ELECTRICAL AND CHEMICAL RESPONSIVENESS OF TRIGEMINAL ROOT
GANGLION NEURONS OF THE GUINEA PIG <u>IN VITRO</u>

by

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Abstract

Intracellular recording techniques were used to investigate the electrical and chemical membrane properties of neuronal somata in guinea pig trigeminal root ganglion (TRG) slices. All impaled neurons (150) remained quiescent prior to square wave current pulse injection through the intracellular recording electrode. The majority of neurons ($\simeq$ 100) exhibited voltage- and time-dependent rectification in response to hyperpolarizing current pulses. Two groups of neurons were distinguished on the basis of action potential characteristics. In one group, action potentials had a plateau (hump) in the repolarization phase. Action potentials evoked from the second group of neurons did not have this characteristic. Average amplitude and duration of spikes and afterhyperpolarizations was larger in the first group of neurons. Bath application of S-glutamate (10^{-2} M) caused small (1-2 mV) depolarizations in 2 of 6 neurons tested. These depolarizations were not associated with changes in membrane conductance. 5-hydroxytryptamine (10^{-3} M) produced a small conductance increase and depolarization in 1 of 6 neurons tested. Similar application of γ-aminobutyric acid (GABA; 10^{-4}–10^{-2} M) produced decreases in membrane resistance (up to 63%) associated with membrane depolarization (2-14 mV) in 30 of 36 neurons tested. Reduction of spike amplitude was observed during GABA-mediated depolarization. These investigations suggest that membrane properties of guinea pig TRG neurons are similar to those reported for other mammalian sensory ganglion neurons.

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I. INTRODUCTION

Vertebrate primary sensory neurons have been the subject of intensive investigation during the last century (cf. review by Lieberman, 1976). The axon fibers of these neurons convey information from specialized receptors that sense changes in the physical or electrical environment of the organism (Gray, 1959). These changes may be perceived as different modalities of sensation i.e., touch, pressure, temperature, pain, sight, hearing, taste and smell. Impulses which are generated by receptors that may be representative of separate cell types or different specialized endings of primary sensory neurons are transmitted via their axons to the central nervous system (CNS). With few exceptions, somata of the primary sensory neurons of vertebrates lie outside the CNS in ganglia. These are located on the dorsal roots between their points of junction with the ventral root and entrance of the dorsal root into the spinal cord (Krieg, 1966). Ganglia of cranial nerves which have a sensory component also are located outside the CNS. Dorsal root and cranial ganglia are collectively referred to as craniospinal, cerebrospinal or sensory ganglia.

Three exceptions to this arrangement are the primary sensory neurons of visual and olfactory systems and the mesencephalic nucleus of the trigeminal nerve which lies within the brainstem (Lieberman, 1976).

Physiological and pharmacological investigations of sensory neuron membrane properties are important for several reasons. Signal transmission of sensory information from peripheral receptors is mediated by ionic mechanisms of the neuronal membrane; the alteration of its electrical properties can influence, profoundly, signal transmission of sensory information to the CNS. Both endogenous substances and therapeutically used drugs may exert their effects through these ionic mechanisms. In addition, many of human pathological conditions appear to involve a disruption of normal membrane function and study of alterations in membrane properties thus may be considered to be clinically important.

The membrane electrical properties of dorsal root ganglion neurons were among the first to be studied using intracellular recording techniques (Svaetichin, 1951). Several features of the sensory neurons of the dorsal root ganglion (DRG) have made them readily accessible to electrophysiological analyses. The cell bodies of these neurons are conveniently isolated from other types of neurons thus allowing for an unambiguous identification. During embryological development, somata of primary sensory neurons may possess several processes. However, only a single process in the adult animal extends from the soma to an axon which runs from the periphery to the CNS, effectively making a T-shaped connection (Krieg, 1966). The presence of only a single stem process which is attached to a spherical or ovoid soma facilitates the analysis of electrophysiological membrane properties when intracellular recording techniques are

employed. This relatively simple geometry obviates some of the difficulties in interpretation of the voltage responses to intracellular current injection which is used to assess certain membrane properties. Synaptic contacts or inputs are virtually absent and this further simplifies analysis and interpretation of results. Kayahara et al (1984) have shown that synaptic contacts of spinal cord fibres with cells of the cat DRG do occur, but the numbers are very small.

In contrast to DRG neurons which have been subjected to extensive electrophysiological examinations, neurons of the trigeminal root (Gasserian or semilunar) ganglion (TRG) have received very little attention. This may have been due in part to the anatomical inaccessibility of the trigeminal ganglion relative to the DRG and in part, to the general assumption that the membrane properties of cranial sensory ganglion neurons are the same as the properties of DRG neurons, an assumption that hitherto has not been verified experimentally.

In humans (and in mammals generally), the trigeminal root ganglion is the largest sensory ganglion in the body and is composed of three divisions: ophthalmic, maxillary and mandibular branches. Each TRG is formed at the point of convergence of its three divisions on the floor of the cranial vault, with the largest or mandibular branch in the most lateral position. These bilateral branches provide sensory innervation to the mucosal lining of the oral cavities, the skin, and muscles of mastication (the latter are innervated by neurons whose somata lie within the trigeminal mesencephalic nucleus of

the CNS). In addition, the mystacial vibrissae (whiskers) possessed by most mammals also are served by fibers of the cell bodies in the TRG. Also, sensory innervation of tooth pulp, surrounding gingiva and periodontal membrane is mediated by the trigeminal nerve (Kelly, 1981).

The need for intracellular studies of TRG neurons arises not only from the fact that their study has been neglected in comparison to DRG neurons but also because of the trigeminal ganglion's relevance to certain human pathological conditions. For example, trigeminal neuralgia, also known also as tic doloureux is a disorder in which the afflicted patient experiences what may be described as lightning-like attacks of pain of extreme intensity that can be precipitated by very light stimuli from the skin, face or gums, in an apparent absence of neurological deficit. Although previously considered to be a disorder of the CNS, recent and more substantial evidence points to peripheral origins of trigeminal neuralgia, with a more likely site of pathology being within the trigeminal ganglion itself or in its sensory root (Kerr, 1979).

Previous investigations using extracellular recording to monitor neuronal discharge activity of the TRG neurons have provided a wealth of information on the functional somatotopic organization of primary afferent neurons in a number of mammalian species (Kerr and Lysak, 1964; Darian-Smith et. al., 1965; Zucker and Welker, 1969). Other investigators have used extracellular recordings from trigeminal ganglion neurons for observations of possible effects of epileptogenic agents on the

excitability of their neuronal membrane (Burchiel et al., 1978). However, extracellular recordings provide insufficient information for the understanding of mechanisms of membrane excitability. Hence, an intracellular analysis is required.

Previous intracellular studies of TRG neurons have been rather preliminary in scope and have dealt with either cultured (Fukuda and Kameyama, 1980) or dissociated and internally perfused cells (Krishtal and Pidoplichko, 1980, 1981; Krishtal and Marchenko, 1984). These experimental preparations are not ideal because extensive modifications of physiological membrane parameters likely have occurred and hence the 'natural' physiological environment of the neuron has been only approximated. The extrapolation of results to the normal cell function is dubious, because of the uncertainty in interpretation of results relative to those obtained from intact physiological systems.

To date, the only intracellular investigation in which TRG neurons were not subjected to extensive manipulation was that by Puil et al (in press) in which membrane electrical properties of TRG neurons were examined in in vivo conditions. Although their guinea pig preparation closely approximated physiological conditions, it did not permit application of pharmacological agents in known concentrations directly to the neuronal membrane.

On the other hand, there are many advantages and disadvantages in studying the behaviour of neurons in vitro . Some of the experimental advantages also may be considered

disadvantages. For example, isolation from the usual physiological environment, possible mechanical deformation of the cell body and loss of the long peripheral and central portions of the neuronal processes render such preparations somewhat unphysiological. However, the advantages outweigh the negative considerations, especially when the results of studies in the _in vitro_ preparation complement those obtained from _in vivo_ preparations. Secondly, problems that cannot be addressed adequately by the latter methods often may be answered by similar investigations _in vitro_ .

An _in vitro_ slice preparation of the TRG of the guinea pig would facilitate stable intracellular recordings from neurons whose cell bodies have not been modified extensively by experimental manipulation. Therefore, this situation may be closer physiologically to _in vivo_ conditions while retaining other advantages inherent to _in vitro_ experimentation.

The present study describes, for the first time, both passive and active membrane properties of guinea pig TRG neurons _in vitro_ allowing a comparison to published reports on the membrane properties of other primary sensory neurons. The modulation of the membrane properties of TRG neurons by neuroactive substances also is presented.

II. <u>METHODS</u>

A. <u>SLICE PREPARATION</u>

Albino guinea pigs of either sex weighing 200-400g were used for all experiments. Anaesthesia was induced with 4% halothane or isoflurane administered in a .2 liter chamber. The trachea was cannulated and the animal's skull hair removed with scissors. The animal then was placed in a stereotaxic head holder. A craniotomy was performed while anaesthesia was maintained endotracheally with 2% halothane. After mid-collicular decerebration with a scalpel blade, the encephalon was aspirated to reveal the underlying posterior fossae. At this stage, the administered halothane concentration was reduced to 0.5%. Heart rate was monitored via electrodes inserted subdermally in the thorax region throughout the surgical dissection procedure in majority of experiments.

The left trigeminal ganglion was carefully isolated from the surrounding connective tissue and cartilagenous bone with the aid of a dissecting microscope and fine instruments (e.g. No. 5 jeweler's forceps). Care was taken not to disturb blood vessels in the vicinity of the ganglion for two reasons: (1) a minimization of the duration of hypoxia was considered desirable, and (2) profuse bleeding made visualization of the ganglion and therefore, dissection, difficult. The central and peripheral ends of the ganglion were severed such that the total length of the excised tissue was 1 to 1.3 centimeters. Usually,

the central end was cut as close as possible to its entry into the brainstem.. Following total excision from the posterior fossa, the ganglion was immersed quickly into cold (4°C) artificial cerebrospinal fluid (Yamamoto's) solution which was oxygenated with 95/5% gaseous mixture of O_2/CO_2. The remaining connective tissue, arachnoidal and dural (capsular) sheaths surrounding the ganglion, and its central and peripheral root stumps, were removed from the ganglion with the aid of a dissecting microscope. The ganglion was placed on a Teflon stage of a mechanical tissue chopper which was covered with artificial CSF-saturated filter paper and then cut into thin (300-500 μm) longitudinally oriented slices; this procedure preserved some of the axon bundles in the stumps of both central and peripheral branches.

Slices were transferred immediately into an incubating chamber where they were maintained in oxygenated Yamamoto's solution at 37°C until needed for recording. The procedures for dissection, slicing, etc. of the second ganglion were identical to those described above and these were carried out within 15 min after preparation of slices of the first ganglion.

B. ELECTROPHYSIOLOGICAL RECORDING SET-UP

For recording, slices were transferred to a superperfusion chamber with a glass bottom. A Nylon mesh was used for retention of the slices in order to prevent their movement or floating in the perfusion fluid. The recording chamber was fixed to the stage of a Leitz microscope equipped with Hoffman modulation contrast optics which allowed visualization of

individual cells at the edges of the slice. A gentle stream of air was blown over the lens in order to prevent condensation of vapour on the objective lens. Sodium S-glutamate, γ-aminobutryic acid and 5-hydroxytryptamine creatinine sulfate (all from Sigma) were applied in appropriate dilutions with the standard perfusion solution which had the following chemical composition (in mM): NaCl, 124; KCl, 3.75; KH_2PO_4, 1.25; $MgSO_4$, 2.0; $CaCl_2$, 2.0; $NaHCO_3$, 26; and dextrose, 10. Continuous bubbling with a 95% O_2 and 5% CO_2 mixture ensured adequate oxygenation and a pH of 7.4. An aluminum heating block, i.e. a Pelletier device, was used to keep the bath fluid temperature at 36 °C throughout the experiment. The total chamber volume was 3 ml in the initial experiments, but after several experiments, was reduced to 1 ml, to facilitate a faster exchange of solution, particularly in the case of drug applications. A steady efflux of fluid from the chamber was achieved by means of a filter paper strip which connected the recording chamber to a small adjacent well from which the fluid was removed by aspiration. The microscope and micromanipulator assembly were housed on a vibration-damping table that operated by an air-cushion arrangement.

