microelectrode penetrations were made in the posterior and most dorsal portion of the nucleus indicated in the cross-section of part A. (These sections were kindly made available by Dr. W. Roth.)

the habenular ganglia lie at the anterior and posterior margins of the nucleus. These structures were clearly visible under mineral oil and, together with the ventricular wall in the midline, they formed a distinctive array of anatomical landmarks which permitted a direct visual identification of the nucleus under low power ($\times 4$ to 16) magnification of a dissecting microscope. The larger cells lie medial, ventral, and posterior just in front of the habenular ganglia (Fig. 2). This portion of the nucleus was the target for the microelectrode penetration.

A second and electrophysiological method of identification was afforded by antidromic activation of the impaled cell following stimulation of the

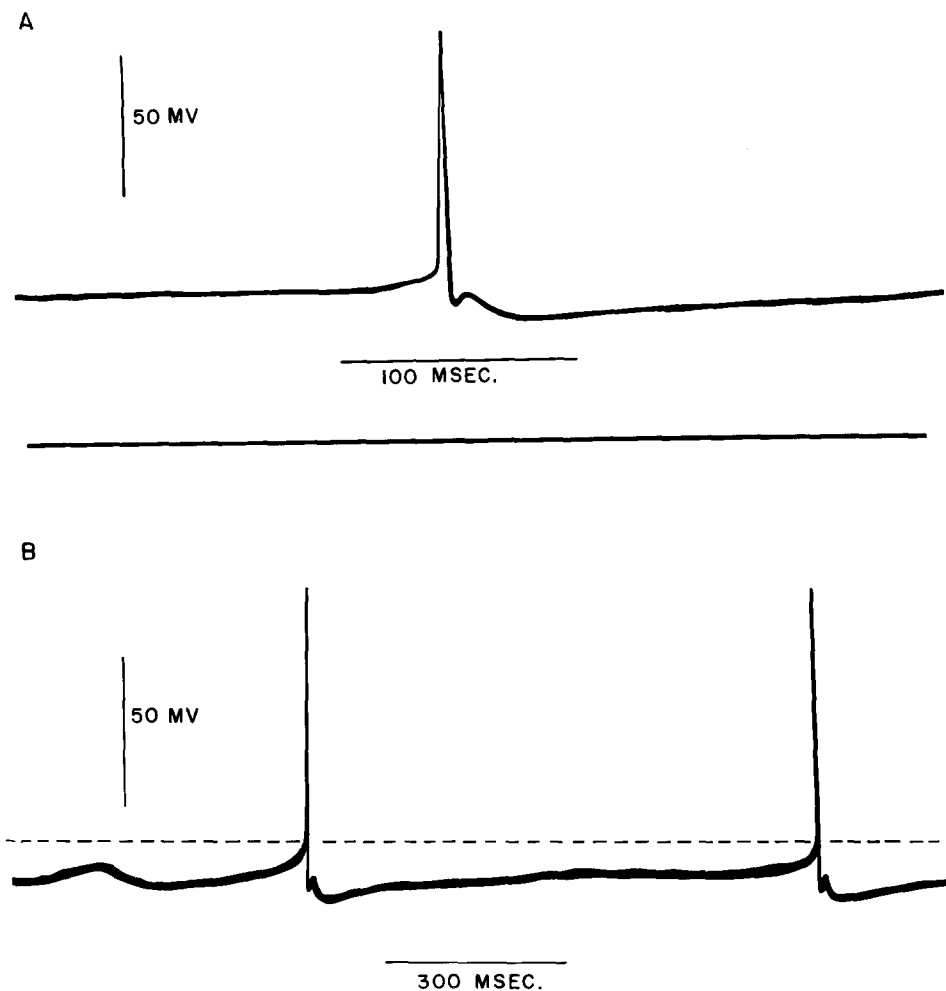


FIGURE 3. Action potential and afterpotential of a neuroendocrine cell at two different sweep speeds. Dotted line in B was drawn to indicate the constancy of the critical firing level. Note subthreshold depolarizing oscillation. In these and all subsequent records, an upward deflection means positivity at the microelectrode.

TABLE I

	R.M.P.	A.P.	S.D.	HAP M ₁	HAP M ₂	HAP D ₁	HAP D ₂	F.L.	Rhb	τ_{tot}	R_{tot}
	<i>mv</i>	<i>mv</i>	<i>msec.</i>	<i>mv</i>	<i>mv</i>	<i>msec.</i>	<i>msec.</i>	<i>mv</i>	10^{-10} amps	<i>msec.</i>	10^7 Ω
	65	91	3.2	2.9	4.8	6.6	170	15.5	5.3	76.3	2.9
	—	117	3.0	2.8	6.6	6.2	63	11.1	1.9	43.7	3.0
	43	55	3.9	5.1	7.6	6.6	100	6.9	1.4	35.6	4.0
	46	78	3.5	1.5	6.3	6.6	165	9.2	1.7	22.6	5.0
	63	77	4.2	6.2	5.5	4.3	78	10.0	1.3	—	3.5
	40	47	3.6	1.5	4.7	5.0	83	7.8	0.9	55.6	1.1
	—	40	2.5	—	2.5	—	30	3.3	1.5	18.1	—
	—	67	3.0	—	4.0	—	60	7.5	2.0	—	—
	—	90	3.5	3.5	6.5	5.4	100	8.8	—	—	—
	—	80	4.5	5.0	7.5	5.0	280	10.0	—	—	—
Mean	51.4	74.2	3.5	3.6	5.6	5.7	112.9	9.0	2.0	41.8	3.3
SEM	±5.2	±6.9	±0.2	±0.6	±0.5	±0.3	±21.5	±0.9	±0.5	±8.1	±0.5

R.M.P., resting membrane potential

A.P., action potential

S.D., spike duration

HAP M₁, hyperpolarizing afterpotential, magnitude 1st component

HAP M₂, hyperpolarizing afterpotential, magnitude 2nd component

HAP D₁, hyperpolarizing afterpotential, duration 1st component

HAP D₂, hyperpolarizing afterpotential, duration 2nd component

F.L., firing level

Rhb, rheobasic current strength

τ_{tot} , total neuron time constant

R_{tot} , total neuron (input) resistance

pituitary gland. This technique also permitted a measurement of conduction velocity in neuroendocrine cell axons. Average latency for antidromic activation was 6 msec. For an average conduction distance of 2.8 mm this yields a conduction velocity of 0.46 m/sec. which is similar to the value of 0.5 m/sec. obtained by Potter and Loewenstein in the pituitary stalk of *Lophius* (41). However, in the present experiments the estimate of conduction distance was made from typical histological sections and was therefore open to error.

Only slightly over 60 per cent of the cells encountered in the preoptic nucleus could be activated antidromically. However, all cells in the nucleus, even those which could not be activated antidromically, showed otherwise identical properties which differed significantly from elements in surrounding structures. Forebrain cells lying dorsolateral to the nucleus produced action potentials which were followed by depolarizing afterpotentials. These cells tended to fire in high frequency bursts both spontaneously and in response to olfactory tract stimulation. Optic chiasm fibers lying ventral to the nucleus produced spikes of very brief duration and showed typical on-off responses to

diffuse retinal illumination. Failure of certain preoptic cells to be activated by pituitary stimuli may perhaps be attributable to two factors: (*a*) not all preoptic cells send axons to the pituitary (1, 36) and (*b*) the teleost pituitary is quite large and the stimulating electrodes may not always have been optimally placed for the activation of all axons.

