

Privileged Communication
Felix Strumwasser

FINAL TECHNICAL REPORT
(July 1969 - June 1973)

In order to avoid redundancy, I include here only the summary of each paper published since the last major Renewal Application, preceded by a list of publications. A list of professional personnel occurs on the last page.

PUBLICATIONS

PAPERS:

- Arch, S. 1972a Polypeptide secretion from the isolated parieto-visceral ganglion of Aplysia californica. J. Gen. Physiol. 59:47-59.
- Arch, S. 1972b Biosynthesis of the egg-laying hormone (ELH) in the bag cell neurons of Aplysia californica. J. Gen. Physiol. 60:102-119.
- Audesirk, G. 1973 Spontaneous and light-induced compound action potentials in the isolated eye of Aplysia: Initiation and synchronization. Brain Research. 59:229-242.
- Berry, R. W., Arch, S. and D. L. Wilson. 1973 "Aplysia nerve proteins" In: Proteins of the Nervous System, (ed. D. Schneider) Raven Press. In press.
- Berry, R. W. and D. A. Weisblat. 1973 The effect of N-ethylmaleimide on membrane conductances in the giant neuron of Aplysia. In preparation.
- Eskin, A. 1971 Properties of the Aplysia visual system: In vitro entrainment of the circadian rhythm and centrifugal regulation of the eye. Zeit. f. vergl. Physiol. 74:353-371.
- Eskin, A. 1972 Phase shifting a circadian rhythm in the eye of the Aplysia by high potassium pulses. J. Comp. Physiol. 80:353-376.
- Jacklet, J. W., Peretz, B. and F. Strumwasser. 1970 Synaptic influences on identified neurons in an aberrant parieto-visceral ganglion of Aplysia. Zeit. f. vergl. Physiol. 66:318-325.
- Jacklet, J. W., Alvarez, R. and B. Bernstein. 1972 Ultrastructure of the eye of Aplysia. J. Ultrastructure Research 38:246-261.
- Meech, R. W. 1972 Intracellular calcium injection causes increased potassium conductance in Aplysia nerve cells. Comp. Biochem. Physiol. 42:493-499.
- Parnas, I., Armstrong, D. and F. Strumwasser. 1973 Prolonged excitatory and inhibitory synaptic modulation of a bursting pacemaker neuron. J. Neurophysiol. (Submitted)

- Parnas, I. and F. Strumwasser. 1973 Mechanisms of long lasting inhibition of a bursting pacemaker neuron. J. Neurophysiol. (Submitted)
- Peretz, B. 1969 Central neuron initiation of periodic gill movements. Science 166:1167-1172.
- Peretz, B. 1970 Habituation and dishabituation in the absence of a central nervous system. Science 169:379-381.
- Strumwasser, F. 1971a The cellular basis of behavior in Aplysia. In: Problems, Practices and Positions in Neuropsychiatric Research (ed. J. V. Brady). J. Psychiat. Res. 8:237-257.
- Strumwasser, F. 1971b Discussion to "Some factors governing entrainment of a circadian rhythm in a single neuron." In: Biochronometry (ed. M. Menaker). Washington, D.C.: National Academy of Sciences, pp. 561-564.
- Strumwasser, F. 1973a Neural and humoral factors in the temporal organization of behavior. (The seventeenth Bowditch Lecture). The Physiol. 16:9-42.
- Strumwasser, F. 1973b Neuronal principles organizing periodic behaviors. In: The Neurosciences: Third Study Program. Cambridge: The MIT Press
- Ward, S., Wilson, D. L. and J. J. Gilliam. 1970 Methods for fractionation and scintillation counting of radioisotope-labeled polyacrylamide gels. Analytical Biochem. 38:90-97.
- Wilson, D. L. 1971 Molecular weight distribution of proteins synthesized in single, identified neurons of Aplysia. J. Gen. Physiol. 57:26-40.
- Wilson, D. L. and R. W. Berry. 1972 The effect of synaptic stimulation on RNA and protein metabolism in the R2 soma of Aplysia. J. Neurobiol. 3:369-379.
- ABSTRACTS:
- Arch, S. 1971 Biosynthesis and processing of a neurohormone in Aplysia californica. The Physiol. 14:104.
- Audesirk, G. 1971 Neuronal interactions in optic nerve impulse production in Aplysia. The Physiol. 14:105.
- Audesirk, G. 1973 Interaction of two circadian oscillators in the isolated central nervous system of Aplysia. The Physiol. 17: 256.
- Eskin, A. and F. Strumwasser. 1971 Properties of the Aplysia visual system: In vitro entrainment of the circadian rhythm; centrifugal regulation. Fed. Proc. 30:666.
- Meech, R. W. and F. Strumwasser. 1970 Intracellular calcium injection activates potassium conductance in Aplysia nerve cells. Fed. Proc. 29:834.