Intracellular recordings were made using potassium chloride (3M) or potassium sulphate (4M) filled glass micropipettes. Resistances of KCl electrodes ranged from 10 to 40 MΩ which were measured in tissue prior to impalement of cells. Electrodes filled with K_2SO_4 had higher values of tip resistances 28-55 MΩ). The microprobe system (M701, WP Instruments) which was

used to measure potentials allowed injection of current via a bridge-balance circuit for conventional measurement of membrane resistance with constant current-pulses. Resting membrane potential of the cell was continuously monitored with a chart recorder (Gould); the zero voltage level was determined upon withdrawal of an electrode from the cell. Amplified potentials were stored on tape using a Hewlett-Packard FM tape recorder (Model 3968A; frequency range flat to 5 KHz at 7.5 ips) and printed on-line on chart recorder paper. For detailed data analysis, intracellularly recorded potentials were played back into the oscilloscope (Tektronix RM565) via a waveform recorder (Biomation 805). This microcomputer digitized the input waveform "sweep" (rate 1/sec) into 2048 points and permitted manual control of digital-to-analogue conversion for display on the oscilloscope and photography with a Polaroid camera equipped with a 75mm lens (Tektronix), or reproduction of the sweep on paper of an X-Y pen recorder (Hewlett-Packard 7015B).

III. RESULTS

A. ELECTRICAL MEMBRANE PROPERTIES OF TRG NEURONS

I. INPUT RESISTANCE MEASUREMENTS

Intracellular recordings were obtained from a total of 150 TRG neurons. Only cells that had stable resting membrane potentials more negative than -50 mV were selected for off-line analysis. Stable recordings could be maintained for as long as 2.5 hours in some neurons. However, most cell impalements were successful for shorter periods at reasonably negative membrane potential levels, depending on the experimental circumstances. In many instances, the input impedance of the electrode increased during stable intracellular recording (presumably due to clogging of the electrode tip) to an extent that currents which were required for a desired voltage displacement (e.g., 5-20 mV) of the membrane potential could not be injected without excessive interference from electrode rectification.

Initially, values of membrane input resistance (R_i) were obtained directly from chart recorder traces. This was achieved by measuring the slope of the linear portion in the I/V curve of the membrane voltage responses to hyperpolarizing current pulses. However, the majority of TRG neurons exhibited a voltage- and time-dependent rectification when hyperpolarizing current pulses (0.5-10 nA) were injected intracellularly. Figure 1 illustrates a current-voltage relationship of one such cell. It is readily apparent from the voltage recording traces

(inset) that only very small membrane potential displacements may provide accurate estimates of input resistance, i.e., voltage- and time-dependent rectification becomes apparent with the larger membrane potential displacements. Thus the R_i values estimated from the slope of the peak voltage response values are associated with a large error, and therefore a different method for obtaining R estimates was used.

A reliable estimate of R_i can be obtained from measurements of the potential near the end of responses to hyperpolarizing current pulses (50-100 ms duration: dotted line in Fig. 1). Although this method was satisfactory in some cells, complications arose in those cells where steady-state values of membrane voltage displacement could not be reached at the termination of the 50 ms current pulse. In addition, many electrodes possessed significant capacitance and rectification which could mask a sag in the membrane response of a neuron which exhibited time-dependent rectification to a hyperpolarizing current pulse injection; this also resulted in overestimates of the steady-state voltage displacement values. Figure 2 illustrates an example of prominent electrode rectification that could result in such overestimates.

Because of the above considerations, R_i in a majority of neurons was obtained using voltage displacements of less than 10 mV. An exception was made for observations of R_i changes due to drug applications. In such cases, changes in R_i due to drug administration were considered to be more important than absolute R_i values because they represented drug action.

Figure 1. Current-voltage relationship of a TRG neuron. Open circles represent steady state voltage responses to 50 ms hyperpolarizing current pulses. Closed circles indicate peak voltage responses to current injection. Electrode rectification becomes evident with large membrane potential displacements. This can be seen in the inset which displays the neuronal hyperpolarizing responses which were measured for the graph.

Figure 2. Control voltage traces obtained with a 50 MΩ, 3 M KCl filled electrode that showed pronounced rectification during passage of hyperpolarizing current pulses. Although the active bridge of the amplifier was well balanced at the onset of the pulses, electrode tip resistance increased during the course of current application as seen by the continuing increases in negativity of potential. This rectification did not include any increase in electrode capacitance because no large capacitive effect (i.e. a large voltage transient) was observed at pulse termination.
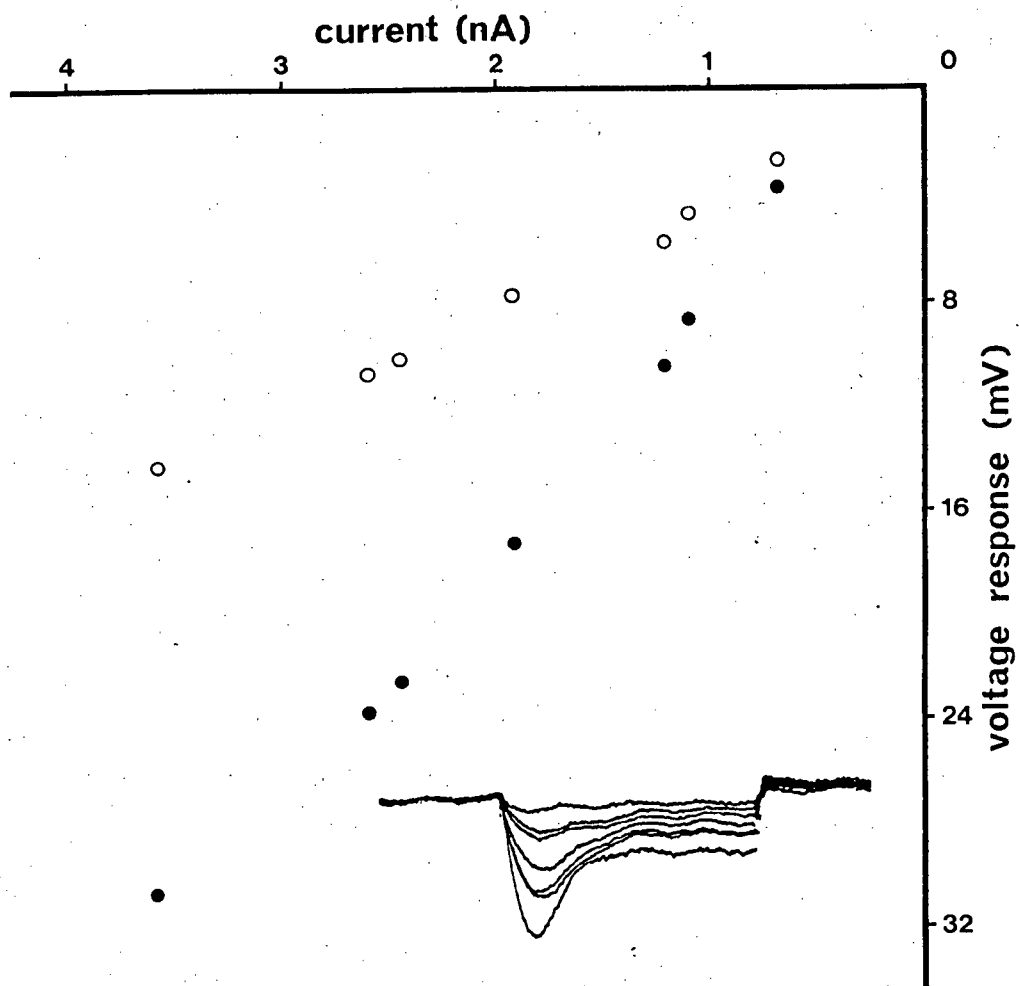
Figure 1

Figure 2
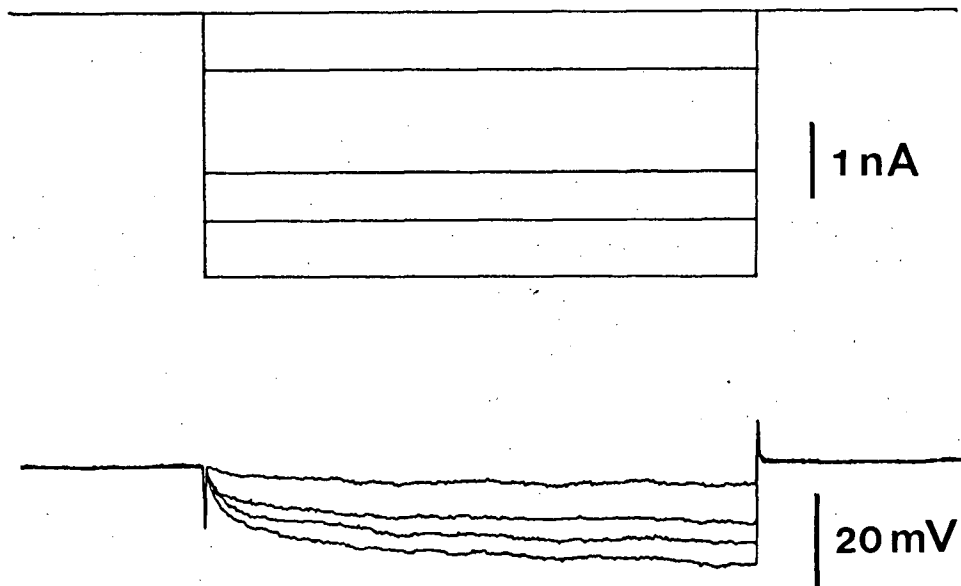
Figure 3 is a histogram of R_i values obtained from 80 TRG neurons. About 65% of this population of neurons fall within the range of 3 to 15 MΩ. The small number of neurons with high R_i values is at least partly a result of significant electrode sampling bias. A large number of neurons with high R_i values were impaled, but such cells could not be recorded from for long periods (e.g. greater than 10 min), presumably because of poor somatic impalements of small cells with coarse electrode tips. The data from such neurons were not used in analyses of input resistance. Use of electrodes with finer tips and, therefore, higher impedances likely would have facilitated stable or prolonged recordings from these neurons. However, high impedance electrodes (> 40 MΩ) did not have adequate current passing capability to allow generation of action potentials in most neurons that were penetrated with such electrodes. Therefore, electrodes with tip resistances less than 40 MΩ were used to achieve a compromise between the ability to successfully impale cells for prolonged recordings and the ability to inject sufficient amounts of current for analysis of membrane properties.

Figure 3. Distribution of measured input resistances in TRG neurons. Values of R_i were obtained by measuring the membrane voltage responses to small hyperpolarizing current pulses (< 10 mV) or by obtaining the slope of the linear portion of the steady-state responses in the I/V curves of neurons that showed a large amount of membrane rectification.

Figure 3
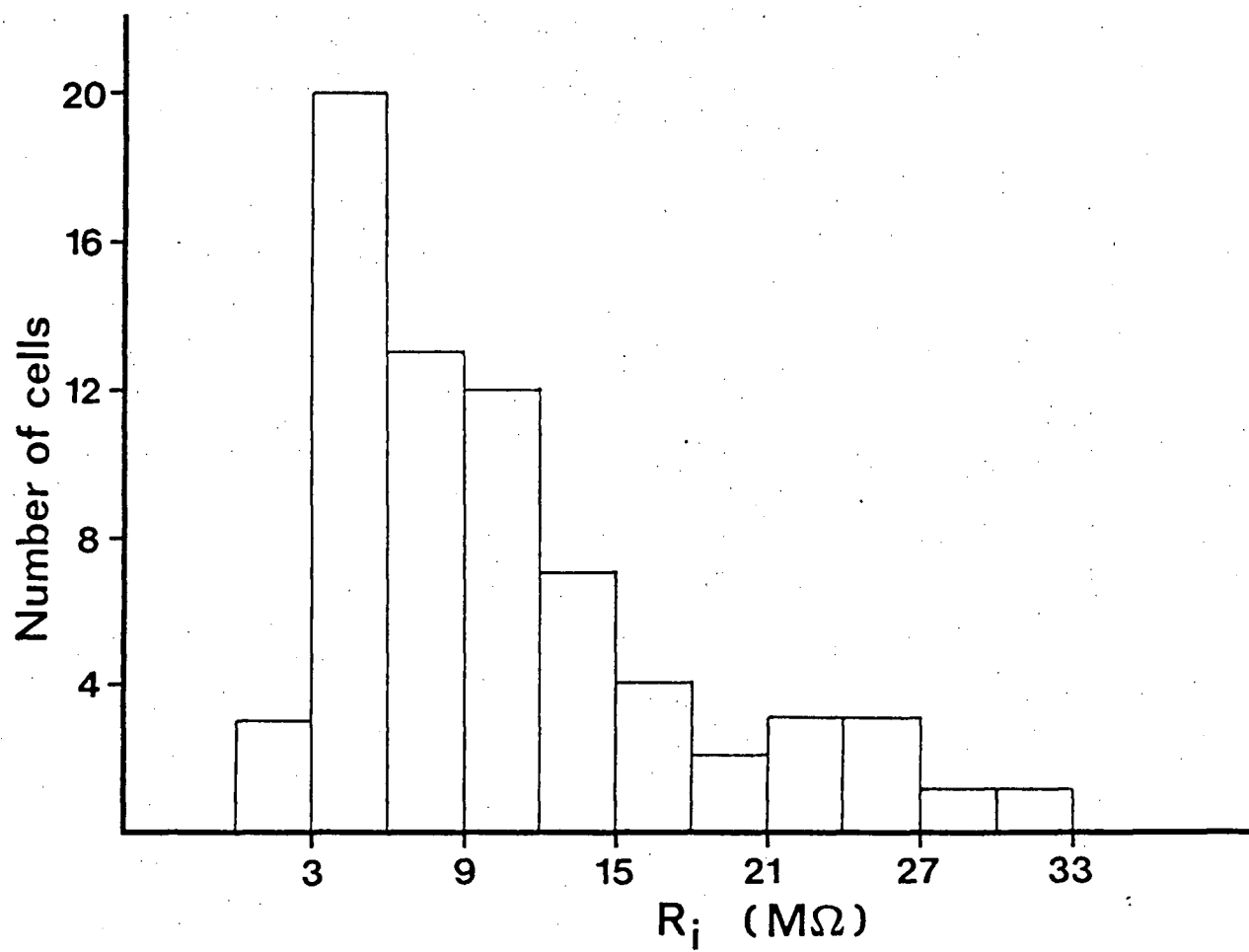
II. MEMBRANE VOLTAGE- AND TIME-DEPENDENT RECTIFICATION