2. Action Potential and Afterpotential

The action potentials of neuroendocrine cells ranged up to 117 mv and were distinguished by their relatively long duration (3.5 msec.) and, in most cells, by a characteristic notched hyperpolarizing afterpotential. The afterpotential had two phases: the first one brief and small and the subsequent one long lasting and larger. Fig. 3 illustrates the action potential and afterpotential of a unit at two different sweep speeds (see also Table I). Resting potentials were not measured in all units but were, on the average, 50 mv.

Impaled cells showed a slow, spontaneous firing rate of 2 to 8 impulses per second, in part determined by the duration of their long afterpotential. The membrane potential also showed some slow spontaneous oscillations but action potentials were only triggered when a relatively critical and constant membrane voltage was reached (Fig. 3B). The firing level varied somewhat from cell to cell (Table I).

A distinctive feature of the action potential of most vertebrate and invertebrate neurons studied so far is a break in the ascending limb of the action potential indicating a two-step invasion of the neuron with a low threshold (usually axonal) trigger area firing before the cell body (12, 16). Under certain circumstances, most clearly noted with antidromic volleys, the neuroendocrine cell action potential showed a similar two-step process. Since here, as with most vertebrate central neurons, the exact anatomical location of the active membrane areas cannot as yet be specified, the notation first introduced by Fuortes *et al.* (16) will be followed. The small initial spike will be designated A and the later, larger spike, B; the areas giving rise to these spikes will be referred to as A and B areas, respectively.

Antidromically triggered neuroendocrine cell spikes showed a slight notch on the ascending limb (upper trace in Fig. 4B) which could be accentuated by using conditioning testing volleys at progressively closer intervals. This is indicated in Fig. 4A. At a separation of about 150 msec. (upper trace) the conditioning and testing responses look alike. But, as the firing interval is shortened (second and third parts of Fig. 4A) the notch on the ascending limb of the test action potential is markedly accentuated. At a critical firing interval (see reference 16), the B spike becomes shorter and broader and occasionally fails to be triggered, permitting an isolated A spike to be recorded (bottom part of Fig. 4A). At an interval less than the critical firing interval, the B spike frequently shows some graded response properties varying in size,

shape, and time-to-peak. This is illustrated in Fig. 4B which is based upon fast sweep records from another unit. The upper and lower parts are superimposed fine line tracings of the conditioning and of the testing action potential respectively.

In spontaneously occurring or orthodromically initiated action potentials, it was usually impossible to detect an inflection on the rising phase even when the action potential was differentiated with respect to time to accentuate changes of slope in the ascending limb. This is illustrated in Fig. 5A which shows a spontaneously occurring and an orthodromically initiated action potential and their simultaneously recorded derivatives. Similarly, directly initiated action potentials, produced by passing current through the microelectrode, failed to show an AB notch. However, if repetitive firing was initiated by long, direct pulses the refractoriness engendered in later members of the train made an AB notch evident in their action potentials. The experi-

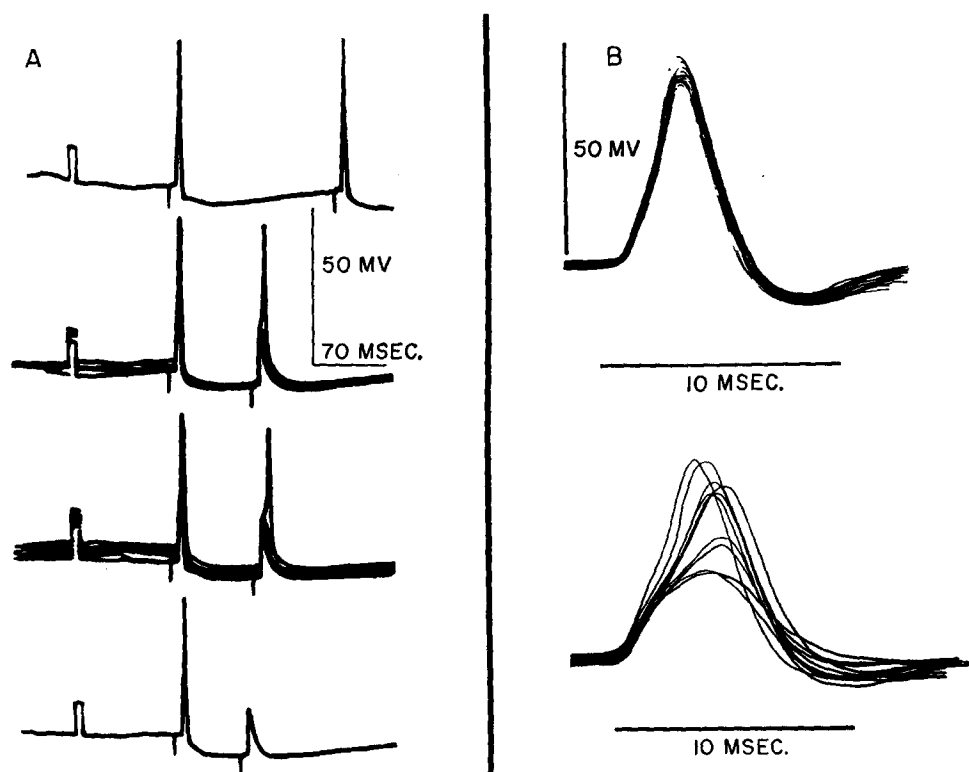


FIGURE 4. Antidromic activation. Part A, conditioning and testing volleys at progressively closer intervals. Note progressive accentuation of the AB inflection (superimposed traces in 2 and 3) and the isolation of the A spike as the B spike fails to fire (trace 4). Part B, from another unit at the critical firing interval (50 msec.). Note "graded" response properties of the B spike.

ment illustrated in Fig. 5B shows the consecutive action potentials produced by a 700 msec. train. The interspike intervals ranged from 52 to 105 msec. and, as indicated in the legend, they have been omitted from the figure in order to illustrate in greater detail the changes in configuration of the action potential. Note that an AB notch is not evident in the early action potentials but that it becomes evident (as indicated by the arrow) in the later ones.

3. Direct Stimulation: Repetitive Firing and the Measurement of Membrane Constants

Action potentials and afterpotentials similar to those occurring spontaneously could also be initiated by direct current pulses (Figs. 5B, 6A, and 7A). Maintained repetitive firing could be triggered by long depolarizing pulses (Figs. 5B and 6A). When firing frequency was examined as a function of stimulus current, both onset and steady-state frequencies were found to be linear