- Parnas, I., Armstrong, D. and F. Strumwasser. 1973 Long-lasting synaptic modulation of spontaneous neuronal activity. J. Gen. Physiol. In press.
- Peretz, B. 1969 Habituation and coordinated movement in the gill of a deganglionated Aplysia preparation. Amer. Zool. 9:1113.
- Ram, J. 1972 Effects of high potassium media on radioactive leucine incorporation into Aplysia nervous tissue. The Physiol. 15:242.
- Rothman, B. and F. Strumwasser. 1973 Aflatoxin B1 blocks the circadian rhythm in the isolated eye of Aplysia. Fed. Proc. 32:365.
- Sener, R. 1972 Site of circadian rhythm production in Aplysia eye. The Physiol. 15:262.
- Strumwasser, F. 1972 Neural and humoral factors in the temporal organization of behavior. Trans. Amer. Soc. for Neurochem. 3:44.
- Strumwasser, F., Schlechte, F. R. and S. Bower. 1972 Distributed circadian oscillators in the nervous system of Aplysia. Fed. Proc. 31:405.

SUMMARY FROM EACH PAPER

Polypeptide Secretion from the Isolated Parietovisceral Ganglion of Aplysia californica. In vitro studies of the secretory behavior of the parietovisceral ganglion in Aplysia californica were performed. The aim of these studies was to investigate the release of polypeptides in response to depolarizing stimuli, and, in particular, to determine if a specific polypeptide known to induce egg laying in the intact animal is secreted into the bathing medium. During continuous perfusion of a ganglion preincubated in leucine-³H the application of either high-potassium medium or a burst of electrical stimuli (via the pleurovisceral connective nerve) evoked a marked increase in the amount of trichloroacetic acid (TCA)-precipitable radioactivity recovered in the perfusate. Enhanced release could be detected within 80 sec of the initial exposure to high potassium; however, incubation of a ganglion in calcium-free media before the application of high-potassium medium abolished the increase of precipitable radioactivity. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of perfusate samples revealed a significant change in the polypeptide species washed from the ganglion during high-potassium depolarization. Bioassays confirmed that egg laying is induced when high-potassium medium used to bathe a ganglion is injected into a recipient animal. These and other results permit the conclusion that the bulk of the polypeptide material secreted from the ganglion in response to depolarization is a specific neurohormone produced by two identified cell clusters, the so-called bag cells.

Biosynthesis of the Egg-Laying Hormone (ELH) in the Bag Cell Neurons of Aplysia californica. Biosynthesis of the egg-laying hormone in the bag cell neurons of Aplysia californica was studied. Bag cells were incubated with leucine-³H in vitro for 30 min and rinsed for variable periods of time in a chase medium. The distribution of incorporated label among proteins within the cells was assayed

by electrophoresis of an homogenate on sodium dodecyl sulfate polyacrylamide gels. Results from rinse times shorter than 30 min revealed that the predominant synthetic product is a 25,000 dalton protein. With longer rinse times, this species was reduced and two species of lower molecular weight became prominent. This redistribution of radioactivity was quantitative and was not prevented by inhibition of protein synthesis during the rinse. A 10°C reduction in temperature (from 15°C) blocked the redistribution. These data are interpreted to indicate that the 25,000 dalton molecule is a precursor which is cleaved enzymatically to yield two lower molecular weight products. One product is a 12,000 dalton molecule which remains in the cell bodies. The other is a molecule of <10,000 daltons, which is exported from the somata into the neurohemal regions of the connective tissue. Perfusion of these regions with high $[K^+]$ medium results in the release of this product into the medium. It is concluded that this product is the 6000 dalton egg-laying hormone (ELH).