The majority of TRG neurons exhibited varying degrees of membrane rectification upon hyperpolarizing current pulse injection. An example of this pronounced "sagging" behaviour of voltage responses is shown in Figure 4a. An opposite extreme in membrane behaviour of another neuron is shown in Figure 4b. Only 3 out of 80 neurons showed no detectable sag associated with hyperpolarizing membrane displacements of up to 35 mV. The "sagging" behaviour of most other neurons was less pronounced than that shown in Figure 4a. A possibility of extensive cell damage was excluded since these cells produced action potentials in response to repetitive injections of depolarizing current pulses and otherwise had stable membrane potentials. Electrode rectification and an inability to pass 'adequate' amounts of current may have resulted in a masking of a fast conductance increase in some neurons. This suggestion was excluded in many cases where the electrode was capable of passing currents of up to 7 nA without noticeable rectification. Some hyperpolarizing responses of one neuron are shown on an expanded time base in Figure 5. These records exhibit a characteristic voltage-dependence of the time-dependent rectification, or "sag" in the voltage response. It is readily apparent that an increase in the amount of hyperpolarizing current causes a faster approach to the steady-state value of membrane voltage displacement.

Figure 4. Two examples of extremes in membrane responses of TRG neurons. Calibration pulse (20 mV, 10 ms) in A applies to traces in A and B. Both neurons fired action potentials with overshoots.

Figure 5. Response of a neurone to hyperpolarizing current pulse injection which was recorded with a K_2SO_4 filled electrode. Note the variation of rate of voltage sag with increasing amounts of injected current. The bridge of the WPI amplifier was well balanced at both onset and termination of pulse. Electrode control upon withdrawal from the cell showed negligible rectification.
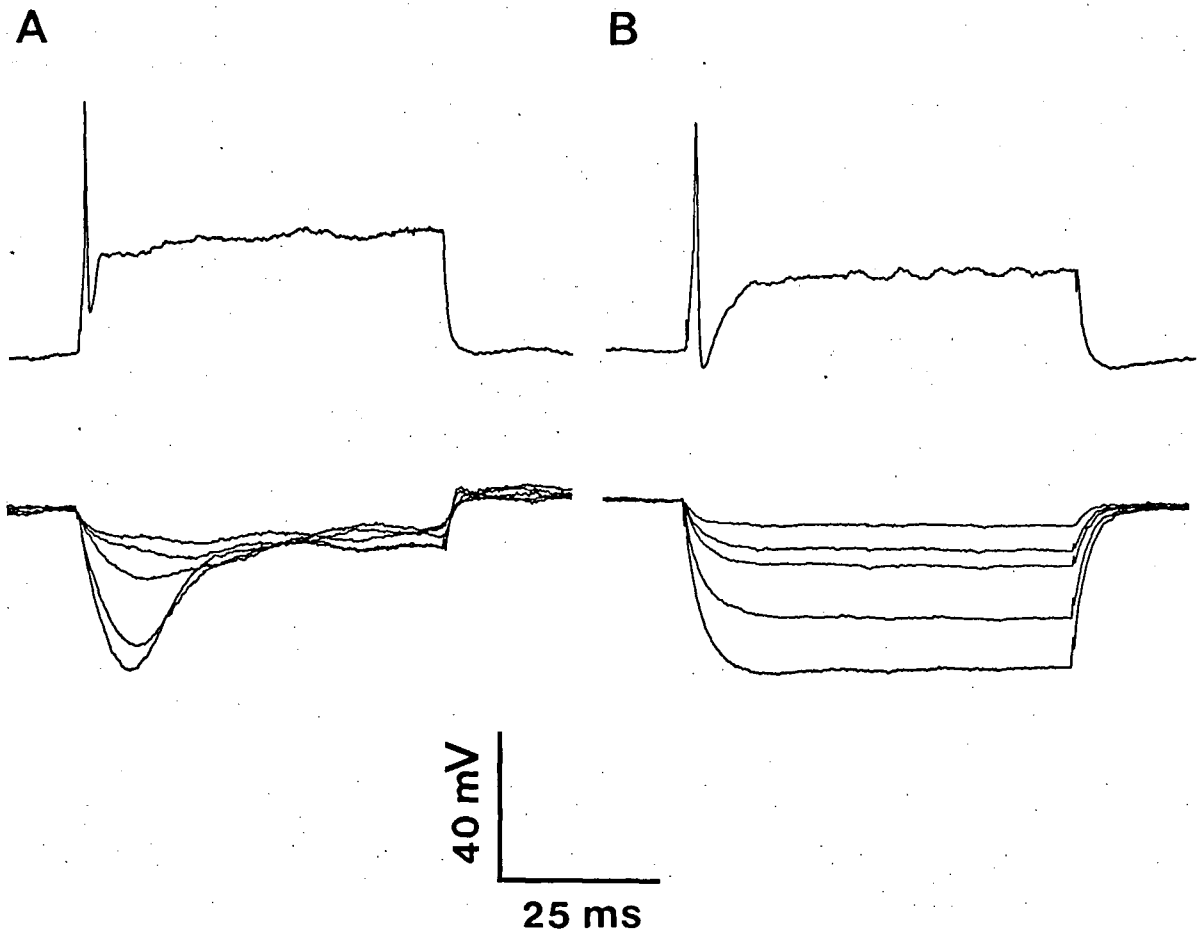
Figure 4

Figure 5
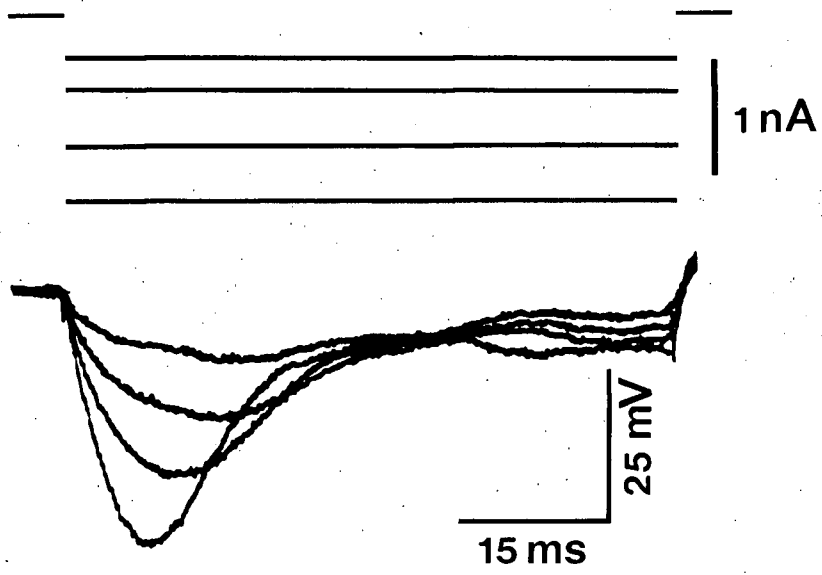
III. ACTION POTENTIALS

Injection of suprathreshold depolarizing current through
the recording electrode resulted in generation of one or more
action potentials.   In most cases and as mentioned previously,
ability to elicit action potentials was limited by the current
passing property of the electrode.   In many instances, the spike
amplitudes were small after initial penetration of the cell, but
later these were increased in amplitude. This increase was
presumably a result of sealing of the cell membrane around the
electrode,   since   membrane   hyperpolarization   occurred
concomitantly with increase of input resistance.   In some
neurons, however, the action potential amplitude remained small
even though resting membrane potential was at stable potentials
of -60 mV or better.

Typical action potentials elicited from two different TRG
neurons are shown in Figure 6.   After an initial membrane
response, a fast-rising and presumably somatic action potential
occurred; this was followed by repolarization and an
afterhyperpolarization (AHP).   Two types of action potentials
were readily distinguishable on the basis of spike durations and
a presence or absence of an inflection in the repolarization
phase.   The spike in Figure 6b had a longer duration and in
addition a "hump" appeared on the repolarizing phase.   Cells
which exhibited action potentials of this type also tended to
discharge action potentials in a repetitive manner in response
to large depolarizing current pulses.   In contrast, cells

exhibiting action potentials of the type in Fig. 6a tended to discharge only single action potentials, in response to injected current pulses, although some repetitive firing sometimes was observed (see Figure 7a).

Neurons which exhibited action potential amplitudes of less than 50 mV were not used for analysis of active properties. The mean amplitude of spikes without a plateau in the repolarization phase was 65 mV ± 1.9 mV S. E. (n=31). The mean amplitude of spikes that possessed a hump during repolarization was 78 mV ± 2.3 mV S. E. (n=7), significantly larger than in the previous group (P< 0.01).

The amplitude and duration of AHPs in neurons where AHPs could be measured without excessive interference from electrode rectification were also different for the two kinds of action potentials. Thus, AHPs following spikes with a hump had a mean amplitude and duration of 16.8 mV and 6 ms respectively (n=6). AHPs following action potentials in the second group had a mean amplitude of 14.1 mV and a mean duration of 2.9 ms (n=6).

Figure 6. Examples of action potentials evoked from two different TRG neurons. Arrows indicate the onset of a 50 ms depolarizing current pulse which was used to evoke the spikes in A and B. Note the presence of a hump during the repolarizing phase of the spike in B.

Figure 7. Action potentials and subthreshold behaviour of a trigeminal ganglion neuron. A: responses to depolarizing current pulses. Note the oscillations of the membrane voltage with injection of a 0.4 nA pulse of current. B: anodal break excitation of the same neuron resulted in generation of a spike.