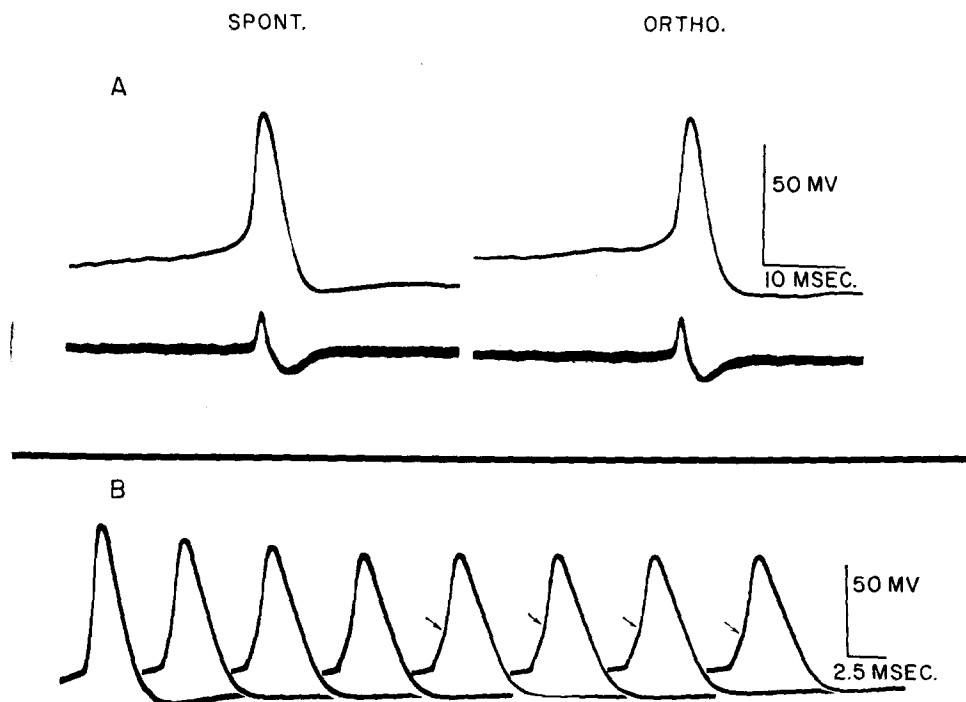


FIGURE 5. AB spikes with orthodromic, spontaneous, and direct stimulation. Part A, spontaneous and orthodromic action potentials and their simultaneously recorded first derivatives (time constant of differentiating circuit 1 μ sec.). Note absence of AB inflection even in differentiated responses. Part B, train of directly initiated spikes. Latency to the first spike 10 msec. Interspike intervals, which have also been omitted are 52, 62, 105, 82, 89, 85, and 89 msec. respectively. Note the accentuation of the AB inflection in later members of the burst (indicated by the arrows).

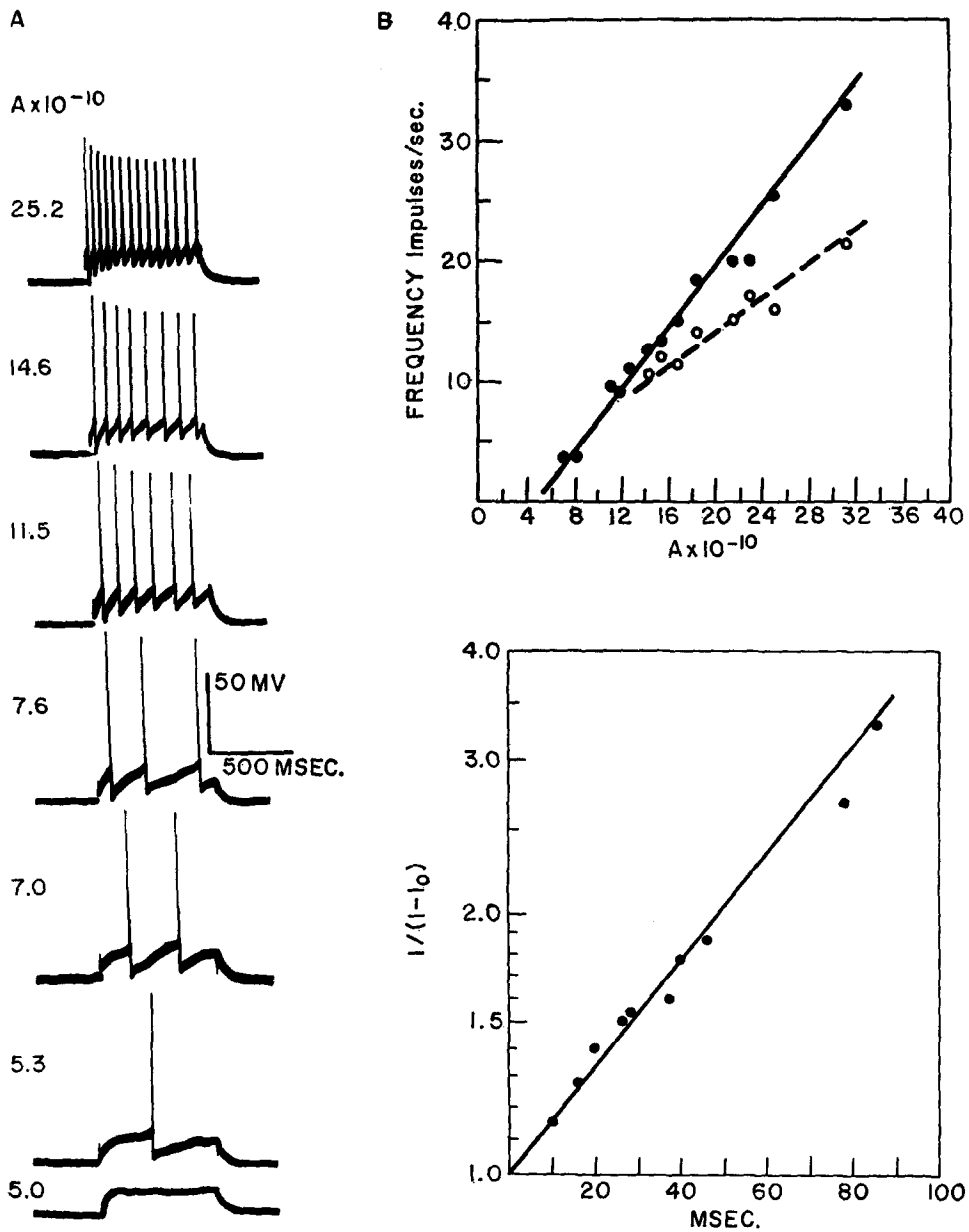


FIGURE 6. Direct stimulation. Part A, patterns of response to progressively increasing direct current stimulation. The intensity of the current used is indicated to the left of the figures. Part B, the upper graph is a plot of the reciprocal interval for the first two spikes (closed circles) and for steady-state firing (open circles) as a function of current intensity. The lower graph is a strength latency curve on a semilog scale. Both graphs are based on the experiment indicated in part A.

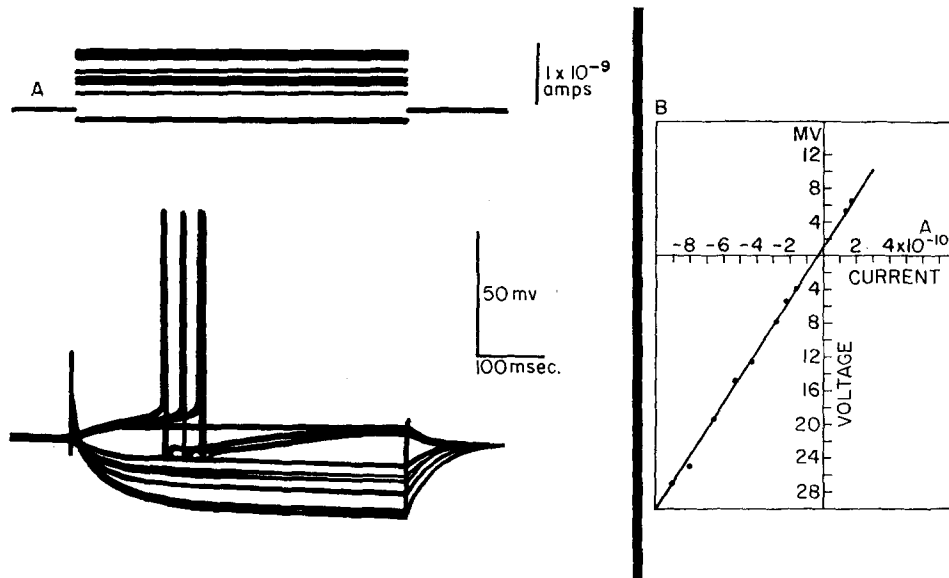


FIGURE 7. Current voltage relationship. Part A, response of a cell to depolarizing and hyperpolarizing currents of varying intensity (lower trace). The voltage imposed across the microelectrode and a current-limiting $10^9 \Omega$ resistor in series with it is indicated in the upper trace. The current injected by the pipette was calculated from the voltage trace ($V_i/10^9$). A downward deflection indicates depolarizing current, an upward deflection, hyperpolarizing current. Part B, graph of current *vs.* voltage based upon the experiment, illustrated in part, in A. R_{tot} for this cell was $3.0 \times 10^7 \Omega$.