Spontaneous and Light-Induced Compound Action Potentials in the Isolated Eye of *Aplysia*: Initiation and Synchronization. The isolated eye of *Aplysia* shows a bursting pattern of spontaneous compound action potentials (CAPs) in the dark. The 'light response', also composed of CAPs, may be separated into a phasic initial response and a tonic late response similar in form to the dark discharges when the illumination is of low intensity. Blockage of chemical synapses with La^{3+} or high Mg^{2+} -low Ca^{2+} stops the dark discharge and tonic light response but not the phasic light response. Synchrony of the CAPs is not affected. Ca^{2+} -free solutions produce continuous rapid firing of CAPs, seldom coordinated into bursts. Replacement of chloride by propionate abolishes all CAPs for several hours, but leaves the ERG intact and the optic nerve electrically excitable. Low sodium levels (about 50% normal) suppress dark discharge and tonic light activity but allow a normal phasic light response. It is concluded that receptors transmit light-induced excitation to a higher order neuron population via electrical junctions, and that synchrony of the CAPs is also due to electrical junctions, interconnecting the higher order population. One or more pacemaker cells are postulated to drive the higher order neurons by chemical synapses, producing the dark discharges and the low intensity tonic light response. The pacemaker mechanism may be sodium dependent.

The Effect of N-ethylmaleimide on Membrane Conductances in the Giant Neuron of *Aplysia*. N-ethylmaleimide reacts irreversibly with sulfhydryl groups in the cell membrane of the giant neuron of the abdominal ganglion of *Aplysia californica*. Physiological correlates of this reaction include: i) a loss of both transmembrane electrical and synaptic excitability, and ii) a five to ten-fold decrease in the soma membrane's input resistance. The resistance decrease is sufficient to account for the loss in excitability, and results from an increase in chloride permeability together with a smaller increase in potassium permeability. Sodium permeability does not appear to be increased. In spite of the drop in resistance, the resting potential is maintained for at least 20'. This appears to be due to a shift in the Nernst equilibrium potentials for chloride and potassium ions.

Properties of the *Aplysia* Visual System: In vitro Entrainment of the Circadian Rhythm and Centrifugal Regulation of the Eye. Properties of the visual system of *Aplysia californica* were studied by recording optic nerve impulses extracellularly from isolated eyes in constant darkness. In vivo entrainment of the circadian rhythm of afferent optic nerve impulses by LD 12:12 cycles phase advance 13 hours was essentially complete after only one exposure of the animal to this light cycle. The impulse rhythms of eyes exposed to the phase advance light cycle

in vitro entrained, but in this case 4 to 5 exposures to the advance light cycle were required before entrainment was complete. The difference in the rates of in vivo and in vitro entrainment obtained whether eyes remained attached to the cerebral ganglion or were cultured in filtered Aplysia blood instead of a modified Eagle's minimal essential medium. Eyes maintained in vitro for 9 days expressed circadian impulse rhythms. The behavior of the eye is influenced by efferent activity from neuronal elements in the cerebral ganglion. The pattern of afferent optic nerve impulses and the waveform of the afferent impulse rhythm varies depending on whether or not the eye remains attached to the cerebral ganglion. Electrical stimulation of either the optic, tentacle, or rhinophore nerves, which emanate from the cerebral ganglion, produces an inhibition of the efferent optic nerve activity. This result indicates that a mechanism exists in Aplysia for the activity of one eye to affect the behavior of the contralateral eye and for other sensory inputs to affect the behavior of the eye as well.

