Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9630–9634, October 1993
Physiology

Single unit components of the hypothalamic multiunit electrical activity associated with the central signal generator that directs the pulsatile secretion of gonadotropic hormones

(gonadotropin-releasing hormone pulse generator/rhesus monkey/single unit activity/synchronization)

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Contributed by Ernst Knobil, July 26, 1993

ABSTRACT **Vertebrate reproduction is dependent on the operation of a central signal generator that directs the episodic release of gonadotropin-releasing hormone, a neuropeptide that stimulates secretion of the pituitary gonadotropic hormones and, thereby, controls gonadal function. The electrophysiological correlates of this pulse generator are characterized by abrupt increases in hypothalamic multiunit electrical activity (MUA volleys) invariably associated with the initiation of secretory episodes of luteinizing hormone. Using cluster analysis, we extracted single units from the multiunit signals recorded from the mediobasal hypothalamus of four intact and four ovariectomized rhesus monkeys. Of the 40 individual units identified in this manner, 24 increased their frequency with the MUA volleys. The onset and termination of these single-unit bursts occurred coincidently with those of the MUA volleys in both intact and ovariectomized animals, indicating that the longer duration of the MUA volleys characteristic of the gonadectomized animals was due not to the sequential activation of different units but to the longer bursts of the individual cells. Four other units showed decreases in firing rate during the MUA volleys, while the frequency of the remainder did not change. All the examined units were active during the intervals between the volleys of electrical activity. The results indicate that the MUA volleys associated with the activity of the gonadotropin-releasing hormone pulse generator represent the simultaneous increase in firing rate of some individual hypothalamic neurons and the decrease in the frequency of others.**

The rhythmic, pulsatile secretion of the pituitary gonadotropic hormones, described in every vertebrate studied in this regard, is governed by a hypothalamic "clock" generally referred to as the gonadotropin-releasing hormone (GnRH) pulse generator. The operation of this signal generator at an appropriate frequency for the species is an absolute requirement for normal gonadal function (see ref. 1 for review).

In the rhesus monkey, this central signal generator fires approximately once an hour and has been localized in the region of the arcuate nucleus (2), but its cellular basis remains in doubt (3). Abrupt increases in multiunit activity (MUA volleys) that are invariably synchronous with the initiation of luteinizing hormone pulses measured in peripheral blood (Fig. 1) have been recorded from this area in the rhesus monkey (4), rat (5), and goat (6). The unitary association between luteinizing hormone pulses and these electrical signals has been observed in a variety of experimental circumstances (4, 7–11) as well as during the normal menstrual cycle (12) and has permitted the conclusion that these MUA volleys are the electrophysiological manifestations of GnRH pulse generator activity.

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The purpose of the present study was to describe the single unit components of the multiunit electrical activity underlying the operation of the GnRH pulse generator. Because the search for single neurons that are activated but once an hour is grossly impractical in a whole animal preparation, we have used the technique of cluster analysis for extraction of single unit discharges from multiunit recordings.

MATERIALS AND METHODS

Four long-term ovariectomized (18–45 months) and four gonadally intact female rhesus monkeys (*Macaca mulatta*; 7–9 kg), the latter with histories of normal ovulatory menstrual cycles, were used. The animals were housed individually in a temperature- and light-controlled room (0700–1900 light phase) and were fed a daily ration of Purina monkey chow, supplemented with fresh fruit three times weekly. Water was available *ad libitum*. The monkeys were fitted with indwelling cardiac catheters and with bilateral chronic electrode arrays, each consisting of nine 50-μm insulated Nichrome wires (California Fine Wire Company, Grover City, CA) implanted stereotaxically in the mediobasal hypothalamus 2–51 months before the experiments as described (4, 13). The electrodes were screened for the characteristic increases in multiunit electrical activity associated with the initiation of each pulse of luteinizing hormone as measured in peripheral blood.

During experiments, the animals were restrained in primate chairs to which they had been extensively habituated. Recordings from gonadally intact monkeys were performed during the follicular phase of the menstrual cycle when the frequency of the MUA volleys is maximal (12). Multiunit signals were recorded from a total of 26 electrodes during 20 recording sessions. Eight of those were performed in intact and six were performed in ovariectomized monkeys. In addition, simultaneous recordings were performed from two different electrodes implanted on the same (four sessions) or on opposite (two sessions) sides of the hypothalamus in ovariectomized monkeys. Only one recording was made from any one electrode. Spikes were acquired during one or two MUA volleys as identified by on-line histogram analysis (see below).