Figure 6

Figure   7

A

10 ms

40mV

0.5 nA

0.4 nA

0.2 nA

B
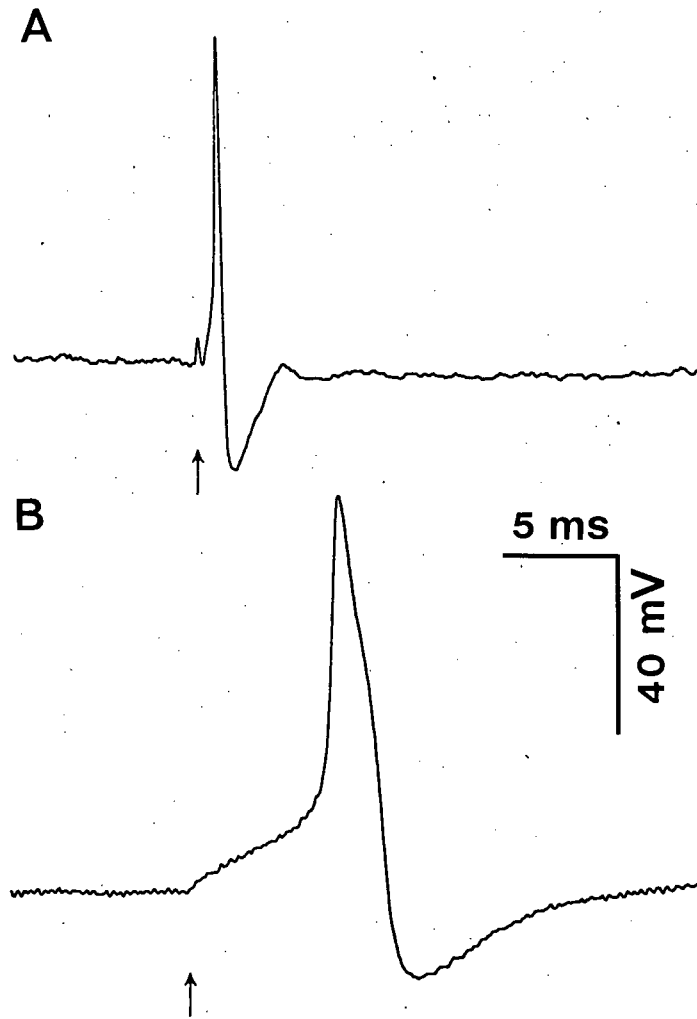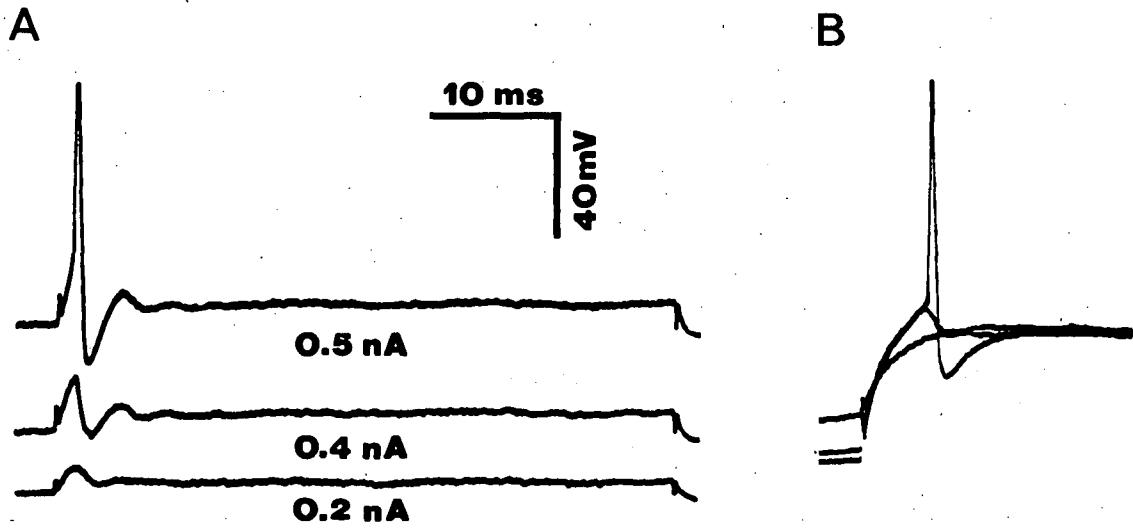
However, these values may not be very good approximations of the AHP parameters. Measurements of AHPs were made difficult by the superposition of action potentials with the intracellular injection of a current pulse (50-100 ms). The mechanism underlying the AHP would be compromised by the depolarizing current pulse. However, if the spike was evoked at the anodal break (e.g. Figure 7b,) the resulting AHP would be expected to be similar to that produced by an action potential that invades the soma from the peripheral axon (i.e. in the absence of intrasomatic current injection).

The size of the AHP was observed to be dependent on the membrane potential level. In neurons with action potentials that could be evoked by injection of depolarizing current pulses the AHP size was seen consistently to increase with depolarization. This phenomenon is illustrated in Figure 8 which shows oscilloscope traces obtained from a TRG neuron at two different values of resting membrane potential. This neuron exhibited 'fast' action potentials without a plateau in the repolarizing phase. At a more hyperpolarized membrane potential, the amplitude of the evoked action potential was larger than at depolarized levels and the peak amplitude of the AHP was reduced. An increased tendency to generate spikes also is evident; the responses in A and B were elicited with identical current pulses. This was similar to the increase in membrane oscillatory behaviour at subthreshold voltages, but here, action potentials (with large AHPs) were evoked and possibly are superimposed on the oscillations.

Figure 8. Effect of membrane potential on size of spike afterhyper- polarization (AHP). In both A and B, responses were elicited with a 2.7 nA depolarizing current pulse. In A, action potential height was smaller than in B, while the AHP is larger. Also note that two spikes were evoked at more depolarized membrane potential in A, whereas an identical current pulse evoked only a single action potential at hyperpolarized membrane potential in B.

Figure 8
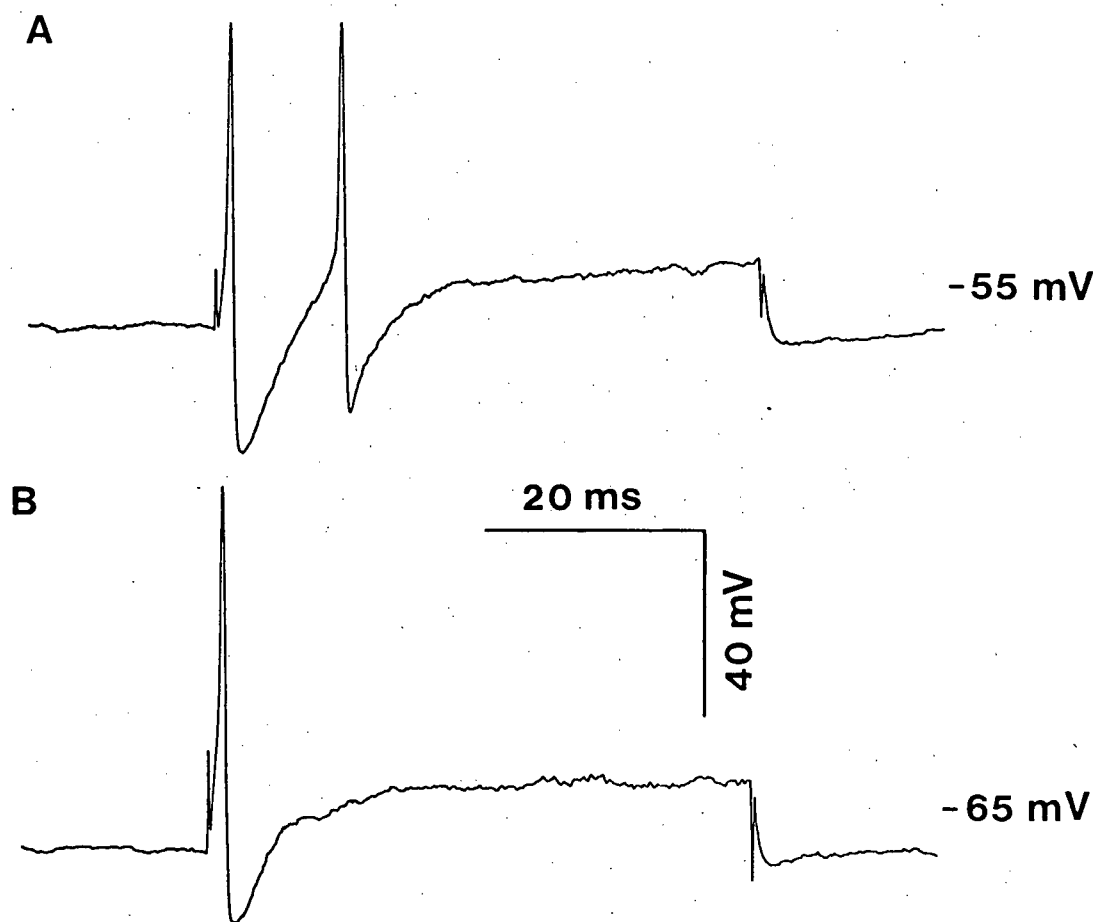
IV. MEMBRANE OSCILLATORY TENDENCY

Many TRG neurons displayed oscillatory fluctuations of membrane potential in response to depolarizing current pulses. Figure 7 illustrates the potential responses to current pulse injections in one such neuron. Oscillatory behaviour is evoked at currents just subthreshold to full spike generation. In such cells, large hyperpolarizing current pulses also evoked oscillations of membrane potential following termination of the current pulse. If membrane potential displacement (or injected current) was large enough, an "anodal break response" or spike followed termination of the pulse, as illustrated in Figure 7b.

Oscillatory membrane behaviour was observed most readily in cells which had a relatively high R_i. A tendency of neurons to exhibit greater potential oscillations at the more depolarized membrane potentials also was observed. Neurons that showed steady membrane depolarization with time, presumably due to a poor impalement or a shunt around the cell-electrode seal, were more likely to display oscillatory responses at depolarized potentials. An example of oscillatory behaviour at a depolarized membrane potential can be seen in Figure 9 where the behaviour is much more pronounced at -52 mV than at -60 mV.

Repetitive firing could be evoked in some neurons with a sufficiently large depolarizing current pulse. The latency of onset as well as the number of action potentials could be changed by varying the amplitude of applied current pulse. An example of this behaviour is shown in Figure 10. Note the

changes in latency of onset of spikes with systematic increases in intensity of injected current. It is readily apparent that the fast rising and repolarizing phases of the somatic action potential are unchanged, but the initial phase of the action potentials becomes longer with each sucessive action potential.

Figure 9. Example of increased oscillatory behaviour of a trigeminal ganglion neuron at depolarized membrane potentials. A: Sub and suprathreshold responses at -60 mV resting membrane potential. B: Subthreshold response of the same neuron at a depolarized membrane potential of -52 mV evoked with injection of the same amount of current as in the case of the subthreshold response in A.

Figure 10. Repetitive firing in a trigeminal ganglion neuron. Increase in amount of depolarizing current led to an increase in spike discharge frequency. The magnitudes of depolarizing currents were 0.16, 0.21, 0.32, 0.41 nA in A, B, C and D respectively. Increase in latency of somatic spike initiation can be readily seen in C and D. Also, note the subthreshold membrane oscillations prior to spike generation.