functions of stimulus current intensity. The upper graph in Fig. 6B is based upon data obtained from the unit illustrated in Fig. 6A. The closed circles represent the reciprocal interval for the first two spikes and the open circles for the last two spikes of the train. Within the range of stimulus currents employed, onset frequencies rarely exceeded 30 to 40 impulses per second. Steady-state frequencies were always lower and their maximum was about 20 impulses per second.

Direct current pulses were also used to measure the electrical membrane constants. Since it was impossible to determine the relative amounts of current drawn by the somatic and dendritic membranes during strength latency determinations and in response to intrasomatically applied rectangular current pulses, the terms total neuron time constant (τ_{tot}) and resistance (R_{tot}) will be used throughout. This convention derives from Rall (42) and has been employed and discussed in the report of an earlier investigation (48).

Data such as those illustrated in Fig. 6A were used to obtain strength latency curves (bottom graph, Fig. 6B). The total neuron time constant (48) was then calculated from the equation $I_0/I = 1 - e^{-t/\tau_{tot}}$ where I_0 represents

rheobasic current strength, I current, t latency to the first spike, and τ_{tot} the total neuron time constant (14). Time constant for the unit illustrated in Fig. 6 was 75 msec.; average value in six experiments was 42 msec. (Table I).

The change in potential caused by different hyperpolarizing and subthreshold depolarizing current pulses produced a family of responses (Fig. 7A) which were used to plot current voltage curves (Fig. 7B). The slope of this curve is a measure of total neuron resistance. This parameter was measured in six experiments and ranged from 1.1 to $5.0 \times 10^7 \Omega$ with an average value of $3.3 \times 10^7 \Omega$ (Table I).

4. *Indirect Stimulation: Excitatory and Inhibitory Synaptic Potentials*

Stimulation of the olfactory tract produced a long latency depolarizing synaptic potential (Figs. 8A and 9A). This excitatory postsynaptic potential (EPSP) was graded and triggered an action potential when a critical firing level was reached. When suprathreshold stimuli were used they produced a faster rising EPSP and a shorter latency spike (Fig. 9A, top trace). The EPSP is multiphasic and therefore probably polysynaptic. Olfactory tract volleys also produced small positive potential changes which could be recorded extracellularly throughout the nucleus and which were similar in configuration to the larger, intracellular EPSPs. When the recording electrode was close to a neuroendocrine cell an extracellular action potential, evoked by olfactory tract volleys, could be noted to fire near the peak of this positive potential. Parts A and B of Fig. 8 permit a comparison of a series of responses recorded intra- and extracellularly to graded olfactory tract stimuli (see figure legend for further details).

The orthodromic EPSPs were frequently followed by small hyperpolarizing synaptic potentials (Fig. 8A, trace 2) which became more prominent and masked the EPSPs when repetitive olfactory tract volleys were used (Fig. 9B). Under these circumstances orthodromic volleys were capable of producing a sustained hyperpolarization.

Stimuli to the pituitary gland activated the neurohypophysial tract and produced antidromic action potentials in the preoptic nucleus (Fig. 4A, 4B, and 10). Shocks that activated the tract but were subthreshold for the axon of the impaled cell consistently produced hyperpolarizing synaptic potentials. This is illustrated in Fig. 10A where the upper trace is the antidromic spike. At slightly weaker stimulus strength only an inhibitory postsynaptic potential (IPSP) was generated (second trace). Part B₁ of this figure illustrates superimposed fine line tracings of the antidromic spike and of the IPSP to show that the latency of the IPSP is only slightly longer than that of the antidromic spike. Occasionally, the IPSP was followed by a late orthodromic spike, perhaps due to postinhibitory rebound excitation (Fig. 10A, traces 3 and 4 and Fig. 10B₂).

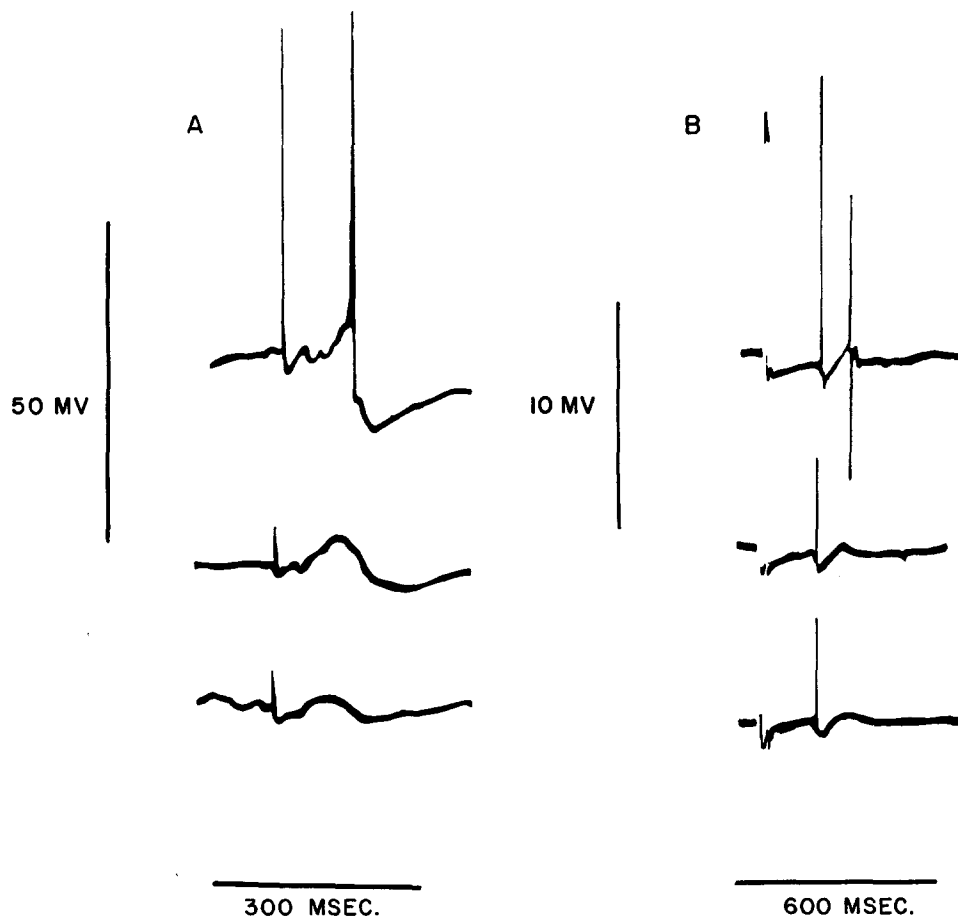


FIGURE 8. Orthodromic responses to single olfactory tract volleys. Series of responses to graded stimuli of increasing intensity (bottom to top). Intracellular recording in A and extracellular recording in B. The polarity convention is the same in both types of record. Note in A that in the intracellular recording the EPSP is followed by a slight hyperpolarization. The first transient in each record in B is a 10 mv and 10 msec. calibration signal which is only fully presented in the top record. The second transient in each record is the shock artifact. The extracellular recorded spike (third transient in top record) is triggered near the peak of the positive wave.