The electrodes were connected through high-impedance probes to Grass P511 amplifiers (Grass Instruments, Quincy, MA) to provide signal amplification (×50,000) and filtering

Abbreviations: GnRH, gonadotropin-releasing hormone; MUA, multiunit activity.

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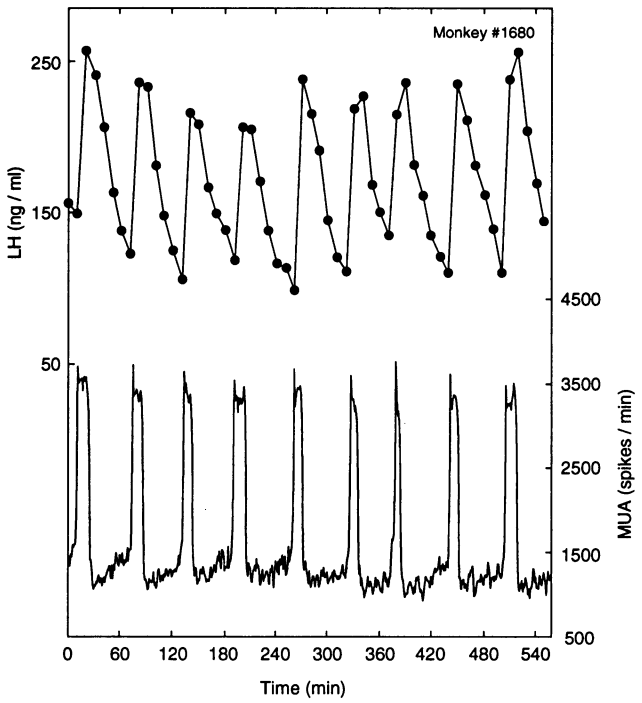

FIG. 1. Hypothalamic MUA volleys and luteinizing hormone (LH) pulses, as measured in the peripheral circulation of an ovariectomized monkey.

(300- to 1000-Hz bandpass). The analog output was split and analyzed with the BrainWave Discovery V2.2 unit recording software (BrainWave Systems, Thornton, CO) implemented in a 20-MHz IBM-compatible personal computer and by Dataquest III (Data Sciences, St. Paul, MN) on an IBM XT computer following amplitude discrimination (WPI model 121; W-P Instruments, New Haven, CT) to obtain on-line activity histograms (13). Spikes were digitized at a sampling rate of 32 or 16 kHz and extracted from the continuous signal when their positive phase exceeded an arbitrary threshold. As this program uses 32 samples to describe the extracted waveforms, each spike represented a 1- or 2-ms section of the continuous signal. The sorting of individual single unit discharges was performed off-line. In the present study, two to eight of the following parameters were used to discriminate and identify the waveforms: peak (maximum excursion of the waveform), peak time (time at which peak occurred), valley (minimum excursion of the waveform), valley time (time at which valley occurred), spike height (peak voltage − valley voltage), spike width (valley time − peak time), as well as the first and second principal components (cf. refs. 14–16 for principal component analysis). The validity of these spike parameters in identifying different waveforms in multiunit recordings has been discussed *in extenso* (16–19). The waveform parameters of up to 4096 spikes are then displayed in pairs using an x/y point plot format. Parameters corresponding to waveforms that share similar features form clusters of points on the display. The boundaries of the different clusters are selected by the user ("cluster cutting"; cf. ref. 16 for more details of cluster analysis); in the present study, cluster analysis was performed on a portion of a signal that had been obtained during a MUA volley. The program then assigns a unique, color-coded identification number to each cluster. The output of the cluster cutting program is processed to display waveforms that correspond to particular clusters as illustrated in Fig. 2*A*, and the spike trains for each of these waveforms (Fig. 2*B*), as well as time-based histograms (bin size, 30 s). The latter were created using the SAS/STAT release 6.03 (SAS Institute, Cary, NC) statistical software.

RESULTS

A total of 40 waveforms were separated from multiunit signals recorded in the mediobasal hypothalamus. Fourteen of these waveforms were identified in intact and 26 were identified in ovariectomized animals.