Figure 9

Figure 10

A

0.16 nA

B

0.21 nA

C

0.32 nA

D

0.41 nA
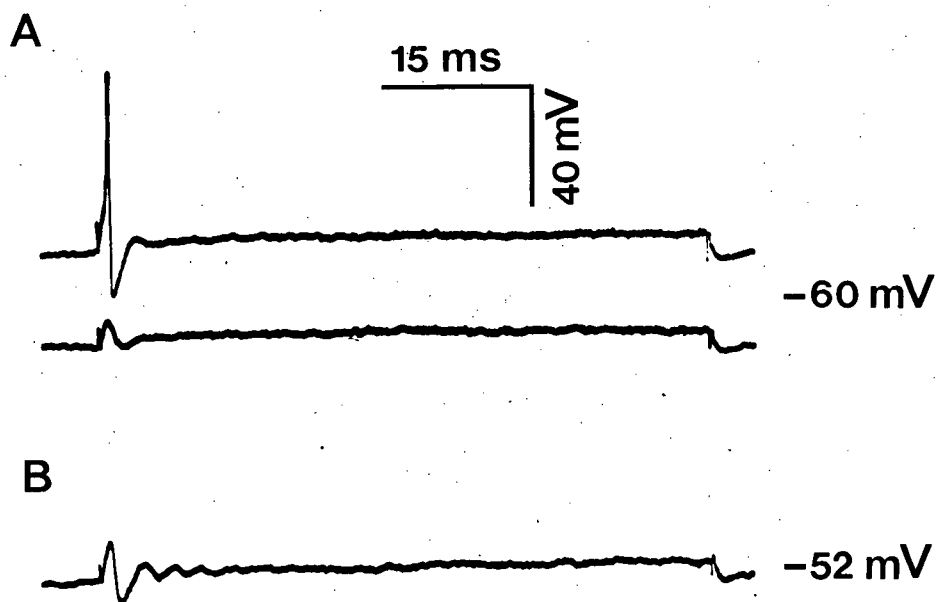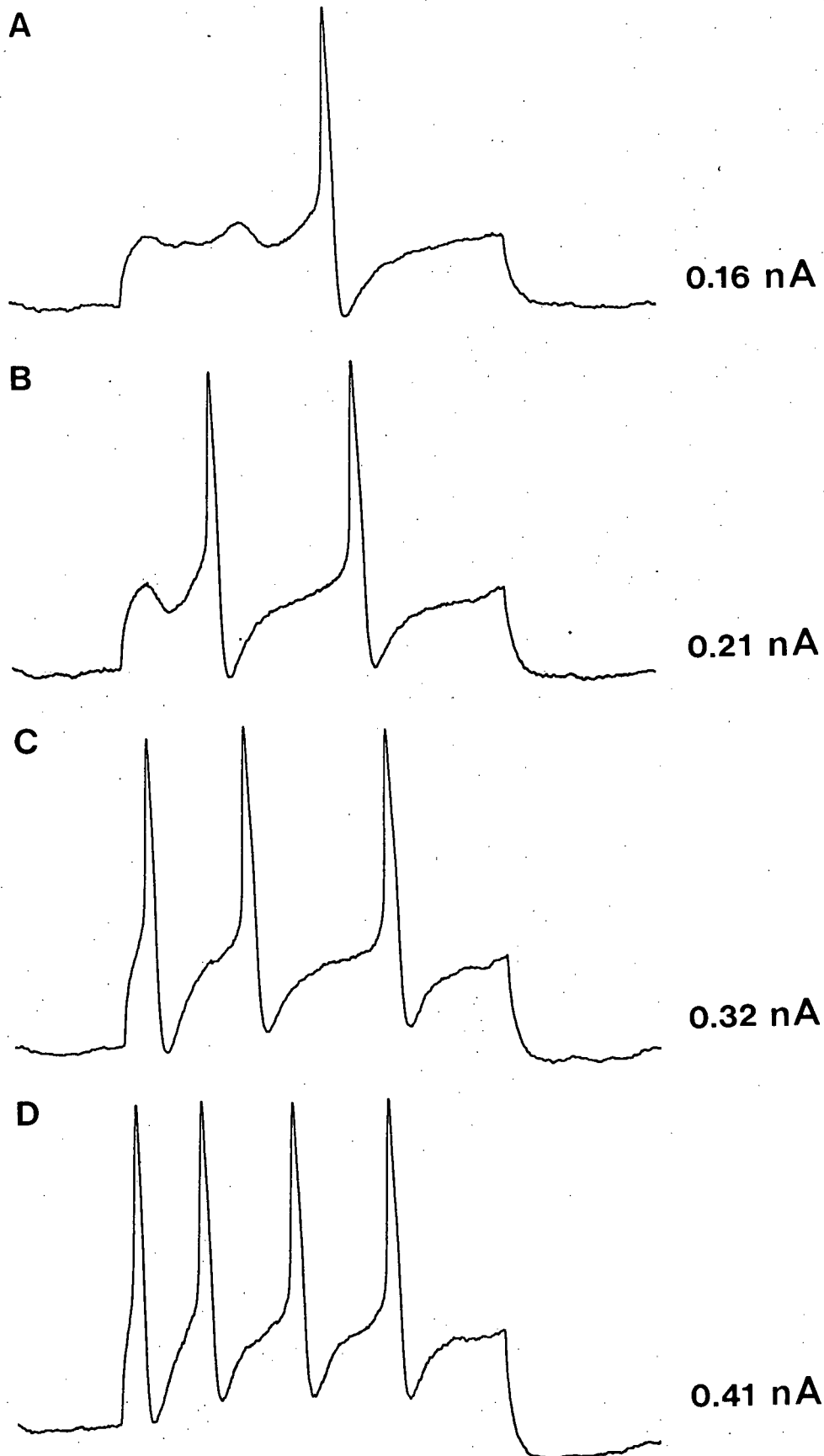
B. PHARMACOLOGICAL PROPERTIES OF TRG NEURONS

   I. EFFECTS OF S-GLUTAMATE

       Effects of S-glutamate were studied in 6 TRG neurons.
Bath applications of glutamate at a concentration of 10^{-2} M did
not elicit changes in membrane potential or input resistance in
four neurons.   In the other two neurons, such glutamate
applications evoked small depolarizations (1-2 mV) which were
not associated with noticeable changes in R_i.   An example of
this response is shown for one neuron in Figure 11a.   In both
cases, glutamate-evoked depolarizations were fully reversible
upon return to the control perfusate.

   II. EFFECTS OF 5-HYDROXYTRYPTAMINE (5-HT)

       Responses to 5-HT (10^{-3} M) were studied in 6 neurons.   In
five of these neurons no changes in membrane potential or input
resistance could be detected with up to 5 minutes of drug
perfusion.   In one neuron (Figure 11b), application of 5-HT
resulted in membrane conductance decrease, measured with
hyperpolarizing current pulses, concomitantly with a small
depolarization of the membrane. Return to the control perfusate
abolished this effect.

III. EFFECTS OF GAMMA-AMINOBUTYRIC ACID (GABA)

GABA in concentrations ranging from 10^{-4} to 10^{-2} M was applied to 36 neurons. In six neurons, GABA application did not elicit detectable changes in either resting membrane potential or R_i. GABA application to the thirty other neurons (some of which failed to respond to glutamate applications) produced a decrease in R_i which occurred concomitantly with membrane depolarization (range: 2-14 mV). The response of one neuron to an application of GABA is reproduced in Figure 11c. Following the initial membrane depolarization and conductance increase which reached their maxima, slight hyperpolarization from peak membrane depolarization was observed, possibly due to receptor desensitization. This decrease in maximum response occurred in all cases where GABA was applied for prolonged time periods. Upon returning the perfusion back to one of control solution, there is a gradual return of both R and membrane potential to levels observed prior to application of GABA. Time for recovery depended in part on the concentration of applied drug and on the rate of perfusate flow. The latency of maximal response to GABA application was decreased when higher concentrations of GABA were applied.

Figure 11. Responses of three neurons to bath applications of glutamate (10^{-2} M), 5-HT (10^{-3} M) and GABA (10^{-3} M). Lines were drawn through the resting membrane potentials in traces of chart records of both A and B. No changes in membrane input resistance were observed in A and the hyperpolarizing pulses therefore were omitted from the trace for better visualization of the change in membrane potential. The resting membrane potential prior to drug application was -60 mV in all three neurons. Note the reduction in membrane depolarization that occurs prior to return to control perfusate in C.

Figure 11

A

glu 10^{-2}M

$|$10 mV

0   1   2

2   3 min   4

B

5-HT 10^{-3}M

$|$10 mV

0   1 min   2

C

GABA 10^{-3}M

$|$10 mV

0   1   2

2   3 min   4
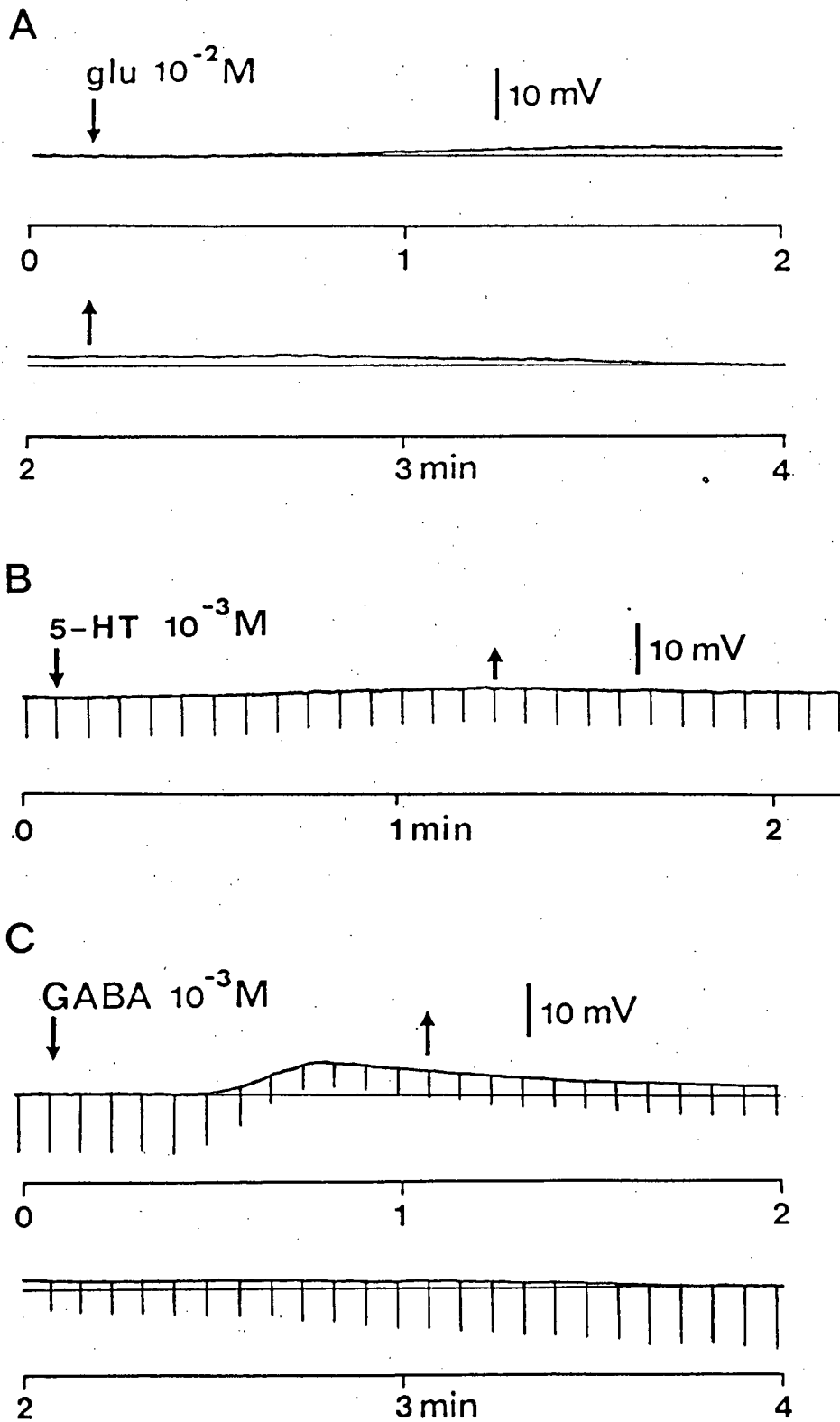
Table I - Effects of bath applied GABA on some membrane
properties of TRG neurons

Cell #	[GABA]	ΔV_m(mV)	ΔR_i (MΩ)	% ΔR_i	V_m(mV)
1	10^{-2}M	5	0.9	19	−70.5
2	10^{-2}M	3.5	4.0	25	−60
3	10^{-2}M	12	5.4	56	−60
3	10^{-2}M	9	5.4	53	−57.5
4	10^{-3}M	7.5	2.6	51	−57
5	10^{-3}M	3	2.6	57	−60
6	10^{-3}M	2.5	1.3	23	−66
7	10^{-3}M	5	5.9	54	−55
7	10^{-3}M	6	7.7	63	−59
8	10^{-3}M	3.5	18.6	51	−60
9	10^{-3}M	14	2.8	27	−54
10	10^{-3}M	3	4.9	51	−65
11	10^{-3}M	5	2.6	35	−62
12	10^{-4}M	2	2.0	35	−59
12	10^{-3}M	2.6	3.7	51	−61
13	10^{-3}M	2	1.1	19	−55
14	10^{-4}M	2.5	1.2	34	−63.5
14	10^{-3}M	5	2.0	51	−65
15	10^{-3}M	4	1.9	40	−61
16	10^{-4}M	3.5	1.4	25	−65
16	10^{-3}M	4	4.0	42	−74
16	10^{-2}M	5.5	3.5	31	−79

Table 1 summarizes the effects of GABA applied in three different concentrations to 16 TRG neurons. Because estimates of R_i obtained during experiments with GABA applications are not very accurate approximations of true (i.e. ideal) R_i, the %ΔR_i gives a better indication of the effect of GABA than ΔR_i. It can be seen from the tabulated results that there is no clear correlation of the resting membrane potential with the amplitude of maximal response to GABA. In addition, although there is a definite relation between %ΔR_i and maximal depolarization produced at different GABA concentrations in the same cell, this does not seem to be the case when a comparison is made between different neurons. For example, in an extreme case, a 27 % change of R_i in cell number 9 produced a 14 mV depolarization while a 54 %ΔR_i in cell number 7 produced only a 5 mV depolarization. In both cases, a 10^{-3} M concentration of GABA was applied when the cells had similar resting membrane potentials and input resistances. These results are suggestive of a differential sensitivity to GABA in TRG neurons.