It seemed quite likely that under the conditions of these experiments the stimulus current would be confined to the antidromic pathway. Since the diaphragma sellae probably acts as an insulator it would tend to limit spread of stimulus current to other fiber systems. In addition, the IPSPs were produced by volleys which were subthreshold for the production of the antidromic spike of the impaled cells; since the volleys were too weak to excite all preoptic axons they were likely to be subthreshold for elements lying above the sella turcica.

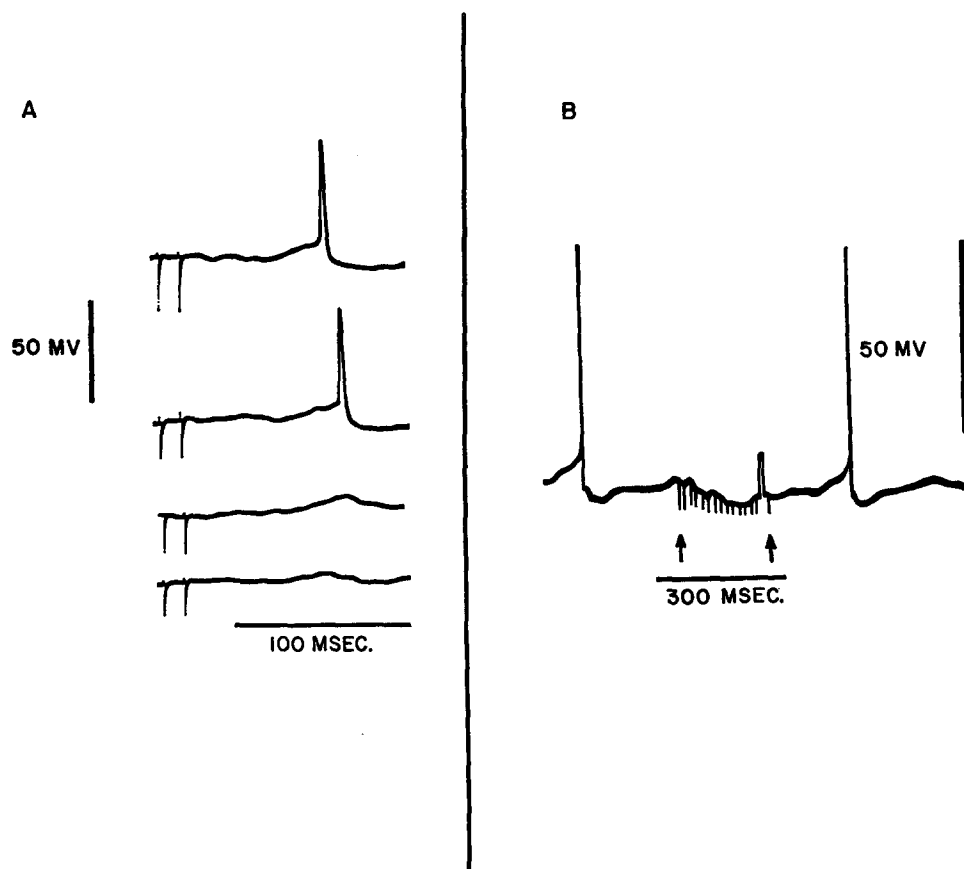


FIGURE 9. Orthodromic responses to repetitive olfactory tract volleys. Part A, double shock of increasing intensity (bottom to top). Part B, train of orthodromic volleys. Calibration signal at end of train is 10 mv and 10 msec.

To test these arguments directly, recordings were obtained from units in the optic chiasm which lies just anterior to the neurohypophysial pathway. The chiasm contains the largest diameter fibers in this region which are presumably also of lowest threshold. In every instance pituitary stimuli which were considerably stronger than those maximal for antidromic activation failed to activate chiasm units although these could be briskly excited or inhibited by diffuse retinal illumination.

Antidromic invasion of neuroendocrine cells and antidromic IPSPs could only be obtained if the exposed tips of the microstimulating electrodes were in the sella turcica or on the aperture of the diaphragma sellae. Other stimulating positions proved ineffective.

An attempt was also made to study the effects of pituitary stimulation in a series of

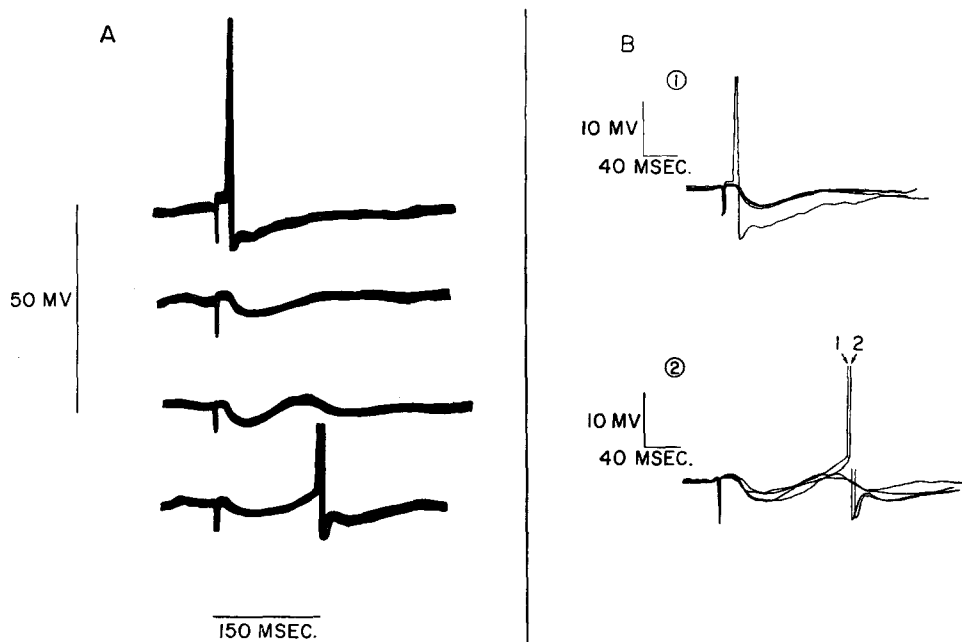


FIGURE 10. Antidromic responses. Part A, series of responses to antidromic volleys of decreasing intensity (top to bottom). Part B₁, superimposed fine line tracings of antidromic spike and IPSP to illustrate that the latency of the IPSP is only slightly longer than that of the spike. Part B₂, superimposed fine line tracings to illustrate late orthodromic action potentials presumably triggered by rebound excitation.

animals in which the stalk had been previously sectioned surgically. However, in the large animals necessary for microelectrode work, this surgical procedure was quite difficult and the mortality high. The few animals who survived were in poor condition and it was not possible to obtain satisfactory intracellular recordings from them.

Further properties of the IPSP are illustrated in Figs. 11 and 12. The IPSP proved to be sensitive to polarizing current (Fig. 11A); the potential change was increased by depolarizing current pulses and decreased and abolished by hyperpolarizing current pulses. The current-carrying capacity of the pipettes used in this study was usually quite limited and this prevented a systematic exploration of the equilibrium potential of the IPSP.

