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THE EFFECTS OF HIGH NITROGEN TENSIONS OF THE PROPERTIES OF NEUR--ETC(U)
JAN 79 J E BLANKENSHIP, R FEINSTEIN N00014-75-C-0547

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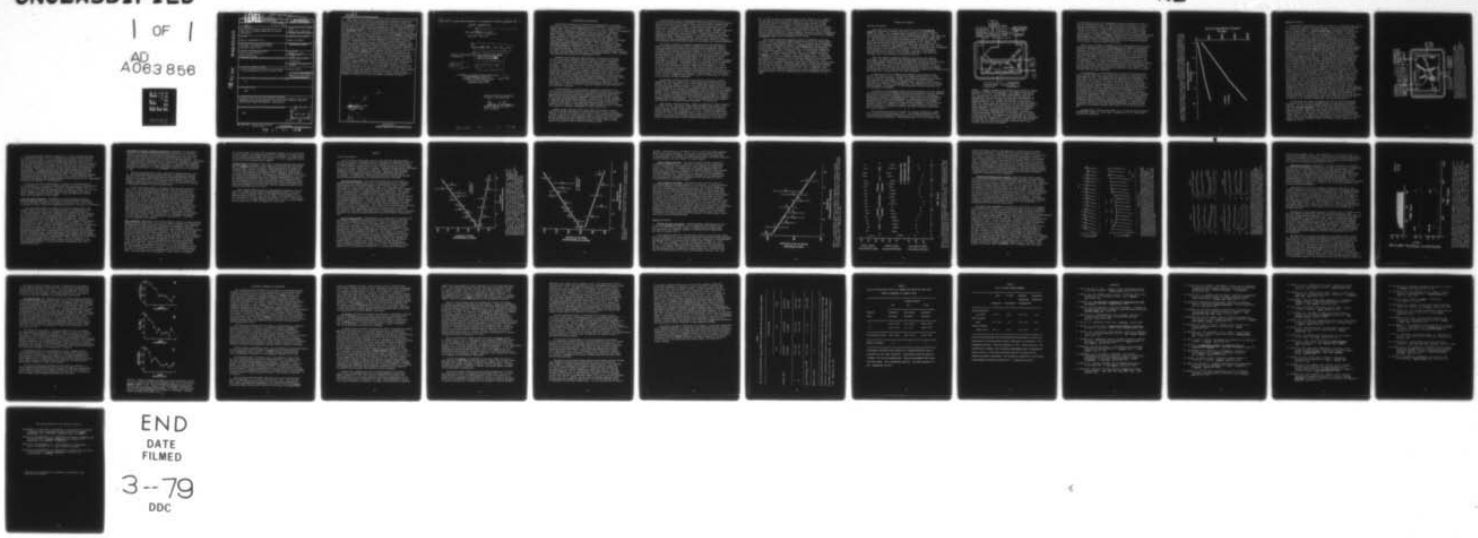

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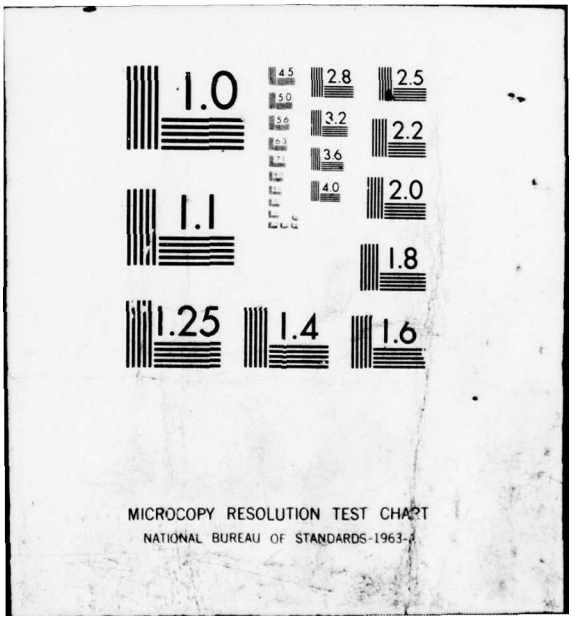
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The effects of increased air, nitrogen and/or hydrostatic pressures to 10 ATA have been studied on electrophysiological properties of identified neurons in two model neurobiological preparations, the marine mollusc Aplysia and the crayfish. Studies were done in vitro in a pressurization chamber with intracellular recordings using multiple microelectrodes in identified neurons in the Aplysia ganglion or from the crayfish giant axon. Ten ATA of air had no demonstrable effect on the resting potential, membrane resistance, time constant or action potential of neurons R2, R14 and R15 Aplysia, and equivalent pressures of nitrogen or hydrostatic force did not affect resting potential of crayfish axon. The rates of depolarization and repolarization of the crayfish axon action potential were increased significantly by nitrogen tensions to 8.6 ATA: depolarization rate, 2.2 ± 0.2%/atm; repolarization rate, 2.1 ± 0.2%/atm. Action potential duration was decreased (0.91 ± 0.19%/atm) under these conditions. Hydrostatic pressure alone had an opposite effect on polarization rates. Thus, hydrostatic pressure slowed action potential kinetics, while the anesthetic gas N_2 augmented action potential kinetics. While action potentials per se of Aplysia neurons appeared unaffected by pressure, air tensions to 10 ATA dramatically altered the spontaneous bursting pattern of the endogenously active cell R15. Numbers of action potentials per burst, burst duration and interburst interval were all shortened at peak pressure by approximately 25%; overall firing frequency did not change. Increased nitrogen, but not oxygen, pressure also caused a significant increase in the amount of frequency-dependent synaptic facilitation in a well characterized cholinergic EPSP in cell R15. When the presynaptic pathway to R15 was stimulated at 10 ATA of air at 2 Hz for 100 pulses the mean size of the last four EPSPs compared to the size of the first EPSP of the train grew by an additional 20% over the control amplitude ratio at sea level. Thus increases in nitrogen tensions equivalent to those encountered by divers who may experience nitrogen narcosis have been shown to cause reversible changes in fundamental properties of action and synaptic potentials in neurons.

(6) THE EFFECTS OF HIGH NITROGEN TENSIONS OF THE PROPERTIES OF NEURONAL MEMBRANES AND SYNAPTIC TRANSMISSION.

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Principal Investigator: (10) James E. Blankenship, ~~Ph.D.~~
Collaborators: Robert Feinstein, ~~Ph.D.~~
Howard J. Bryant, ~~Ph.D.~~

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Division of Comparative Marine Neurobiology
~~Marine Biomedical Institute~~
University of Texas Medical Branch
200 University Boulevard
Galveston, Texas 77550

New
411041

Approved by Official Authorized to Sign for the Institution

George T. Bryan, M.D.
Dean of Medicine

INTRODUCTION AND BACKGROUND

Application of pressure to isolated neural preparations is of interest not only as a means of elucidating some of the mechanisms of pressure/anesthesia interaction at the excitable membrane (Bennett et al., 1974; Johnson and Flager, 1950; Lever et al., 1971; Miller, 1977) but also as a means of investigating the etiology of neurologic dysfunctions which may occur when compressed gases are used in diving or hyperbaric treatments (Bennett, 1966; Hills, 1977). Symptoms of neurological dysfunction in diving can appear at air pressures as low as 2 ATA (Poulton et al., 1964) to 6.4 ATA (Behnke et al., 1935; Kendig et al., 1975). These symptoms range from the euphoria of nitrogen narcosis (Hill and McLeod, 1903) to the muscle tremor encountered in the high pressure nervous syndrome (Braur, 1968). It is very likely that these observable symptoms of neurological dysfunction will be ultimately traceable to the modification by pressure of one or more of the basic properties of the excitable membrane. Several workers using single cell in vitro techniques have shown that increased hydrostatic and helium and hyperbaric nitrogen pressures can affect excitable membranes and synaptic transmission (Bennett, 1966; Grundfest, 1936; Henderson and Gilbert, 1975; Spyropoulos, 1957b).

Grundfest (1936) found that under hydrostatic conditions excitability and action potential amplitude increased initially as pressure was applied to frog sciatic nerve. However, upon continued application of pressure (up to 15,000 psig ≈1021 ATA) excitability was depressed and the amplitude decreased. Action potential duration increased throughout the application of pressure. Using single nerve fibers from the toad under hydrostatic pressures, Spyropoulos (1957a) confirmed the increase in duration found by Grundfest (1936) and observed an increase in action potential amplitude in most cases. Squid single axons studied by Spyropoulos (1957b) under hydrostatic pressures to 16,000 psig (≈1089 ATA) showed an increase in duration of the action potential but only slight effects on amplitude and membrane impedance. Spyropoulos (1957b) was also the first to demonstrate that pressure could ameliorate, at least partially, the effects of ethanol-induced narcosis in a single nerve cell. Axons whose action potential amplitude had been markedly depressed in 3% ethanol were exposed to 7,000 psi hydrostatic pressure whereupon the spike amplitude was appreciably increased.

A slight decrease in action potential amplitude, prolongation of the time course of sodium and potassium action potential currents, and an increase in action potential duration have been reported for squid axon subjected to gaseous helium pressure to 158 ATA by Henderson and coworkers (1975, 1977). These results are qualitatively comparable to Spyropoulos' (1957b) findings in squid axon using hydrostatic pressure. Analysis of the voltage clamp data by Henderson and Gilbert (1975) indicated that the effects of pressure are to slow the time course of the sodium and potassium currents of the action potential, most likely by affecting gating current kinetics and not by changing total numbers of channels, conductance or sodium inactivation.

Kendig, Trudell and Cohen (1975) have studied the effects of helium pressure and gaseous anesthetics on isolated rat superior cervical ganglion. Pressure to 137 ATA of helium had little effect on the compound action potential in normal preparations, though duration tended to increase. The same treatment applied to ganglia partially anesthetized with halothane served to restore the depressed action potential amplitude almost to normal. Beyond 137 ATA, pressure and anesthetic had an additive depressant effect.

Recent studies with the voltage clamped squid axon (Shrivastav et al., 1978) indicate that hydrostatic pressures of 300 ATA broaden the action potential and reduce threshold, pure pressure effects reported by others (Henderson and Gilbert, 1975; Spyropoulos, 1957b). Pressure also induced a shift of a few millivolts in the apparent soldium reversal potential toward more positive values and a reduction in the amount of peak inward current. Ketamine anesthetic results in a negative shift in the apparent sodium reversal potential and a reduction in excitability (Shrivastav, 1977). Pressure applied to ketamine anesthetized axons resulted in a return of the reversal potential back toward the control values. Time discrepancies in onset of the two effects and differences in sodium accumulation in the axon led these authors to postulate that anesthesia and pressure exert effects by two separate but opposing mechanism.

The effect of pressure on synaptic transmission has been examined in a variety of preparations using intra- and extracellular electrodes. Studies using extracellular electrodes at the curarized frog neuromuscular junction by Yeandle (1977) indicate that helium pressures to 31 ATA have no effect on poststimulus facilitation, depression, or shape of the evoked synaptic potential in this preparation. Kendig and Cohen (1976) found that the indirectly evoked EMG amplitude in rat diaphram was unchanged by helium pressures to 137 ATA. Reduction of the calcium concentration present in the bath had no effect on the EMG at 1 ATA but upon pressurization to 137 ATA with helium the indirectly evoked EMG was depressed. They suggest that this depression is due to decreased junctional transmission. A depressent effect of helium pressure at 35-100 ATA on synaptic transmission was also demonstrated by Kendig et al., (1975) in the rat superior cervical ganglion. Athey and Akers (1978) in studies on the frog neuromuscular junction reported that helium pressures from about 14 to 69 ATA depressed the extracellularly recorded muscle compound action potential induced by nerve stimulation by almost 60% at peak pressure. They and Friess et al. (1971) have suggested that pressure effects at synapses may be involved with postsynaptic receptor mechanisms.

Intracellular electrode studies of the effects of pressure on synaptic transmission have been carried out in three invertebrate preparations, squid, lobster, and crab. Henderson et al., (1977) showed that the time course of the excitatory postsynaptic potential in the squid giant synapse was slowed by helium pressures as low as 35 ATA. Increased synaptic fatigue and an increase in the threshold of the synaptically generated action potential were also observed. Campenot (1975), using the lobster neuromuscular junction, found that hydrostatic pressure to 200 atmospheres depressed the amplitude of the endplate potentials. In crab, however, the amplitude of these potentials was depressed by pressure at low stimulation frequencies but was relatively unchanged when the stimulation frequency was increased (Campenot, 1975). This frequncy effect with pressure was attributed to a pressure-induced enhancement in facilitation.

These previous studies have been carried out generally at higher pressures (> 31 ATA) than are required to induce some neurological dysfunction in man. In addition, the pressure medium in most cases has been either a hydrostatic fluid or gaseous helium. Since helium is used to alleviate the symptoms of nitrogen narcosis in diving (Bennett et al., 1974; Johnson and Flager, 1950; Lever et al., 1971), and hydrostatic fluid systems do not increase the partial pressure of the gases inthe system, these pressure media may not produce the narcotic responses induced in man and animals breathing air at less than 10

ATA. We are not aware of published studies on the effects of air pressures in this range at the cellular level, despite the fact that this represents the typical conditions necessary for inducing nitrogen narcosis in human divers and workers. In an attempt to study the effects of low pressure nitrogen we have examined changes in action potential amplitude and duration, rates of depolarization and repolarization and resting potentials in crayfish giant axon under hydrostatic and air pressures from 1 to 8.6 ATA. In addition, we have studied alterations in passive membrane properties, synaptic transmission, bursting patterns, and action potential parameters in the marine mollusc Aplysia californica brought about by hyperbaric air to 10 ATA.

