Intracellular Recording of Rat Neuron Activity at the Medial Preoptic Area of the Hypothalamus Using Triangular Wave Microelectrode Oscillation

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ABSTRACT. A triangular wave microelectrode oscillating apparatus was constructed to evaluate an intracellular recording of rat neuron activity in the medial preoptic area (POA) of the hypothalamus. In this apparatus, electrodes passed a current with a frequency of 1.0 to 1.8 kHz and a voltage of 2.2 to 3.2 V and produced micro-oscillation of the electrode tip. The electrode was inserted into a neuron of the rat POA in vivo. In vivo recording of the activity of the rat POA neuron was possible. By means of electrical stimulation of the median eminence arcuate of the hypothalamus, an intracellular recording of antidromic, orthodromic or non-responding neuron was also possible. As a result, various components of the action potential such as the resting, threshold and spike potentials, and depolarization and repolarization such as after-hyperpolarization and after-depolarization were observed. The resting potentials ranged from 45 to 90 mV, and POA neurons possessed action potentials of almost the same magnitude. Several problems, however, remain to be solved. In general, the time available for the intracellular recordings is too short. The cells survive only for 15 minutes at the longest and may die in only a few minutes. An improvement of the apparatus was mandatory.—KEY WORDS: intracellular unit, rat POA neuron, triangular wave oscillation.

Electrophysiological studies of rat neurons in the hypothalamus have been performed primarily using extracellular recordings. Using this method, however, it is almost impossible to analyze quantitatively the generation of membrane potential and the changes of membrane potential because of shifts in the ionic composition of the intracellular fluid, to give only one example. The nerve and myocytes actually used for intracellular recording studies include giant nerve fibers from cuttlefish (loligo and sepia), preparations of nerves and muscles of crustaceans and frogs, and ootid, extracted cells of mammalian skeletal muscle, cardiac or smooth muscle, and their isolated cell derivatives. Some years ago, a method for the direct insertion of electrodes into these cells was developed [2]. This method consisted of the introduction of electrodes into cells by penetrating the cell membranes with micro-oscillation of the microelectrode tips using sine wave current.

This microelectrode oscillation using sine wave current was applied in vivo to the intracellular recording of rat neurons in the medial preoptic area (POA) of the hypothalamus. However, favorable results were not obtained despite initial expectations. Consequently, a microelectrode oscillating apparatus using a triangular wave was manufactured, specifically for the use of intracellular recording in the rat POA. With this apparatus, the microelectrode was inserted into POA neurons in vivo. The triangular wave current was passed through the electrode, creating a micro-oscillation of the tip. As a result, the insertion of the electrode into the cell was realized by combining certain degrees of frequency and voltage. This method enabled the intracellular recording in vivo of a rat POA neuron, as described below.
MATERIALS AND METHODS

Microelectrode Oscillating Apparatus:
This apparatus is composed of the following input and output specifications (Fig. 1).

Power Source: 18 V DC (2 9V dry cells (6AM6))
Frequency Output: 0.2 to 2.2 kHz
Voltage Output: 0 to 10 V
Timer Range: 10 to 1000 msec

Figure 2 shows the actual images of the waveforms photographed using a digital memory oscilloscope (DM-1562A, Toshiba digital memoryoscope, Tokyo Electric Ind. Co., Ltd.). This corresponds to the triangular wave transmitted into the rat POA using a glass microelectrode.

Application of Microelectrode Oscillation:
In these experiments, 200 to 300 g Sprague-Dawley strain rats were used. They were in proestrus of a natural sexual cycle. Urethane (1.0 to 1.2 g/kg) was injected into the abdominal cavity and the heads were fixed in a brain stereotaxic apparatus (SR-6, Narishige, Tokyo). The parietal region was dissected along the median line, and the parietal bone as well as dura mater (endocranium) was removed only to the extent required for inserting the electrodes. Bipo-
lar stimulating electrodes of 0.125 mm (outer diameter) nichrome wire were inserted into the median eminence arcuate (ME) and then fixed on a screw fixed into the parietal bone with dental cement. The fenestrated portion was covered with a 2% agar solution dissolved in physiological saline solution to prevent it from drying and to reduce cerebral tissue agitation due to respiration.