Sixteen of the 26 units identified in ovariectomized and 8 of the 14 units found in intact rhesus monkeys had bursts with onsets and terminations invariably coincident with those of the MUA volleys, indicating that the longer duration of the MUA volleys characteristic of the gonadectomized animals [10–25 min, in contrast to the 1- to 3-min duration that can be found in intact monkeys (20)] was due not to the sequential activation of different units but to the longer bursts of the individual cells contributing to the MUA volleys (Fig. 3, units 1L, 2L, and 4R; Fig. 4, unit 1; Fig. 5, units 1 and 2). Thus, when more than one single unit of this type could be identified in the same recording obtained from one (Fig. 5, units 1 and 2) or two electrodes at a time—regardless of whether they were implanted ipsi- or contralaterally (Fig. 3, units 1L, 2L, and 4R)—the volleys of the individual units were also coin-

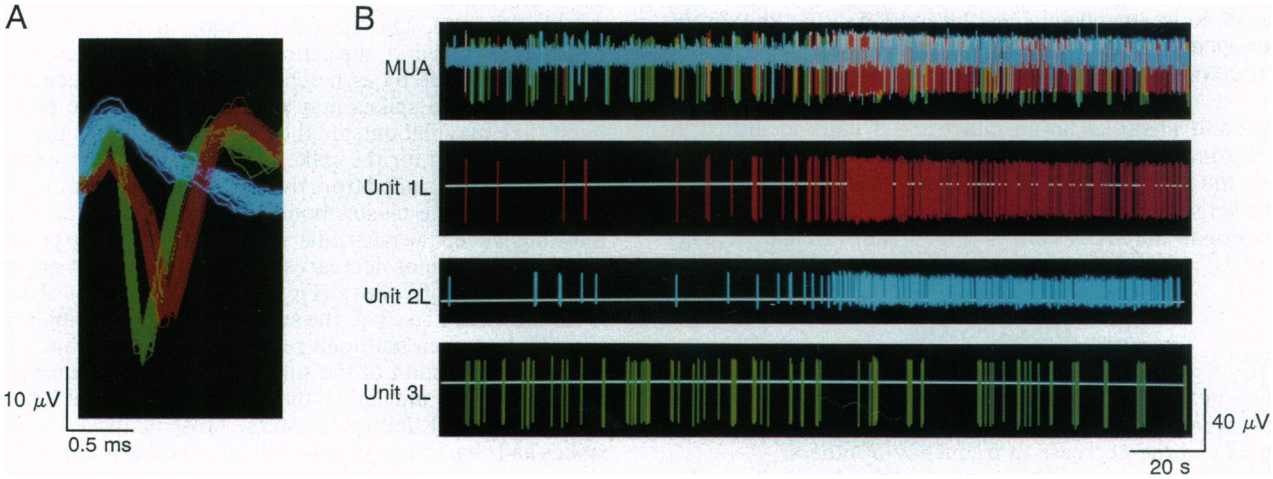

FIG. 2. Three different waveforms extracted by cluster analysis from one multiunit recording from the mediobasal hypothalamus of an ovariectomized rhesus monkey. (*A*) Each waveform is composed of ≈100 spikes aligned by their first positive excursion. (*B*) A 200-s portion of the multiunit spike train acquired during the onset of a MUA volley (top trace). Bottom three traces represent discharges of the individual units shown in *A*. Their activity histograms are shown in Fig. 3 *Left*.

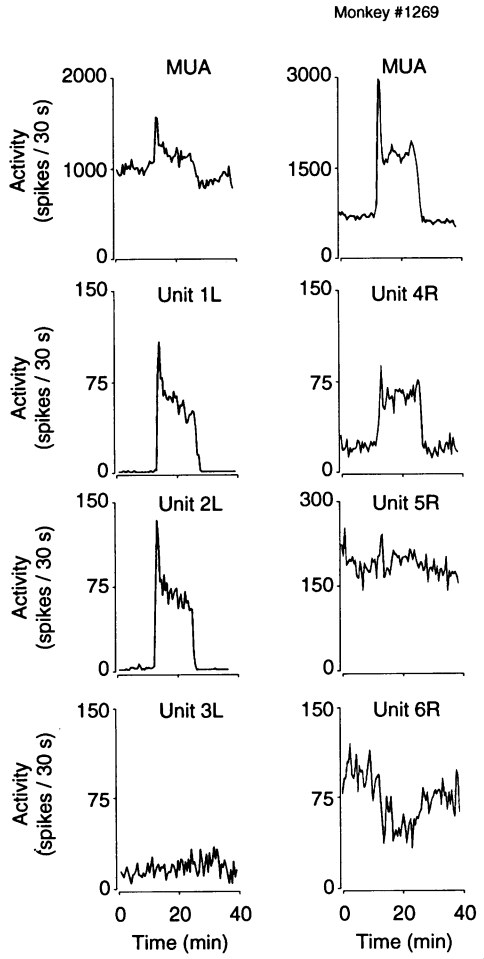

FIG. 3. Activity histograms of multiunit spike trains (MUA) recorded simultaneously from two electrodes implanted bilaterally (*Left*: L, left side) (*Right*: R, right side) in the mediobasal hypothalamus of an ovariectomized rhesus monkey. Bottom three pairs of histograms show activity of the individual units extracted from the corresponding MUA. (The waveforms of units 1L–3L as well as their spike trains are shown in Fig. 2.) Units 1L, 2L, and 4R increased their frequency coincidently with the MUA volleys, while unit 6R showed a simultaneous decrease in firing rate. The activity of units 3L and 5R remained unchanged during the volley.