The increase in membrane conductance evoked by application of GABA resulted in diminution of the amplitude of action potentials produced by injection of depolarizing current pulses. The decrease in membrane potential cannot account for the much larger decrease in action potential height because amplitudes of spikes generated by 50 ms depolarizing current pulses, concomitantly with DC current injection to depolarize the resting membrane potential, are not reduced to the extent seen with GABA-mediated depolarizations. Effects of GABA on the

spike height are illustrated in Figure 12. At a maximal membrane depolarization of 10.5 mV evoked with a 10^{-3} M concentration of GABA, the corresponding decrease in spike amplitude is approximately 50 mV. Therefore depolarization of the membrane alone apparently was not entirely responsible for the reduction in size of the action potential.

It is important to note that in all cases where drug applications were made, none of the impaled neurons were observed to discharge action potentials that had a hump in the repolarization phase of the spike. Neurons that were sampled during the pharmacological tests either could not be induced to fire spikes due to limited current passing ability of the electrode or discharged spikes without a hump in the repolarizing phase of the spike.

Figure 12. Plot of membrane potential and action potential amplitude changes resulting from bath application of 10^{-3} M GABA. A clear correlation can be observed between the GABA-mediated membrane depolarization and the decrease in the amplitude of the spikes evoked by suprathreshold (1.5 X) depolarizing current pulses. Note that the decrease in spike height is much greater than the corresponding decrease in membrane potential suggesting a shunting effect of the GABA-evoked increase in membrane input conductance.
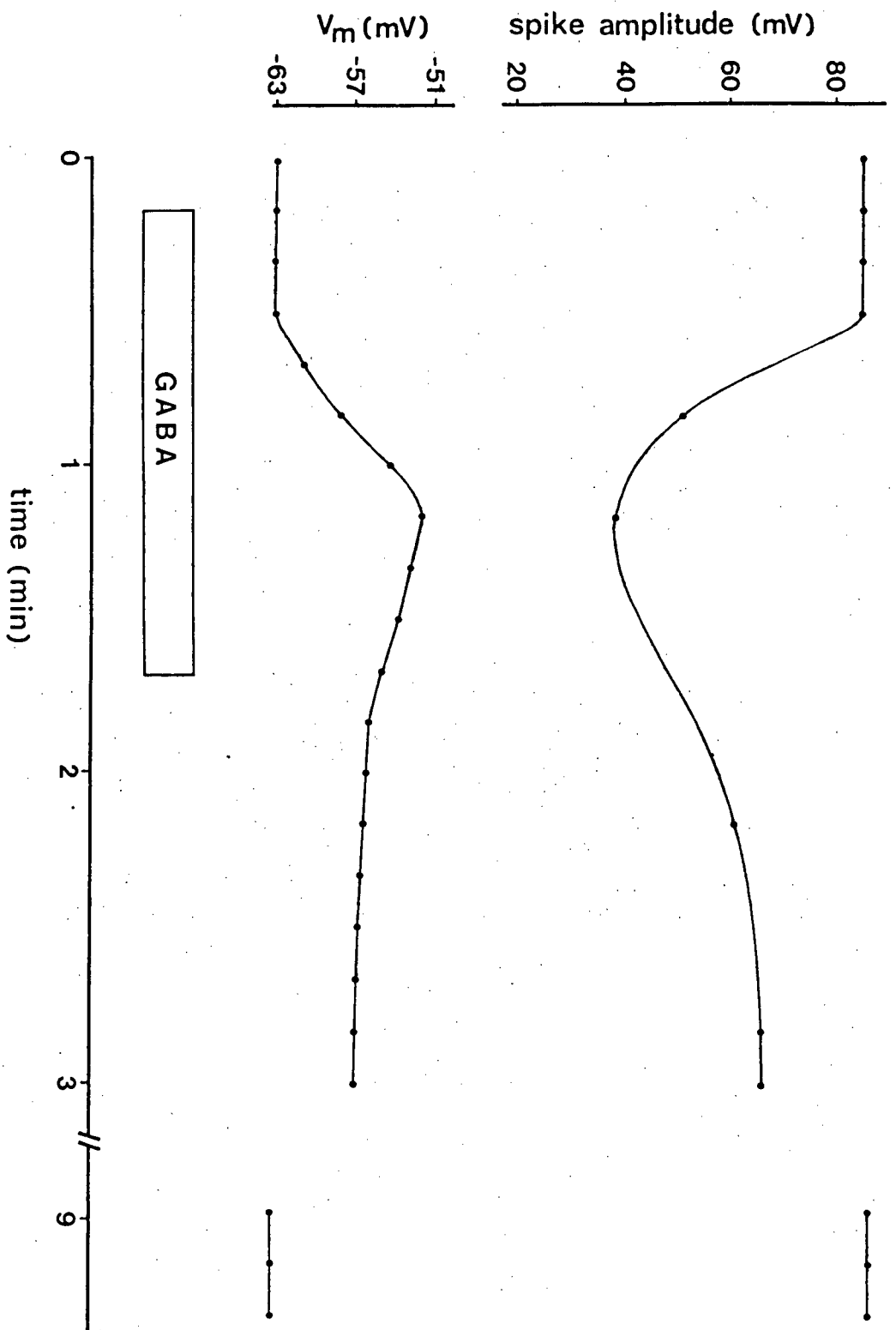
Figure 12

IV. <u>DISCUSSION</u>

A. <u>PHYSIOLOGICAL ROLE OF THE SOMATA OF PRIMARY AFFERENT NEURONS</u>

The offstream anatomical position of the perikarya of the cerebrospinal ganglia with respect to their axonal processes that transmit sensory information from periphery to the CNS has led to the view that ganglion cell bodies are of limited significance in terms of an electrophysiological role in the intact animal (Lieberman, 1976). This view is supported by the evidence that the majority of somata in cerebrospinal ganglia appear to be devoid of synaptic contacts. However, a small number of synaptic bouton terminations have been demonstrated conclusively to impinge on perikarya in the cat DRG. The origin of axons that synapse on these sensory cell bodies have been located in the spinal cord (Kayahara et al, 1984). Spontaneous electrical activity was not observed in our intracellular recordings from freshly isolated TRG cells which invariably were quiescent prior to passage of current through the intracellular electrode or stimulation of axonal processes. Also, Darian-Smith (1973) has shown that the centrally located terminals of large myelinated axons are depolarized following peripheral stimulation, before excitation is observed within the ganglion. Thus, most of the available evidence would not indicate that synaptic activity is significant in sensory ganglion function. However, the presence of receptors for GABA as indicated in our

investigation of TRG cells and synaptic contacts in DRG cells would suggest that caution should be exercised before ruling out a synaptic modulating function in either ganglia.

In addition to the above mentioned observations, the perikarya of the primary sensory neurons synthesize compounds necessary for the overall functioning of the cell, including its central and peripheral terminals. Substances synthesized in the perikaryon travel via fast and slow axoplasmic transport to both terminal ends where they may be utilized for the various functions including neurotransmitter release. The synthesizing or "factory" role of the soma is supported by the reported presence of putative transmitters such as S-glutamate and substance P in the somata of primary sensory neurons. The presence of these substances has also been demonstrated in neurons of the TRG (Hokfelt et al, 1975).

In spite of the evidence suggesting an electrophysiologically passive role of the sensory neuron cell bodies, some evidence indicates a possible modulatory role in sensory signal transmission. An invariable finding of intracellular electrophysiological studies of DRG is that an action potential at the periphery always invades the soma regardless of the species and the degree of complexity of the initial glomerular segment of the stem process (Svaetichin, 1951; Ito, 1957; Sato and Austin, 1961; Scott et al, 1969). Also, there is a delay of impulse transmission that consistently occurs for arrival of a spike in the ganglion, presumably at the junction of peripheral, central, and stem processes (Dun, 1955).

More importantly, some experimental evidence implies that the cell body and stem process may function as a filter and modulator of incoming signals by damping signals that lie outside the natural resonant frequencies (cf. Puil et al, in press). Tagini and Camino (1973) have shown that under conditions of fatigue induced by high frequency stimulation a second action potential is generated in the vicinity of the ganglion following the electrically induced spike, measured intra-axonally in the frog spinal nerve. Surprisingly, the second action potential travelled antidromically towards the periphery, apparently without invading the central process. This phenomenon subsequently was shown to occur not only as a result of artificially induced fatigue but also as a consequence of action potential propagation through nonhomogeneous regions (Ramon et al, 1975). Rebound action potentials of long duration were demonstrated when a spike travelled from a region of fast conduction, to a region of slow conduction velocity.

Perikarya of amphibian DRG neurons do not possess extensive stem process convolutions (glomeruli), which are much more prominent in mammals, especially carnivores (Chase, 1909). This would suggest that secondary action potential generation would be much more prominent in mammalian sensory neurons. Unfortunately, no studies have been yet made to test this possibility. In the trigeminal system, antidromic stimulation of the sensory fibers is known to produce vasodilation and plasma extravasation as it does in other neuronal systems (Couture and Cuello, 1984). It seems quite possible that this

phenomenon may occur as a result of rebound action potentials that travel antidromically, following intensive sensory receptor activity, in normal physiological circumstances.

Normally, cell bodies of neurons in cerebrospinal ganglia do not generate action potentials in absence of input, but spontaneous activity has been recorded in dorsal roots of cat and rabbit spinal ganglia one or more days after transection of the spinal nerve root distal to the ganglion (Kirk, 1974). Unlike the findings of Tagini and Camino (1974), the action potentials subsequent to axotomy, propagated only along the centrally directed processes and not to the periphery.

It is conceivable that in certain conditions such as trigeminal neuralgia, degeneration of the peripheral axons may be involved in the induction of abnormal activity in sensory ganglion cells. Alternatively, excessive reduction in conduction velocity of stem processes could result in a production of abnormal rebound activity. This in turn may invoke a release of algogenic substances as a reflex consequence of cutaneous vasodilation and extravasation, thereby eliciting painful sensations. The latter suggestion seems less likely since the pain experienced by patients afflicted with trigeminal neuralgia is fast in onset; indeed, its termination is unlike the slowly developing protracted pain experienced after peripheral administration of compounds such as histamine or 5-HT.

B. ACTION POTENTIAL CHARACTERISTICS OF PRIMARY AFFERENT NEURONS

Many previous studies have contributed to a better understanding of membrane properties and function of the primary sensory neurons of different mammalian species. Use of intracellular recording techniques combined with extracellular stimulation of the peripheral processes of the neurons has allowed classification of different types of sensory neurons according to their conduction velocities and action potential characteristics; these studies would include DRG (Bessou et al, 1971), nodose ganglion (Gallego and Eyzaguirre, 1978), and petrossal ganglion of the cat (Belmonte and Gallego, 1983), as well as mouse DRG (Yoshida and Matsuda, 1979), or rat DRG (Harper and Lawson, 1985).

In our investigations, two types of action potentials could be distinguished readily on the basis of their durations and the absence or presence of a hump in the repolarizing phase. Other investigators have shown that an influx of calcium, in addition to that of sodium, plays a role in the genesis of action potentials which exhibit long durations with a hump in the repolarizing phase (Yoshida et al, 1978). Such action potentials in rat DRG are produced in unmyelinated C-fibers with conduction velocities less than 1.4 m/s (Harper and Lawson, 1985). The sodium component in these types of action potentials is only partly sensitive to blockade with tetrodotoxin (TTX), whereas the spikes without a hump are blocked completely by TTX administration (Yoshida et al, 1978; Gallego, 1983). The

proportion of neurons with TTX-resistant to TTX-sensitive action potentials varies greatly between different species and preparations. The main cause of this variability is the differences in primary afferent neuron populations. For example, in mouse DRG, TTX-resistant spikes are present in 33% of sampled neurons (Yoshida et al, 1978), whereas in cat petrossal ganglion, 90% of cells with myelinated axons have spikes resistant to TTX (Gallego, 1983).

Because stimulation of central or peripheral axons was not employed in the present study, it was possible to classify the TRG neurons only in 2 groups on the basis of action potential differences. Surgical and anatomical constraints limit the length of the TRG slices that can be obtained. Further classification would require axonal stimulation and measurements of conduction velocities. This would be accomplished most easily under in vivo exerimental conditions where intact axons can be stimulated at their peripheral receptors or at the central processes. One also may expect the sampled populations of sensory neurons in TRG to be more homogeneous than those found in the dorsal root ganglia of mammalian species, partly because the cell bodies of myelinated, fast conducting fibers of spindles and tendon organs of the the jaw musculature are located in the mesencephalic nucleus in the brainstem (Lieberman, 1976). Thus, there should be proportionally fewer cell bodies of the fastest conducting fibers in the ganglion proper.