A single unit formed the recording subject after introduction into a single active neuron in the POA. The recording and stimulating electrodes were inserted in the POA and ME according to the coordinates of Fessard et al. [1]. The electrodes used for recording the single unit of the POA neuron consisted of glass tubes of 2 mm in diameter. The tips of the electrodes were narrowed to 0.1 μm or less using a glass tube puller (PW-77, Narishige, Tokyo). These glass capillary electrodes were filled with 3M-KCl solution to which pontamine sky blue 6B was added to maintain a 2% concentration and to allow local staining at the recording locus. The resistance of these electrodes was 10 to 60 megohms.

For unit recording, the glass microelectrode was mounted in the micromanipulator of the stereotaxic instrument and action potentials were led from the microelectrode to an amplifier. The recording electrode was connected to the input of a differential amplifier (ME-3241, ME Commercial Co., Tokyo) on one channel of a dual-beam amplifier through the high impedance probe, and differential input was used with indifferent lead connected to the ear bar of a stereotaxic apparatus. Action potentials were monitored visually and acoustically, displayed on a digital memory or monitoring oscilloscope (VC-9, Nihon Kohden, Tokyo). The amplified outputs were also led to an audio-amplifier and loudspeaker, oscilloscope, spike counter and to the external trigger of a square-wave pulse generator (ME-2100, MEAC System, ME Commercial Co., Tokyo). Triggered 1 msec biphasic square waves were supplied from the pulse generator to the driver amplifier of one channel of a polygraph (Nihon Kohden, Tokyo) and to a tape recorder (DFR-3515, Sony data recorder, Sony Magnescale Inc., Tokyo). The data were stored on magnetic tape for later analysis, as well as a digital memory oscilloscope from which spikes were photographed directly with a polaroid camera (M75D, King CRT, Asanuma & Co., Ltd., Tokyo) using an instant black and white film (FP-3000B, Fuji Film Co., Tokyo).

The electrical stimulus to the ME was single biphasic rectangular wave of 1 msec (ME-6102, ME Commercial Co., Tokyo). Principally, the intensity of the current for stimulation was twice the threshold value, and the stimulation was applied at a frequency of once a second for 30 sec. The response of the POA neuron to the ME stimulation was classified as antidromic (AD), orthodromic (OD) or non-response (NR). Intracellular insertion of electrodes was attempted using these AD, OD and NR neurons. The recording electrode was introduced into the cell after the response was confirmed by an extracellular recording method. The active and spontaneous extracellular potentials of POA neurons began to be recorded after the observations of electrocardiogram (ECG) and electroencephalogram (EEG) to make sure that they were ordinary. ECG was led from the forelegs, and EEG was taken from bipolar leads positioned from the frontal cortical area of the brain and connected to an AC preamplifier of the polygraph. ECG and EEG were monitored throughout the experiment. The electrode was stereotaxically inserted so as to pass through the POA. Initially, extracellular action potentials were recorded with glass microelectrodes from neurons in the POA. The electrode was
pressed against the neuron at its initial position or at a position 2 to 10 μm lower. A triangular wave was then applied once to 5 times using the following conditions.

- **Frequency:** 0.8 to 1.8 kHz
- **Voltage:** 2.5 to 6.0 V
- **Continuation:** 50 to 500 msec

After introduction of the microelectrode into a single active neuron in the POA, a baseline activity of the cell decreased to the polarizing level (the sum of tip potential and resting potential). It was established by the DC bucking regulator of an amplifier to ±zero volt on the X-axis of the oscilloscope and intracellular active spikes riding on the slow waves of membrane potential oscillations were then spontaneously observed.