Phase Shifting a Circadian Rhythm in the Eye of Aplysia by High Potassium Pulses. The circadian rhythm of spontaneous optic nerve impulses from the isolated eye of Aplysia californica was studied. A general hypothesis was investigated: light-dark cycles, which can entrain in vitro the rhythm from the eye, couple to the intracellular clock mechanism through membrane depolarization. This hypothesis was tested by exposing isolated eyes for fixed durations of time to a depolarizing stimulus, namely, elevation of K^+ (hi-K pulses). Depending on the phase of the rhythm at which the eyes were treated, hi-K pulses produced either advance or delay phase shifts in the rhythm. A phase response curve was thus generated for 107 mM K^+ pulses of 4 hr duration. If the duration of the hi-K pulse was varied, keeping the phase at which the pulse started constant, the magnitude of the phase shift varied in a linear manner with the duration of the pulse. To examine the involvement of transmitter and neurohormone release in the production of the phase shifts, eyes were exposed to hi-K pulses in the presence of a medium (high Mg^{++} , low Ca^{++}) which should inhibit such release. Since only slight differences were observed in the phase shifts produced by hi-K and by hi-K in the presence of high Mg^{++} , low Ca^{++} , transmitter and neurohormone release do not appear to be involved in phase shift production by hi-K. It is likely that membrane depolarization alone is responsible for the phase shifts.

Synaptic Influences on Identified Neurons in an Aberrant Parieto-Visceral Ganglion of Aplysia. One aberrant parieto-visceral (abdominal) ganglion in Aplysia californica was found in which the right hemiganglion was separated from the left hemiganglion by a commissure approximately 1 cm long. Simultaneous records were made from R15 (intracellular) and the commissure in the isolated right hemiganglion; from L11 (intracellular) and the genital and pericardial nerves in the isolated left hemiganglion; in addition, intracellular recordings were made from R14. These and other cells were found in their respective hemiganglia in the position they usually occupy in the normal ganglion. The recordings have allowed us to indicate the relative influence or lack of influence of L10 and Interneuron II on R15 and L11 in their respective hemiganglia and the position of Interneuron II as being in the right hemiganglion. R15 was shown to send an axon into the interganglionic commissure. R15's bursting activity was modulated by an ILD-E input from Interneuron II in that hemiganglion.

Ultrastructure of the Eye of Aplysia. The eye and optic nerve of the sea hare, Aplysia californica, were examined with the light and electron microscopes. The eye is a closed vesicle type with a spheroidal lens nearly completely surrounded by the retina. It contains several thousand receptor, pigmented, and

other cells. Receptor cells are replete with 500-700 Å clear vesicles, pigment granules, and have a diffusely organized microvillous rhabdomere that projects to the lens. The fibers of the receptor cells and other cells form a retinal neuropile where synapses occur between these fibers. Pigmented cells have a short microvillous distal segment and associated rudimentary cilia. Cellular processes which contain dense vesicles (900-1200 Å) are prominent in the retinal neuropile. The majority of the processes appear to be the neurites of the receptor and other cells that contribute to the optic nerve. Some of these cellular processes end in microvillous processes on diffuse vascular spaces at the base of the retina near the epineural sheath. Receptor fibers and perhaps others contribute to the several thousand optic nerve fibers (0.3-3.0 μ diameter).

Intracellular Calcium Injection Causes Increased Potassium Conductance in Aplysia Nerve Cells. Small quantities of calcium salt were injected into Aplysia neurones. The membrane potential was hyperpolarized because of a specific increase in potassium conductance. The hyperpolarization was reversibly blocked by tetraethylammonium chloride. Combination of calcium with the cell membrane may be an important step in potassium activation.

Prolonged Excitatory and Inhibitory Synaptic Modulation of a Pacemaker Neuron. Aplysia has a circadian rhythm of locomotor activity that is entrained by the eyes (Strumwasser, 1973). A neurosecretory neuron, R15, in the isolated PVG also shows a circadian rhythmicity of impulse activity but the mechanism of its entrainment is still unknown. Since any nervous signals from the eye to R15 must pass through the pleuro-visceral connectives, we were interested in discovering whether changes in bursting activity could be produced by synaptic inputs to R15. We find that activation of the d-TC sensitive excitatory input of the right connective at 1-4/sec for only 3 minutes leads to a dramatic increase in the number of spikes and average frequency per burst that persists for hours. Nevertheless, the mean frequency after stimulation remains constant because the longer bursts are associated with longer interburst intervals. Hyperpolarizing the cell to block all impulse generation for several minutes abolishes the change, and the number of spikes per burst returns almost immediately to the prestimulation level. This long-lasting excitatory effect cannot be produced by stimulating the cell with depolarizing current, whether steady or with waves of the same amplitude and duration as the epsp. This suggests that the critical stimulus is either binding of transmitter or the conductance changes associated with it rather than electrogenesis itself. This notion is supported by the effectiveness of synaptic stimulation in producing the excitatory effect despite sufficient d-TC to reduce the epsp to 20% of its control amplitude (~2 mv).

