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A Study on the Identified Neurons Related to the Visceral Nerve in the Terrestrial Slug, *Incilaria fruhstorferi daiseniana*.

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육생 민달팽이 *Incilaria fruhstorferi daiseniana*의 내장신경과 관련이 있는 동정된 뉴우런에 관한 연구

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요 약

*Incilaria fruhstorferi daiseniana*의 뇌는 쌍을 이루고 있는 뇌(cerebral), 측(pleural), 노(parietal), 족(pedal)신경절과 단일 복부(abdominal)신경절로 구성되어 있으며, 이들은 융합된 신경고리를 이루고 있었다. 복부신경절로부터 나오는 내장신경은 생식, 장, 심장, 신장신경등으로 분지되어 이들 각 기관에 신경을 공급하고 있었다. 다음 3가지 방법, 즉, 내장신경을 Ni^{2+}로 역방향으로 충진하여 세포체를 염색하는 방법, 형광화합물 Lucifer yellow를 세포내로 주입하는 방법, 세포내활동전위와 내장신경의 세포외 활동전위를 동시에 측정하는 방법으로 적어도 12가지의 동정된 뉴우런이 내장신경과 관련되고 있음을 알았다. 알아낸 각 세포의 전기생리학적인 특성을 검지하고, 이것과 각 세포의 돌기의 분지 상태와의 관련 및 일부 세포간의 상호관계를 검사하였다. 이들중 8가지 세포가 내장신경에 축색돌기를 내보내는 효과신경세포라는 증거를 얻었다.

INTRODUCTION

In quest for the better understanding of the mechanism of nervous functions invertebrate preparations are frequently used. Lately the gastropods have been studied by many because of their simplicity of neural organization (several hundred to a few thousand neurons, Boyle *et al.*, 1983), and larger identifiable nerve cells (cf. Kandel and Kupfermann, 1970). Same identifiable neurons from different individuals of same species offer remarkable

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constancy in location, shape, size, coloration, projections of neurites into nerves, pharmacology, and electrical activities. These characteristics have, in fact, served as criteria for identification and further studies (*Aplysia:* Arvanitaki and Chalazonitis, 1958; Frazier *et al.*, 1967; *Tritonia:* Willows, 1967; *Helix:* Kerkut *et al.*, 1975; *Helisoma:* Kater and Kaneko, 1972; *Limax:* Parmentier, 1973; *Pleurobranchaea:* Siegler *et al.*, 1974; *Lymnaea:* Benjamin *et al.*, 1979; *Incilaria:* Hong, 1981; Leem, 1982).

Among the land slugs, *Limax maximus* has been more extensively studied, including calcium flux during action potentials of neurons (Chang *et al.*, 1974), associative learning by slug (Gelperin, 1975; Sahley *et al.*, 1981), neural control of feeding motor program (Gelperin *et al.*, 1978), *in vitro* learning by isolated brain (Chang and Gelperin, 1980), activities of bursting neurons (Copeland and Gelperin, 1983), neurotransmitters (Osborne and Cottrell, 1971), collective computational properties of neurons (Hopfield, 1984), and neural modeling (Gelperin *et al.*, 1985). However, relatively little was done on identification of its neurons and their characterization.

Incilaria fruhstorferi daiseniana Azuma is a terrestrial slug common in Korea. Works on *Incilaria*, other than systematics, have only recently been started. They include, mapping identifiable neurons (Hong, 1981), responses of neurons to 5-hydroxytryptamine (Choi, 1981), identification and acetylcholine responses of abdominal neurons (Leem, 1982), chemical composition (Kim *et al.*, 1983), culture of foot and mantle cells (Furuta and Shimozawa, 1983), identification and electrical studies of neurons in the buccal ganglia (Jeong, 1985), and post-ingestive avoidance learning (Lee and Chang, 1986).

Studies on the function of the visceral nerve (VN), one of the most important nerves, of terrestrial gastropods has been very limited, most of them being in connection with the heart (*Ariolimax;* Carlson, 1905; *Helix;* S.-Rózsa and Perényi, 1966; *Limax;* Carlson, 1905; MacKay and Gelperin, 1972). This study presents initial basic information on: the anatomy of the brain and of the visceral nerve (VN), which of the identified neurons are related to the VN, their individual morphology and electrophysiological characteristics, and 4) their possible functions and some interrelations. The approach using multi-dimensional techniques employed in the present work appears to be a more positive method for identification of neurons.

MATERIAL AND METHODS

Biological material. The terrestrial slug, *Incilaria fruhstorferi daiseniana* Azuma was collected in and around mushroom cultivating fields, in the mountainous regions of Muju, Chollabukdo from June to September. Collected slugs were maintained in flat plastic containers with wet paper lining the bottom and perforations on the cover. They were kept under a light: dark cycle of 10 : 14 hr at 12~14°C. The slugs were fed with fresh carrot and potato. One or two days before the experiment slugs weighing 7~12 g were

transfered individually into separate petridishes and kept in dark at 18°C.

To isolate the brain, the animal was cold anesthetized by burying in crushed ice or placing in a freezer for about 10 min, and dissected in a circulating cold (0~4°C) saline solution. The saline solution (Chang and Gelperin, 1980) had a composition in mM: Na 55.6, K 7.2, Ca 7.0, Mg 4.0, Cl 80.3, H_2PO_4 0.2, HCO_3 2.5 and dextrose 5.0. The pH was adjusted to 7.6 before use. When recording or injection was to be made intracellularly the isolated brain was subjected to elastase solution (Sigma, Type I, diluted by a factor of 10 with saline solution) for about 10 min. The epineural sheath was then carefully removed. Physiological experiments were carried out at temperatures between 15 and 19°C.