At the end of each recording session, a current of 30 μA/30 sec was applied to the recording and stimulating electrodes to permit an outflow of dye at the recording region. Subsequently, iron ion was deposited at the stimulated region. Perfusion fixation was then performed with 10% formalin solution containing 3% potassium ferrocyanide and herricyanide prior to brain removal. The recorded and stimulated regions were reconfirmed by examining histological sections of the stained, fixed brain.

**RESULTS**

The initial frequency, voltage and continuation were 0.8 to 1.8 kHz, 2.5 to 6 V, and 50 to 500 msec respectively. As the frequency was increased, the voltage decreased parabolically. Figure 3 shows the frequency and voltage gain when an electric current was applied through the glass tube electrodes, and the results of electrode insertion into the POA neuron. A voltage of 2.3 to 3.2 V (measured values) was applied to the electrode with a frequency of 1.0 to 1.8 kHz, and the electrodes were inserted into the cells by micro-oscillation of the electrode tips. This made it possible to perform an intracellular recording of POA neuron activity.

Figure 4 shows the potential variation before and after the insertion of the glass tube electrode by repeated oscillations. The decreased baseline (arrow part) on the left in the photo A represents the changes in the electric current through the oscillating apparatus. The right portion illustrates the decrease of the baseline activity of the cell to the resting potential after the insertion of the electrode into the cell. B is an example in which the electrodes, after oscillation, are instantaneously inserted into cells through the cell membranes. C shows the changes of
Fig. 4. Changes of the action potential before and after insertion of the electrode into the rat POA neuron. The lower portion (arrow part) of the baseline on the Photo A corresponds to variation during current application by the microelectrode oscillating apparatus. The right hand implies that the electrodes have been inserted into the cells and the baseline has decreased to the resting potential level. B represents an example of instantaneous penetration of electrodes into the cell while C represents a rather slow penetration of the electrode into the cell. Calibration bars: 0.1 sec and 50 mV.

action potential when the electrodes inserted relatively slowly through the cell membranes.

The intracellular recording from the POA neuron was performed after the spontaneous spike potential had become constant with a stabilized baseline activity of the cell following insertion of the electrode into the cell. As a result, the action potential of the POA neuron could be recognized as antidromic, orthodromic or non-response. It was composed of a variety of factors including the resting, threshold and spike potentials, and depolarization and repolarization such as after-hyperpolarization and after-depolarization (Fig. 5). Intracellular recordings from rat POA neurons showed that these cells possessed resting potentials of 45–90 mV, and action potentials of almost the same magnitude.

The intracellular recording of the rat POA neurons was realized using the microelectrode oscillating apparatus with a triangular wave current, but certain problems remain to be solved in the future. In general, the time available for the intracellular recordings is too short. The cells survive only for 15 minutes at the longest and may die in only a few minutes. In this study, the microelectrodes were inserted into cells only after observation and identification by extracellular recording of an AD, OD or non-response. The cells generally died during intracellular recording because of electrical stimulation of ME.

DISCUSSION

Recently, a number of studies on the function of the brain, the center of the cell, have been performed. This is primarily due to the development of microtechniques. The intracellular recording method has made it possible to record the generated difference in potential by inserting a small diameter microelectrode into each cell. This method has become indispensable to studying the mechanism of resting or action potential development as well as presynaptic conduction and synaptic transmission of post-nerve cells.

However, direct application of intracellular recording in vivo to the hypothalamic
neurons in rats to measure intracellular potentials is not completely practical for the following reasons: 1) Hypothalamic neurons differ from each other in size; 2) difficulty in production of fine-tip electrodes: 0.1 μm or less for glass tube electrodes and 1 μm or less for metal electrodes; 3) when the electrodes are forcibly pressed against cell membranes, the tips may penetrate completely the cells, and 4) by blood pressure, pulse pressure, or respiration, the cells may oscillate during recording and may eventually die. For these reasons, few reports have been available regarding intracellular recordings in vivo of rat POA neurons. On the other hand, there are many reports describing in vitro recordings from hypothalamic slice and other in vivo preparations. Kandel [3] reported intracellular recordings of goldfish POA neurons. Koizumi and Yamashita [4–5], and Yamashita and Koizumi [6–7] reported the intracellular recording of the activity of neurosecretory cells in the supraoptic and paraventricular nuclei of the hypothalamus in cats and dogs.