The crayfish single axon preparation offers an advantage for this type of study in that it generally inhabits shallow water and is not known normally to dive to the depths simulated here (250 feet of seawater) and is presumably not preadapted to function at pressure. The Aplysia offers several advantages for the study of membrane properties, synaptic transmission and endogenous bursting behavior. There are many cells that can be identified consistently in the abdominal ganglion by topography, size, or firing pattern (Frazier et al., 1967). Numerous studies have been done on the membrane properties of Aplysia (Tauc, 1966; Carpenter, 1967; Marchiafava, 1970). In addition, the behavior of one of the synaptic connections between an axon in the right pleurovisceral connective and cell R15 has been extensively studied (Halstead and Jacklet, 1974; Schlapfer et al., 1974, 1976; Woodson et al., 1976). This particular connection demonstrates facilitation, depression, and posttetanic potentiation and provides a convenient model system to study these parameters as a function of pressure. The bursting behavior of the identified cell R15 has also been well documented (Frazier et al., 1967; Strumwasser, 1965). This cell produces relatively regular bursts of action potentials and has been suggested to play a role in controlling water balance (Kupfermann and Weiss, 1976).

METHODS AND MATERIALS

Crayfish experiments:

Giant axons from the ventral nerve cord of the crayfish Procambarus acutus were dissected according to the method orginally described by Dalton (1958) for lobster. The circumesophageal connectives were ligated caudal to the cerebral ganglion and rostral to the circumesophageal ganglion. The connectives were dissected free of the animal and mounted in the experimental chamber (Fig. 1). The connective tissue sheath surrounding the axon bundle was removed and the bundle rotated until the giant axon was uppermost in the chamber. The giant axon was penetrated with two microelectrodes (5-40 MΩ, filled with 2.7 M KCl), one at each end of the preparation. Electrode spacing was 5 mm. Action potentials were evoked by stimulating the axon through one electrode with supramaximal square pulses of 5 ms duration at a frequency of 2 Hertz (WPI Digipulser) and recorded from the other electrode through a unity-gain high-impedance amplifier (WPI M4A or WPI 701). The reference electrode for the system was a Ag-AgCl coil of wire immersed in the bathing solution.

Since the changes in membrane parameters with pressure were expected to be small, the action potential was signal-averaged by means of a Nicolet 1072 signal averging computer in order to improve both the accuracy and consistency of the measurements. The averaging system sampled the analog signal every 20 microseconds, digitized it, and recorded the average in memory. The stored average to 32 action potentials was then differentiated by the Nicolet computer and the average values of the action potential amplitude, maximum rate of depolarization, and maximum rate of repolarization were read from memory.

At least four sets of 32 samples were obtained in this manner for each axon at all pressures tested. The mean and standard deviation (SD) of these 4 sets of data was calculated. Changes in the measured parameters with pressure were calculated as percentages of their respective 1 ATA control values. An analysis of variance including a weighted linear regression (Sokal and Rohlf, 1969) was then performed on the combined data. The significance of any pressure-induced changes, the presence of a linear regression, and the deviations from the regression were examined.

In addition to the averaging system, the action potentials were also displayed on an oscilloscope and the resting potential was monitored with a digital voltmeter. The duration of the action potential was taken as the period of time elapsed between the occurrence of the maximum rate of depolarization and repolarization. The response time of the recording system to a square pulse was 50 microsecond (measured from 0 to 67% of pulse height). Resting potentials were averaged on-line by a DEC GT-40 computer. The computer was programmed to take 100 resting potential samples at 2 samples per second, calculate averages and variances, plot the results as a function of pressure, and output the data on paper tape for further analysis.

The pressure system (Fig. 1) consisted of a one-man decompression chamber with a total volume of approximately 670 l. The system was pressurized with air either from a compressor fitted with filters to remove water vapor or form a bank of 2200 psi gas bottles. Pressurization from 1 to 7.8 ATA without stops

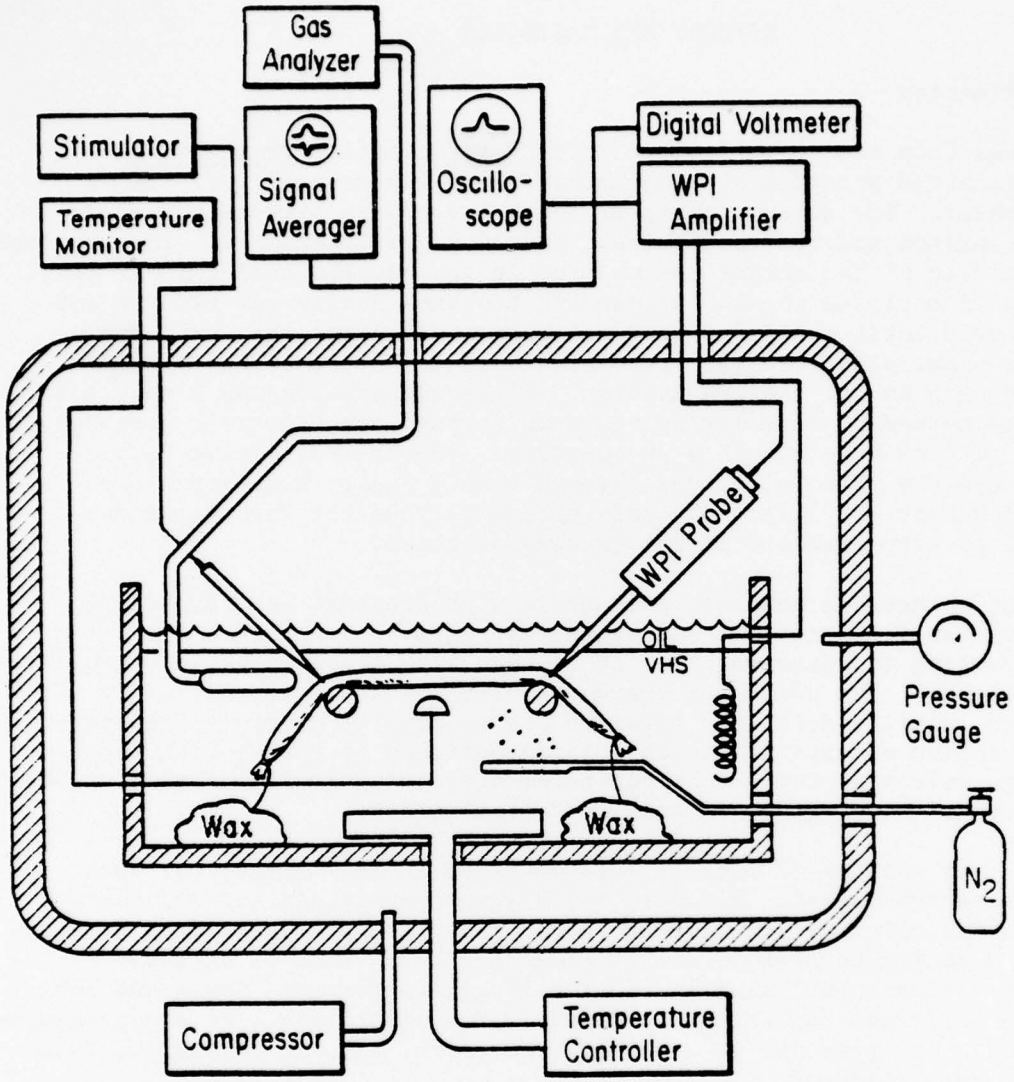

Figure 1. Schematic diagram of the pressure and electronic recording systems for crayfish axon experiments. The chamber containing the axon was 7 x 8 x 5 cm. For hyperbaric measurements it was filled with van Harraveld's solution (VHS) until the fluid level was approximately 2 mm over the axon. For hydrostatic measurements the solution level was 5 to 7 mm above the axon and 1 to 1.5 cm of degassed mineral oil was layered over the VHS. A dissecting microscope (not shown) which was rotated out of the way when the pressure vessel door was closed and bolted was used to view the preparation during impalement. All electrical cables penetrated the pressure vessel through one electrical feed-through and not at various positions as shown diagrammatically in the figure. Separate gas tension measurements were made with the analyzer probe at the same location as the axon. The thermistor was located near the axon and at approximately the same depth. A 1 mm polyethylene tube, perforated near the end, was cemented to the chamber floor. This tube was used to bubble gas into the solution through a metering valve from a cylinder located outside the chamber.

at intermediate pressures for measurements required a minimum of 1.3 hours when the compressor was used. Pressurization from the gas bottles to 8.6 ATA required approximately 8 minutes. When the effects of hyperbaric air were examined the fluid level in the chamber was adjusted so the axon was about 2 mm below the surface. Pressure was applied slowly over a period of hours to allow the gas partial pressures in the solution near the axon to assume steady values. In separate experiments gas parital pressures (oxygen and nitrogen) were measured as a function of chamber pressure (Fig. 2) by mass spectrometry (Perkin Elmer Medical Gas Analyzer Model 1100) with the probe located at the axon position. Thirty to 60 minutes were required for the partial pressures in the solution to achieve steady state values.

Since it is of interest to determine if the observed effects on the action potential parameters are due to pressure per se or to changes in gas partial pressures, the system was also pressurized hydrostatically. For hydrostatic compression the axon bathing solution was degassed and the recording chamber was filled until the solution depth over the axon was 5 to 7 mm. One to 1.5 cm of degassed mineral oil were then carefully layered on top of the bathing solution. The chamber with the layered solutions was then compressed by air from the gas bottles at a rate of 0.3 atm/min with stops every 1.5 to 3 atm for measurements. Gas analyzer measurements as a function of pressure with the probe located in the soltuion at the axon position indicated that the mineral oil layer effectively isolated the axon-containing solution from changes in dissolved gas concentrations. Provision was also made in the chamber to bubble selected gases into the solution below the mineral oil layer after the system had been hydrostatically compressed.

The temperature of the preparation was controlled by circulating water from a constant temperature unit (Lauda K-2R), through an epoxy-coated coil surrounding the axon. The bathing solution temperature was maintained at all times within $\pm$ 0.5^{o}C of the stated value and monitored with a thermistor probe (YSI 43 TD) located in the bath at the same depth as and less than 1 cm laterally from the axon. In order to insure the widest band width and to minimize noise, the high impedance probe of the WPI amplifier was mounted inside the pressure vessel. Pressurization of this system with the electrode in the bath did not show any significant pressure-dependent effects on the recording system. This conclusion is based on two independent criteria. The response of the recording system to various sized current pulses applied through the electrometer bridge circuitry revealed no differences in response rise time or amplitude at 1 ATA and 10 ATA. Furthermore, the differences in response of axons at equivalent hydrostatic and air pressures eliminate the possibility that the effects of increased air pressure can be accounted for by pressure effects on the electrical recording system. A preparation was included in the data set only when measured parameters returned to control values after the complete pressure cycle from 1 to 7.8 or 8.6 and back to 1 ATA.

van Harraveld's (1936) solution (VHS) with 1 mM Tris (Tris(hydroxymethyl) aminomethame; Sigma, St. Louis) added to stablize the pH at 7.55 was used to bathe the preparation.