Retrograde staining of neurons through nerves (back-filling). A short front portion of the animal containing the brain and the nerve to be stained through, e.g., the visceral nerve, was cut transversely. Then, a minimal amount, i.e., only the length of the nerve to be filled, was dissected. The free cut end of the nerve was sucked into a suction pipette made of plastic tubing drawn out to an appropriate tip diameter which will snugly accommodate the nerve. The saline in the suction pipette was replaced by 0.2~0.3 M $CoCl_2$ or $NiCl_2$ made up in saline. Then the preparation was placed in the saline solution, and "incubated" for 18~36 hr at 10°C or 24~48 hr at 4°C. After incubation the brain and attached nerves were isolated, pinned, rinsed with saline, and placed in dithiooxamide (rubeanic acid) solution saturated with 70% ethanol for the development of yellow to deep orange color for Co^{2+} and violet to blue-black for Ni^{2+}. After 20 min the preparation was rinsed a few times with 70% ethanol, fixed in Carnoy's solution (ethanol 6 parts: chloroform 3 parts: glacial acetic acid 1 part) for 2~3 hr, dehydrated with ethanol, cleared with methyl salicylate, then wholemounted between a coverslip and a slide.

To trace the innervation of a nerve, the free end of the nerve distal to the cut was fitted into a suction pipette and the nerve and its branches were filled orthodromically using the procedure similar to the one just described above for back-filling.

Staining by intracellular injection. Intracelluar injection of $CoCl_2$, $NiCl_2$, or Lucifer yellow CH (Sigma) was carried out by pressure. Lucifer yellow (5%) in 0.5 M LiCl, 0.2~0.3 M $CoCl_2$ or $NiCl_2$ was allowed to move up to the tip of the micropipette pulled from a glass capillary with an internal fiber. The stem of the micropipette was filled with the same solution or in case of Lucifer yellow with 0.5 M LiCl. For pressure injection, the micropipette was filled into an electrode holder with a side port (WPI, Inc.). The holder was connected to a DC preamplifier for monitoring membrane potential during impalement and pressure injection. The pressure was applied through the side port by giving pressure pulses of 10~150 msec at 3~10 bars through a solenoid valve (Automatic Switch Co.) driven by an electrical pulse generator (Tektronix, Type 161). Co^{2+} or Ni^{2+} filled preparations were incubated for 1~3 hr at 4°C. They were then developed, fixed, dehydrated, cleared and mounted as above. Preparations with Lucifer yellow injected neurons were preferably cleared and wet mounted in 80% glycerol made with 4 M carbonate or

phosphate buffer for immediate observation and photography. Alternatively, the preparation was fixed either in an aqueous 4% formaldehyde for 10~24 hr, dehydrated, cleared in methyl salicylate, then observed in methyl salicylate. Lucifer yellow marked neurons were observed either under an epiillumination fluorescence microscope (Leitz Ortholux with Ploemopak and fluorescence objectives) with a 390 nm band pass exciter filter, a 510 nm chromatic beam sliter, and a barrier filter of long pass above 515 nm, or under a transillumination fluorescence microscope (Zeiss WL with large fluorescence illuminator and a dark field condenser of either NA 0.63 or 0.80) which provided good dark images with BG-12 exciter filter and 470 nm, and 530 nm barrier filters. The Lucifer yellow injected neurons were photographed without delay on Kodak Ektachrome (ISO 400) film, since Lucifer yellow faded within a few hours.

Electrophysiological measurements. For electrophysiological experiments the brain with attached nerves were fixed on the transparent resin (Sylgard, Type 184, Dow Chemical Co.) which formed the bottom of a chamber with fine tungsten wires. The intracellular glass microelectrodes filled with either 3 M KCl or 4 M potassium acetate, had resistances 10~50 MΩ. They were connected to DC preamplifiers (own make) with a gain of 10 input impedance of $10^{12}\Omega$, negative capacitance compensation, and a bootstrap bridge circuit for passing current into or out of cells through the recording microelectrode. Suction electrodes for extracellular recording were made by pulling 2~3 mm diameter plastic tubing (Tygon) over a small flame. These electrodes were connected via Ag-AgCl wire to differential AC preamplifiers (own make) which had a gain of 1,000 input impedance of $10^{12}\Omega$, and common mode rejection ratio of 80 dB or better. Amplified signals were displayed on dual trace storage oscilloscopes (Tektronix, Types 468 and 5110+5D10), and recorded with a 35 mm close-up camera.

RESULTS

Anatomy of the brain and the relevant nerves.

The brain of the terrestrial slug, *Incilaria* was a fused ring of ganglia surrounding the esophagus. It consisted of a pair of cerebral ganglia dorsal to the esophagus, and ventrally, paired pleural, parietal, pedal ganglia and an unpaired abdominal ganglion. The five subesophageal ganglia forming the visceral chain were highly condensed and appeared as one fused mass. However, the brain ganglia were connected to their neighboring ganglia by means of short connectives and commissures. Such connectives and commissures were not visible unless the surrounding tissues were carefully dissected away. Such dissections were made and the result is schematically presented in Fig. 1. In addition to this main mass of brain there are a pair of buccal ganglia connected to the cerebral ganglia via a pair of relatively long cerebro-buccal connectives.