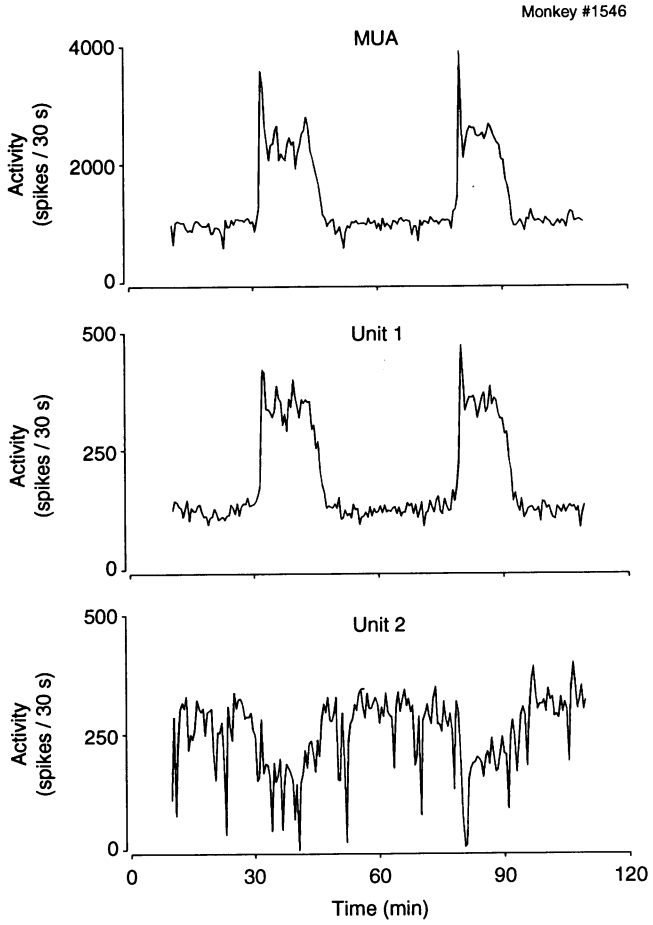

FIG. 4. Time-based activity histogram of a multiunit spike train (MUA) and of 2 component units recorded from one electrode in the mediobasal hypothalamus of an ovariectomized rhesus monkey. Unit 1 increased its frequency coincidently with the MUA volleys; unit 2 exhibited a decreased firing rate during the volleys.

cident. All the units examined were also active during the intervals between volleys (Figs. 2*B* and 3–5)—i.e., the abrupt increases in neuronal activity during the MUA volleys were not the consequence of the activation of previously silent cells.

One unit identified in an intact and 3 units identified in ovariectomized monkeys exhibited decreases in firing rate during the MUA volleys (Fig. 3, unit 6R; Fig. 4, unit 2).

The activity of the third type of single units did not change in relation to the MUA volleys (Fig. 3, units 3L and 5R; Fig. 5, unit 3).

DISCUSSION

The results indicate that the MUA volleys associated with the activity of the GnRH pulse generator represent the simultaneous increase in firing rate of some individual hypothalamic neurons and the decrease in frequency of others.

The interpretation of the results obtained with the spike separation method described here depends on the validity of the conclusion that each identified waveform represents the activity of a single neuron. Because the waveform parameters depend on the distance of the recording site from the firing neuron, the position of the electrode tip relative to the axon hillock, as well as on the morphological characteristics of the firing cells such as their diameter and the spatial configuration of their dendritic tree (16, 21, 22), the probability of having the same waveform from different neurons is negligibly low (16), thus supporting the view that each spike in the same cluster can be considered as the output of a single neuron (16, 23). On the other hand, in the course of a high-frequency burst, the action potentials of a given cell can vary in amplitude by as much as 50% (24–26). Because the amplitude of the spikes may decrease toward the end of a burst they may fall outside the cluster boundaries that have been determined for the spike height occurring between the bursts and be excluded from the spike train. Therefore, in the present study the cluster boundaries were adjusted so that they might keep the particular single unit even in the presence of occasional minor decreases in spike height that occurred after the onset of volleys (Fig. 2*B*, MUA and unit 1L).