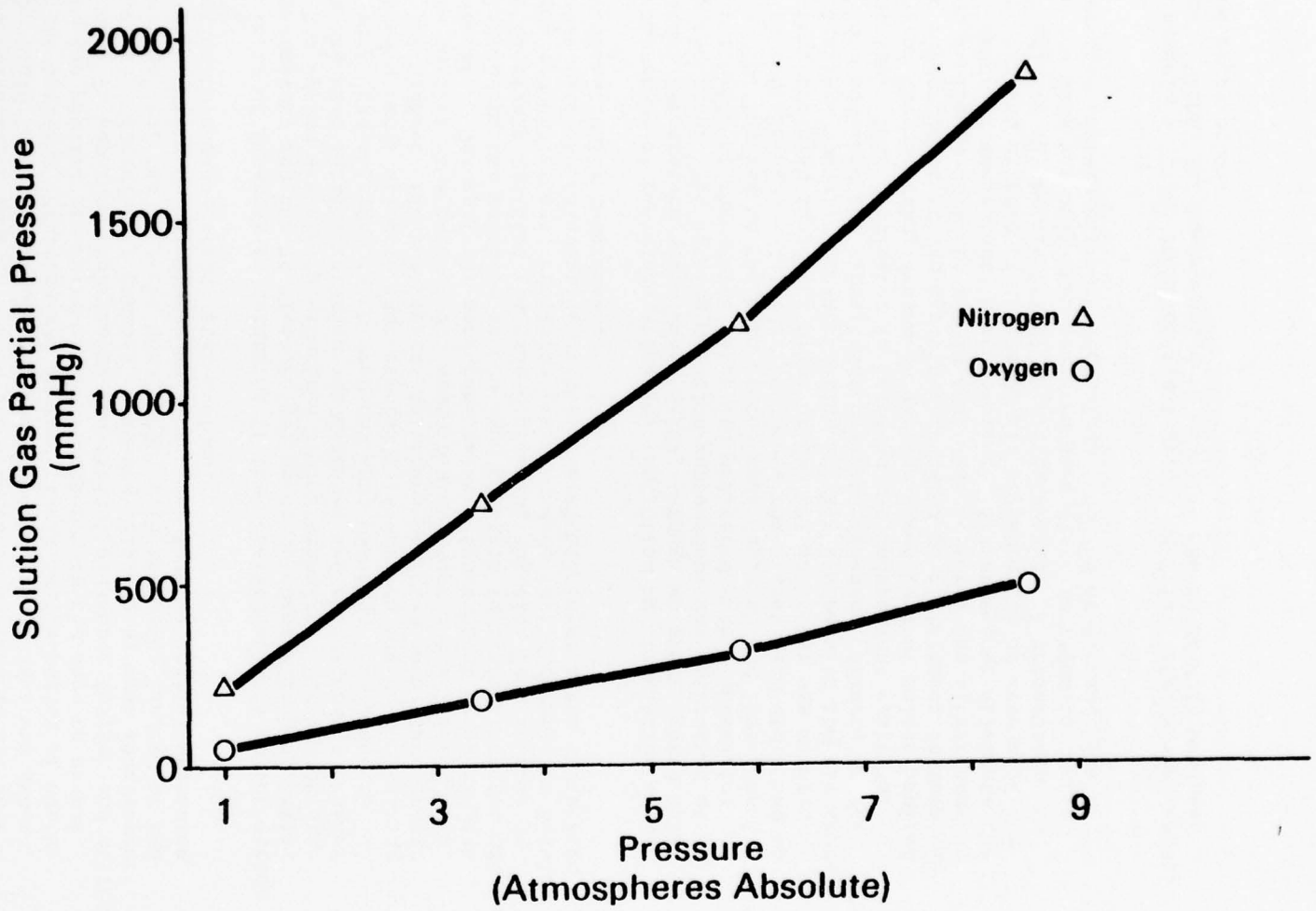

Figure 2. Nitrogen and oxygen partial pressures. Nitrogen (triangles) and oxygen (circles) partial pressures were measured in van Harraveld's solution as a function of chamber pressure. Plotted points were obtained after the partial pressures achieved steady state values (from 30 to 60 minutes after a chamber pressure step).

Aplysia experiments:

We performed 40 experiments to study the effects of increased introgen tensions on certain of the passive electrophysiological properties of identified neurons in the abdominal ganglion of Aplysia californica. The ganglion was pinned onto the Sylgard base of a lucite recording chamber filled with sea water which was then secured onto a platform in the pressurization chamber. Temperature control was maintained as in crayfish experiments (Figs. 1 and 3). A single identified neuron, usually either cell R2 (the giant cell) or cell R14 (large neurosecretory neuron), was impaled with two independent microelectrodes. A third microelectrode was placed in the bath as an indifferent for voltage measurements. All electrodes were filled with 2.7 m KCl and matched initially for similar impedance values. The hyperbaric chamber was sealed and control values of resting potential, membrane resistance, time constant, action potential amplitude and duration, and temperature were obtained. The GT40 computer sampled the differential recording of membrane voltage for one minute, taking 300 data points, and calculated and plotted the mean resting potential and standard deviation. A current (I)-voltage (V) curve for membrane resistance was then obtained by passing 5 sec pulses of DC current through the second intracellular electrode and measuring the amount of current given with each stimulation and the resulting induced voltage change. The computer plotted this information on its graphic display as an I-V relationship using the resting potential as the zero current point. The computer also sampled the rising phase of some of the induced voltage steps and calculated the time to achieve 67% of the final voltage as the time constant. Temperature was measured by a thermistor probe in the sea water bath near the ganglion. The initial temperature was between 14° and 17°C and was maintained to within ±1°C throughout a run. After completion of control measurements, the pressure in the chamber was increased in one atmosphere steps of pressure, and the measurements above taken at each step through 10 ATA and back to 1 ATA. The pressure was changed over a two to five minute period in order to minimize temperature changes and mechanical disturbances. After a new pressure level was reached we waited 5-10 minutes before taking data to allow gas and temperature equilibration with the sea water bath. Data acquisition time at each pressure took 5-10 mins, and a complete experimental run normally required a total of 5-6 hours. After the completion of a run, the electrodes were withdrawn from the cell, the DC potential recorded and the resting potential corrected for drift or offset in the measurement system. In addition, in most experiments the pressure was again taken to 10 ATA and back with the electrodes in the bath to observe any fluctuations in electrode impedance or differential DC level that might occur due to the influence of pressure on the recording system. These types of changes were minimal and corrections of the biological measurements were usually unnecessary.

An additional large number of Aplysia were used for further experiments on pressure effects on action potential, synaptic transmission and bursting patterns. For these studies abdominal ganglia were obtained from over 30 (200-300g) Aplysia californica. The left and right pleurovisceral connectives were cut near their respective pleural ganglia and remained attached to the abdominal ganglion. Other nerves were severed within 5 mm of the ganglion. The preparation was pinned onto a Sylgard surface in a 7x8 cm lucite recording chamber (Fig. 3). The chamber was filled with artificial sea water until the solution depth over the ganglion was approximately 1 mm and mounted on a platform inside the hyperbaric pressure system. The solution volume contained in the chamber was ~18 ml.

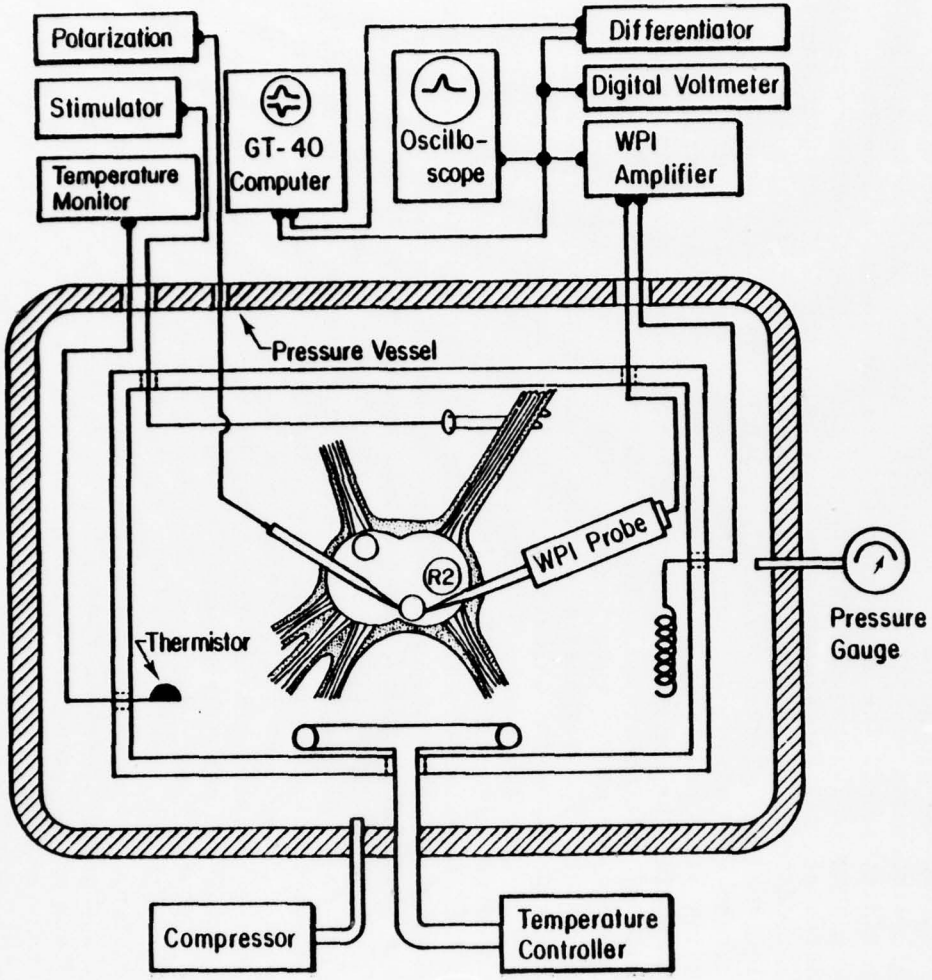

Figure 3. Schematic of pressure and electrical recording systems for Aplysia experiments. Two electrodes were usually inserted into the cell. For synaptic facilitation experiments a third microelectrode (not shown) attached to a WPI probe was placed in solution near the ganglion for differential recording. Constant bipolar polarization current was supplied by a battery across a potentiometer and 100 MΩ resistor. The differentiator was a passive RC device. All electrical connections penetrated the pressure vessel wall through one multi-pin connector and not at various points as shown in the diagram. Solution depth over the ganglion was 1 mm or less to improve gas transfer.

Two microelectrodes (10-30 MΩ) filled with 2.7 M KCl were positioned over the identified cell (R2 or R15) to be impaled and a Ag-AgCl reference electrode was placed in the chamber. A third microelectrode was placed in the solution near the ganglion when differential recordings were employed. Either one (for the R15 burst experiment) or two (for the R2 action potential and R15 facilitation experiments) electrodes were inserted into the selected cell. Connective tissue over R15 was carefully cut away with a sliver of razor blade before penetration was attempted. Electrodes inserted in R2 were tapped through the connective tissue capsule. Transmembrane potentials from these electrodes were recorded through a unity-gain high-impedance amplifier (WPI, M4A) and displayed on an oscilloscope or a strip-chart recorder (Gould 2400). A GT-40 (Digitial Equipment Corporation) laboratory computer was connected to the system for on-line data collection and analysis. The recording system was direct coupled for all measurements. Stimulation was provided by a WPI Digipulser.

Artificial sea water (Instant Ocean) was used as the bathing solution for the preparation. A solution containing one-third the normal Ca^{++} was employed in the R15 facilitation experiment. The loss of osmolarity brought about by removal of 2/3 of the $CaCl_2$ was compensated by the addition of NaCl. All solutions had an osmolarity of 1000 ± 50 mOsm. Solutions were exchanged by flowing at least 5 chamber volumes through the system. During the experiments, however, there was no solution flow in the chamber.

Action Potential Parameters. Measurements of resting potential and action potential amplitude, and rates of depolarization and repolarization employed two microelectrodes inserted into the soma of the giant cell R2. One of these electrodes was used to record the transmembrane potential; the other for stimulation.

Data was acquired under the control of the GT-40 computer system. The computer sampled 100 values of the resting potential at 2 samples per second. It then calculated and printed the average value and standard deviation of the resting potential. After this calculation was complete, an action potential was elicited from the normal resting potential by stimulating the cell through the second electrode with a supramaximal 100 ms square pulse. The evoked action potential and its first time derivative obtained from a passive RC differentiator (R = 1 KΩ, C = 0.02mf) were fed into two channels of the computer. Both signals were sampled within 30 microseconds of one another at a rate of one pair every 0.3 ms. A total of 500 samples were taken, 250 from the derivative and 250 from the action potential, for a total sample time of 75 ms. After the signals were displayed on the GT-40 terminal screen, the action potential amplitude and maximum rates of depolarization and repolarization were calculated from the respective traces. These values were stored in memory and the entire process, including resting potential measurement, was repeated every 90 seconds for a total of 20 repetitions. Averages and standard deviations of all quantities were calculated and these values were plotted. After a suitable set of 1 ATA control measurements were obtained, pressure was then returned to 1 ATA and measurements repeated. All action potential measurements were taken in artificial sea water.

Measurement of Synaptic Potential Facilitation. Measurements of postsynaptic potential (PSP) amplitude employed three microelectrodes two of which were inserted into the soma of the parabolic bursting cell R15. One of the intracellular electrodes was used for potential measurement while the other was used to inject current into the cell to control the membrane potential. The third microelectrode was positioned in the solution near the ganglion. Differential recording between the indifferent and the intracellular electrode was employed to reduce the overall noise level in the system. An extracellular stimulating electrode was attached to the right pleurovisceral connective. This electrode allowed the intact nerve to be held en passant in mineral oil for long periods of time.

After all electrodes were in place, cell R15 was hyperpolarized to -100 mV. This holding potential was always sufficient to suppress the bursting activity of the cell. Stimulating current of increasing intensity was then applied to the extracellular electrode on the right connective until a PSP appeared in the soma of R15. Once threshold for the PSP was established, the stimulus voltage was increased 50% to prevent loss of the PSP due to variation in presynatpic threshold or electrode polarization.

A 2 Hz train of 100 stimuli (1 ms square pulses) was used to evoke the PSPs in R15. The computer digitized 160 points from the first PSP of the train at a rate of one ssmple point every 2 ms. The first digitized PSP was stored in memory and the computer repeated the digitization and storage procedure on the second PSP. It then digitized and stored the 97th through 100th PSP of the train using the same sampling parameters as used for the first two PSPs. After the termination of the train, the holding potential, PSP amplitude, and time to peak were printed out for each individual PSP. These quantities for the last four PSPs were then averaged. Facilitation ratios were calculated as the average amplitude of the last four PSPs of the train divided by the amplitude of the first PSP ($f_{100} = PSP_{100}/PSP_1$) or the amplitude of the second PSP divided by that of the first ($f_2 = PSP_2/PSP_1$). This procedure was repeated at 1 ATA a minimum of three times at intervals of at least 30 minutes to obtain control values. The pressure was then raised to 10 ATA over a period of 80 to 100 minutes. The experiment was repeated 3 or more times at 10 ATA. The pressure was then returned to 1 ATA where control measurements were repeated.