From the midventrally located abdominal ganglion emanated five nerves, i.e., the VN,

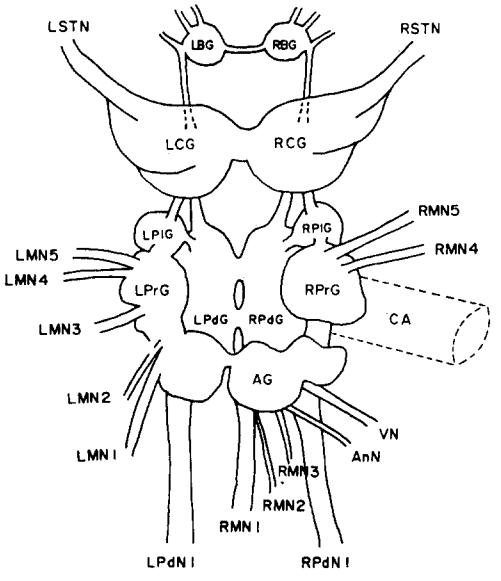

Fig. 1. Schematic drawing (dorsal view) of the CNS of *Incilaria* after removing sheaths and much of connective tissues. Many nerves radiating from the cerebral and pedal ganglia are not shown. L- left, R-- right, --G ganglion, --N nerve, A abdominal, An anal, B buccal, C cerebral, M mantle, Pd pedal, Pl pleural, Pr parietal, ST superior tentacular, V visceral.

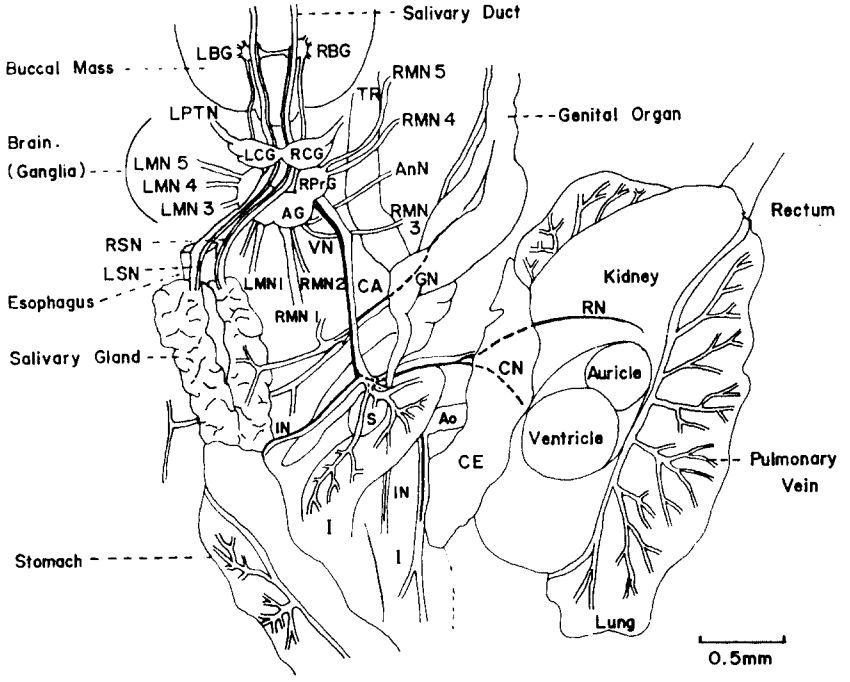

Fig. 2. The visceral nerve of *Incilaria* and its branches (disproportionately drawn thick, for clarity). Ao aorta, CA cephalic aorta, CE coelomic epithelium, I intestine, S spermatotheca, TR tentacle retractor muscle; CN, GN, IN, RN, SN cephalic, genital, intestinal, renal, and salivary nerves, respectively. Other abbreviations as in Fig. 1.

the anal nerve (AN) and three right mantle nerves (RMN1, 2, 3). The VN left the brain at the dextro-posterior edge of the abdominal ganglion. Dissections revealed that the main trunk of the VN ran posteriorly along the cephalic aorta, then split into several branches. Staining by orthodromic filling of the main trunk of this VN with Ni^{2+} revealed that its major branches were the intestinal, genital, cardiac and renal nerves and that they, in turn, innervated the respective organs as shown in Fig. 2.

Other nerves leaving from the visceral chain were two remaining right mantle nerves (RMN4, 5) from the right parietal ganglion and five left mantle nerves (LMN1, 2, 3, 4, 5) from the left parietal ganglion.

