Behavioral Acts Elicited by Stimulation of Single, Identifiable Brain Cells

Abstract. *By stimulation of and recording from all of the nerve trunks and from over 50 of the large nerve cell bodies in the isolated brain of the nudibranch* Tritonia gilberti *a map of the axonal paths and synaptic connections has been constructed. The nervous correlates of sensory and motor activities can be monitored in single cells of the intact animal. Similarly, discrete responses in local muscles of the body wall and complex behavioral sequences such as turning and swimming are triggered by stimulation of single identifiable units.*

Attempts to bridge the gap between unit-level analyses of input and output of nerve cells and studies of complex behavior in intact animals have not yet been successful, partly because of the lack of suitable preparations (*1*). Limitations are invariably imposed upon the animal's freedom to engage in normal activities (to allow stimulating or recording apparatus to function properly) or upon the ability of the researcher to identify nervous units from animal to animal (to permit testing of hypotheses generated by previous work). Nevertheless, aspects of the problem have been explored in both vertebrate and invertebrate species.

In particular, the brains of vertebrates have been probed with intra- and extracellular microelectrodes to determine the sensory, motor, or intermediate integrative functions assumed by various central nervous regions (*2*). Unique and useful features in the nervous anatomy of crustaceans and insects have been exploited in analyses of the electrical events accompanying sensory-motor functions in particular cells or cell groups (*3*).

My report briefly indicates the sources of synaptic input, the paths of axons, and some of the cell interactions for a number of central nerve cells in a nudibranch mollusk, and some of the behavioral acts elicited by stimulation of known cells in the intact animal are described.

Direct comparisons of the neurography of single cells from animal to animal are usually difficult because, in most animals, the small size of the cells and the homogeneous appearance of masses of cells frustrate identification of specific nerve units. Evidence obtained in unit-level studies of a few invertebrate preparations suggests, however, that within a species there is a marked uniformity of neural structure from individual to individual. Exploiting the segmentally ganglionated nerve cord and the esophageal commissures of the crayfish, Wiersma and co-workers (*3*) have succeeded in identifying several hundred primary sensory units and interneurons. Each has been specified by its location in nerve cross sections and in a characteristic sensory field. In the ganglia of some opisthobranch mollusks, a few large cell bodies can be identified visually, and the axonal paths for some of these cells have been described (*4*). To my knowledge, no detailed map has yet been reported of the input-output relations and functional roles of central nerve cells in any species.

The complex of cerebral, pleural, and pedal ganglia in this opisthobranch has several hundred large, pigmented nerve cell bodies conspicuously distributed over its surface (*5*). Over 100 of these cells have been identified by comparisons of their size, pigmentation, and location with respect to the exit points of certain nerve trunks. A map of the sources of synaptic input and the paths followed by the axons of these cells was made to determine whether the physiological connections of these cells were similar from animal to animal.

The isolated ganglia, with lengths of all nerve trunks, connectives, and commissures intact, were placed in the center of an array of suction electrodes (Fig. 1). Each electrode was constructed of a length of polyethylene tubing attached to a hypodermic syringe; the open end of the tubing was pulled out until its inside diameter closely approximated the diameter of one of the nerve trunks. A silver-silver chloride wire was inserted into the base of each syringe, and this wire passed to a switching network from which it could be connected to stimulating or recording equipment. Two glass intracellular micropipettes, made and filled in the usual way, were direct coupled through cathode follower amplifiers to a dual beam oscilloscope; they were also connected, in parallel, through 40-megohm resistors to another stimulator. A 2-minute immersion of the ganglia in a 5 percent solution of the enzyme Pronase softened the epineural sheaths, permitting microelectrode penetrations of the cell bodies be-