Burst Parameters. Measurements of the burst parameters employed one microelectrode inserted into the soma of R15. The cell was allowed to produce its characteristic bursting pattern without artificial polarization or stimulation. Burst duration was defined as the time between the occurrence of the threshold voltage of the first action potential and approximately the same voltage on the repolarization phase of the last action potential in the burst. The burst interval was taken as the time between the last action potential in a burst and the first action potential in the next burst. The computer counted the number of action potentials in each burst and timed the burst duration and interval between bursts to within ± 10 ms. This time-count procedure was repeated for 30 bursts. The average values and standard errors of the parameters were calculated and displayed at the terminal screen. Several sets of these 30 bursts measurements were obtained at 1 ATA. The pressure was raised in 1 to 3 ATA increments to 7.8 ATA, the measurements repeated at each pressure step, and the pressure lowered to 1 ATA to obtain another set of controls. The entire experiment generally required 6 to 10 hours. All burst measurements were taken in artificial sea water. Data were expressed as percentages of control values. An analysis

of variance (Sokal and Rohlf, 1969) including a weighted, linear least square fit was performed on the combined data from all preparations. Weight factors were the number of measurements obtained at each pressure. The significance of any pressure-induced changes, the presence of a linear regression, and the deviation from the regression were examined.

Pressure System. The pressure vessel (Fig. 3) was the same used with crayfish and other Aplysia experiments. The system was pressurized with filtered air from a compressor or with a specially prepared gas mixture (2% oxygen, 98% nitrogen) from a 3000 psi gas bottle. Pressurization of the system from 1 to 10 ATA without stops at intermediate pressures generally required about 1.5 hours. Bath temperature was monitored with a thermistor probe located in the solution near the ganglion. The temperature was maintained at 15.0 ± 0.5°C by circulating refrigerated water from a constant temperature bath (Lauda K-2R) through an epoxy-coated copper coil submerged in the bathing solution. The high-impedance probe of the electrometer was mounted inside the pressure vessel to minimize noise and input cable capacity. Control experiments with electrodes submerged in the bath did not show any significant effects as the pressure was cycled from 1 to 10 ATA.

The 2% oxygen mixture was used to investigate the respective roles of oxygen and nitrogen in any observed pressure-dependent effect. Air at sea level (~1 ATA) is composed roughly of 20% oxygen and 80% nitrogen with a resulting partial pressure of 0.2 ATA for oxygen. Pressurization to 10 ATA with air results in a final oxygen partial pressure of 2 ATA. Pressurization to 10 ATA with the 2% oxygen mixture, however, will result in a final oxygen partial pressure of 0.2 ATA, the same as in 1 ATA of air.

RESULTS

Crayfish experiments:

Action potential records were taken at 1 ATA after the resting potential and the action potential parameters (rates of polarization, amplitude) stabilized following impalement. Air pressure was then increased to 7.9 ATA and returned to 1 ATA in steps of between 0.6 and 7 ATA. Data was recorded at various stages both on the increase and decrease of pressure. Threshold voltage was not consistently changed by pressure. There were no cases where threshold voltage decreased, spontaneous firing at pressure was not observed. Experiments were performed on 20 axons to determine the change in resting potential with hyperbaric air pressure. No significant, consistant variation with pressure was observed. Action potential amplitude was not consistently affected by pressure.

Rate of Depolarization. The effect of hyperbaric air on the rates of depolarization and repolarization of the action potential was very consistent. Combined data from multiple measurements of depolarization rate in 12 axons under hyperbaric air pressure are shown in Fig. 4 (circles, upper curve). An analysis of variance (Sokal and Rohlf, 1969) with a weighted linear regression shows that the presence of a linear regression was significant at the $p < 0.001$ level. Deviations from the regression line were not significant. The maximum rate of depolarization, calculated from the regression line increased with hyperbaric air pressure by a factor of $2.2 \pm 0.2\%$/atm (average ± SE). The behavior of the maximum depolarization rate under hydrostatic conditions, however, was considerably different (Fig. 4, squares, lower curve). Analysis of data combined from multiple measurements on 9 axons indicates that the presence of a linear regression is significant at the $p < 0.025$ level and that deviations from the regression line are not significant. The maximum rate of depolarization decreased with the application of hydrostatic pressure at a rate of $-0.57 \pm 0.13\%$/atm. The results of these experiments indicate that while hydrostatic pressure in this range has a very small, depressant effect on depolarization rate, an equivalent increase in air pressure clearly accentuates the action potential depolarization rate.

Rate of Repolarization. The magnitude of the maximum rate of repolarization increased as a function of hyperbaric air pressure. All axons studied under these conditions demonstrated this behavior. Analysis of combined data from 12 axons (Fig. 5, circles, upper curve) showed that the presence of a linear regression was significant at the $p < 0.001$ level and that deviations from the regression line were not significant. The change in the magnitude of the maximum rate of repolarization with hyperbaric air pressure was $2.1 \pm 0.2\%$/atm calculated from the regression line (Fig. 5). This results in a change in the repolarization rate of about 14% at 7.8 ATA, the maximum pressure applied under these conditions. Hydrostatic compression of the system, as in the case of the depolarization rate, produced an opposite change in the maximum rate of repolarization. Analysis of pooled data from 9 axons under hydrostatic pressure (Fig. 5, squares, lower curve) indicates that the presence of a linear regression is significant at the $p \approx 0.05$ level and that deviations from the regression line are not significant ($p > 0.2$). The magnitude of the maximum rate of repolarization decreased $-0.9 \pm 0.3\%$/atm under hydrostatic

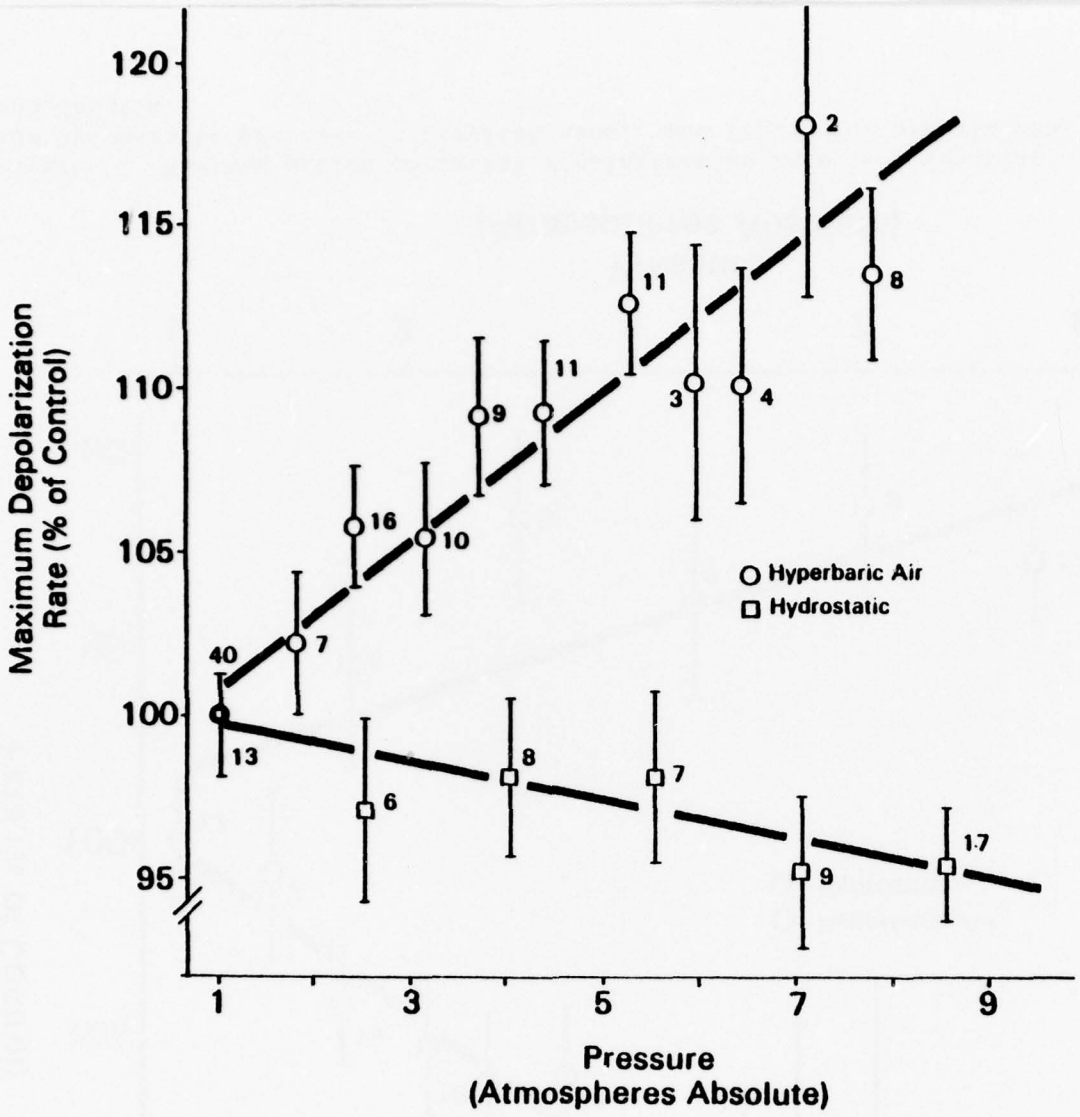

Figure 4. Maximum action potential depolarization rates as a function of hyperbaric and hydrostatic pressure in crayfish axon. Hyperbaric data (circles, upper curve) were collected from 12 axons. Hydrostatic data (squares, lower curve) were collected from 9 axons. Control values at 1 ATA are averages including both pre- and post-pressurization measurements. The numbers near each point indicate how many measurements were included in the particular average. Bars represent ± one standard error. Lines through the data were calculated by linear regression where the number of measurements was employed as the weight factor. Temperature was 20°C.

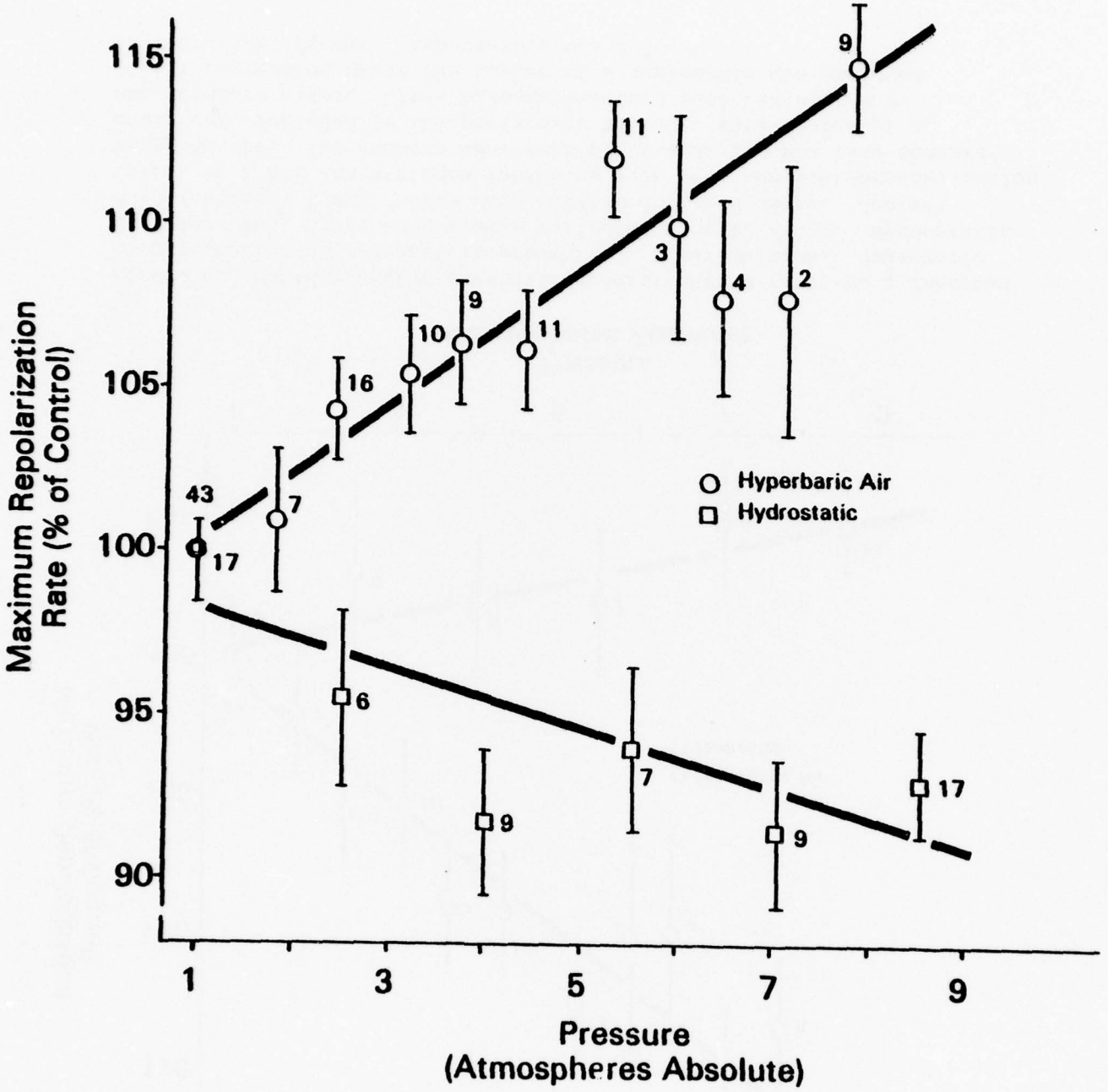

Figure 5. Maximum action potential repolarization rate as a function of hyperbaric and hydrostatic pressure in crayfish axon. See Fig. 4 for experimental conditions and details.

pressure. This results in a decrease of 6.1% at 7.8 ATA, the maximum pressure applied under these conditions. As with rate of depolarization, the effects of air and hydrostatic pressures were opposite with respect to maximum rate of repolarization. Air pressure accentuated repolarization rate while hydrostatic pressure depressed the rate, though to a smaller degree.

