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Technical contribution

DEVELOPMENT OF THE SINGLE MICROELECTRODE CURRENT AND VOLTAGE CLAMP FOR CENTRAL NERVOUS SYSTEM NEURONS

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The use of conventional voltage clamping techniques to investigate the membrane events of central nervous system neurons has often been precluded by the inability to simultaneously penetrate the cell with two microelectrodes. Recently, a single microelectrode current and voltage clamping technique ** was developed by Wilson and Goldner (1975) and employed successfully in molluscan systems. The operation of this single microelectrode system is based primarily upon electronically switching the microelectrode between current passing and potential recording modes, during which sample and hold modules acquire and store the amount of injected current and resultant membrane potential. Such a system is not only attractive in use for voltage clamping, but also for current clamping *** in order to circumvent the difficulties associated with a single microelectrode Wheatstone bridge balance system, i.e., bridge imbalance resulting from variations in electrode resistance upon cellular impalement, during the course of prolonged intracellular recording, and during the passage of current (Wilson and Goldner 1975), and also noncompensation of the local potential fields emanating from a point source of current (Peskoff and Eisenberg 1973).

The extension of this system for use in central nervous system preparations, however, is not necessarily straightforward. This is primarily due to the more stringent constraints imposed upon the clamping system, e.g., current demands by cells of more complex neuronal geometries with membrane time constants on the order of 1—5 msec.

In this communication, we present both the considerations involved in employing the single micr electrode current and voltage clamp circuit to invest gate slowly varying events in mammalian central ne vous system neurons and our experimental resul which demonstrate the efficacy of this technique in central nervous system preparation.

Methods

To evaluate the capability of this technique to cu rent and voltage clamp central nervous system ne rons, we chose to measure the membrane propertie of cat spinal motoneurons. In our experiments, hig spinal (C-1 level transection) adult (2—4 kg) cat pre arations (Somjen 1970) were used. Motoneuror located in lumbrosacral segments L7 and S1 wer identified via the antidromic stimulation of variou leg nerves and impaled with beveled glass microele trodes. The electrolyte concentration, tip diamete and resistance of the microelectrodes (0.5 *M* K citrat 0.5—2.5 μm; 1—10 MΩ) were adjusted to minimiz the electrode rectification effects of ionic redistribu tion (Barrett 1976) † and to maximize the circui switching rate. To allow for comparison with a established technique, a conventional Wheatston bridge balance (BB) circuit (Nelson and Frank 1967 was incorporated into the single electrode clam (SEC) system. For details of SEC circuitry see Wilso and Goldner (1975).

Results

The system was first used in the current clam mode to measure the input resistances and membran time constants of normal motoneurons. These dat were compared to those obtained using a bridge bal ance circuit for the same cells. Prior to cellular pene tration, we adjusted the SEC circuit to the maximun

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** Brennecke and Lindeman (1974) present the mathematical theory for this electronically discontinuous system.

*** Muller (1973) utilized a method of current clamping quite similar to this technique.

† Barrett, J.N., personal communication, 1976.

