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Research Notes

Phasic Discharge in Supraoptic Neurones Recorded from Hypothalamic Slices

E. W. Haller[1], M. J. Brimble[2] and J. B. Wakerley

A. R. C. Institute of Animal Physiology, Babraham, Cambridge CB2 4AT, England

An intriguing feature of recordings from the supraoptic (SO) nucleus in vivo is that many of the neurones exhibit a phasic firing pattern when excited by appropriate osmotic (Walters and Hatton, 1974; Arnauld et al., 1975; Brimble and Dyball, 1977; Wakerley et al., 1978) or hypovolaemic (Poulain et al., 1977) stimulation. Phasic firing, which seems to be associated with vasopressin release (Dreifuss et al., 1976; Poulain et al., 1977) is characterized by high frequency (6–12 Hz) trains of spikes, lasting 5–120 sec and alternating with periods of electrical silence of 4–80 sec duration. At present it is not known whether the pacemaker mechanism for timing the intermittent bursts during phasic firing lies in the region of the SO nucleus, or distant from it. To resolve this question, we have investigated the occurrence of phasic firing in SO neurones recorded from thin (300 μ) hypothalamic slices in vitro. In this preparation, it is possible to activate the neurones in the SO nucleus by direct chemical stimulation, and to study their firing characteristics in the absence of connections from other brain areas.

Brains were removed from decapitated male rats and a tissue block containing the hypothalamus was prepared. This tissue block was then sectioned at 300 μ in a coronal plane, using an 'Oxford' vibratome. Throughout sectioning, the tissue remained immersed in standard incubation medium, kept at 37° C. Hypothalamic slices containing parts of the SO nucleus were immediately transferred to an incubation chamber. Their upper surfaces were exposed to a humidified gas mixture (95% O_2–5% CO_2) while their undersurfaces were supported on a nylon grid and perifused with Yamamoto's solution (pH 7.3–7.5) (Yamamoto, 1972) at a flow rate of 1.4–1.6 ml/min. The osmotic pressure of the medium was in the range 296-300 mOsm/kg, determined using

[1] Permanent address: School of Medicine, University of Minnesota, Duluth, Minnesota, 55812, U.S.A.
[2] Present address: Dept. of Physiology, University of Manchester, Manchester M13 9PL, England
Offprint requests to: J. B. Wakerley (address see above)

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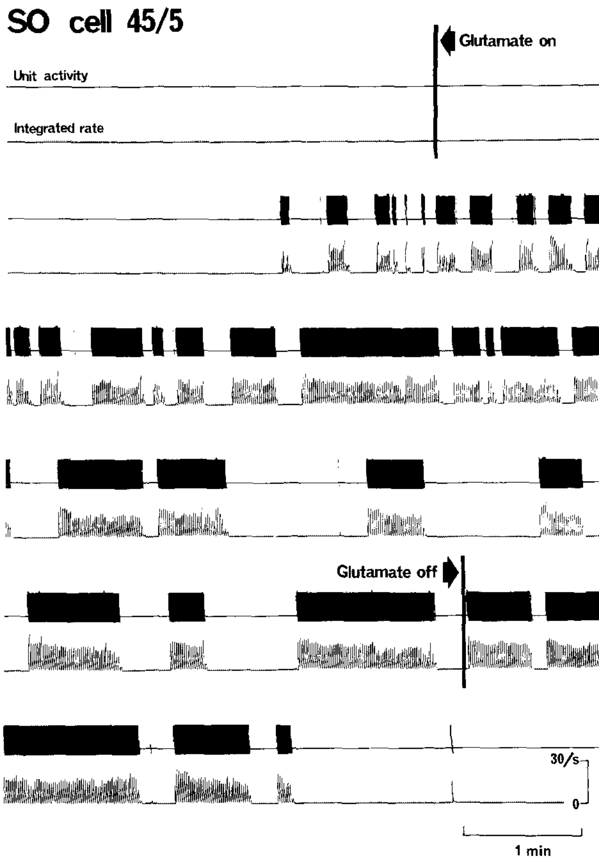

Fig. 1. Continuous polygraph record of unit activity (upper trace) and integrated firing rate (lower trace) showing phasic activity in a SO neurone in vitro during perifusion with medium containing glutamate (5×10^{-3} M)

the freezing point method (Knauer-Semimicro-Osmometer). The dead space in the perifusion system was approximately 4.5 ml, so that it took 3–4 min for a complete exchange of the medium within the incubation chamber. The temperature in the chamber was thermostatically controlled at 37° ± 1° C. A 2 hr incubation period for stabilization of the tissue was allowed before recording was begun. Extracellular potentials were recorded with 4M NaCl-filled glass micropipettes (tip 1–2 μ; resistance 10–20 MΩ) connected to conventional recording apparatus. The electrodes were placed in the SO nucleus under direct visual control. Supraoptic units were activated by perifusing the slices with medium containing L-glutamic acid at a concentration of 2.5 or 5.0 mM. Each unit was recorded in glutamate-free medium for 5 min and then tested with glutamate-containing incubation medium for 15–20 min, followed by a 10 min washout period.

Complete tests were performed on 15 SO neurones from 7 hypothalamic slices. Most of these cells were quiescent (spontaneous activity <0.01 Hz).