Action Potential Duration. The action potential duration decreased as a function of hyperbaric air. An analysis of variance on the combined data (Fig. 6) indicates that the presence of a linear regression is significant at the $p < 0.001$ level. Deviations from the weighted regression were not significant. Application of hyperbaric air pressure produced a small decrease in the duration of the action potential of $-0.91 \pm 0.19\%$/atm as calculated from the regression line. This results in a reductioon in the duraation at 7.8 ATA of 6.2% which is consistent with an increased rate of depolarization and repolarization. Hydrostatic compression of the axon showed no effect on the duration of the action potential.

Direct Effects of Nitrogen. This system allows the direct effect of dissolved nitrogen on the axon parameters to be examined. In three experiments axons were compressed hydrostatically to 8.6 ATA. The observed changes in the rates of polarization, action potential amplitude, duration, and resting potential were all consistent with the previous results. Pure nitrogen was then bubbled into the solution bathing the axon while maintaining the chamber pressure at 8.6 ATA. Gas analyzer measurements indicated that nitrogen tensions in the solution increased rapidly and if bubbling was prolonged the solution saturated. After bubbling the nitrogen for several minutes the magnitudes of both rates of polarization increased in agreement with the hyperbaric results. The changes in the action potential amplitude, as in the hyperbaric case, were mixed. Variability in the action potentials duration were too large to discern a significant trend under these conditions. These results confirm in an independent manner our conclusions that increased gas pressure, not hydrostatic pressure, accounts for the increase in polarization rates observed with air compression. Furthermore, since only pure N_2 was bubbled into solution at 8.6 ATA, we may conclude that increased nitrogen tension as opposed to increased oxygen tension can account for the changes observed.

Aplysia experiments:

Resting and Action Potentials. Resting potential measurements from cells R2 and R14 in Aplysia were made in over 50 experiments with pressures from one to 7-10 ATA. No consistent effect of nitrogen tension at these levels was observed on this parameter. Examples of resting potential measurements are given in Figures 8 and 9.

Action potential and first time derivative traces were recorded from neuron R2 by the computer after the cell had recovered from the trauma of double impalement. Multiple measurements of action potential amplitude, maximum rate of depolarization, and maximum rate of repolarization were obtained from 19 animals at 1 ATA and 7.8 ATA of air pressure. Data from a representative animal are shown in Fig. 7. All measured parameters showed some variation with time and pressure. In Fig. 7, for example, the magnitude of the maximum rates of depolarization and repolarization show a tendency to increase steadily as a function of time while the resting potential (not shown) was essentially constant. Data from

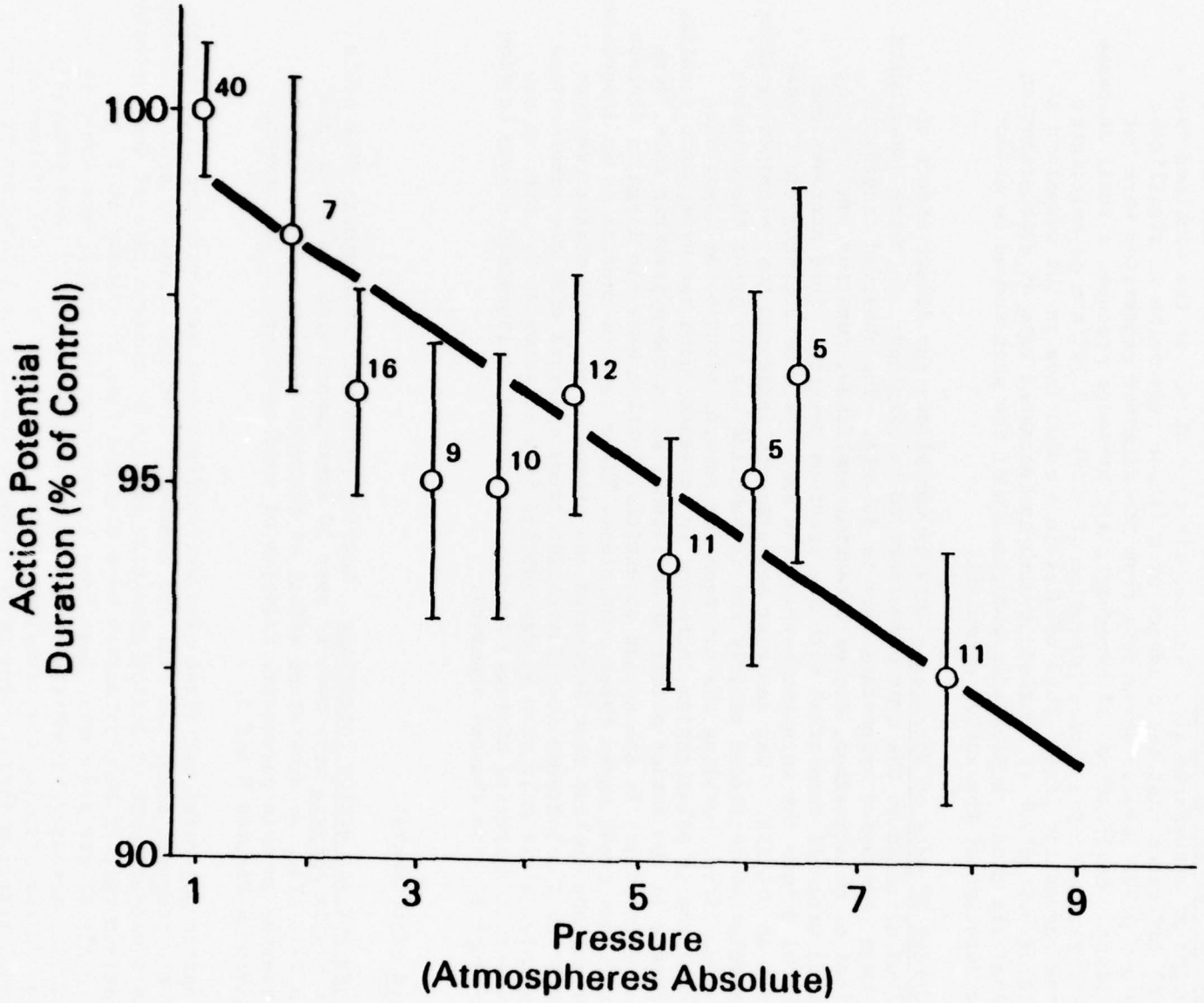

Figure 6. Action potential duration as a function of hyperbaric air pressure in crayfish axon. Pooled data from 12 axons. Control values at 1 ATA include pre- and post-pressurization measurements. Line through points was calculated by weighted linear regression. Bars represent ± one standard error. Temperature was 20°C.

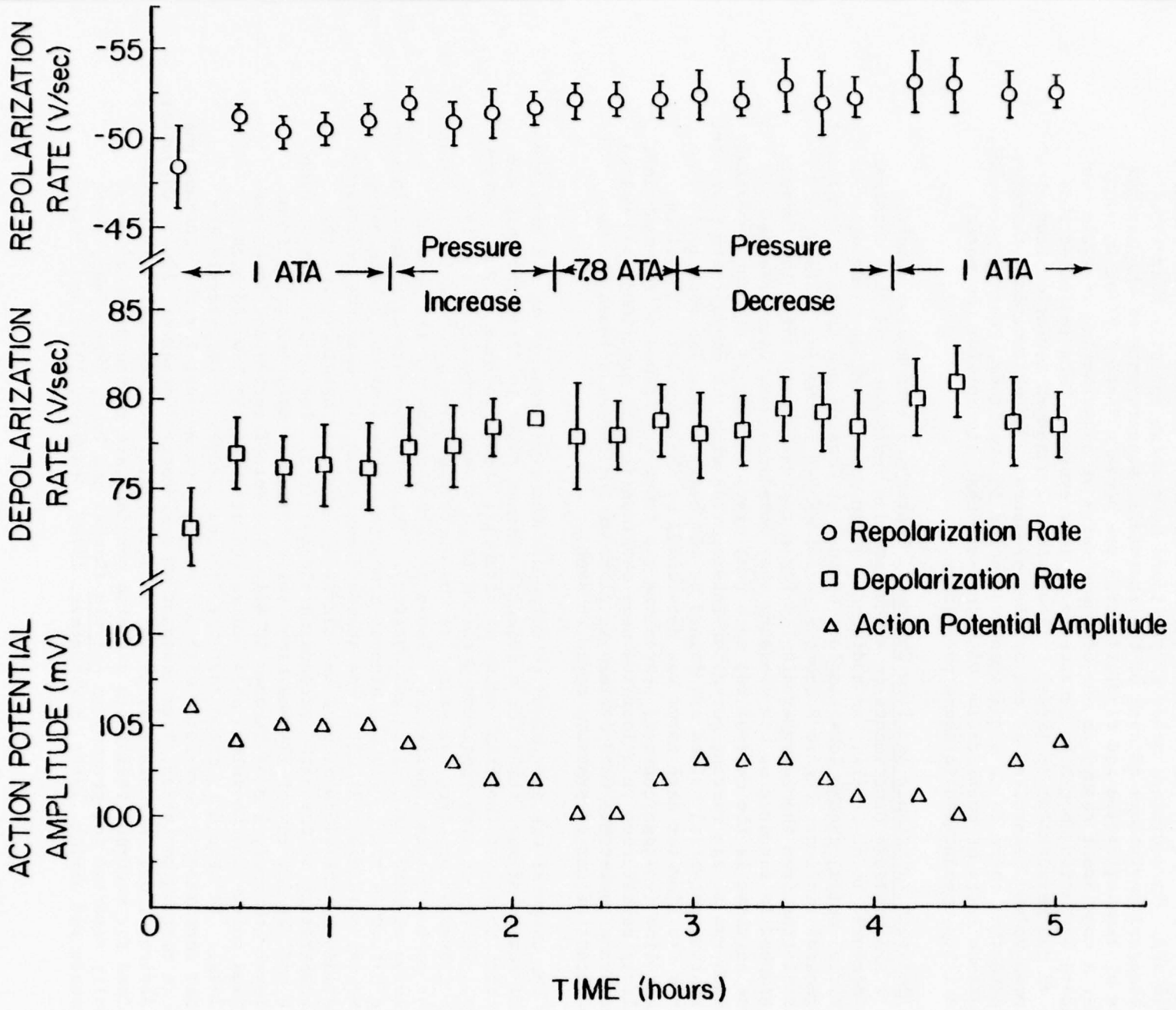

Figure 7. Action potential parameters for Aplysia cell R2. Each data point is an average of the results from 20 separate action potentials. Error bars are ±1 standard deviation. Time zero was the time the animal was killed. ATA=atmospheres absolute. Representative data from one preparation. Temperature was 15°C.

another preparation, however, show generally the opposite type of behavior with similar magnitudes. Although the action potential amplitude in Fig. 7 decreased upon the initial application of pressure, the effect is not sustained at 7.8 ATA. In addition, other preparations have shown opposite behavior. The standard deviations of each of the individual measurements of the maximum rates of depolarization and repolarization are between 2 and 3 V/sec in most cases. A consistent change in one of these rates of polarization of twice the standard deviation should be detectable in this system. Data indicated that most of the measurement do indeed fall within a 2 standard deviation band about the mean values regardless of the applied pressure. Our observations therefore indicate that there is no significant variation in amplitude, resting potential, or maximum rates of polarization with pressure that lie outside the normal variability encountered in these parameters.

Membrane Resistance and Time Constant. There have been twelve complete 1-10-1 ATA pressure experiments in which membrane resistance and time constant were measured in R2 or R14. An additional seven experiments on these or other identified cells, though less complete because of loss of penetration, complement our general findings. Figure 8 shows a series of I-V curves for cells R2 and R14 replotted from the computer display for a typical experiment. The curves are graphed in sequence at the pressure they were obtained. Also represented on the abscissa is the current for each run; zero current is plotted separately for each run at the resting potential intersection with the appropriate pressure. The resting potential values are joined by the heavy line. The best least squares fit line for each curve was determined by the computer. The slope of this line (voltage/current) represents the input resistance of the membrane. Similarly constructed relationships were obtained in each experiment and values of membrane resistance were defined as slope of the I-V relationship. No significant effect of pressure could be seen.