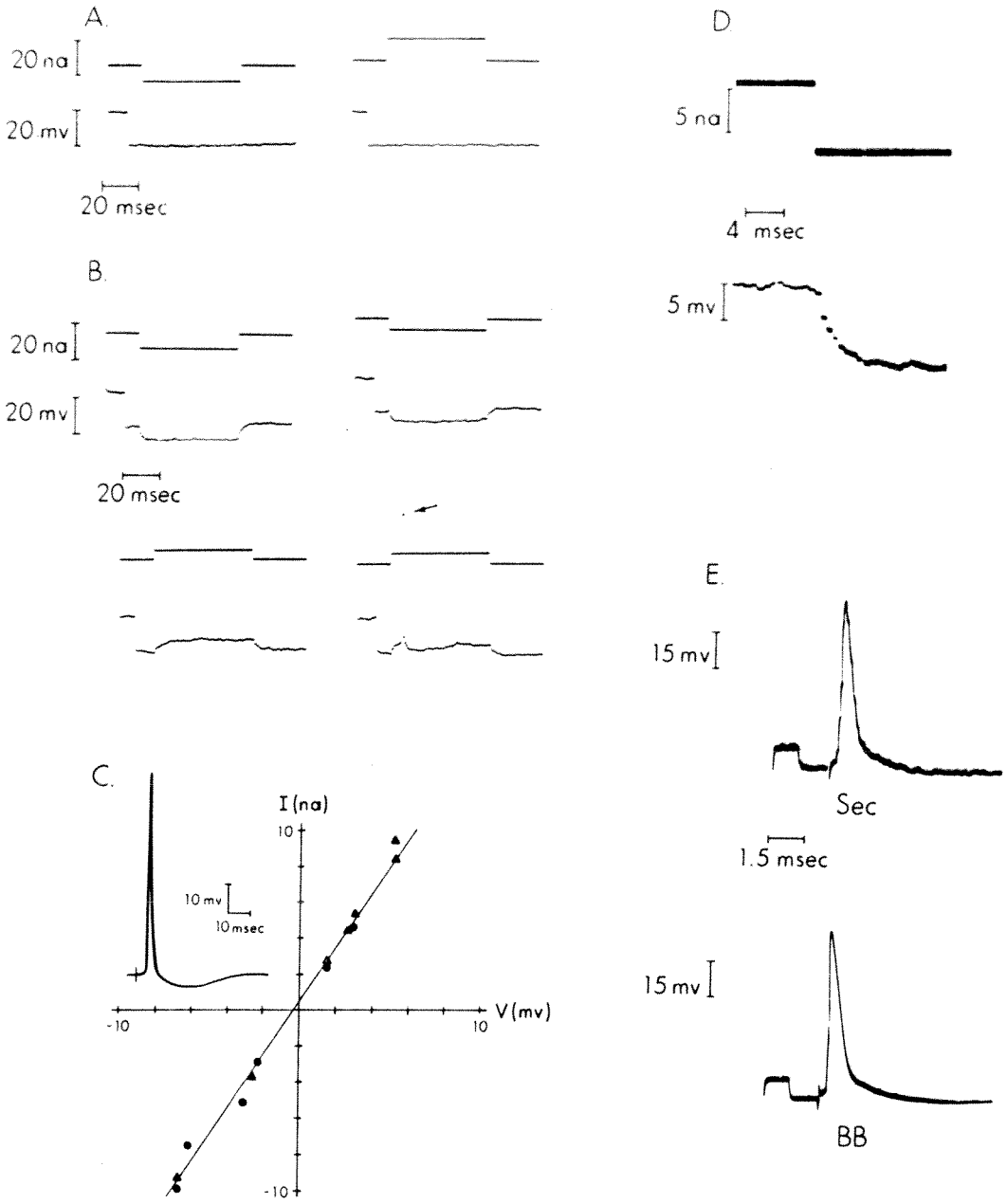

Fig. 1. Data showing the operation of the single electrode clamp circuit and comparison with bridge balance cir cuitry in the current clamp mode. A, oscilloscope records of a "zero" extracellular control at 1.32 kc/sec switch ing frequency (to show that the voltage trace is not changed by current flow through the microelectrode). Top trace is current sample and hold output; bottom trace is voltage sample and hold output containing a 20 mV cali bration pulse. B, intracellular motoneuron oscilloscope records at 1.32 kc/sec. Input resistance is determined from passive current and voltage sample and hold displacements. The action potential peak in the active voltage sample and hold trace is denoted by an arrow. C, I—V plot comparison of bridge balance (●) and single electrode clamp (▲). Results obtained at a switching frequency of 1.72 kc/sec. Resting potential, —70 mV; 81 mV spike. A least-squares fit of data from each circuit revealed a 2.2% difference between input resistances. Composite data yielded an input resistance of 0.68 MΩ. D, oscilloscope records of current (top trace) and voltage (bottom trace) sample and hold outputs used to determine the membrane time constant of a motoneuron subjected to a hyper polarizing pulse (time constant 4.3 msec). E, oscilloscope records of a 10 mV calibrator pulse followed by a 78 mV motoneuron action potential recorded via the single electrode clamp voltage sample and hold output at 10 kc/ sec and via the bridge balancing circuit. Both records agree within 1.5%.