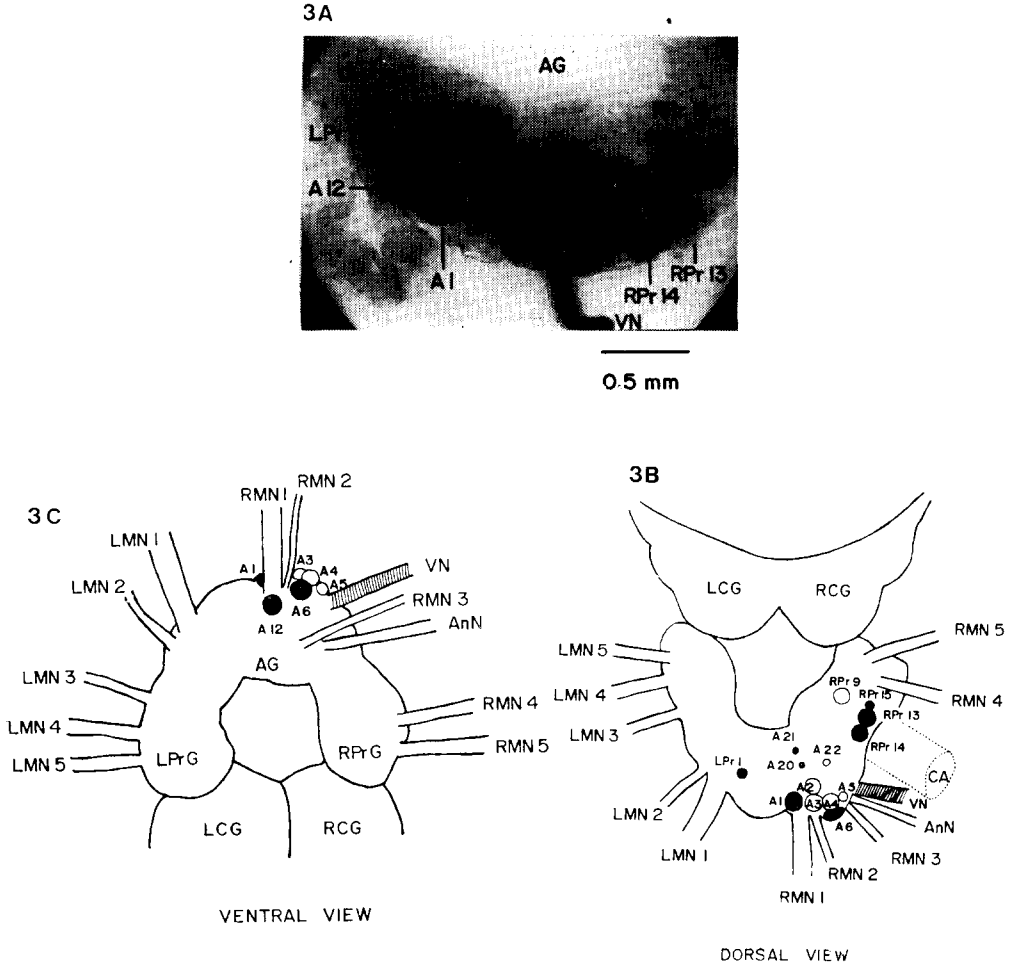

Fig. 3. Results of Ni^{2+} back-filling of the VN. 3A: Photomicrograph of a dorsal view of the visceral chain after staining and clearing. 3B, 3C: Schematic mappings of identified neurons stained and related to the VN by this method (filled circles). Abbreviations as in Fig. 1.

Antidromic staining of the visceral nerve.

As in other gastropods, *Incilaria* contained on their outer surfaces many large neurons, besides more numerous smaller ones on the outer surface of ganglia. Antidromic stainign (back-filing) of the VN with Ni^{2+} was carrie out several times. The region surrounding the base of the VN contained many densely packed small neuronal cell bodies. Identifiable cells stained by these fillings, and therefore related to the VN, were A1, A6, A12, A20 and A21 neurons in the abdominal ganglion, RPr13, RPr14 and RPr15 neurons in the right parietal ganglion and LPr1 neuron in the left parietal ganglion (Fig. 3). Neurons A20, A21 and RPr15 were newly identified in this study. A20 and A21 cells were located on the mid-dorsal surface of the abdominal ganglion, and had diameters of around 50 μm. They were transparent in appearance. RPr15 neuron was on the dextro-anterior side of RPr13, about 70 μm in diameter and tranparent.

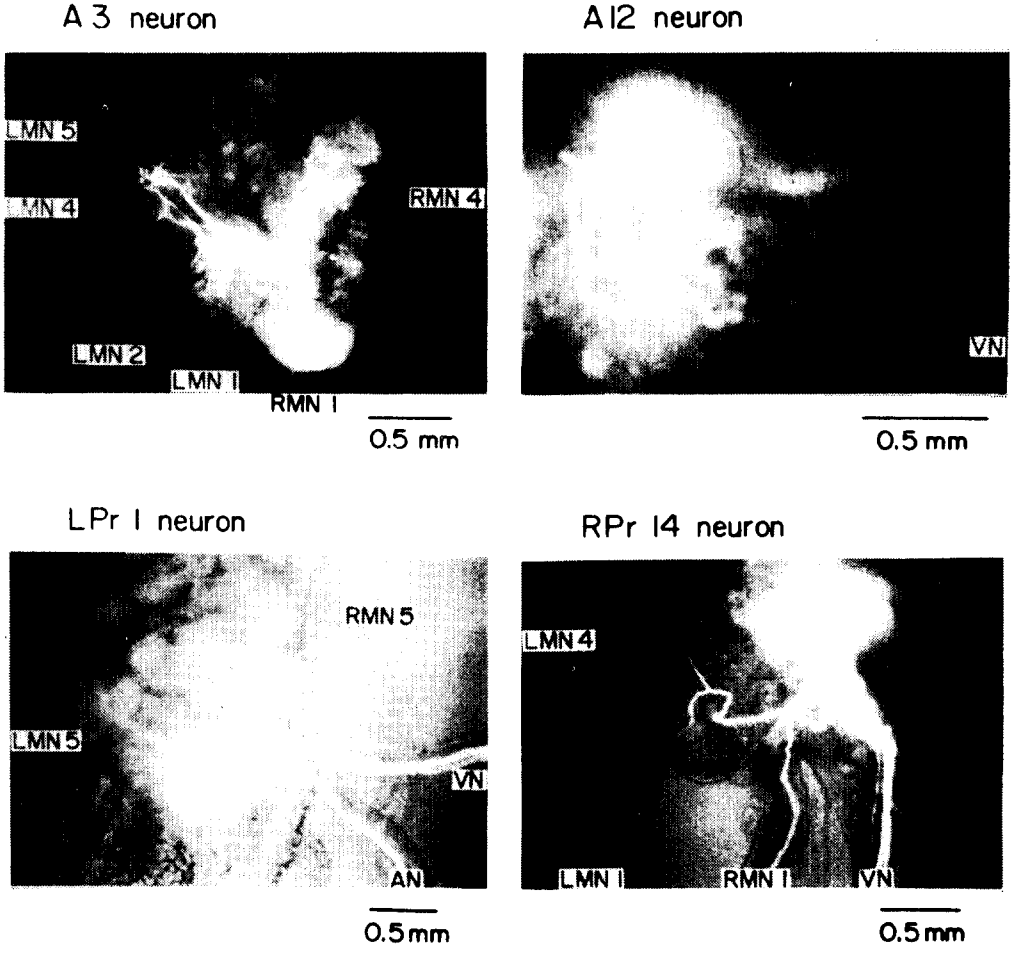

Fig. 4. Photographs of individual identified neurons after each soma was intracellularly injected with Lucifer yellow subjected to fluoresce.