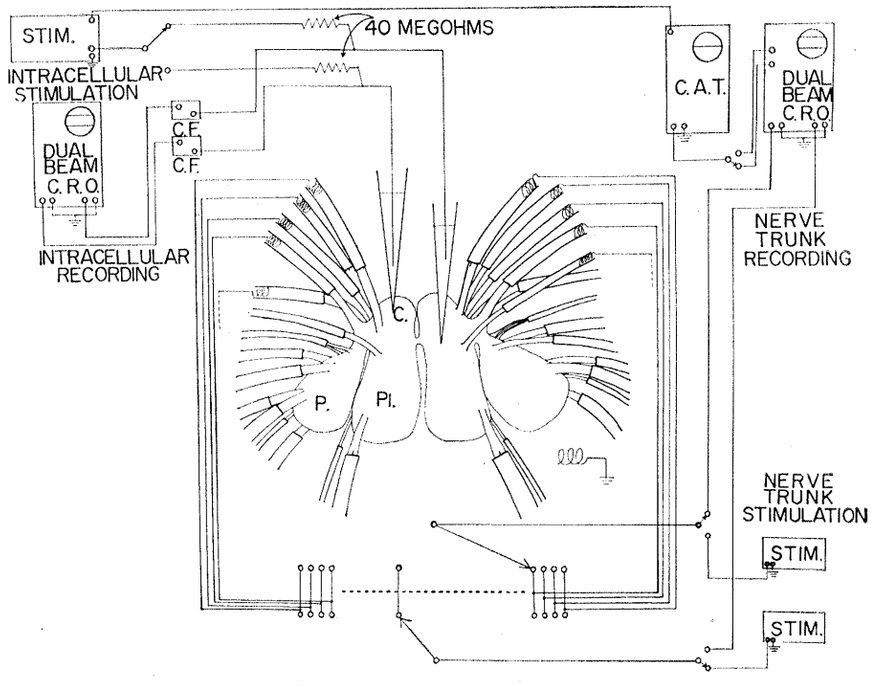


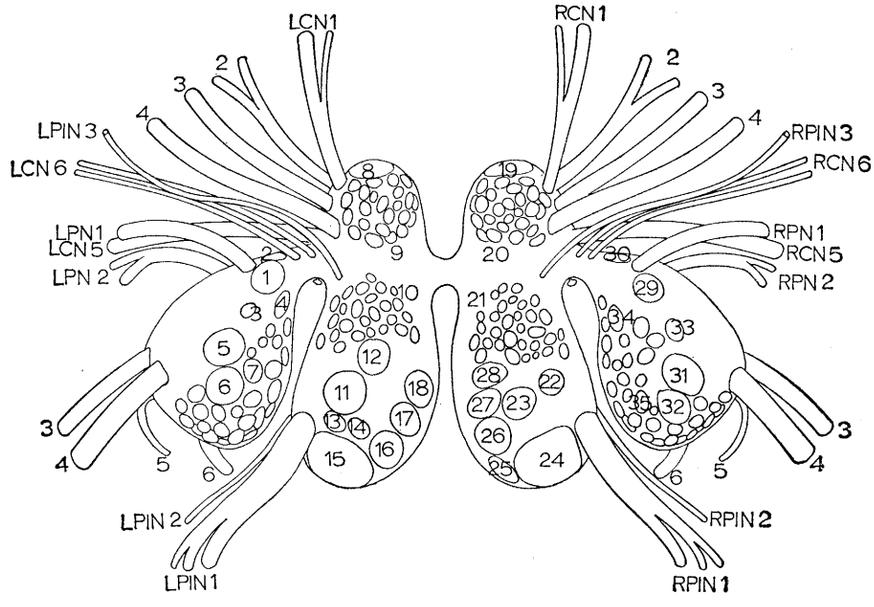
Fig. 1. Thirty stimulating-recording suction electrodes arranged around the ganglia and two microelectrodes placed intracellularly were used to determine the source of synaptic input, axonal paths, and cellular interactions in 54 identifiable cell bodies.

neath. Enzymic digestion caused no appreciable long-term reduction of the excitability of the cells.

To determine the sources of synaptic input I stimulated specified nerve trunks through the suction electrodes while recording intracellularly from selected cells. A shock delivered to a whole nerve trunk produced one of three responses in any particular cell body. Often, a single excitatory or inhibitory postsynaptic response or a burst of such synaptic responses was seen. Less often, this burst was either preceded or accompanied by a spike or evidence that a spike had occurred some distance away in an axon. Occasionally, no response whatever was seen. If either of the first two alternatives occurred, the stimulating-recording connections were reversed, and depolarizing shocks were applied to the cell body while a record of the electrical responses in the nerve trunk was made. To improve the ratio of signal to noise in the extracellular record, and to emphasize any response in the nerve trunk that was phase-locked to the stimulus, I averaged a series of stimulus-response cycles in a Mnemotron computer of average transients. If a phase-locked response was observed, then it was assumed that the cell had an axon in that particular trunk. Just under twenty thousand such measurements were made for the 30 nerve trunks and 54 cells (Fig. 2) (*5*).