Another method for intracellular recording of the rat POA neuron has recently been developed using microelectrode oscillation with a sine wave. This in vitro application was made both on samples of neurons and muscles, however almost all the cells died. In consequence, the author proceeded to manufacture a microelectrode oscillating apparatus using a triangular wave to insert these electrodes into POA neurons. As a result, the intracellular recording of POA neurons has become possible. By applying a certain frequency and voltage to the apparatus, the microelectrodes could be inserted into cells through micro-oscillation of the electrode tips. The difference between the sine wave electrode oscillation method previously described [2] and the triangular wave microelectrode oscillation method is the use of an AC amplifier; the former method can rarely produce oscillation from zero. The triangular microelectrode oscillation uses a dry cell (DC) battery and initiation of oscillation from zero is always possible. For this reason, cell injury may be minimized during the intracellular recording.

Using this apparatus, it has become possible to record intracellularly the activity of the POA neurons. Several problems, however, remain to be solved. The cells may die in 15 minutes at most and in several minutes at least, after introduction of the microelectrodes into neurons. Several causes may be responsible. Insertion of the electrodes into cells by penetrating the cell membrane with oscillating electrode tips may be detrimental to the cell surface. As is clear from Fig. 4 (A, B and C), after electrode insertion, the intracellular potential spike has not risen to the baseline activity of the cell established at the time of the extracellular recording. The injury current caused by cell membrane injury from the non-injured region towards the injured region may explain this phenomenon. Further severe injury may cause leakage of intracellular fluid. An electric stimulus was applied to the ME cell penetration by the electrodes to classify the POA neuron into AD, OD or NR (non-response). Seventy-five percent (108/144 neurons) of the cells die with the stimulus, probably due to minute oscillation onto the recording electrodes or cells themselves. An improvement of the apparatus was mandatory.

Although a few of these problems remain, the improved model made it possible to record intracellularly the activity of the rat POA neuron. This should be useful not only for electrophysiological studies of neurons, but should also contribute to the measurement of electrolytes in a single neuron through insertion of an ion-sensitive microelectrode.
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REFERENCES


要約

三角波微小電極振動法によるラットの視床下部内側視索前野ニューロンの細胞内記録の試み：渡部 敏（日本大学農獣医学部獣医生理化学教室）ラットの視床下部内側視索前野（POA）ニューロンの細胞内記録を行う目的で三角波による微小電極振動装置を試作した。この装置は、一定の三角波の周波数と電圧をガラス管電極に通電して電極の先端を微細に振動させることによってin vivoでラットのPOAニューロン内に電極を刺入するものである。この装置を用いて視床下部弓状核（ME）の電気刺激に対して antidromic（AD）, orthodromic（OD）または non-response（NR）を示すPOAニューロンの細胞内記録を試みた。その結果、細胞内記録の成功率は44中36細胞で25％と低かった。更に細胞内記録の可能な時間が短く、長く15分、一般的には数分であった。また今回の実験では予め AD, OD 反応または NR を細胞外記録法によって観察、同定してから微小電極を細胞内に刺入した。その理由は細胞内記録時にMEを電気刺激した場合、細胞が死滅してしまう例が多かったからである。これ等の点については未装置を更に改良する必要があるが、静止電位、閾値電位、spike および overshoot 等の興奮時膜電位、陰性および陽性後電位、後脱分極および脱分極などの活動電位の各要素が観察されたことからラットのPOAニューロンの細胞内記録の可能性が示唆された。