As shown in Figs. 3–5, the sums of activities of single units identified in given multiunit recordings represent but a relatively small fraction of the total activity (5–17% during the MUA volleys), indicating that most spikes could not be assigned to well defined clusters. Most of these excluded spikes had characterless, low-voltage waveforms, rounded in shape, indicating that they represented the discharges of cells located relatively far from the recording electrode (27). Because the parameters of these "distant" spikes did not form clusters clearly distinguishable from each other, they were excluded from further analysis. A small number of

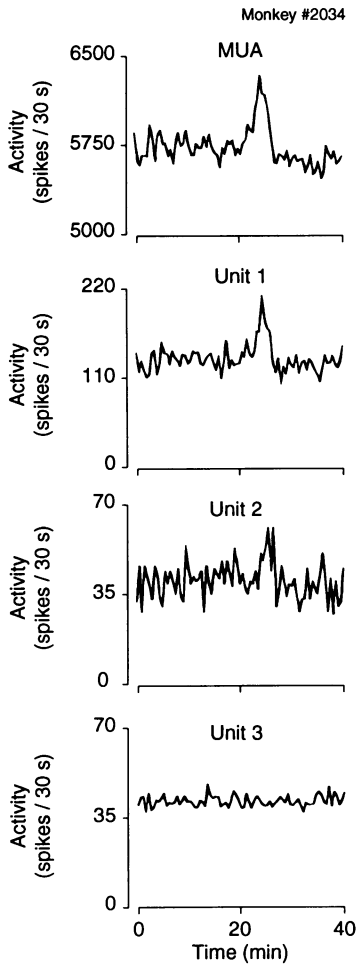

FIG. 5. Activity histograms of MUA and reconstructed single unit spike trains (units 1–3) obtained from one electrode implanted in the mediobasal hypothalamus of a gonadally intact rhesus monkey. Activity of units 1 and 2 increased coincidently with the MUA volley, while the firing of unit 3 did not change. Note the shorter duration of the volleys when compared to those of the castrated animals in Figs. 3 and 4.

waveforms were polyphasic, most probably resulting from the simultaneous discharges of different neurons, and were also excluded (27, 28).

Histograms of the individual spike trains revealed three types of units. The majority (24 of 40) of the identified units increased their firing frequency coincidently with the MUA volleys (Fig. 2*B*; Fig. 3, units 1L, 2L, and 4R; Fig. 4, unit 1; Fig. 5, units 1 and 2). It should be noted, however, that in the present paper "coincidence" only means that the increases/decreases in the firing rate of the corresponding spike trains took place within the same 30-s time bins. However, in view of the low firing frequency of the identified units (2- to 20-Hz peak firing rate during the MUA volleys; cf. Figs. 3–5), it would have been largely impractical to use shorter time bins to describe single unit activity. The onset time lag between bursts of individual units (29) could also not be determined for these reconstructed spike trains because the variability of the basal firing rate prevented us from precise identification of the time that a burst actually began. Despite these methodological limitations it can be concluded with confidence that the MUA volleys represent an increase in firing rate of these neurons rather than recruitment of units that are inactive during the interval between volleys.

The second largest group of waveforms (12 of 40) consists of units showing no variations in association with the MUA volleys (Fig. 3, units 3L and 5R; Fig. 5, unit 3). This finding not only indicates that they were unrelated to GnRH pulse generator activity but also provides confidence in the ability of the method to identify single neurons that do participate in the operation of the system.

The units in the remaining group (4 of 40) decreased their firing frequency during the MUA volleys (Fig. 3, unit 6R; Fig. 4, unit 2). Although these periods of decreased activity unambiguously represented the "mirror image" of the MUA volleys, the considerable variability of the basal firing rate prevented us from determining the time bins during which the onset and termination of the decreased activity took place. The role of these units can only be speculated at present. Although they might be intrinsic regulatory elements of the GnRH pulse generator indicating the complexity of this neuronal oscillator, these "negative volleys" may also reflect the functioning of another system secondarily coupled to the pulse generator. In view of the coincident pulses of luteinizing hormone and prolactin described in both anesthetized rhesus monkeys (30) and women during certain phases of the menstrual cycle (31, 32), it may be that these "dips" in single unit activity are related to the periodic inhibition of the tuberoinfundibular dopaminergic cells resulting in the synchronous appearance of prolactin pulses and those of luteinizing hormone.

The authors are grateful to S. Pate for his expert technical assistance and to J. Pastore for preparation of the figures. This study was supported in part by Grants HD 08610 and HD 17438 from the National Institutes of Health and by the Clayton Foundation for Research. H.C. received a postdoctoral fellowship from the Rockefeller Foundation.

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