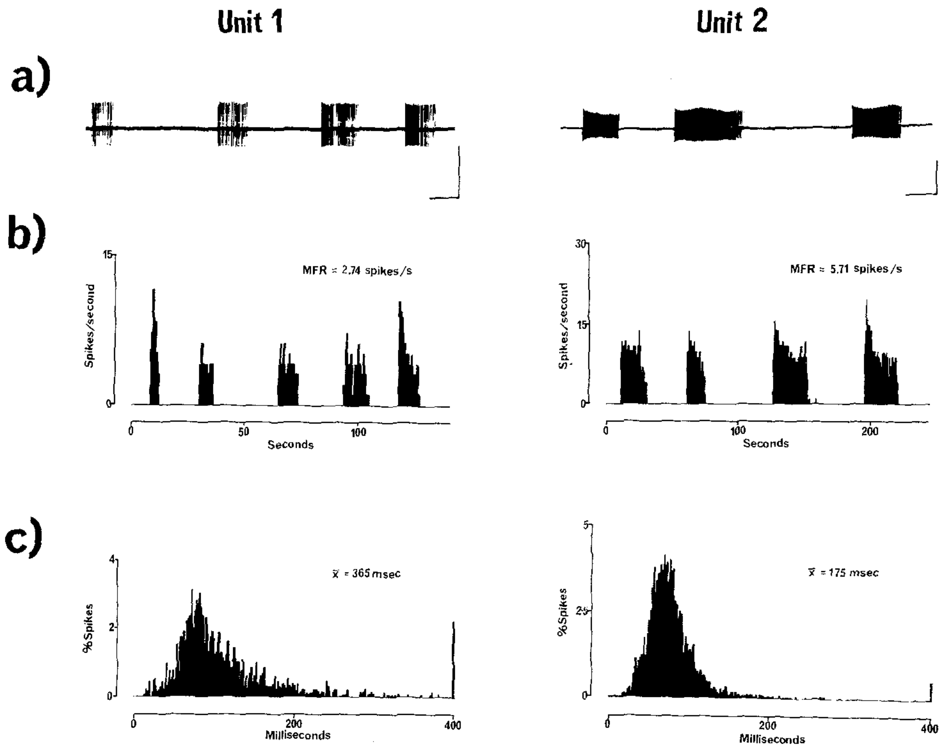

Fig. 2. Analysis of the phasic firing activity of two (unit 1 and unit 2) SO cells during activation with glutamate (5×10^{-3} M): **a** Photographs of oscilloscope traces of spike trains showing the phasic pattern of activity (calibrations: horizontal 9 sec, vertical 0.2 mV). **b** Sequential histograms of firing rates of the same cells during the glutamate response, the mean firing rate (MFR) over the test period of each cell is indicated. **c** Interspike interval histograms of the same cells during the glutamate-induced response, the mean interval ($\bar{X}$) is indicated

When tested with glutamate, 13 of the cells showed sustained activation and over the test period their average firing rate was 8.4 Hz. As defined by the simple criterion of bimodality of firing rates (Poulain et al., 1977), phasic activity was observed in 5 of the SO cells during glutamate stimulation. Within 4 min of the onset of the glutamate perifusion, electrical activity was initiated with the appearance of phasic bursts of firing (Fig. 1). For individual cells, the mean duration of the bursts during the response ranged from 14.3 ± 6.1 sec (mean ± S.E.) to 73.6 ± 9.4 sec, with intervening silent periods ranging from 8.0 ± 1.1 sec to 59.2 ± 13.2 sec. The intermittent bursts had a sudden onset and often followed a characteristic pattern, with a higher firing rate during the first 2 sec than during the remainder of the burst (Fig. 2a, b). The mean firing rate within the bursts ranged from 7.3 ± 0.7 Hz to 14.1 ± 1.1 Hz. Thus a high proportion of spikes in these phasic cells occurred with intervals of under 100 msec (Fig. 2c). With the end of the glutamate perifusion, the phasic cells reverted to electrical quiescence within 4 min, a lag time comparable to that of the cell's initial activation (Fig. 1). A further 5 of the SO units had a normal distribution of firing

rates throughout their glutamate response and were classified as continuous cells (Poulain et al., 1977). The remaining 3 cells fired in an ambiguous pattern which could neither be classified as continuous nor phasic.

While glutamate is not known to be a specific synaptic transmitter in the hypothalamo-neurohypophysial system, it served in these experiments as a reliable excitant of hypothalamic neurosecretory cells. When activated with glutamate in vitro, the SO cells exhibited phasic spike trains remarkably similar to those previously recorded in the intact brain. Thus the durations of the bursts and silences, the rate and pattern of firing within the bursts, and the interspike interval histograms all resembled those of activated SO neurones in vivo (Arnauld et al., 1975; Brimble and Dyball, 1977; Wakerley et al., 1978). Since our hypothalamic slices were prepared by coronal section at a thickness of 300 μ, it is likely that only those afferents to the SO nucleus which run exactly in the same coronal plane would escape section. In fact, few of the transverse fiber systems within the hypothalamus are oriented in such a precise plane; most move partly caudally or ventrally as they course across the hypothalamus (Kreig, 1932). Longitudinally oriented fibers, such as the medial forebrain bundle which forms a major component of the SO afferents (Zaborsky et al., 1975), would be completely transected in our preparation. The current observations therefore suggest that within, or very close to the SO nucleus, there exists a pacemaker mechanism capable of generating phasic firing patterns in response to continuous chemical stimulation.

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