Table 1. Results of Lucifer Yellow Injection into Somata.

Nerve \ Neuron	A1	A3	A4	A5	A6	A12	A20	A22	RPr13	RPr14	LPr1
VN	←		←	←	←	←	←	←	←	←	←
AN					←		←	←			←
RMN1		←	←	←			←			←	
RMN2				←							
RMN3											
RMN4		←			←				←		
RMN5									←		←
LMN1	←	←	←	←			←			←	
LMN2		←									
LMN3	←		←								
LMN4		←			←					←	
LMN5		←									←

When the cardiac nerve, a branch of the VN, was back-filled with Ni^{2+}, identifiable neurons A12, A20 and A21 were stained, relating them with the heart.

Intracellular staining with Lucifer yellow.

The reasons for intracellularly staining identified neurons in this study were: to determine the fine morphology of each neuron, to find those neurons which were not stained by the back-filling of the VN but still sent processes out to the VN, and to verify whether those neurons stained by back-filling indeed sent processes out to the VN. Somata of numerous neurons belonging to at least 12 different identities were intracellularly injected with Lucifer yellow. Each of the Lucifer yellow injected neuron showed brilliantly fluorescing soma and extremely elaborate fine branching of the processes. Some examples of Lucifer yellow injected neurons are shown in Fig. 4. As shown in Table 1, 7 cells reaffirmed the results of the Ni^{2+} back-filling showing processes running into the VN. Three neurons, A4, A5 and A22, whose somata were not stained by VN back-fill, showed neuronal processes clearly entering into the VN, thus adding these cells to those previously related to the VN. The cell A3 was filled well by Lucifer yellow giving off arborations running into many nerves, but did not show processes proceeding to the VN. The shapes of the neurons bearing the same identity from different individual slugs bore some similarities despite their variations.

Electrophysiological studies.

One of the reasons for obtaining electrophysiological characteristics was to use them as one of the criteria for identifying a particular cell. If cells from different individuals had characteristics matched electrically as well as morphologically, they are most likely to be the cells bearing the same identity (identification number or name). Some electrophysiological studies on *Incilaria* have previously been made in connection with identification

Table 2. Electrophysiological Characteristics of Neurons Related to the VN.

Neuron \ Data	Resting Potential (mV)	Action Potential (mV)	Firing Pattern	Firing Interval (sec)	Possible Relation to the VN	Size of Soma (μm)
A1	40-75	80-90	irregular	—	non-effector	150-200
A4	55-60	80-85	regular	3-7	effector	180-220
A5	70-75	85-90	irregular	—	effector	100-150
A6	50-60	80-90	regular	3-7	effector	175-230
A12	50-60	80-90	irregular	—	effector	140-170
A20	60-70	85-90	regular	ca. 15	effector	40-50
A22	70-75	85-90	regular	1.3-1.7	effector	80-100
RPr14	55-60	70-80	irregular	—	effector	150-200
RPr15	40-50	60-70	regular	1.8-2.3	non-effector	40-50
LPr1	45-55	60-70	irregular	—	effector	70-80

or responses to neurotransmitters (Hong, 1981; Choi, 1981; Leem, 1982).

In this study, two kinds electrophysiological studies were used. One was to identify and elaborate electrical characteristics of individual neurons using intracellular recording methods. The other was to establish the relationship between a cell in question and the VN, using simultaneous intracellular recording from the cell and extracellular recording from the VN. Table 2 shows the basic electrophysiological data obtained on the cells studied. When simultaneous extracellular recordings from the VN showed one-to-one correlation, after set delays, with the action potential in the cell, the cell was concluded to be an effector neuron sending an axon or axons into the VN (Fig. 5).

In addition to these basic data, some cells showed particular aspects and these are described individually below.

A1 cell. This cell showed fairly regularly occuring membrane potential oscillation at an average interval of 16 s. Also large excitatory postsynaptic potentials (EPSPs) were regularly observed, sometimes at a frequency of 1/s.

A4 cell. Some preparations of this cell beat as slowly as at 15 s intervals.

A6 cell. This large neuron beat spontaneously and showed practically no postsynaptic potentials (PSPs) on its membrane potential. Extracellular recordings showed spikes following intracellular action potentials with a fairly long delay of 30~50 ms.

A12 cell. This neuron normally beat irregularly with long periods of silence. But firing could be easily induced by applying depolarizing outward currents through the recording electrode (Fig. 6).

A21 cell. This small cell, although related to the VN through back-filling of that nerve, was not electrophysiologically studied because of its small size.

RPr13 cell. Spontaneous firing pattern of this neuron was mostly regular but it was interposed with irregularly beating periods and silent periods. The cell produced spikes in

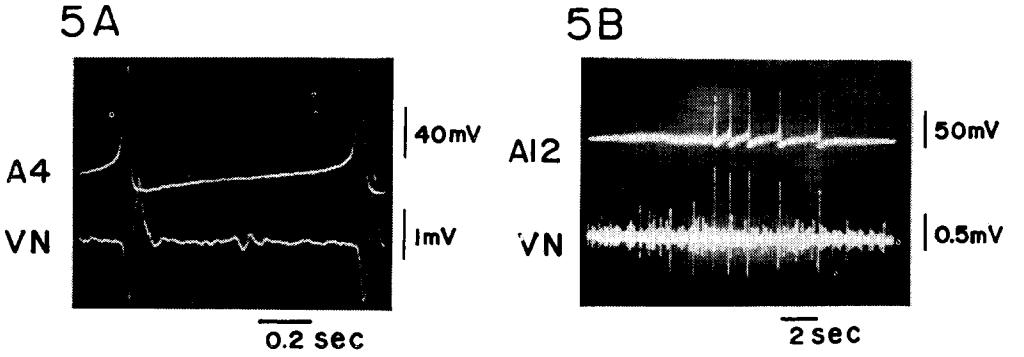

Fig. 5. Simultaneous recordings of intracellular action potentials from spontaneously firing A4 neuron (5A) or A12 neuron (5B) and of extracellular action spikes from the VN showing one-to-one correlation.