The computer was programmed to calculate the time constant of the membrane as the number of msec taken for a selected current pulse to drive the membrane potential to 67% of the final value of potential change induced by that current pulse. Values of time constants obtained in this manner are shown in Fig.9. These illustrations (Fig. 9) summarize the battery of data obtained for representatives of our most complete experiments. Shown on each abscissa are the absolute pressures at which each value was obtained. The data for each experiment include (from bottom to top): (1) the elapsed time, in minutes, at which each value of RMP was obtained, (2) RMP ± one standard deviation, (3) membrane resistance, as defined by the slope of each I-V curve, (4) the time constant, and (5) the temperature of the bath surrounding the ganglion. The solid lines of each curve and the top row of elapsed times refer to values obtained going from sea level (control, 1 ATA) through 10 ATA. The dotted curves and lower row of times correspond to values obtained as the pressure was lowered back to sea level. It should be noted that the limited changes in temperature are neither consistently correlated with nor adequate to account for the fluctuations seen in resting potential, time constant or resistance (Carpenter, 1967; Carpenter and Alving, 1968; Marchiafava, 1970; Marmor, 1971). In addition, the values obtained for membrane resistance and time constant are in the range of those normally reported for neurons in Aplysia (Kandel and Tauc, 1966; Tauc, 1966; Carpenter and Alving, 1968; Marchiafava, 1970; Graubard, 1973) and closely

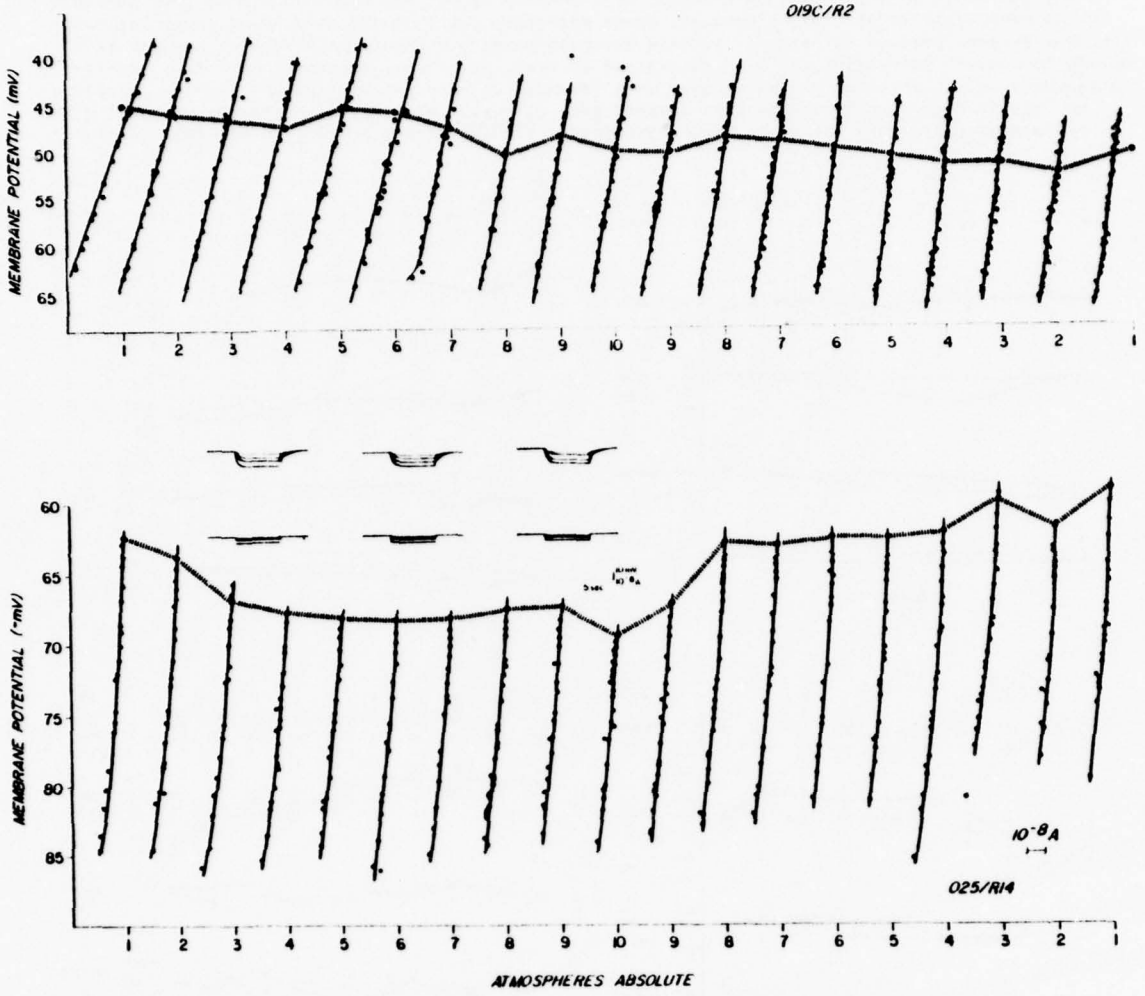

Figure 8. Plots of current-voltage (I-V) relationships for Aplysia cells R2 (above) and R14 (below) from two experiments in which nitrogen tensions was increased from one to ten ATA and back (abscissa). Heavy hatched line in each graph represents resting potential value (mV on ordinate). At each ATA, an I-V plot was generated by passing varying amounts of current through one of two intracellular microelectrodes and plotting voltage change induced on ordinate and current applied on abscissa. Current calibration for both experiments is given at lower right of bottom graph. Zero current was set at the resting potential at each respective pressure. A computer was used to plot curves, obtain the best fit (least squares) line and compute the slope of each I-V plot. Insets over lower plot are representative records from the experiment showing voltage changes induced (upper records) for various applied currents (lower records) at the control 1 ATA, at 10 ATA and at 5 ATA on descent (respectively from left to right).

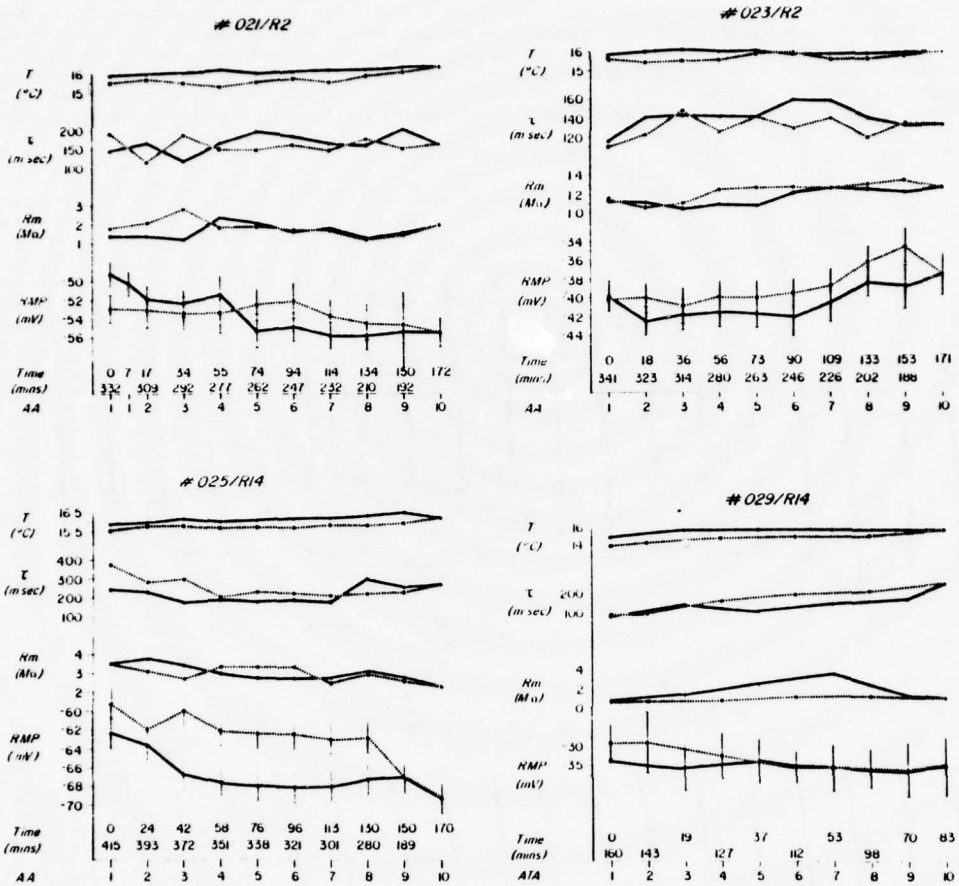

Figure 9. Results of four different Aplysia experiments (cell R2 above, R14 below) showing in each case the following data (from bottom to top) plotted against nitrogen tension as ATA on abscissa. Lowest record, elapsed time in minutes, that each value was obtained at the respective pressure. Top row of numbers (and solid lines in all curves) are for increasing pressure, bottom row of numbers (and hatched lines) are for decreasing pressure. Next is plotted resting membrane potential (RMP) in mV with one standard deviation shown for each value. Input resistance of the membrane (Rm) is given in megohms and is derived from computer determination of slope of I-V relationships. The time constant, τ, plotted in msec, was also determined by computer (see Methods), and the temperature of the bath surrounding the ganglion is given in degrees centrigrade.

related species (Marmor, 1971). Most importantly, it is seen that there is no clear-cut correlation between any of these biological parameters and pressure. Thus, over experiments it has become evident that the passive electrophysiological properties of the neurons of the abdominal ganglion which we have studied are not affected by increased nitrogen tension to 10 ATA.

Synaptic Facilitation. Stimulation of the right pleurovisceral connective produces a unitary excitatory postsynaptic potential in the soma of the identified cell R15 (Fig. 10, and Frazier, et al., 1967; Halstead and Jacklet, 1974; Schlapfer et al., 1974). With the stimulus paradigm employed (100 stimuli, 2 Hz) there was a slight depression in the amplitude of the second PSP followed near the end of the stimulus train by a marked amplitude increase. There was a large amount of variability in the PSP amplitude between animals. At a holding potential of -100 mV the largest initial PSP encountered was 35 mV while the smallest was about 3 mV. However, when facilitation ratios are calculated and the responses are paired at 1 ATA and 10 ATA in the same preparation this variability becomes manageable.

Application of 10 ATA of air pressure can alter the plasticity of this synaptic connection in response to repeated stimulation. The average amplitude of the initial PSP of the stimulus train measured in the 11 preparations bathed in artificial sea water at 1 ATA was 14 ± 2 mV (average ± SE). By the end of the stimulus train the PSP amplitude (averaged over the last four responses) had grown 64%, or the facilitation ratio of the one-hundredth to the first PSP (f_{100}) was 1.64 (Table I). Raising the pressure to 10 ATA of air increased this facilitation ratio by 17%. This increase in the f_{100} ratio was found to be significant at the $p < 0.01$ level by means of a t-test for related measures (Bruning and Kintz, 1977). Raising the pressure with the 2% oxygen, 98% nitrogen gas mixture produced about a 30% increase ($p < 0.05$) in the facilitation at pressure (Table 1).

Replacing the normal seawater bathing the ganglion with a 1/3 normal calcium solution reduced the amplitude of both the first and one-hundredth PSP. In this low calcium medium the amplitude of the first PSP was about 1/3 the amplitude in normal seawater. The facilitation ratio, however, was increased about 60%. This increase was significant at the $p < 0.01$ level. Increasing the pressure to 10 ATA of air increased the f_{100} ratio 20%. Significance tests performed between the various pressure-induced changes indicated no difference between the effect of pressure in air, low calcium, and 2% oxygen. The changes produced in the f_{100} facilitation ratios ranged from 17% to 30%. The average increase in the f_{100} ratio brought about by a pressure change of 9 ATA from all methods of pressure application (weighted by the number of preparations) was 2.3% per atmosphere.

The amplitude of the second PSP produced by the stimulus train in normal sea water (Fig. 10) was slightly less than the amplitude of the first PSP at 1 ATA (Table 2). This facilitation (depression) ratio (f_2) was unaltered when the preparation was pressurized to 10 ATA with air. Pressurization of the system in normal artificial sea water with the 2% oxygen, 98% nitrogen gas mixture also did not significantly alter the facilitation ratio between the first and second PSP. Bathing the ganglion in sea water containing 1/3 the normal calcium increased the facilitation (f_2) at 1 ATA 21% ($p < 0.05$) over the value obtained in normal sea water. The second PSP of the train was now larger than the first. Although the f_2 ratio is greater than 1 in the low-calcium solution compared to a value of less than 1 for f_2 in normal sea water, there is no significant change in this facilitation ratio when the system is pressurized.