Mechanisms of Long-Lasting Inhibition of a Bursting Pacemaker Neuron. In addition to the biphasic psp, bpsp (described above) of the third input, there is a delayed (30 to 60 seconds) hyperpolarization after even a single bpsp. With a short train of pulses at 1/sec this "delayed hyperpolarization" (DH) is very pronounced and can last for 20 minutes up to 4 hours depending on the number of pulses in the train (3 to 60 pulses). This long hyperpolarization completely blocks spontaneous activity in R15 and is associated with an increase in membrane conductance. This DH cannot be due to reverberatory synaptic action since application of tetrodotoxin and zero Ca-80 mM Mg immediately after stimulation does not block the effect. Neither is the DH dependent on pump activity since the long-lasting conductance increase persists in ouabain (10^{-3} M). The effects of lithium could not be evaluated since it alone reduced membrane resistance by 50%. The DH can

be selectively blocked by intracellular injection of tetraethylammonium; therefore, we suspect that the DH is due to increases in potassium conductance whereas the hyperpolarizing phase of the bpsp is caused by an increase in chloride, as well as potassium conductance. However, chloride must be present to produce the DH although its direction of movement is not important. By hyperpolarizing the cell beyond E_{Cl} , presumably we caused chloride to move out of the cell during the psp, but the DH still occurred. Intracellular injection of sodium or chloride produces hyperpolarization together with a conductance increase, but the effect only lasts a few minutes. Thus we demonstrate two synapses, one excitatory and one inhibitory, that can alter the spontaneous output of R15 for hours after relatively few pulses. It is possible that these synapses might be involved in the entrainment of R15 to different light-dark cycles. This hypothesis is supported by G. Audesirk's demonstration that the first input epsp can be evoked by stimulation of the optic nerve.

Central Neuron Initiation of Periodic Gill Movements. In Aplysia periodic spontaneous gill movements are controlled by activity endogenous to the abdominal ganglion. These movements were still observed when only the ctenidio-genital nerve was left intact between the ganglion and the gill. One kind of spontaneous gill movement (one per 5 minutes at 15°C) was correlated with the expression of activity of Interneuron II; others were not. With reference to this kind of spontaneous gill movement, four types of central neurons in the ganglion send processes to the gill via the nerve. Two cell types (ii, iii) are inhibited and the other two (i, iv) are excited. Two types (i, ii) elicited gill movement -- one type activating large gill areas elicited spontaneous gill movements, and the other activating specific gill regions did not participate in the spontaneous gill movements. The value of this preparation in studying the role of central neurons eliciting specific patterned movements and the temporal organization of their activity is shown.

Habituation and Dishabituation in the Absence of a Central Nervous System. Habituation and dishabituation have been observed in a semi-intact Aplysia preparation in which the central nervous system is removed. The amplitude of withdrawal responses in the gill decreases in proportion to the rate of water drops applied, 1/0.5 to 1/2.5 min at 15°C. The effects of habituation last for at least two hours. A dishabituated response is elicited by stopping water drops or electrically stimulating the preparation. Further, the gill was found to contain nerve cell bodies and habituation and dishabituation appear to be properties of these peripheral neurons.

The Cellular Basis of Behavior in Aplysia. This essay includes some of the more important findings of our continuing research on the nervous system of the sea hare, Aplysia. My associates and I have tried and are trying to understand the temporal organization of behavior in terms of cellular processes and macromolecular metabolism which can be readily studied in the large and identifiable neurons of Aplysia. The long-term cyclic processes which we have discovered in some of these neurons may underlie behaviors such as sleep and waking, reproductive cycles, and periodic feeding. In view of the conservative nature of neuronal physiology, as reviewed earlier, it would be surprising if our investigations of these processes in Aplysia did not have relevance for the vertebrates including man.