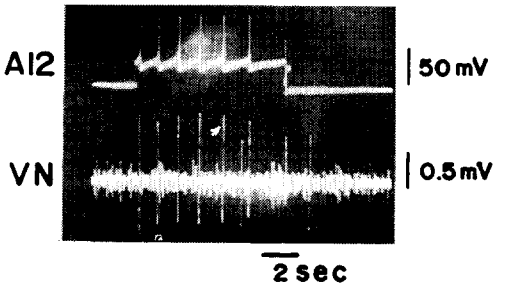

Fig. 6. Action potentials induced by intracellularly applied depolarizing current during a silent period of A12 cell. Simultaneous recording shows corresponding extracellular spikes in the VN.

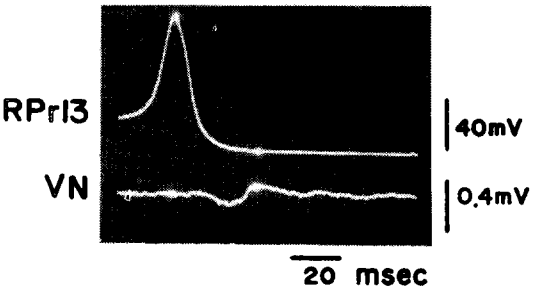

Fig. 7. Simultaneous recording of RPrl3 cell action potential and corresponding extracellular spike in the VN. The correlation was resolved by increasing the sweep speed and analysing many such recordings.

the extracellular recording which was very small, but the correlation was resolved by increasing the sweep speed of oscilloscope displaying simultaneous recordings and by repeated reproducible observations (Fig. 7).

RPr14 cell. The spontaneous firing pattern of this cell was irregular and seemed to be considerably influenced by EPSPs coming from other cell(s).

RPr15 cell. This was among the smallest of identified neurons in this study. Although this neuron was related to the VN by antidromic staining and Lucifer yellow injection, the extracellular recording of VN showed no spikes corresponding with the intracellular action potentials.

LPr1 cell. This neuron's spontaneous beating was very irregular, infrequent and often silent. Particularly, it received large number of EPSPs and inhibitory postsynaptic potentials (IPSPs). Oftentimes the spontaneous action potentials were triggered by EPSPs superimposed on the spontaneous depolarizations.

Other cells. Several other identified cells were electrophysiologically studied to relate

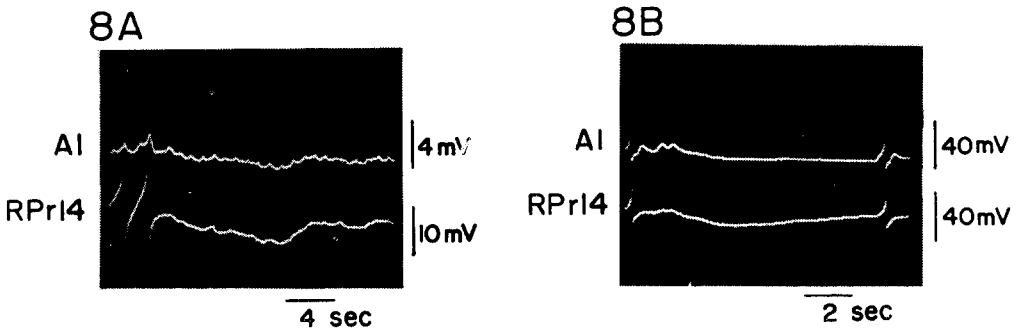

Fig. 8. Two examples of intracellular recordings from Al and RPr 14 cells showing closely related simultaneous slow membrane potential vacillations. In 8B, both neurons fired almost simultaneously, whereas in 8A only RPrl4 cell fired.

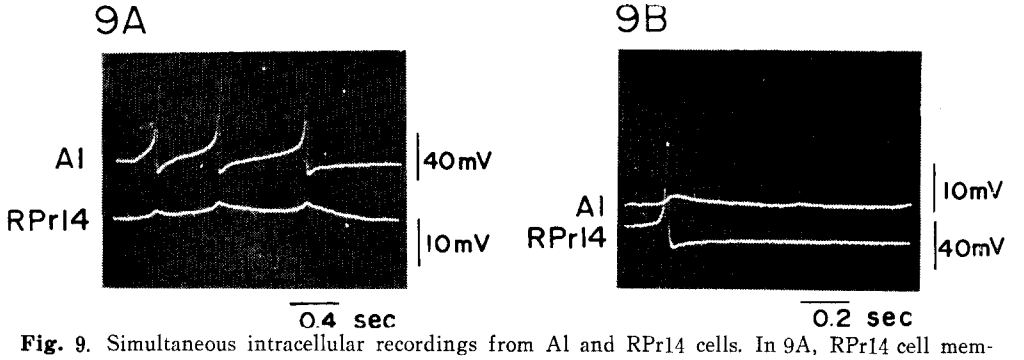

Fig. 9. Simultaneous intracellular recordings from Al and RPrl4 cells. In 9A, RPrl4 cell membrane potential shows EPSPs which are correlated with action potentials of Al cell. In 9B, membrane potential of Al neuron shows an EPSP from RPrl4 action potential.

them to the VN by measuring resting potentials, action potentials, beating patterns and simultaneous intra- and extracellular recordings. But they showed no relations with the VN either anatomically or electrophysiologically. Among them were A3, A8, A9, A18, RPr6 and RPrl0 neurons.