The IPSP was graded; repetitive volleys produced a membrane hyperpolarization which lasted for the duration of the stimulus train (Fig. 11B). This hyperpolarization also produced a sustained inhibition of spontaneous or evoked firing. This is illustrated in Fig. 12. The upper part of this figure shows the response of a cell to a constant current pulse of 6.6×10^{-10} amps. The lower part illustrates the interruption of this directly initiated repetitive firing by the generation of a train of pituitary stimuli. The onset and termination of this train are indicated by the arrows.

5. Physiological Stimulation with Dilute Sea Water

As described in the Methods section, sea water was introduced into the tubing that bathed the gills. By appropriate adjustment of the sea water flow rate, it was possible to use a wide range of dilutions. The dilution ultimately employed was determined by selecting the weakest sea water stimulus (0.18 to 0.35 per cent) that would produce effects localized to the preoptic nucleus. Stronger stimuli tended to produce slow, positive extracellular potential changes in surrounding areas of the brain.

Dilute sea water was injected intermittently, for periods generally ranging from 10 to 60 seconds, during the course of extra- or intracellular recordings from neuroendocrine cells. Frequently, single injections had little or no effect, but multiple injections always produced a significant slowing of spontaneous

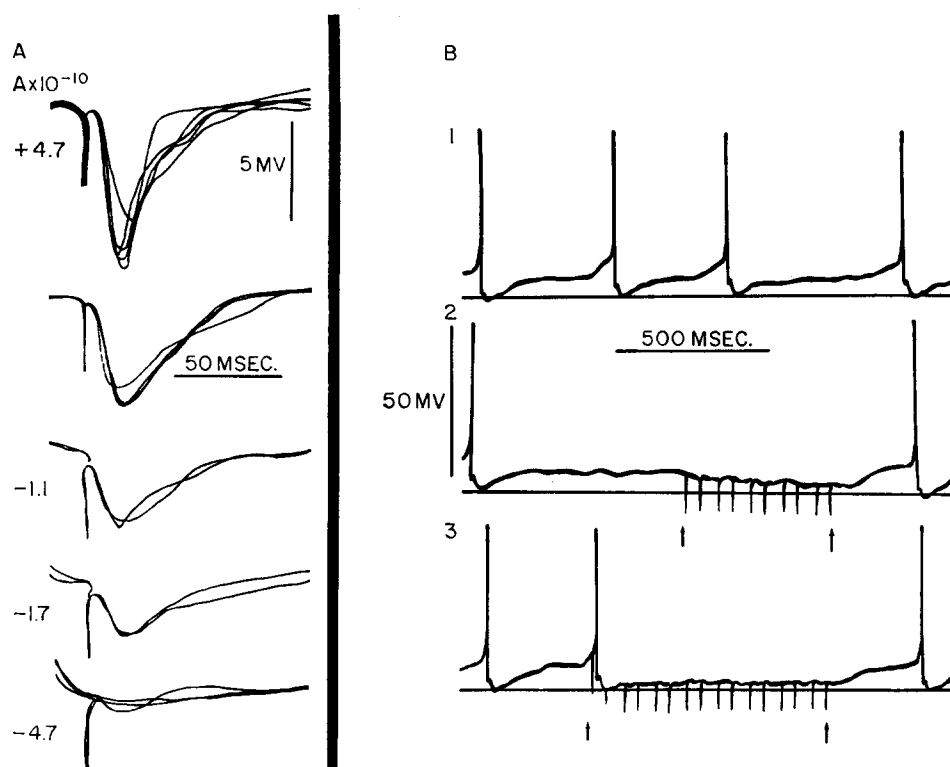


FIGURE 11. Properties of the antidromic IPSP. Part A, superimposed fine line tracings to indicate changes in IPSP amplitude with depolarizing (+) and hyperpolarizing (-) currents. Part B₂, graded and sustaining properties of IPSP produced by a train of pituitary stimuli (B₂ and B₃). Spontaneous firing of this unit, in absence of pituitary volleys, is illustrated in B₁. Onset and termination of the train are indicated by the arrows. The second beam of the oscilloscope has been used as a reference line to permit measurement of changes in membrane polarization.

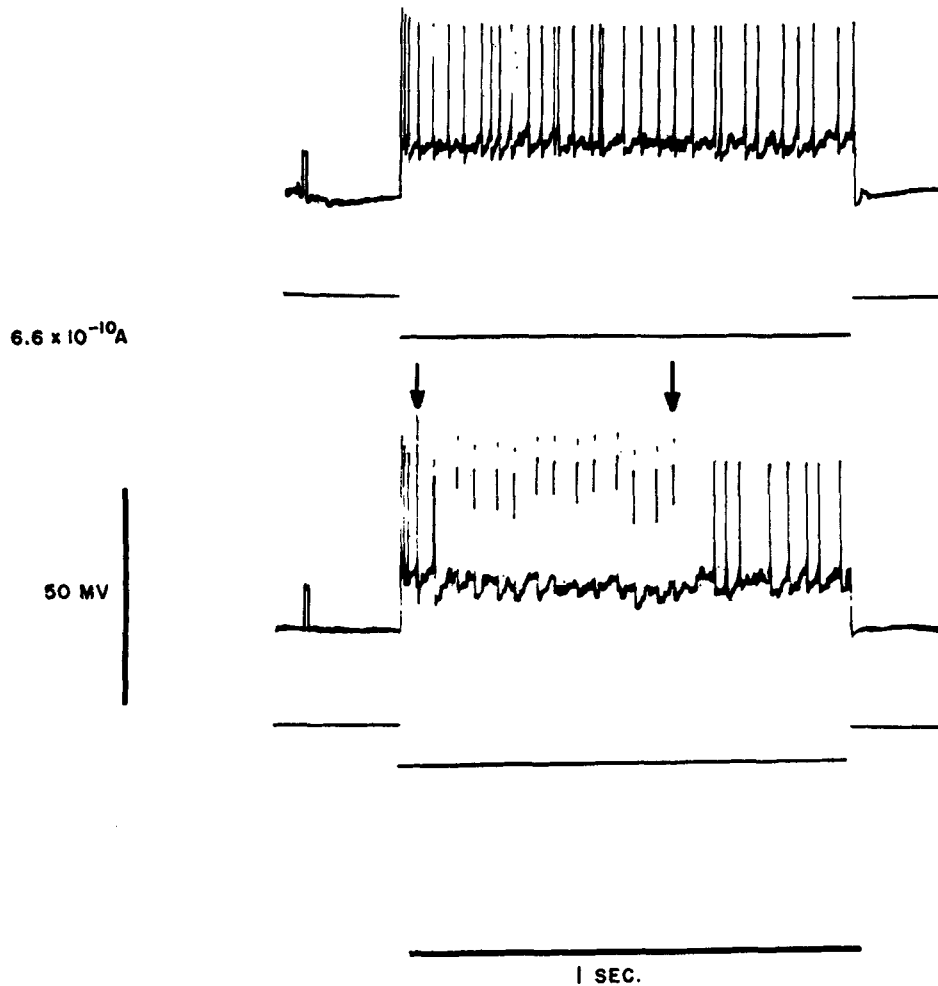


FIGURE 12. Inhibitory action of pituitary volleys. Interruption of repetitive firing in response to a prolonged direct stimulus by a train of pituitary volleys whose duration is indicated by the arrows (second trace). Directly initiated train in the absence of pituitary volleys is indicated in the upper record.

activity. Fig. 13 summarizes data from two different experiments; part A is from an extracellular recording and parts B₁ and B₂ from an intracellular one.