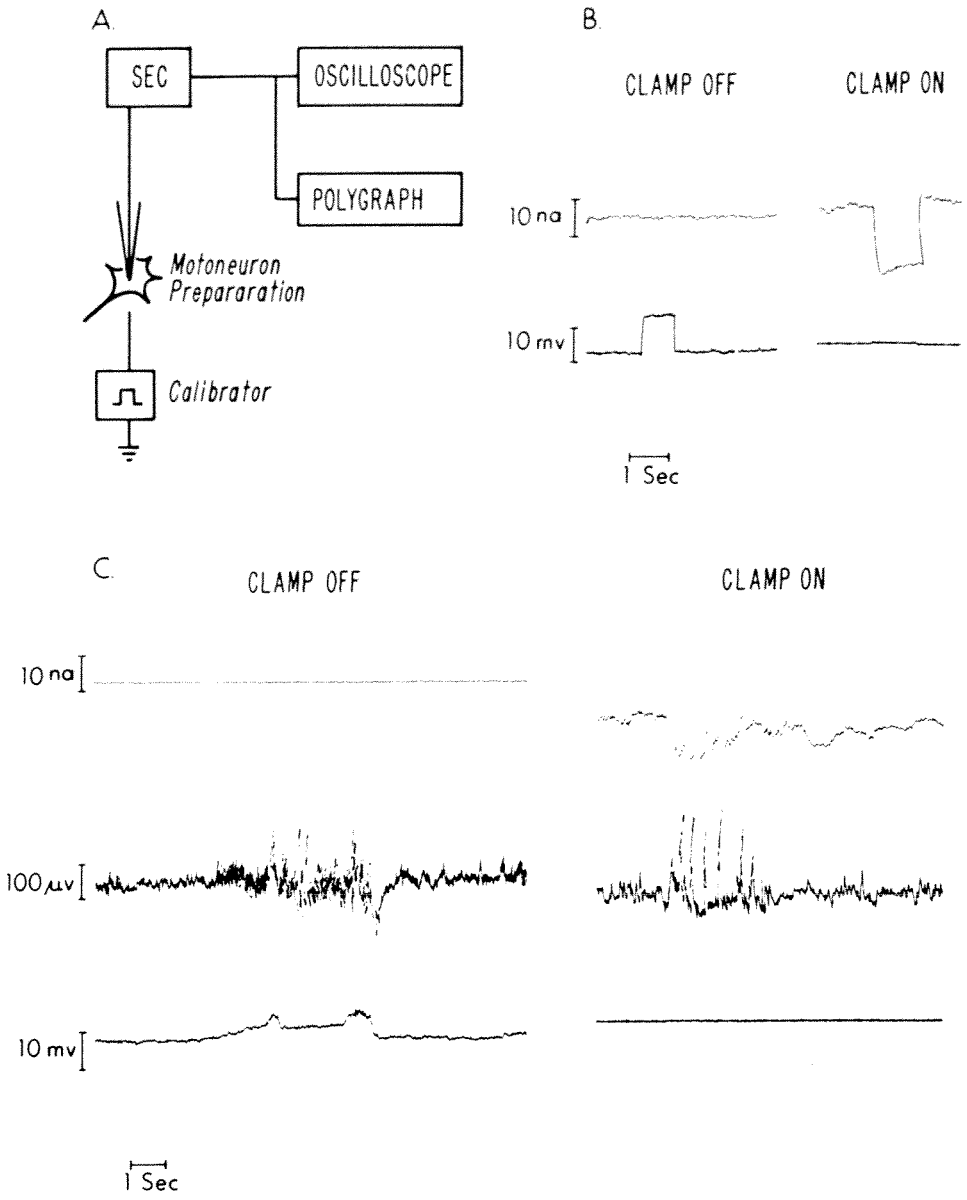

Fig. 2. Results obtained utilized the SEC circuit in the voltage clamp mode. A, schematic of configuration for voltage clamp testing. B, polygraph recording of current (top trace) and voltage (bottom trace) sample and hold outputs when the 10 mV calibration pulse was applied with the circuit in the normal recording mode (clamp off) and the voltage clamp mode (clamp on) while impaled within the same motoneuron (switching frequency = 2.5 kc/sec). C, clamp off: polygraph recording of the current clamp of the same motoneuron in part B undergoing a Na-penicillin induced paroxysmal depolarizing shift. Top trace is the current sample and hold output, middle trace the L7 ventral root recording, and bottom trace the voltage sample and hold output (at a resting potential = −65 mV). The sharp high frequency lines in the voltage sample and hold record are action potentials attenuated by the response characteristics of the polygraph. Clamp on: polygraph recording of the voltage clamp of the same motoneuron at a holding potential of −66 mV. Inward (excitatory) current is displayed as a negative deflection in the top trace.

switching rate allowable for proper current injection and sampling, and the recording of the resultant potential change (for the extracellular case, a "zero" membrane potential change as shown in Fig. 1A). The best electrodes were found to carry maximum currents of 50 nA at a maximum switching rate of 8 kHz without showing signs of electrode rectification or other artifacts. Typical electrodes passed up to 20 nA (average current) at 2 kHz. A series of hyperpolarizing current commands were given initially via the bridge circuit, then via the SEC circuit in the current clamp mode (Fig. 1B). Input resistances were calculated from a least-squares fit of the current—voltage (I—V) data obtained using each circuit. From the seven impalements in which I—V plots could be acquired using both circuits, input resistances were found to differ by no more than 6%. A typical comparison is shown in Fig. 1C. The input resistances versus conduction velocities for 14 cells were also plotted and compared with those obtained by other investigators (Barrett and Crill 1974) and found to agree within experimental error (Dunn and Somjen 1977). Membrane time constants were determined from the slope of final exponential decay (Rall 1969) (Fig. 1D). They were found to vary between 1 and 6 msec for normal motoneurons, a range similar to that found by previous investigators using bridge balance techniques (Barrett and Crill 1974).