The mean size and duration of AHPs was different in TRG

neurons which discharged spikes with a hump compared to neurons whose action potentials did not have this property. In the frog DRG, action potentials of C-neurons are followed by AHPs with amplitudes and durations which exceed those of A-neurons (Holz et al, 1985). In the rabbit nodose ganglion, AHP amplitude is similar in C-neurons and A-neurons, but the duration of AHPs is much greater in C-neurons (Stansfield and Wallis, 1985). A calcium component is present in cat petrosal ganglion neurons that exhibit action potentials with a hump, and because Ca^{+2} - channel blockers decrease the amplitude and duration of the long AHPs in these neurons, it was proposed that the long AHPs are produced by activation of the Ca^{+2}-dependent K^+ conductance (Gallego, 1983). However, it remains to be shown whether a Ca^{+2}-dependent K^+ conductance is responsible for the AHPs in those TRG neurons that have long duration spikes with a hump. In TRG, the observation that AHP size in spikes without a hump increases with membrane depolarization implies a dependence of these AHPs on K^+ conductance.

C. VOLTAGE- AND TIME-DEPENDENT RECTIFICATION IN SENSORY NEURONS

Most of the TRG sensory neurons were shown to possess membrane rectification in response to hyperpolarizing current pulses. This phenomenon, first described in frog DRG neurons by Ito (1957) has been called "time-dependent rectification". Czeh, Kudo and Kuno (1977) proposed an increase in sodium conductance (g_{Na}) produced by the removal of partial

inactivation of Na and/or Ca channels, to account for this phenomenon. An involvement of g_{Na} also has been supported by the data of Gallego (1983) who showed the rectification to be either partially or completely abolished in sodium free solutions in the presence of TTX. Mayer and Westbrook (1984) who used a voltage-clamp analysis of cultured mouse DRG neurons found time-dependent rectification to be due to a "mixed" Na^+ and K^+ current.

Results of the present study indicate that the rectification in TRG neurons may be both time- and voltage-dependent. This proposition stems from two observations made consistently in this study. First, the time course of membrane depolarization from the intital peak hyperpolarizing response to a steady state value depended largely on the amount of membrane displacement by current pulse injection in a given neuron. Secondly, the extent of membrane rectification also seemed to be dependent on the resting membrane potential of the neuron. Thus membrane rectification was easier to elicit with hyperpolarizing current pulses in cells with very negative resting membrane potentials. To verify these observations, current pulses of 50 ms duration were superimposed on tonic DC currents and the degree of membrane rectification was compared for depolarized and hyperpolarized membrane potential values on the same neuron. Larger sagging hyperpolarizing responses could be obtained at relatively hyperpolarized resting potentials. This may be explained partially on a basis that resting conductance is higher in TRG neurons at depolarized membrane potentials (Puil

et al, unpublished observations) thus requiring larger hyperpolarizing current inputs for membrane potential displacement. Another possible contributing factor may be that sodium channel activation occurs more quickly at more negative membrane potentials or that there is simply a larger driving force for sodium ions [defined by $(V_m - E_{Na})$] that accounts for a faster time course of the sag at more negative resting potentials.

Sag behaviour appeared to be absent in a small proportion of TRG neurons but this does not mean necessarily that these cells represent a distinct or separate group of sensory neurons. Czeh, Kudo and Kuno (1977) have shown that somata of DRG neurons undergo significant changes in membrane electrical properties following axotomy of the peripheral fibers. In particular, at two weeks following axotomy, the number of cells exhibiting sag behaviour was reduced drastically. The amount of current required to induce rectification also was much greater on the average in cells which showed rectification. It is not clear, however, if the change in membrane properties occurs over a short period of time, i.e. within hours of axotomy which was usually within the time period that intracellular recordings were made following excision from the animal in the present investigations. Interestingly, neurons that showed no sag behaviour also are encountered on occasion during recordings from cells in in vivo experiments where the peripheral axons are presumably intact (Puil, et al., in press).

D. ACTIONS OF GLUTAMATE IN SENSORY GANGLION NEURONS

S-Glutamate is recognized as the major impermeant intracellular anion in the CNS of vertebrates (Puil, 1981). In the spinal cord it was proposed as a neurotransmitter of the primary afferent fibers implicated in monosynaptic excitation (Graham et al, 1967). The highest concentrations of glutamate in the spinal cord occurs in the dorsal horns, but the dorsal roots and the DRG also contain higher concentrations than those found in the ventral horns (Johnston and Aprison, 1970). It was of interest to observe possible effects of glutamate on TRG neurons especially in view of past reports where glutamate was without effect on DRG (DeGroat et al, 1972) and superior cervical ganglion (Bowery and Brown, 1974) neurons. Also, other investigators have reported weak effects in the DRG (Gallagher et al, 1978) as well as in the primary afferent mesencephalic neurons of the trigeminal nerve (Henderson et al, 1982).

In our investigations, glutamate applications resulted in either small membrane depolarizations or no effect on membrane electrical properties of six TRG neurons. The results indicate a similarity between somatic responses to glutamate of TRG neurons and neurons in the trigeminal mesencephalic nucleus or in the DRG. An absence of significant effects of glutamate on membrane electrical properties of these neurons suggests an absence of glutamate receptors such as those demonstrated in the CNS.

Also, perikarya of rat DRG neurons have been shown to lack

an uptake system for glutamate. Instead, glutamate is taken up by the surrounding glial cells (Schon and Kelly, 1974), while the perikarya of DRG neurons exhibit high affinity uptake for glutamine (Duce and Keen, 1983). Similar observations were made with inner hair cells and glial cells in the guinea pig cochlea (Eybalin and Pujol, 1983). These investigations provide strong evidence for the presence of a neuronal-glial glutamine cycle in sensory neuron ganglia (cf. Shank and Aprison, 1981). This cycle provides neurons with a substrate for synthesis of glutamate and its subsequent utilization in various cellular functions, including the formation of neurotransmitter pools. Interestingly, the small neurons that have been shown immunohistochemically to contain other transmitter candidates such as substance P or somatostatin (Hokfelt et al, 1976) take up glutamine at six times the rate seen in large diameter DRG neurons (Duce and Keen, 1983). This observation seems to contradict the proposed neurotransmitter candidacy of glutamate in large diameter primary afferent neurons that are involved in monosynaptic reflexes (Graham et al, 1967) because one would expect greater uptake of a substrate for glutamate synthesis in neurons that utilize it as transmitter.

E. ROLE OF 5-HYDROXYTRYPTAMINE IN SENSORY TRANSMISSION

An algogenic compound, 5-hydroxytryptamine (5-HT), has been implicated in a variety of biological reactions, for example, it causes vasoconstriction when released from platelets following

injury (Mills et al, 1968). 5-HT also is thought to be the neurotransmitter of neurons whose cell bodies are grouped mainly in the midbrain raphe nuclei (Consolazione and Cuello, 1982). When injected beneath the skin, 5-HT causes a pronounced and long-lasting discharge in sensory nerves of the rat. Mast cells of the rat contain 5-HT in addition to histamine which did not cause neuronal discharge (van Gelder, 1962). Application of 5-HT to isolated bullfrog DRG has been shown to produce slow maintained and fast transient depolarizations in both fast conducting A-neurons and slow C-neurons (Holz et al, 1985). Also, slow conducting C-neurons in the rabbit nodose ganglion recently have been shown to produce predominantly depolarizing responses in response to 5-HT application, whereas A-cells were not affected (Stansfield and Wallis, 1985). In the guinea pig TRG, 5-HT has been biochemically and immunohistochemically located in the mast cells, but not in the neuronal cell bodies (Lehtosalo et al, 1983).

If the release of chemicals such as 5-HT from the mast cells occurs in the skin it is possible that similar release of 5-HT from mast cells in the TRG may produce responses in the cell bodies, provided receptors for 5-HT are present on TRG neurons. Another important reason for studying 5-HT effects on these neurons is the proposed modulation of sensory information by primary afferent terminals of neurons containing substance P and 5-HT.

Demonstration of 5-HT and substance P in the cell bodies of raphe nuclei neurons provided the first example of coexistence

of neuropeptide and neurotransmitter in the CNS (Chan-Palay et al, 1978; Hokfelt et al, 1978). Stimulation in the region of medullary nucleus raphe magnus and dorsal raphe nucleus results in profound surface analgesia, whereas interruption of descending axons of these neurons blocks the analgesic effect of stimulation (Kelly, 1981). The analgesic effect is very specific in that other sensory modalities remain unaffected during stimulation, suggesting a close interaction of raphe neurons with pain pathways, which led to the model proposed by Iversen (1978). According to this model, 5-HT-containing fibers excite the enkephalin-containing interneurons which in turn inhibit the release of transmitter (substance P) from the terminals of the pain fibers. A possibility still exists that inhibition of nociceptive pathways is mediated by direct influence of the raphe neurons on primary afferent terminals.

Unlike neurons in frog DRG or C-neurons in rabbit nodose ganglion, the six primary afferent neurons in the TRG that were tested seemed to be little affected by bath application of 1mM 5-HT. In view of the fact that 5-HT depolarizes only C-neurons in the nodose ganglion, it is quite likely that this also may be the case in TRG. The number of stable recordings from cells with long duration spikes was limited, presumably because of the small cell size which makes it more difficult to successfully record from C neurons using relatively coarse tipped microelectrodes.

The only response observed following 5-HT application was a membrane depolarization with a relatively fast recovery

following discontinuation of 5-HT perfusion. This type of response is similar to that observed for glutamate administration on TRG neurons. It is possible that the slight depolarizations observed with glutamate and 5-HT are a consequence of electrogenic uptake of these agents by the cell rather than due to any action on specific receptors for glutamate or 5-HT. However, if TRG neurons possess an uptake mechanism for 5-HT, the compound must be rapidly metabolized within the cell since no 5-HT can be localized within the neurons (Lehtosalo, 1983). An alternative explanation would suggest a lack of an uptake system for 5-HT in TRG neurons and absence of specific receptors.

F. ACTION OF GABA ON PRIMARY SENSORY NEURONS

Gamma-aminobutyric acid has been implicated as a possible neurotransmitter in a number of neuronal pathways in the CNS of vertebrates. In the mammalian CNS, presynaptic inhibition is associated with a depolarization of primary afferent terminals (Eccles, 1964). Reduction of presynaptic inhibition by GABA antagonists picrotoxin and bicuculline led to the suggestion that GABA may be a mediator of presynaptic inhibition (Eccles, et al, 1963; Schmidt, 1964). The role of GABA in primary afferent depolarization has been shown conclusively in numerous studies (cf. review by Levy, 1977). Several independent investigations have revealed that exogenous GABA also depolarizes the somata of amphibian and mammalian DRG primary

afferent neurons (Feltz and Rasminsky, 1974; Gallagher et al, 1974; Nishi et al, 1974). In DRG neurons, GABA-mediated depolarizations are caused by increases in chloride conductance (Nishi et al, 1974; Gallagher et al, 1978).

In the present study, GABA depolarized a majority of TRG neurons to which it was applied (30/36). All depolarizations were associated with decreases in input resistance. These results indicate that somata of TRG primary afferent neurons are similar to those of DRG in their responses to GABA applications.

Decreases in amplitude of action potentials during GABA-mediated depolarizations further corroborate this contention. Depolarization of the membrane apparently was not entirely responsible for shunting of the action potential. The reduction in spike amplitude could involve either inactivation of voltage-dependent channels responsible for spike generation or, more likely, be a result of an effective membrane "voltage clamp" that occurs when 10^{-3} M GABA increases membrane conductance which "fixes" the membrane potential near the chloride equilibrium potential.

Because the relationship between depolarizations and conductance increases was not the same for all cells tested, that is small conductance changes sometimes resulted in large depolarizations, and large conductance changes sometimes produced small depolarizations, a possibility exists that receptor sensitivity for GABA is not the same for different populations of TRG neurons. Although some recordings were made with potassium sulphate electrodes, a majority of GABA

applications were performed during recording with potassium chloride filled electrodes. This may have increased appreciably intracellular chloride concentrations, depending on the amount of leakage due to ionic concentration differences of electrolyte and intracellular milleu and also on the intensity of applied hyperpolarizing current pulses. More importantly, significant leakage would occur from the electrode tip due to hydrostatic pressure, although this may be somewhat reduced in this study because of the acute angle which the electrode makes with the chamber floor ($\simeq$ 50°). Another possible, but less likely reason for the differences in sensitivity to GABA could be due to a compromised chloride pump. In view of the fact that GABA-mediated depolarization is chloride-dependent, the internal chloride concentration of DRG primary afferent neurons must be higher than that of the outside and an inwardly directed chloride pump mechanism should be present in these neurons (Nishi et al, 1974). Therefore, if the internal chloride concentration is low it will result in a reduced depolarizing response to GABA. Alternatively, sensitivity differences may be, as Feltz and Rasminsky proposed (1974), due to the surrounding satelite cells acting perhaps as a site of GABA uptake.