The unit in Fig. 13A showed considerable sensitivity to a first brief (11 seconds) injection of dilute sea water. The constant voltage calibration signal on the micropipette trace was triggered at a rate of 1 per second. The duration of the sea water stimulus is indicated by the double beam on the second trace. After a latency of about 7 seconds there was a slight slowing of unit activity

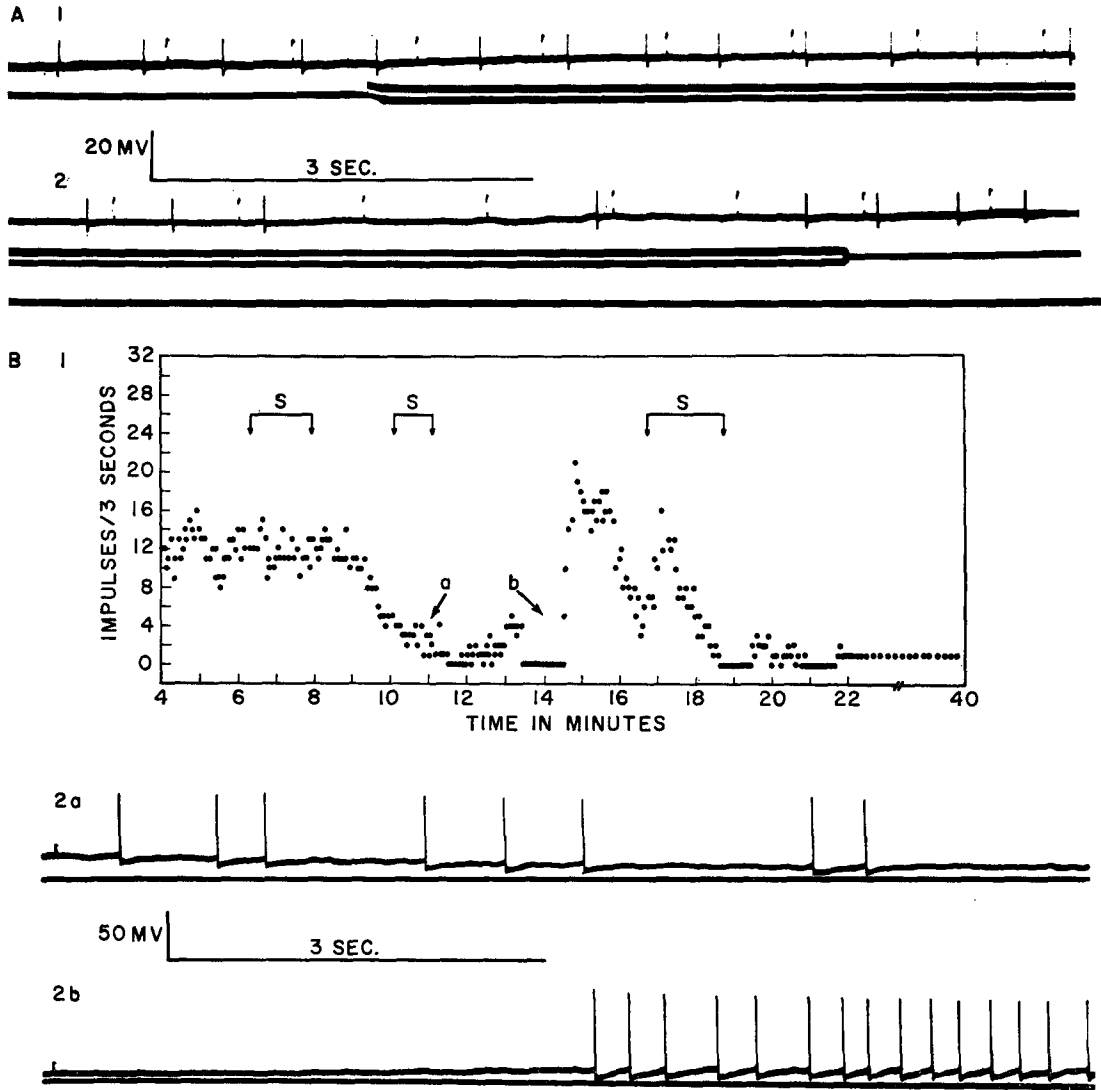


FIGURE 13. Response to weak solutions of sea water. Part A, extracellular recording. A_1 and A_2 are a continuous record. The calibration pips recorded by the microelectrode are 10 mv and 10 msec. and occur every second. The duration of the stimulus is indicated by the double beam in the lower trace. Part B, similar experiment as in part A but with intracellular recording and multiple injections. B_1 , graph of frequency (impulses/3 sec.) vs. time. The three injections indicated (s) represent stimuli Nos. 3, 4, and 5. There was no response to stimuli 1 and 2 and these are not included in the graph. All stimuli were of the same concentration (0.3 per cent sea water). B_{2a} and B_{2b} are the intracellular records during the periods indicated by arrows a and b in the graph B_1 . These two periods represent the maximum rates of change in firing frequency in response to the 4th stimulus (2nd s on the graph).

and, after the cessation of the stimulus, there was a slight increase in firing frequencies.

Similar effects are illustrated more strikingly in Fig. 13B₁ which is a graph of data from a unit's response to multiple injections. During the period of time from zero to 4 minutes, not indicated on the graph, two injections of 0.3 per cent sea water had produced no significant change in over-all firing frequency. The responses to injections three, four, and five are illustrated on this graph. The third injection produced a significant slowing; after this slowing had clearly begun, another injection (number four) was given. The cell's firing continued to decrease and for two periods (11.5 to 12 minutes and 13.5 to 14.5 minutes) spontaneous firing completely stopped. Following the second cessation of spontaneous firing, unit activity rose transiently to 23 impulses per 3 second period, a frequency almost twice that of the control period. Following this rebound there were some oscillations in firing frequency and a fifth injection, made during this period of oscillations, produced another decrease in spontaneous firing and two periods of silence (18.5 to 19.5 minutes and 20.8 to 21.8 minutes). Following the silent period, unit activity returned but to a new and lower level (1 impulse per 3 seconds) for the remainder of the impalement (20 minutes).

The cessation of spontaneous firing was associated with a hyperpolarization and the resumption of spontaneous firing with a return of the membrane potential to its earlier level. Figs. 13B_{2a} and B_{2b} show the membrane potential changes and alterations in firing pattern that correlate with the times indicated as a and b on the graph (Fig. 13B₁). The lower reference line has been drawn to facilitate the measurements of membrane potential in 2a and 2b.

At the onset of the stimulus, there was in this unit and in several others a slow positive shift of about 5 to 10 mv that could also be recorded, with equal magnitude, extracellularly. This shift was not associated with a change in unit firing and the elements responsible for its generation are still obscure. However, it clearly did not represent a transmembrane change. By contrast, at least a major portion of the hyperpolarization represented a transmembrane potential change, although the exact magnitude of this shift could not be accurately specified because of the occurrence of smaller and inconstant extracellular negative shifts.

Despite many attempts there have been few successful experiments with multiple injections and long periods of intracellular recordings, and the data are fragmentary and should be considered preliminary. The data also fail to specify the nature of the extracellular potential changes and whether the change in firing pattern is a result of a primary effect of the sea water stimulus on the neuroendocrine cell or on a population of elements that drive these cells. Finally no attempt has yet been made to differentiate osmotic from ionic effects.