Neural and Humoral Factors in the Temporal Organization of Behavior. It appears that both slow and even circadian oscillators can occur as special mechanisms within single neurons. In the parabolic burster neuron (and the eye) of

Aplysia, the slow 1/minute and circadian oscillations already span three orders of magnitude, in terms of frequency; therefore, it seems that single mechanisms governing both oscillators are unlikely. There are weakly electric fish (certain fresh water gymnotids) that generate electric pulses, used in navigation and communication, at rates around a few hundred Hz for all of their life (Lissmann, 1958). It seems unlikely that this high frequency oscillation would have a mechanism similar to that of the parabolic burster slow pacemaker oscillator which is four orders of magnitude lower in frequency. It appears likely that different aspects of cellular organization (pumps and channels in membranes, transcriptional and translational controls in macromolecular metabolism) have evolved to cover these seven orders of magnitude of frequency. The functional significance of oscillators can only be worked out when there is enough specific information in favorable cases. The eyes of Aplysia, from the evidence presented in this paper, have a circadian rhythm of optic nerve impulses which appears to serve to synchronize other circadian oscillators. Macrobehaviors, such as sleep, waking and cycles of sexual activity, can be imagined to be controlled by such systems of circadian oscillators, but the details clearly need to be worked out before most of us will be convinced. Some circadian oscillators are neurosecretory. The nature of neurosecretory products and their physiological and behavioral effects is an area with sparse information but again in favorable cases, such as Aplysia, a single polypeptide neurosecretory product (Toevs and Brackenburg, 1969; Toevs, 1970; Arch, 1972) is known to organize behavioral egg-laying (Kupferman, 1967; Strumwasser et. al., 1969). The conservative nature of neuronal and glial mechanisms discussed in the introduction of this paper is a constant reminder that the chances of encountering general principles from special cases, where the system is more accessible to analysis, are quite good indeed.

Neuronal Principles Organizing Periodic Behaviors. How do neurons produce and read out the free-running (nonreflexive) programs that control behavior? The answer to this question may involve certain specialized neurons that possess oscillators with long periods (minutes to hours). When these neurons proceed into the active phase of their cycle, they may turn on and turn off different populations of neurons controlling or triggering different behaviors. The property that behavioralists refer to as motivation may be associated with the activity of such sophisticated neurons.

All of the work that I will describe has been performed on the well-known marine mollusc, Aplysia. The behaviors that I study are periodic, e.g. sleep-waking and sexual cycles. The sleep-waking cycle is circadian in period, while sexual activity has a pronounced annual cycle in Aplysia. With the help of my colleagues, we are beginning to learn about the nature of the secretions that control one aspect of reproductive behavior, i.e., egg-laying (Toevs, 1970; Arch, 1972). Egg-laying behavior is entirely under the control of a 6000 dalton polypeptide synthesized by several hundred neurosecretory neurons, the bag cells, in the parieto-visceral ganglion.

Sleep-waking behavior has been studied by time-lapse photography and, more recently, by an automatic tracking system involving a TV camera, a special video encoder that recognizes the animal, and a computer that stores the animal's position and computes its movements. The short- and long-term effects on the sleep-waking system of removing or ligaturing several parts of the nervous system (the eyes, the parieto-visceral, and the cerebral ganglion) are described, which contain neurons possessing circadian oscillators. The results, in brief, suggest that the

eyes mediate both entrainment and synchronization of the sleep-waking cycle and that the motor commands for movement emanate from the cerebral ganglion.

The frequency of optic nerve impulses in the isolated eye of *Aplysia* oscillates with a large-amplitude circadian period when kept in darkness (Jacklet, 1969; Eskin, 1971). The eye is now a second example of a circadian neural oscillator, the other being the parabolic burster neuron (R15) in the parieto-visceral ganglion (Strumwasser, 1965). The neural organization within the eye has been deduced, so far, from experiments involving blockers of chemical and electrical synapses (Audesirk, 1971, 1972) and from tissue reduction and microstimulation experiments (Sener, 1972). These findings will be reviewed, as they provide the best available (but indirect) evidence that special pacemakers with a circadian oscillator mechanism drive a larger population of electrically coupled follower output cells. Eskin, this volume, demonstrates that membrane depolarization influences the circadian oscillation in the eye, which suggests that processes in the membrane are coupled to the internal oscillator. Other experiments have shown that in R15 a circadian cycle of membrane activity can be expressed when synaptic transmission and impulse production is blocked in the entire ganglion (Strumwasser, 1971). The circadian rhythm in this neuron is expressed as a modulation of faster pacemaker waves. The available evidence for the mechanism of pacemaker wave production will be reviewed at both the membrane electrophysiological and biochemical levels. The macromolecular constitution of some of these specialized pacemaker and circadian neurons has been studied by separating on gels the proteins synthesized in single identifiable neurons (Wilson, 1971).