Cell to cell relationships. In the course of this study, some cell to cell interrelationships were observed. The neurons Al and RPrl4 showed simultaneous slow membrane potential vacillations closely related to each other (Fig. 8A). Sometimes they fired almost, but not exactly, simultaneously (Fig. 8B). These observations indicated that they were sharing a common source of input from without. Also action potentials of Al caused EPSPs in the membrane potentials of RPrl4 (Fig. 9A), and *vice versa* (Fig. 9B). RPrl4 cell also demonstrated some relationship with RPrl0 cell (which was located anterior to RPrl4) sharing simultaneous membrane potential variations including some common PSPs. They both seemed to receive common inputs. But their action potentials had no influence on each other.

DISCUSSION.

The ganglia of *Incilaria*, like in other well evolved pulmonates, are highly concentrated in a circumesophageal ring. Bargmann (1930) has classified the CNS of the Pulmonata into 8 types according to the degree of compactness of the ganglia in the visceral chain. She gives *Philomycus bilineatus*, quoting Hoffmann (1924), as one of the type examples of the "*Vaginula* type" in which all the visceral ganglia form a fused mass. From the observations made in this study and from the consideration that the genus *Philomycus* was synonymous with *Incilaria* in these species (Hoffmann, 1924), the CNS of *Incilaria fruhstorferi daiseniana* could be assigned to the "*Vaginula* type". But, through careful dissections, it could be seen that each ganglion was separated by epineural sheath, and was connected to other ganglia by short commissures or connectives (Fig. 2). Careful histological work is in order for further clarification.

"There are staggering number of descriptive accounts of nerves of gastropods. ...most authors have not accurately determined the area of distribution of the respective nerves..." (Bullock and Horridge, 1965). Since staining by orthodromic filling with Ni^{2+} or Co^{2+} proved to be more effective than methylene blue or other general nerve staining, this method was extremely useful for tracing peripheral nerves in unsectioned preparations. In the present study, however, bifurcation of the cardiac nerve into auricular and ventricular branches was not found (Fig. 2), whereas such branches have been described in *Limax* (MacKay and Gelperin, 1972) and *Tapes* (Phillis, 1966). Orthodromic filling of the cardiac nerve at a location closer to the heart of *Incilaria* might give clues whether such branching occurs. Silver intensification or sectioning of Ni^{2+} or Co^{2+} filled preparation would also reveal much more detail of fine innervations of organs or tissues. The renal nerve was clearly observed in *Incilaria* as a branch of the VN, whereas no mention of such a branch was given by MacKay and Gelperin (1972) for *Limax*.

By back-filling individual branches entering various organs it will be possible to find particular neurons in the CNS which are connected with a particular organ. Although this was not very easy because the branches were fine and distant from the CNS requiring long filling time and causing sometimes cell deaths before the filling was complete, it was possible in this study to relate A12, A20, A21 and LPr1 cells and other smaller neurons with the heart by this method. Central connections of other organs should also be investigated in the future.

In the present work 17 identified types of neurons were examined by three different methods, and 12 of them showed relationship with the VN at least by one method. Each of these methods supplied different yet mutually complementary results. The antidromic staining of the VN or its branches filled somata of certain identified neurons and many smaller cells. Nine types of identified neurons were first related to the VN by this method.

The next method, intracellular injection with Lucifer yellow of selected identified neurons, revealed that 10 types of them sent neurites into the VN. Of these, 8 were those already related to the VN by the first method but the remaining 3 were newly related to the VN by this method. Finally, simultaneous intracellular recording from the soma and extracellular recording from the VN showed that 10 identified types of neurons had their processes in the VN. Six types overlapped with those related to the VN by the first method, 9 overlapped with those by the second method, and 1 identified type was newly related to the VN by this electrophysiological method. These results indicated that employing different modes of examination could not only find greater number of identified neurons related to the VN than using only one method, but it could also confirm the results obtained by one method or another.

It will be of interest in the future to investigate further interrelations among the neurons found by these methods as well as their role in the functioning of the organs involved.

ABSTRACT

The brain of the slug, *Incilaria fruhstorferi daiseniana* consisted of paired cerebral, pleural, parietal, pedal ganglia and an abdominal ganglion. The visceral nerve (VN) leaving the abdominal ganglion branched into the intestinal, genital, cardiac and renal nerves, and innervated respective organs. At least 12 different types of identified neurons were found to be related to the VN by three methods, i.e., antidromic staining of the VN with Ni^{2+}, intracellular injection with Lucifer yellow, and simultaneous recording of intracellular potential from the soma and of extracellular spikes from the VN. Electrophysiological characterization of these identified neurons was made, and its relationship between the electrical data and the branching pattern of these neurons as well as some interrelations among them were observed. Eight of these showed evidences to be possible effector neurons sending axons into the VN.

REFERENCES

Arvanitaki, A. and N. Chalazonitis, 1958. Configuration modales de l'activité, propres à différents neurones d'un même centre. *J. Phisiol. Paris* **50**:122-125.

Bargmann, H.E., 1930. The morphology of the central nervous system in the Gastropoda Pulmonata. *J. Linn. Soc. (Zool.)* **37**:1-59.

Benjamin, P.R., R.M. Rose, C.T. Slade and M.G. Lacy, 1979. Morphology of identified neurones in the buccal ganglia of *Lymnaea stagnalis*. *J. exp. Biol.* **80**:119-135.

Boyle, M.B., L.B. Cohen, E.R. Macagno and H. Orbach, 1983. The number and size of neurons in the CNS of gastropod molluscs and their suitability for optical recording of activity. *Brain Research* **266**:305-317.