DISCUSSION

The results obtained from this study of a vertebrate neuroendocrine cell permit comparison with data available from other central neurons and from three types of salivary gland cells. The gland cells do not generate an action potential, have a resting potential that is either lower (20 mv) or higher (80 mv) than that of the neuroendocrine cell, and give rise to PSPs which are relatively insensitive to polarizing current (28). In maintaining a resting potential of 50 mv, developing a full sized action potential, and generating an IPSP which has a specific equilibrium potential, the neuroendocrine cell differs significantly from salivary gland cells and is similar to other central neurons. These data therefore extend the findings of earlier studies based on tract (3, 9, 41) and extracellular recordings (8, 11) all of which have tended to confirm Scharrer's original suggestion that these hypothalamic cells have a completely dual function. As glandular cells they are capable of producing, storing, and releasing hormones, while as nerve cells they are also capable of receiving, transforming, conveying, and probably also of transmitting neural information.

The specific responses of hypothalamic neuroendocrine cells to several different types of stimulation will now be considered in turn.

1. Active Membrane Areas and Electrical Membrane Constants

Consistent with their central neuronal electrical properties, the action potential of neuroendocrine cells can, under certain conditions, occur in two steps demonstrating a heterogeneity of spike-generating areas within the cell. The finding that the critical firing level for orthodromic as well as for direct stimulation is that which is threshold for the A (3 to 15 mv) rather than for the B area (15 to 40 mv) indicates that the A area is a low threshold trigger zone for all modes of activation. Since, however, an AB inflection cannot be demonstrated in single orthodromic or spontaneous action potentials, it must be concluded that under most conditions of activation, particularly those that most resemble naturally occurring conditions, AB coupling is tight and the two areas fire in synchrony.

The slow sustained firing with relatively limited accommodation typical of neuroendocrine cells in response to constant currents is similar to the response patterns of spinal motoneurons and *Limulus* eccentric cells, both of which also have prominent hyperpolarizing afterpotentials (17). This pattern is in clear contrast to that of hippocampal neurons which have a depolarizing afterpotential and generate high frequency self-limiting bursts (25).

The data on electrical membrane constants reveal an average total neuron time constant of 42 msec. and a total neuron (input) resistance of 3.3×10^7 ohms. Taking 25μ as an average diameter of the neuroendocrine cell, the specific membrane resistance can be estimated to be about $600 \Omega\text{cm}^2$ and

the specific capacity $70 \mu\text{f}/\text{cm}^2$. These calculations are based on the simplifying assumption that the neuron is spherical and lacks dendritic extension. Although the processes are short, these neurons do have dendrites (38). Therefore the estimated specific resistance and capacitance should only be considered as rough approximations (42). These values are, however, similar to those obtained for a non-secretory teleost ganglion cell, the supramedullary neuron of the puffer (4).

2. *Synaptic Potentials to Orthodromic and Antidromic Stimulation*

The data on synaptic input confirm the previous and less direct findings that neuroendocrine cells are under reflex control of other central nervous system structures. The efficacy of the olfactory input is evident from the anatomical data which show that olfactory tract afferents, particularly the nervus terminalis, constitute the major inflow to the teleost preoptic nucleus (46). This relatively direct influence is of further interest since in higher vertebrates osmotic stimuli which trigger the release of posterior pituitary hormone produce simultaneous EEG alterations both in the olfactory bulb and in the anterior hypothalamus (50). Diffuse light was also used to test the efficacy of visual input but proved ineffective.

Of particular interest to the current concept of the neuroendocrine cell are the data on antidromic inhibition. Antidromic inhibition, which constitutes electrophysiological evidence for inhibitory recurrent collaterals, has been noted in several systems: motoneurons (12), hippocampal pyramidal cells (49), neocortical Betz cells (39), olfactory bulb mitral cells (20), and Mauthner's cells (18). In the present experiment inhibition following pituitary stimulation could perhaps represent stimulus escape beyond the confines of the antidromic pathway causing activation of an intrahypothalamic pathway. The finding that the IPSP could be produced with stimuli which were below threshold for some of the neurohypophyseal tract fibers, as well as the results of the control experiments which were performed to evaluate the limitation of stimulus current spread, makes the alternative of an intrahypothalamic pathway unlikely. The results are therefore consistent with a recurrent collateral system.

The probable existence of recurrent collaterals in preoptic neurons suggests that the current definition of neuroendocrine cells (6, 7, 10) as neurons whose axons end only on blood vessels and not on other neurons or effector cells may be too restrictive. It appears that preoptic neuroendocrine cells do form synaptic junctions with nerve cells. The more restricted definition may still hold for the neurosecretory cells of the caudal neurosecretory system which is found in the tail of most fishes. These caudal neurosecretory cells have recently been studied with intracellular electrodes by Morita *et al.* (32) and by Bennett and Fox (5) and have been shown to generate spike and excitatory synaptic

activity. These cells do not possess recurrent collateral axons as tested with antidromic volleys.

Attempts to demonstrate these collaterals histologically must await the use of silver stain since the Gomori stain is not taken up with sufficient intensity in the goldfish to outline the whole neuron (Roth and Kandel, unpublished observations). However, the stain is taken up quite intensely by the preoptic nucleus of *Fundulus heteroclitus* and, in this fish, Sokol has demonstrated the existence of abundant recurrent collaterals (47). In Sokol's study it was, however, impossible to specify whether or not these collaterals ended on other neuroendocrine cells.

The existence of neuroendocrine axons that do not go to the pituitary but go to the third ventricle or to other nuclear groups has also been demonstrated by the anatomical work of the Legaits (27). Finally, degeneration studies in mammals have also suggested the existence of collaterals between the supra-optic and paraventricular neuroendocrine nuclei (33).

The relatively brief latency of the IPSP and the absence of other cell types in the magnocellular portion of the preoptic nucleus suggest the possibility that the IPSP may not involve an intercalated neuron in the recurrent pathway. Since the IPSP is sensitive to polarizing currents and has an equilibrium potential, it has the characteristics attributable to a chemical inhibitory synapse. This, therefore, introduces the possibilities that (a) the hormone may serve as an inhibitory transmitter at certain junctional sites or that (b) neuroendocrine axons may be capable of releasing transmitter substance in addition to neurohormones from their terminals. A dual secretory role is suggested by the existence of "synaptic vesicles" as well as neurosecretory profiles in the electron microscopic studies of the neuroendocrine axon terminals which end on blood vessels in the posterior pituitary (13, 19, 37).

3. *Response to Physiological Solutions of Dilute Sea Water*

The preliminary data on the response of neuroendocrine cells to perfusion with dilute sea water solutions which are within the physiological range of this species, show a membrane hyperpolarization and inhibition of spontaneous spike activity.

In the goldfish, teleost posterior pituitary hormones have been shown to promote active sodium influx across the gill membrane (29–31). Physiological manipulations that increase extracellular sodium cause a decrease of Na^+ uptake by the gills presumably by decreased release of posterior pituitary hormone while procedures that decrease extracellular sodium promote increased Na^+ uptake by the gills presumably by augmented release of hormone (29). The finding of sustained neuroendocrine cell inhibition and the absence of spike generation in response to a three-to sixfold increase of Na^+ concentration of the gill perfusate suggests that inhibition of posterior pituitary hor-

hormone may be associated with, if not caused by, inhibition of neuroendocrine cell electrical activity. Further experiments are clearly necessary to specify directly the relationship among the sea water stimulus, the electrical response of the neuroendocrine cell, and its release of hormone.

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