The presence in neurons of two types of receptors for GABA was shown first in cultured embryonic sensory neurons of the chick (Dunlap and Fischbach, 1978) and subsequently in mammalian peripheral (Desarmenien et al, 1982) and central (Bowery et al, 1980) neurons. In sensory neurons, the second type of GABA

effect was a decrease in the somatic Ca^{+2} action potential duration that resulted from a selective decrease in voltage-sensitive Ca channel conductance which did not involve a change in resting membrane permeability (Dunlap and Fischbach, 1981). Baclofen, a GABA analogue used clinically as a muscle relaxant, recently has been found to be efficaceous in the treatment of trigeminal neuralgia (Fromm et al, 1980; Steardo et al, 1984); this analogue is selective for the second effect which is not antagonized by picrotoxin or bicuculline.

In TRG neurons, a Ca^{+2} dependent component of the action potential may be present only in neurons which discharge spikes of long duration. In order to adequately estimate effects of GABA (or baclofen) on the shape and duration of action potentials in these neurons one would need to artificially prolong the Ca^{+2} -component of the spike by blockade of K channels and/or to increase the extracellular Ca^{+2} concentration. Alternatively, a voltage clamp analysis could be used for more exact estimation of changes in Ca^{+2} current.

A physiological role for GABA-induced reduction of Ca^{+2} component of somatic action potential is unclear. In sympathetic ganglia, GABA depolarizes both pre- and post-ganglionic axons. However, when depolarization is blocked entirely with bicuculline, no change in transmitter release can be detected either with GABA or baclofen (Koketsu et al, 1974; Kato and Kuba, 1980). Also, GABA and its synthesizing enzyme glutamic acid decarboxylase are both absent in sympathetic ganglia (Nagata et al, 1966; McBride and Klingman, 1972).

Whereas a functional role of GABA-mediated depolarizations in presynaptic inhibitions is implied by sensitivity of primary afferents to bicuculline (Curtis et al, 1971), no selective antagonist of baclofen induced effects is available to assert a physiological role of the second GABA receptor type in presynaptic inhibition.

G. MEMBRANE OSCILLATORY BEHAVIOUR AND ACCOMODATION

Subthreshold oscillatory behaviour is observed in a variety of excitable membranes including squid giant axon (Chandler et al, 1962) and embryonic cultured heart cell aggregates (Clapham and De Felice, 1982). Using equivalent electrical circuit models this oscillatory activity can be described by the resistive, capacitive and inductive characteristics of the membrane (Mauro et al, 1970).

When oscillatory currents are applied to excitable membranes they may exhibit resonant characteristics, i. e. oscillatory input signals that most closely resemble the natural frequency of oscillation are more efficaceous in preturbing the membrane potential than the signal frequencies that lie outside the natural resonant frequency(ies) of the membrane.

The observation made in the present study using time-domain analysis techniques was that oscillatory behaviour is more pronounced at depolarized levels of membrane potential. These findings are fully corroborated using frequency-domain analysis techniques, where resonant behaviour was shown to depend

dramatically on the resting membrane potential (Puil et al, in press). The ability of somata of TRG neurons to act as a filter of subthreshold input signals by damping those signals that lie outside the natural resonant frequency poses an intriguing problem. The somata of TRG neurons are not known to possess synaptic contacts and being located away from the main axon do not seem to impede action potential transmission, except for a small delay at the junction of the stem process and the main axon (Dun, 1955).

Oscillatory membrane behaviour is dependent on the activity of voltage-dependent ionic channels that govern membrane excitability. Another phenomenon that is dependent on the ionic channel activity is that of spike accomodation.

Accomodation is an important property of neurons which is usually defined as an increase in action potential threshold due to application of slowly rising current (Koestler, 1981). Accomodation has been shown to be much less prominent in spinal motoneurons than in peripheral nerve (Araki and Otani, 1959; Frank and Fuortes, 1960). It would seem that in motoneurons, decreased accomodation is due to the activity of synapses impinging on the neuron (cf. Hubbard et al, 1969). Later investigations have shown the presence of a persistent inward sodium current in motoneurons (Schwindt and Crill, 1980; 1982) and neocortical neurons (Stafstrom et al, 1982) which countered the outward potassium current and thus may effectively reduce accomodation.

Perikarya of primary sensory neurons are mostly devoid of synapses and should exhibit more accomodation than motoneurons. Accomodation can be observed readily in TRG neurons that discharge repetitively in response to injection of depolarizing current pulses. It is evident from Figure 10 of Results that the fast rising component of the spikes seems to remain unchanged but the initial phase becomes more prolonged with each successive action potential. A number of models have been put forward to explain this phenomenon. Early work on squid axon (Hodgkin and Huxley, 1952) and frog myelinated fibers (Frankenhauser and Vallbo, 1965) implicated differences in sodium current inactivation rates to be responsible for variations in accomodation seen in the excitable neurons. In motoneurons, it is based mainly on the rate of recovery from potassium conductance since recovery from sodium inactivation in these neurons is rapid and therefore can be neglected (cf. Jack et al, 1983).

The relative contributions of sodium and potassium currents to the accomodation observed in TRG neurons remain to be shown. The possible presence of non-homogeneous membrane in the perikaryal region of TRG neurons may further complicate experimental interpretations. Shifts in the location of impulse generation sites have been shown to occur upon electrical stimulation in several types of excitable membranes. For example, Ringham (1971) showed that at higher current injection strengths, the site of impulse generation moved closer to the soma of the crayfish stretch receptor. In TRG neurons,

accomodation characteristics may be different for the stem segment compared to the rest of the somatic membrane (i.e. the stem process may be analagous to the spike initiating zone of the motoneuron).

There is no good reason to assume that the subthreshold oscillatory phenomenon that occurred as a result of voltage-dependent ionic channel activity is not present during the generation of suprathreshold events which are probably mediated by these same channels. Therefore, it is not sufficient to exclude a modulatory role of the cell body on the basis of an electrophysiological observation that large hyperpolarizations of the cell body membrane do not impede the passage of an action potential from the periphery to the central terminals.

It is conceivable that the modulatory role of TRG neuron cell body becomes important during the transmission of repetitive action potentials, where accomodation is an important factor in determining frequency and duration of the discharge. Thus hyperpolarization of the cell body which can result in large decreases of oscillatory tendency (resonance) and an increase in accomodation may significantly reduce the duration or frequency of the action potential discharge.

Conversely, depolarization of cell body membrane may enhance spike discharge by increasing oscillation and reducing accomodation in a manner analogous to the decrease of accomodation in the motoneurons and neocortical cells by synaptic inputs.

H.   DIRECTIONS FOR FUTURE RESEARCH

One compound that also has been proposed as a putative neurotransmitter in primary terminals of afferent neurons is substance P. This undecapeptide which was discovered by Euler and Gaddum (1931) has been since shown to be localized in specific neuronal tracts of the mammalian nervous systems, particularly in DRG neurons and in areas of the spinal cord where terminals of these neurons are localized (Takahashi et al, 1974). Specifically, Substance P was found almost exclusively in the small cell bodies of the DRG and TRG neurons as well as in their central and peripheral processes (Hokfelt et al, 1977). Neurophysiological studies of excitatory actions of substance P in spinal neurons provided evidence for facilitation of discharges induced by nociceptive stimulation in the spinal cord with iontophoretic substance P application (Henry, 1976). In addition, neurons in the ophthalmic division of cat trigeminal nerve were shown to contain substance P and to innervate the arteries of the circle of Willis, one of the few pain sensitive areas within the cranium (Mayberg et al, 1981). These findings point to the possibility that substance P may be a neurotransmitter at CNS terminals of nociceptive primary afferent neurons in the trigeminal and spinal ganglia, in addition to its possible role as a vasodilator substance at the peripheral sensory terminals (Lembeck et al, 1977).

It would be of interest to test substance P on the TRG

slices to see whether the compound can modulate the somatic membrane properties of sensory neurons. Fetal trigeminal ganglion neurons of the rat, studied under conditions of internal perfusion and voltage clamp were observed to repond (40/110) with inward Na^+/K^+ currents to rapid pH changes in the extracellular medium. Preliminary tests showed such neurons to be insensitive to putative transmitters such as GABA, histamine, glutamate, 5-HT and substance P applied at pH 7.4 (Krishtal and Pidoplichko, 1981).

Observations made with fetal neurons and the present study of S-glutamate and 5-HT effects are rather preliminary. A more extensive study is needed to evaluate 5-HT action on TRG neurons. In view of recent reports of 5-HT action on the C-neurons of the cat nodose ganglion, use of fine-tipped electrodes, for prolonged recordings from neurons that exhibit action potentials with a repolarization phase plateau, is indicated.

V. SUMMARY AND CONCLUSIONS

1. The methodology for obtaining viable thin slices of the trigeminal root ganglion of the guinea pig was developed in order to allow prolonged (stable) intracellular recordings with electrophysiological techniques, from somata of primary afferent neurons. This in vitro slice preparation permitted the bath application of pharmacological agents at known concentrations which facilitated investigations of the influence of endogenously found compounds and drugs on membrane electrical properties of trigeminal root ganglion neurons.

2. These investigations have permitted, for the first time, a description of both passive and active (voltage- and time-dependent) membrane properties of trigeminal root ganglion neurons in vitro . The modulatory actions of several neuroactive substances on these membrane properties also were studied in these investigations.

3. All 150 neurons which were impaled with microelectrodes remained quiescent prior to intracellular injection of depolarizing current square-wave pulses. Only cells that maintained steady membrane potentials more negative than 50 mV and displayed action potentials greater than 50 mV were used in the detailed analysis of membrane properties. The mean resting membrane potential of TRG neurons was -61.7 ± 0.4 mV S. E. (n=50). Injection of hyperpolarizing current pulses (0.5-10 nA)

evoked "time-dependent rectification" in a majority of sampled neurons (about 100). This membrane response of TRG neurons, described previously in a number of investigations of other types of primary sensory neurons, was found to have a marked voltage dependency. Oscillatory behaviour of the membrane potential of TRG neurons was observed during intracellular injections of depolarizing current pulses or following termination of similar injections of hyperpolarizing pulses. This phenomenon also was shown to be voltage-dependent, with more pronounced oscillations evident at depolarized levels of membrane potential relative to the initial resting state.

4. Two groups of neurons could be distinguished on the basis of their action potential characteristics. A group which typified a majority of sampled neurons exhibited fast-rising spikes with mean amplitude of 65 ± 1.9 mV S. E. (n = 31) and afterhyperpolarizations that ranged from 4.5 mV to 25 mV in size (mean 14.1 ± 1.1 mV S. E.; n = 22). Peak amplitude of the afterhyperpolarization was partly dependent on the value of the resting membrane potential, and was largest at depolarized levels of membrane potential. Action potentials evoked by intracellular injection of depolarizing current pulses in the second group of neurons had a plateau in their repolarization phase. These action potentials had a mean amplitude of 78 ± 2.3 mV S. E. (n = 7) and afterhyperpolarizations ranging from 13 to 25 mV (mean 16.8 ± 2.7 mV S. E. n = 6) in amplitude. Repetitive firing could be evoked in both groups of neurons if

adequate suprathreshold amounts of depolarizing current were applied through the recording electrode. Accomodation of action potentials often was observed in such cases.

5. Bath applications of S-glutamate (10^{-2} M) resulted in small (1-2 mV) membrane depolarizations in two of the six neurons which were tested for responses to S-glutamate. These depolarizations were not associated with noticeable changes in membrane conductance and disappeared completely upon return to control perfusate. Similarly, 5-hydroxytryptamine (5-HT) had little effect on six TRG neurons. A small (1-2 mV) membrane depolarization not unlike that observed for S-glutamate was seen in one neuron, although a slight increase in membrane conductance was observed.

6. Bath perfusion of gamma-aminobutyric acid (GABA 10^{-4} - 10^{-2} M) resulted in membrane depolarization (2-14 mV) in 83% of tested neurons (n = 36). Reduction of maximal response, presumably due to receptor desensitization, was observed readily when 10^{-3} M concentrations of GABA were applied. GABA-mediated conductance increases resulted in a decrease in the height of action potentials evoked by intracellular injections of depolarizing current pulses. This effect could not be accounted for by membrane depolarization alone and also was attributed to a GABA-induced shunt of the spike-generating mechanism. These investigations suggest a physiological relevance for the modulatory role of GABA in TRG neurons similar to that described

in DRG neurons.

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