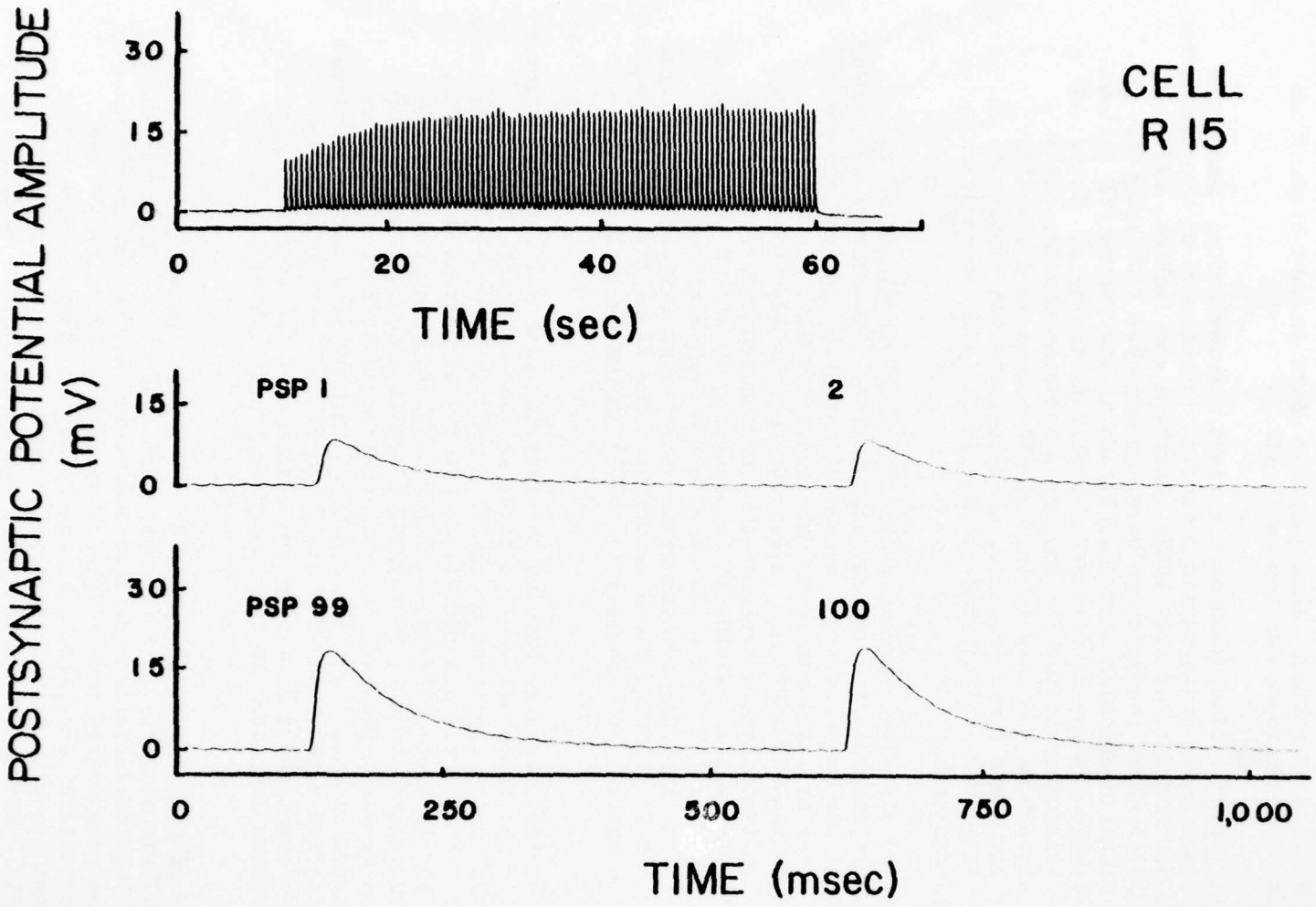

Figure 10. Postsynaptic potentials in Aplysia cell R15. The right pleurovisceral connective was stimulated by means of an extracellular electrode to elicit excitatory postsynaptic potentials (EPSP) in the soma of R15. Current was injected into the cell through one of two intracellular electrodes to hold the membrane resting potential at -100 mV. Traces were recorded on a Gould chart-paper recorder for illustration only. All analysis was from the computer digitized data. Time scale origin is arbitrary. Temperature 15°C. Pressure 1 ATA. Upper trace: Response to a 2 Hz train of 100 stimuli. Lower traces: Responses to a subsequent train of stimuli with the time scale expanded. Only the first, second, 99th and 100th EPSPs are shown. Trains of stimuli were separated by at least 30 min. to allow EPSP amplitudes to return to control values.

The time for the PSP voltage to achieve its maximum value was measured by the computuer system at 1 ATA and 10 ATA for the first, second, and the 97th through the 100th PSP of the train. The time-to-peak PSP voltage was constant at 1 ATA for the first, second, and one-hundredth PSP. At 10 ATA of air the time-to-peak appears to be slightly shorter in all cases than at 1 ATA of air but this difference is not statistically significant.

Bursting Patterns. The identified cell R15 is almost always spontaneously active, generally producing a characteristic burst of action potentials followed by a period of silence (Frazier et al, 1967; Strumwasser, 1965) The computer system was programmed to clear and restart data collection again when the number of action potentials per burst was less than 3 or more than 50. Data from 16 preparations are reported in Table 3. At 1 ATA the average number of action potentials in an R15 burst was 24 (Table 3). The application of air pressure in approximately 1 ATA steps to 7.8 ATA reversibly reduced the number of action potentials in the burst. The differences between the number of action potentials per burst were significant at the $p < 0.001$ level (Sokal and Rohlf, 1969). A pressure coefficient (Table 3) of -3.7% per atmosphere was obtained from a linear regression on data from the 16 individual preparations (Fig. 11A). The presence of a linear regression was significant at the $p < 0.001$ level and deviations from the regression were not significant (Sokal and Rohlf, 1969). At 7.8 ATA this results in approximately 6 fewer action potentials in each burst than at 1 ATA.

The reduction in the number of action potentials per burst as pressure was increased was accompanied by a concurrent reduction in the burst duration (Fig 11B). The burst duration was reduced by 4.6% per atmosphere as pressure was elevated. The differences between durations at various pressures was significant at the $p < 0.001$ level. This resulted in a decrease in burst duration from 9.1 seconds at 1 ATA to approximately 6.3 seconds at 7.8 ATA. The intraburst frequency (number of action potentials divided by burst duration) was 2.6/sec and 2.9/sec at 1 ATA and 7.8 ATA respectively. These rates are not significantly different. Thus, within the burst, the frequency of action potential generation was essentially unchanged by the application of pressure.

Pressurization with air also reduced the interval between bursts of action potentials (Fig. 11C). This interval was reduced from 20 seconds at 1 ATA to about 14 seconds at 7.9 ATA (Table 3). The interval between bursts was reduced by 4.2% per atmoshere as pressure was elevated (differences of values significant at $p < 0.001$ level). The overall action potential generation rate (number of action potentials divided by (interval + duration) was not significantly changed by 7.8 ATA of air pressure.

For both the duration and interburst interval data the presence of a linear regression was significant ($p < 0.001$) and this explains a large portion of the range of variation with pressure. However, in both cases derivations from the linear regression were significant ($p < 0.005$) above ~5 ATA where the rate of change for both parameters decreases (Fig. 11B and C).

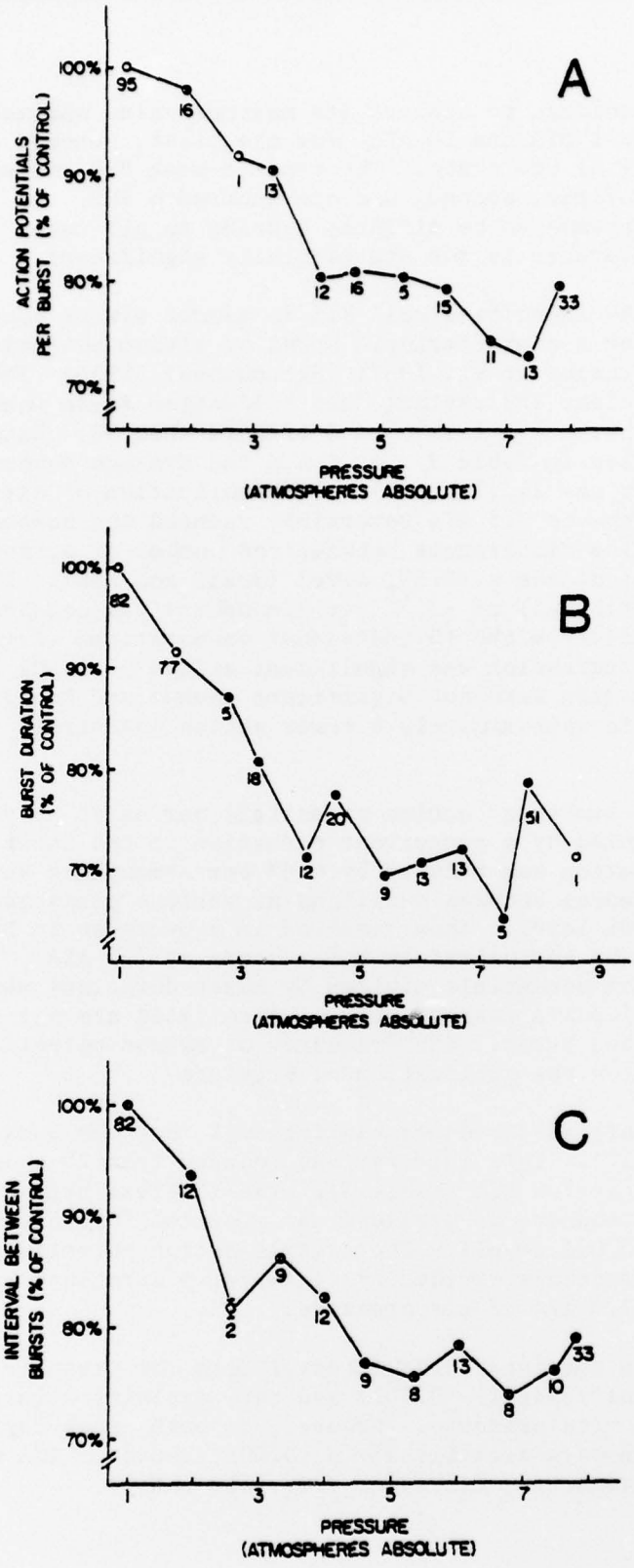

Figure 11. Effect of pressure on number of action potentials per burst (A), burst duration (B) and interval between bursts (C) for cell R15 in Aplysia. Summary of data from 16 preparations expressed as per cent of control (100% at 1 ATA). Values at 1 ATA are averages including both pre- and post-pressurization measurements. The numbers near each point indicate how many measurements are included in the average. Standard errors are smaller than symbol size.

DISCUSSION, SUMMARY AND CONCLUSIONS

One consistent finding throughout the literature is that membrane resting potential is unaffected by any of the reported types of pressure application, a finding with which we concur for both crayfish and Aplysia neurons. The effect of various pressures on threshold appears to be small and variable. Under hydrostatic conditions a decrease in threshold has been reported in squid axons at pressures below 100 psi (≈7.8 ATA) (Spyropoulos, 1957b) and at 300 ATA (Shrivastav et al., 1978). Spyropoulos (1957b) reported spontaneous firing of squid axon at over 200 ATA. Squid axons under normoxic helium (Henderson and Gilbert, 1975; Henderson et al., 1977) do not show significant changes in threshold at 155 ATA. At hydrostatic pressures below 1,000 psi (≈68 ATA), threshold was decreased as shown by Grundfest (1936) from extracellular records in frog sciatic nerve. Kendig et al. (1978) have recently reported that helium at 35-200 ATA causes repetitive and spontaneous firing of crayfish claw nerves. We could not demonstrate a consistent effect of either hydrostatic or air pressure on crayfish axon or Aplysia neuron threshold at the pressures employed in this study. The pressure effects on bursting in R15 are not strictly threshold phenomena since the underlying burst oscillator potential and not spikes per se was affected.

Our results also indicate that the passive electrophysiological properties and action potentials of neurons in the abdominal gangion of Aplysia are unaffected by increases in nitrogen tensions to 10 atmospheres absolute. Since no significant or consistent alteration occurred in resting potential, action potential amplitude and polarization rates, membrane resistance or time constant, it is concluded that these mambrane parameters and their underlying conductance states (for sodium and potassium) are not affected by nitrogen tensions through ten atmospheres. This would indicate that these conductance states are stable and well controlled in these neurons to this degree of pressure. Whether such stability would persist at greater nitrogen tensions, or whether vertebrate excitable cells demonstrate the same stability remains to be tested.

Besides synaptic transmission (below), the one parameter of electrophysiological activity that was altered by pressure in Aplysia was the bursting pattern of the endogenously active cell R15. Increased nitrogen tensions may thus alter the mechanism of generation of the pacemaker potential of this cell.

The principle effect of hyperbaric air on the endogenous firing of R15 is on the firing pattern and not on the firing frequency. At pressure there are fewer action potentials generated in each burst. Were this the only effect, the overall firing frequency would decrease with pressure. The burst, however, is shorter and the intra-burst frequency is unaltered by pressure. The interval between bursts is decreased as well. The overall effect is that, while the number of action potentials within a burst period (from the beginning of one burst to the beginning of the next) is decreased, overall action potential frequency (number of action potentials divided by (the interval + duration)) is constant.