Results from the organ-cultured parieto-visceral ganglion (PVG) will be described. Multiunit discharges can be recorded from the nerve trunks of the PVG for periods of up to 6 weeks. A special digital template-sorter has been developed and is capable of reliably sorting the discharges of up to 8 single neurons recorded with a single electrode. Thus, the long-term activities of several neurons can be selectively and simultaneously tracked 24 hours a day for many weeks. Some of these single neurons show regular circadian periods in their discharge rates, allowing a study of the organization of a circadian system by direct observation of the unit elements.

Methods for Fractionation and Scintillation Counting of Radioisotope-Labeled Polyacrylamide Gels. This paper describes two polyacrylamide gel fractionators. One can be easily constructed from disposable syringes and used for fractionating standard-size analytical gels. The second is more complex but can be used for fractionating miniature gels. A rapid method of preparing gel fractions for liquid-scintillation counting is also presented. The method gives high counting efficiency, does not require hydrolysis of the gel prior to counting, and allows gel fractions to be collected directly in scintillation vials.

Molecular Weight Distribution of Proteins Synthesized in Single, Identified Neurons of *Aplysia*. Parietovisceral ganglia from *Aplysia californica* were incubated in medium containing leucine-³H. Single, identified nerve cell somas were isolated from the ganglia, and their proteins extracted and separated by electrophoresis on 5% SDS-polyacrylamide gels. The distribution of total or newly synthesized proteins from the single neurons was determined by staining or slicing and liquid scintillation counting of the gels. Experiments showed that: (a) a number of proteins were being synthesized in abundance in the nerve cells; (b) different, identified neurons showed reproducibly different labeling patterns in the gels;

(c) cells R2 and R15, which showed different distributions of radioactivity in the gels, had similar staining patterns; and (d) there was significant incorporation into material of high (>75,000) molecular weight in most of the cells.

The Effect of Synaptic Stimulation on RNA and Protein Metabolism in the R2 Soma of Aplysia. Excitatory synaptic stimulation of the R2 neuron in the abdominal ganglion of Aplysia californica causes an increased incorporation of ^3H -uridine into RNA. However, this could be the result of a change in precursor specific activity rather than an increase in RNA synthesis. We find that at low external uridine concentrations (1.5 μM) there is no increase in ^3H -uridine incorporation correlated with synaptic stimulation. In addition, no change in incorporation of ^3H -leucine into total protein or in the pattern of newly-synthesized proteins, resolved by electrophoresis on SDS-polyacrylamide gels, was detected with stimulation. Since the R2 neuron can be stimulated without a detectable change in RNA or protein synthesis, we conclude that the increase in incorporation observed at high external uridine concentrations (100 μM) could be caused by increased specific activity in a precursor pool rather than by an RNA synthesis change.

STAFFING

<u>Professional Personnel</u>	<u>Title</u>	<u>Period of Appointment</u>
Stephen Arch	Research Fellow	3/70 - 8/72
Robert W. Berry	Research Fellow	7/71 - 12/72
Douglas Eaton	Visiting Associate	7/73 - 12/73
Arnold Eskin	Research Fellow	4/69 - 9/71
Robert W. Meech	Sr. Research Fellow	1/69 - 12/69
Itzchak Parnas	Visiting Associate	9/72 - 11/73
Bertram Peretz	Research Fellow	2/68 - 9/69
Robert Sener	Research Fellow	7/72 - 6/73
Mary Weir	Research Fellow	3/69 - 4/70
David Wilson	Research Fellow	7/69 - 3/72