Switching rates greater than 5 kHz usually prohibited the passage of currents greater than 20 nA. To adequately sample rapid events, higher switching rates must be used. However, only small currents (<5 nA) could be injected at these rates. In Fig. 1E, an action potential recorded at a switching rate of 10 kc/sec is accurately reproduced when compared to a recording obtained in the same cell using the bridge system.

Next, we tested the SEC circuit in the voltage clamp mode. In order to ascertain the circuit's ability to clamp motoneuron events, we placed a calibrator in series with the system ground as depicted in Fig. 2A. With the cell held in voltage clamp mode, a 10 mV pulse was applied. As shown in the voltage sample and hold record of Fig. 2B, the applied calibrator pulse is rapidly attenuated. Fig. 2C displays a recording of a motoneuron undergoing a paroxysmal depolarizing shift (during a penicillin-induced seizure) and the voltage clamp record of a similar event in the same cell.

Discussion

In the present study we found the SEC circuit useful for the study of slowly varying events. It was less effective for the study of rapid events, since high transient currents were required to clamp fast events. On occasion, it was necessary to either reduce the switching rate and/or filter the voltage feedback to attenuate faster events in order to reduce current demands on the electrode. The use of the circuit in other central nervous system neurons may not impose such high current demands, thereby permitting the clamping of faster events. It should be noted, however, that in all preparations the effectiveness of the circuit is dependent on the attainment of a switching rate faster than the time constant of the membrane event to be studied. Thus, when using this technique to voltage clamp events in central nervous system neurons, one must especially consider the neuronal geometry and the membrane time constant. For the case of a neuron with extensive dendritic processes, the system voltage clamps only the membrane area electrically local to the microelectrode tip (presumably most of the soma). Yet, such a partial voltage control may be adequate to clamp the soma membrane potential during slowly varying events (such as demonstrated herein), to prevent the summation of post-synaptic currents at the soma (Wilson and Wachtel 1970), or to study iontophoretic responses.

An improvement in the ability to clamp faster events rests in the reduction of the time constant of the input circuit, thereby increasing the switching rate. This is accomplished by a reduction in electrode resistance and input capacitance. For investigations in central nervous system preparations, electrode resistance is optimized by choosing a tip diameter sufficiently small so as not to cause membrane damage upon penetration, yet large enough to permit the necessary passage of current through the microelectrode. In the present experiment, we tested beveled electrodes filled with electrolytes of various concentrations (0.2—3.0 *M* KCl, 0.5 *M*—3 *M* K acetate, 0.5 *M*—2 *M* K citrate) with beveled tip diameters ranging from 0.5 to 4.0 μm. The best results were obtained using beveled electrodes filled with 0.5 *M* K citrate.

Some performance limitations result from microelectrode capacitance produced by deep electrode placement in the conducting spinal tissue. Although negative capacitance feedback can usually minimize this problem, it would be desirable to directly eliminate this effect by shielding the microelectrode to a point near its tip and driving the shield at the same potential as the microelectrode.

Our results illustrate the ability of the SEC circuit to current and voltage clamp neurons in a central nervous system preparation. A circuit with the capability of controlling particular spinal motoneuron events at switching frequencies as high as 8 kc/sec provides the investigator with a powerful tool to precisely determine the membrane resistance and capacitance of many central nervous system neurons, to study their variations over extended periods of time independent of changes in electrode resistance,

and to investigate slow membrane events under voltage clamp conditions. This may prove useful for a variety of studies in preparations where voltage clamping is desirable but cells are only accessible to one microelectrode.

Summary

The development of a single microelectrode clamping circuit for central nervous system preparations is presented. The efficacy of the circuit in both current and voltage clamp modes is demonstrated by investigating the membrane properties of cat spinal motoneurons. Results are compared with those obtained using conventional techniques and reveal that this technique can be more readily applied to central nervous system neurons. The constraints involved in employing this system to study time-varying events are also discussed.

Résumé

Developpement d'une microélectrode isolée à courant constant et tension constante pour l'étude des neurones du système nerveux central

Les auteurs présentent un circuit de microélectrode à tension imposée destiné à être utilisé dans des préparations du système nerveux central. L'efficacité de ce circuit dans ses deux modalités, courant constant et tension constante, est démontrée par l'étude des propriétés de membrane des neurones moteurs de la moelle épinière du chat. Les résultats sont comparés à ceux obtenus par des méthodes conventionnelles et montrent que cette technique peut être plus aisément appliquée aux neurones du système nerveux central. Les contraintes impliquées par l'emploi de ce système à l'étude des évènements qui varient avec le temps, sont également discutées.

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