Cell R15 has several properties indicating that it has a neurosecretory function (Coggeshall et al., 1966; Frazier et al., 1967). This cell may play a role in regulating water balance in the animal since Kupfermann and Weiss (1976) have shown that homogenates of R15 injected into intact Aplysia can produce weight increases with relatively brief delays even against hyper-

osmotic environmental solutions. Spike activity of R15 is inhibited when hypo-osmotic solutions are applied to the osphradium of the animal (Jahan-Parwar et al., 1969; Stinnakre and Tauc, 1969). The firing of R15, which is normally an endogenous bursting pattern, may thus cause release of a substance(s) that facilitates water uptake or retention. Inhibition or reduction of firing under conditions where water gain is possible, such as exposure to hypo-osmotic sea water, could reduce the tendency for the animal to absorb additional water. The net loss of numbers of R15 spikes under increased pressure conditions could represent a similar response. To the extent that increases in ambient pressure, such as occur when Aplysia are at depth, could shift net water movement into the animal, it would seem appropriate for R15 to lower its overall output of action potentials or reduce its own contribution to water gain. The extent to which this osmo-conforming organism actually experiences water shifts with increasing depth in sea water remains to be tested.

Our Aplysia work differs from other investigations of hyperbaric influences on synaptic transmission at the cellular level in that we have examined the effects of increased nitrogen tensions and have used pressures of only a fraction of the hydrostatic/helium pressures used by others. We have found that in Aplysia (1) short term facilitation (f_2) of a synaptic potential in cell R15 is unchanged; (2) longer term facilitation (f_{100}) is increased; and (3) PSP amplitude and duration are unchanged.

Although we did not directly investigate the effects of hydrostatic pressure per se in this preparation as we did with crayfish, we are confident that the changes induced are due to increased nitrogen tension and not pressure alone for three reasons: (a) direct measurements of nitrogen and oxygen tensions have shown that the partial pressures of these gases are increased in the bathing medium under these conditions (Fig. 2); (b) since no one has shown a direct effect on synaptic transmission in any preparation at hydrostatic/helium pressures of less than around 30 ATA, we doubt that hydrostatic pressure at 10 ATA or less could produce the changes we observed; (c) if the effects we observed are not due to pressure alone, they must be caused by N_2 since O_2 was eliminated as a direct contributing factor. Since oxygen has been shown to effect the size of the PSP in Aplysia fasciata (Nahas et al., 1965) it is important to eliminate changes in oxygen partial pressure as a possible source of the pressure-induced facilitation increase. Pressurization to 10 ATA with 2% oxygen, 98% nitorgen produced the same pressure-induced facilitation (f_{100}) change as did normal air (20% oxygen) at 10 ATA. Thus, an increase in the partial pressure of oxygen is not required to produce these effects. If the effect is caused by pressure alone and not increased nitrogen tensions, then this study illustrates for the first time that certain aspects of synaptic transmission and endogenous bursting patterns of central neurons can be altered by pressures much lower than those heretofore studied and in the range that has significance for animals that experience narcotic symptoms.

Yeandle (1977) examined the effect of helium pressure on two closely spaced (20 ms) endplate potentials at the frog neuromuscular junction and found no changes to 31 ATA. This result is similar to the outcome of the measurement of f_2 in the current study where no pressure modification of this short term facilitation or depression was found. The neuromuscular junction in rat also is resistant to pressure. In normal calcium solutions no pressure-

induced effect was observed at helium pressures to 137 ATA (Kendig and Cohen, 1976). The neuromuscular junction in some invertebrates has been influenced by pressure. Pressure (200 ATA) depresses junctional transmission in crab and lobster (Campenot, 1975). In a crab known to inhabit depths to 1600 m this depression can be reversed by increasing the stimulus frequency. It was suggested that this frequency-dependent effect might be due to a pressure-induced facilitation. In Aplysia a pressure-induced facilitation is observed (Table 1) but in a central neuron and not at the neuromuscular junction.

When one looks elsewhere from the neuromuscular junction, the effects of pressure on synaptic transmission are more apparent. Synaptic transmission at the squid giant synapse is slowed and the junction fatigues much more rapidly at pressure (helium to 200 ATA) (Henderson et al., 1977). The superior cervical ganglion is rat (Kendig et al., 1975) also shows severe depression at pressures to 137 ATA of helium. The behavior of the Aplysia PSP studied here at pressure is dependent, somewhat as in the crab, on the stimulus regime employed, although depression was not observed. Synaptic transmission brought about by closely spaced stimuli (500 ms) showed no change with pressure (Table 2). Continued stimulation, however, does produce facilitation which grows above control levels as pressure is increased. Since the effects of hydrostatic/helium pressure are depressant on synaptic transmission, it is possible that increased nitrogen tensions may tend to facilitate such transmission under certain conditions. Increased nitrogen tensions cause an opposite effect (increased kinetics) on action potential characteristics as compared to the effects of pressure alone (Figs. 4 and 5). This is difficult to reconcile with the additive depressant effects of halothane and high helium pressures on synaptic transmission observed by Kendig et al. (1975).

Since we could find no change in input resistance in Aplysia induced by pressure (Figs. 8 and 9), this suggests that a membrane conductance change in the postsynaptic cell is probably not the prime cause of the observed increase in facilitation with pressure. Schlapfer and coworkers (1974, 1975; Woodson et al., 1976) have argued that the mechanism responsible for the facilitation phenomena for the R15 PSP studied here at 1 ATA is presynatpic. At the rat neuromuscular junction (Kendig and Cohen, 1976) it was suggested that the depressant pressure effect in low calcium was due to a reduced safety factor in the amount of transmitter released. Campenot's (1975) results also indicate that pressure affects presynaptic mechanisms.

The fact that Aplysia is considered an intertidal animal that has not been reported at depths greater than 200 feet ft. (Eales, 1960; Miller, 1960), indicates that we have only mildy "stressed" this animal's nervous system by taking it to depths equivalent to some 300 feet of sea water. At higher pressure some effects in addition to reduced bursting and improved synaptic facilitation might be demonstrated.

The data on pressure effects on action potential parameters in crayfish axon might indicate that one advantage of this preparation is that it is not known normally to dive to the depths simulated in this study (250 feet of seawater) and is presumably not preadapted to function at pressure. We have shown that low hydrostatic pressures can alter the action potential properties of crayfish axons and are in keeping with the findings of others using squid

(Henderson and Gilbert, 1975; Henderson et al., 1977; Spyropoulos, 1957b), frog (Grundfest, 1936) toad (Spyropoulos, 1957a) and mammalian excitable tissues (Kendig et al., 1975). The small but significant decrease in the rates of depolarization and repolarization of the crayfish action potential closely parallel the voltage clamping results of Henderson and coworkers (Henderson and Gilbert, 1975; Henderson et al., 1977) who demonstrated a prolongation of the time course of sodium and potassium currents in the squid axon action potential under high helium pressure. Because of the variety of preparations used, techniques employed (extracellular, intracellular and voltage clamp recording), and pressure conditions (hydrostatic or helium at 1 to 2 orders of magnitude greater pressure than used in this study), it is difficult to ascertain whether the lack of clear effect on action potential duration and amplitude in our hydrostatic studies are contradictory to previous findings. Since changes in these parameters were sometimes slight or variable in other species at much higher pressures, we feel that our inability to detect such changes reflects the use of relatively low pressures.

The most significant effects of increased air (nitrogen) pressure found were on the rates of depolarization (Fig. 4) and repolarization (Fig. 5) of the action potential. The magnitude of the pressure-induced change in depolarization rate (12 V/sec·atm) is larger than that in the repolarization rate (7.2 V/sec·atm). However, when the percentage or relative change in these rates with pressure is examined, the changes in the rates of polarization are approximately the same. Spyropoulos (1957b) reported that 5,000 psi hydrostatic pressure had a relatively greater effect in slowing of repolarization than of depolarization. The data from the current study indicate that the action potential kinetics are speeded up as nitrogen pressure is applied. Both depolarization and repolarization occur faster under air pressure, the opposite effect of increased hydrostatic pressure.

If both rates of polarization of the action potential are increased with the amplitude remaining constant, this should naturally lead to an overall decrease in the total action potential duration, which we observed (Fig. 6). Decreases in both rates of polarization should lead to an overall lengthening of the action potential duration. This effect has been observed in other preparations under hydrostatic or helium pressures (Henderson and Gilbert, 1975; Shrivastav et al., 1978; Spyropoulos, 1957b). There was a tendency for crayfish axon action potentials to lengthen under hydrostatic conditions in this study. Presumably, had we gone to higher pressures the effect would have become significant.

The results of this study clearly indicate that increased nitrogen tensions cause changes in action potential characteristics which are opposite to those induced by increases in hydrostatic or helium pressures. The magnitude of the effects is greater with air pressure based on our own results, but the work of others at higher hydrostatic or helium pressures confirms that the direction of our "control" hydrostatic results were correct. Although the same parameters, that is, depolarization and repolarization rates, were oppositely influenced by air and hydrostatic pressures, it is not warranted to state that such results unambigously support the "critical volume" hypothesis (Miller, 1974, 1977). Whether the two types of pressure oppositely affect the same molecular biophysical properties of the excitable membrane cannot be determined from the these results alone. It is of interest that the effects

of increased nitrogen tensions are of the sort that resemble "excitatory" rather than "depressant or anesthetic" actions. Typically, anethetics tend to slow and block action potential currents. Perhaps higher gas tensions would have lead to such an effect in our system, and in the less than 10 ATA range of air pressures only an anomolous facilitatory effect is observed. One could also argue that the effects seen could lead to earlier fatigue and thus eventually to depression of the system, but this is not a compelling argument. It is also of interest to note that several workers (see Bennett et al., 1967, for example) have suggested that increased inert gas tensions should lead to increased cation permeability. There are, however, peculiar aspects to most of the previous neurophysiological studies dealing with the "antagonistic" effects of pure pressure and anesthetics. All workers have observed that increased hydrostatic or helium pressures lead to a fundamentally detrimental conditons of a reduced amplitude, prolonged duration action potential. There may or may not be changes in threshold under these conditons. At the same time, the application of pressure has been shown to ameliorate the suppressive effects of anesthetics (Kendig et al., 1975; Shrivastav et al., 1978; Spyropoulos, 1957b). Until further data is available it is too early to know how two presumably depressive effects might work through a common mechanism to antagonize one another. Recent evidence (Shrivastav et al., 1978) would suggest that anesthesics and pressure may not affect precisely the same membrane processes.

The present findings do illustrate the occurrence of reversible alterations in the membrane properties of single nerve cells under hyperbaric gas conditions. These conditions simulate those under which human divers may experience neurologic abnormalities associated with nitrogen narcosis. We have also found that similar conditons cause changes in endogenous bursting patterns and synaptic facilitation in the marine mollusc Aplysia. Whether or not any of these alterations actually represent a basis for neural dysfunction in diving man remains to be tested, but at least it is now clear that such subtle effects can be detected in living systems.

TABLE 1

CELL R15 FACILITATION RATIOS (f_{100}) BETWEEN THE ONE-HUNDREDTH AND FIRST POSTSYNAPTIC POTENTIAL

	Pressure Medium		
	Air	Air	2% O_2
Pressure (ATA)	Artificial Sea Water	Low Calcium Sea Water	Artificial Sea Water
1	1.64 ± 0.11	2.65 ± 0.27	2.03 ± 0.12
10	1.92 ± 0.16	3.23 ± 0.56	2.64 ± 0.14
f_{100} (10 ATA)/f_{100} (1 ATA)	1.17 ± 0.05	1.2 ± 0.1	1.30 ± 0.08
Number of animals	11	5	4

Postsynaptic potentials were produced in a hyperpolarized R15 cell by stimulation of the right connective. Facilitation ratios are given as (average ± SE) and are dimensionless. Multiple measurements were taken from each animal. ATA = atmospheres absolute. Sea level pressure is 1 ATA. Temperature was 15°C.

TABLE 2

CELL R15 FACILITATION RATIOS (f_2) BETWEEN THE SECOND AND FIRST POST-SYNATPIC POTENTIALS IN STIMULUS TRAIN

	Pressure Medium		
	Air	Air	2% O_2
Pressure (ATA)	Artificial Sea Water	Low Calcium Sea Water	Artificial Sea Water
1	0.94 ± 0.03	1.14 ± 0.07	0.99 ± 0.02
I0	0.93 ± 0.03	1.1 ± 0.1	1.08 ± 0.03
f_2(10 ATA)/f_2(1 ATA)	0.99 ± 0.03	0.96 ± 0.09	1.09 ± 0.05
Number of Animals	11	5	4

Postsynatpic potentials were produced in a hyperpolarized R15 cell by stimulation of the right connective. Facilitation ratios are given as (average ± SE) and are dimensionless. Multiple measurements were taken from each animal. ATA = atmospheres absolute. Sea level pressure is 1 ATA. Temperature was 15°C.

TABLE 3

CELL R15 BURST PATTERN SUMMARY

	1 ATA (measured)	7.8 ATA (calculated)	Pressure Coefficient (%change/ATM)	Correlation Coefficient
Action Potentials per Burst	24 ± 3	18.0	-3.7 ± 0.4	0.81
Burst Duration (sec)	9.1 ± 0.5	6.3	-4.6 ± 0.6	0.81
Interval Between Bursts (sec)	20 ± 1	14.3	-4.2 ± 0.6	0.77

Cell R15 was penetrated with one microelectrode and allowed to produce its characteristic bursting pattern without polarization or stimulation. ATA = atmospheres absolute. Sea level pressure is 1 ATA. Values quoted at 1 ATA represent the (average ± SE) of multiple measurements from 16 preparations. Values quoted at 7.8 ATA were calculated from the 1 ATA data and the respective pressure coefficient. The correlation coefficient is the Pearson product-moment correlation coefficient (7). Temperature was 15°C.

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