Bullock, T.H. and C.A. Horridge, 1965. Structure and Function in the Nervous System of Inverte-

brates. W.H. Freeman, New York.

Carlson, A.J., 1905. Comparative physiology of the invertebrate heart. III. Physiology of the cardiac nerves in molluscs. *Am. J. Physiol.* **14**:16-53.

Chang, J.J. and A. Gelperin, 1980. Rapid taste aversion learning by an isolated molluscan central nervous system. *Proc. Natl. Acad. Sci. USA* **77**:6204-6206.

Chang, J.J., A. Gelperin and F.H. Johnson, 1974. Intracellularly injected aequorin detects transmembrane calcium flux during action potentials in an identified neuron from the terrestrial slug, *Limax maximus. Brain Research* **77**:431-442.

Choi, J.K., 1981. Responses of identifiable giant neurons to 5-hydroxytryptamine in the brain of terrestrial slug, *Incilaria fruhstorferi.* Master's thesis, Sogang Univ., Korea.

Copeland, J. and A. Gelperin, 1983. Feeding and a serotonergic interneuron activate an identified autoactive salivary neuron in *Limax maximus. Comp. Biochem. Physiol.* **76**:21-30.

Frazier, W.T., E.R. Kandel, I. Kupfermann, R. Waziri and R.E. Coggeshall, 1967. Morphological and functional properties of identified neurons in the abdominal ganglion of *Aplysia californica. J. Neurophysiol.* **30**:1288-1351.

Gelperin, A., 1975. Rapid food-aversion learning by a terrestrial mollusk. *Science* **189**:567-570.

Gelperin, A., J.J. Chang and S.C. Reingold, 1978. Feeding motor program in *Limax.* I. Neuromuscular correlates and control by chemosensory input. *J. Neurobiol.* **9**:285-300.

Gelperin, A., J.J. Hopfield and D.W. Tank, 1985. The Logic of *Limax* Learning. *In:* Model Neural Networks and Behavior (A.I. Selverston, ed.), Plenum Publ. Corp., New York, pp. 237-261.

Hoffmann, 1924. Zur Anatomie und Systematik der Philomyciden. *Jena. Z.f. Naturwiss.* **60**:363-396.

Hong, S.W., 1981. Identification, mapping and electrophysiological survey of giant neurons in the brain of Korean terrestrial slug, *Incilaria fruhstorferi.* Master's thesis, Sogang Univ., Korea.

Hopfield, J.J., 1984. Neurons with graded response have collective computational properties like those of two-state neurons. *Proc. Natl. Acad. Sci. USA* **81**:3088-3092.

Jeong, S.W., 1985. Electrophysiological studies on the buccal ganglia cells and feeding motor program of the Korean terrestrial slug, *Incilaria fruhstorferi daiseniana.* Master's thesis, Sogang Univ., Korea.

Kandel, E.R. and I. Kupfermann, 1970. The functional organization of invertebrate ganglia. *Ann. Rev. Physiol.* **32**:193-258.

Kater, S. and L.R.S. Kaneko, 1972. An endogenously bursting neuron in the gastropod mollusc, *Helisoma tribolis;* characterization of activity *in vivo. J. comp. Physiol.* **79**:1-14.

Kerkut, G.A., J.D.C. Lambert, R.J. Gayton, J.E. Loker and R.J. Walker, 1975. Mapping of nerve cells in the suboesophageal ganglia of *Helix aspersa. Comp. Biochem. Physiol.* **50**:1-25.

Kim, S.-K., S.-H. Moon, E.-H. Lee and O.-J. Kang, 1983. Studies on the chemical composition of Korean slug, *Incilaria fruhstorferi*, and land snail, *Acusta despecta* Gray. *Bull. Natl. Fisheries Univ. Pusan (Nat. Sci.)* **23**:15-21.

Lee, J.H. and J.J. Chang, 1986. Learning of post-ingestive food-aversion by the land slug, *Incilaria fruhstorferi daiseniana* to amino acid deficient diets. *Soc Neurosci. Abstr.* **12**:in press.

Leem, J.W., 1982. Identification and acetylcholine responses of the neurons in the abdominal ganglion of the terrestrial slug, *Incilaria fruhstorferi.* Master's thesis, Sogang Univ., Korea.

MacKay, A.R. and A. Gelperin, 1972. Pharmacology and reflex responsiveness of the heart in the

giant garden slug, *Limax maximus*. *Comp. Biochem. Physiol.* **43**:877-896.

Osborne, N.N. and G.A. Cottrell, 1971. Distribution of biogenic amines in the slug *Limax maximus*. *Z. Zellforsch.* **112**:15-30.

Parmentier, J., 1973. Mapping studies of a gastropod brain. *Brain Research* **59**:201-210.

Phillis, J.W., 1966. Innervation and control of a molluscan (*Tapes*) heart. *Comp. Biochem. Physiol.* **17**:719-739.

Sahley, C.L., J.W. Rudy and A. Gelperin, 1981. An analysis of associative learning in a terrestrial mollusc: higher order conditioning, blocking and transient US pre-exposure effect. *J. comp. Physiol.* **144**:1-8.

Siegler, M.V.S., G.J. Mpitsos and W.J. Davis, 1974. Motor organization and generation of rhythmic feeding output in buccal ganglion of *Pleurobranchaea*. *J. Neurophysiol.* **37**:1173-1196.

S.-Rózsa, K. and L. Perényi, 1966. Chemical identification of the excitatory substance released in *Helix* heart during stimulation of the extracardial nerve *Comp. Biochem. Physiol.* **19**:105-113.

Willows, A.O.D., 1967. Behavioral acts elicited by stimulation of single, identifiable brain cells. *Science* **157**:570-574.