In some instances, contrary observations of neuronal paths were made in different animals often enough to prohibit an unambiguous statement of the result, but these observations could usually be associated with uncertainties in the identification of either cell or nerve trunk. Certain trunks have overlapping sensory-motor fields in the periphery and tend to distribute a number of axons between themselves in a nonspecific fashion. This being the case, some variation in the paths of certain synaptic inputs and axons is expected. But in general, whenever visual identification of both cell body and nerve trunk was reasonably certain, the neuronal circuitry was highly predictable. For instance, two large cells (8 and 19) with two or less axons and a restricted synaptic input field had an average predictability over all 30 nerve trunks of 87 percent and in specific nerve trunks of 95 percent.

By using two intracellular microelectrodes placed in different cells, I could observe the effect on one cell of a stimulus in another, and thus the presence or absence of synaptic connections between them could be inferred. In only two cases were such connections found. Each cell in a group of 25 to 30 cells located adjacent and anterior to cells 11 and 12 on the left pleural ganglion made reciprocal excitatory synaptic connections with every other member of the group. The same applied to an analogous group near cell 22. However, these cells never made synaptic contact with other identifiable cells nearby. While none of the other recognizable cells interacted synaptically, in a few instances pairs of cells were observed to receive excitatory synaptic input in common. The intracellular records of ongoing activity in such pairs had sequences of



NERVE TRUNK	CELL NUMBER	1	5	6	8	11	12	15	19	22	24	29	31	32	36	37	39	45	52	53
	RCN1	so	0	0	0	S	so	os	os	so	S	S	0	0	S	os	S	S	so	0
	RCN2	S	0	0	S	0	0	S	S	S	S	S	0	S	S	S	sa	S	S	so
	RCN3	S	0	0	S	0	0	S	S	0	S	S	0	S	S	so	S	S	S	0
	RCN4	so	0	0	sa	os	0	S	A	0	0	S	os	os	S	os	S	S	so	0
	RCN5	0	0	0	0	0	0	os	sa	0	0	0	0	os	so	so	S	so	so	0
	RCN6	so	0	0	0	0	0	S	0	so	S	S	0	0	S	os	S	S	so	0
	RPN1	S	0	0	0	0	0	S	0	so	S	S	os	so	S	S	A	S	0	0
	RPN2	S	0	os	0	0	0	S	0	S	S	S	S	A	A	S	as	sa	0	os
	RPN3	S	0	0	0	so	os	as	0	S	A	S	A	sa	S	S	A	S	so	so
	RPN4	S	0	os	0	as	sa	A	0	S	A	S	A	sa	S	A	A	S	S	S
	RPN5	0	0	0	0	0	0	so	0	0	0	os	S	S	S	A	os	os	so	0
	RPN6	os	0	os	0	0	0	so	0	0	os	os	S	S	S	S	so	os	so	0
	RP1N1	S	0	0	0	sa	as	A	0	A	A	A	0	os	S	S	A	A	S	0
	RP1N2	S	0	0	0	so	ao	S	0	A	S	S	0	0	os	0	S	S	so	0
	RP1N3	S	0	0	0	os	os	so	0	os	S	S	0	os	so	so	sa	S	os	0
	LCN1	S	0	0	os	S	0	S	0	os	sa	S	0	os	S	0	S	S	so	0
	LCN2	S	so	S	S	0	0	S	S	so	S	S	os	0	S	os	S	as	S	so
	LCN3	S	os	0	S	0	so	S	S	so	S	S	0	0	S	so	S	S	S	os
	LCN4	so	os	S	sa	0	os	so	as	0	S	so	0	0	S	0	S	S	S	os
	LCN5	0	0	0	A	0	0	so	0	0	0	0	0	0	0	0	os	so	0	0
	LCN6	S	0	0	0	0	0	so	0	so	S	so	0	so	os	0	S	S	so	0
	LPN1	S	os	so	0	0	so	S	0	0	S	S	0	0	os	0	S	S	S	so
	LPN2	S	0	A	0	0	0	so	0	0	as	so	0	0	S	os	S	as	A	S
	LPN3	S	A	A	0	so	so	S	0	so	sa	S	os	os	S	S	S	A	S	S
	LPN4	S	A	S	0	as	A	as	0	so	as	S	S	0	S	S	S	A	S	A
	LPN5	S	S	S	0	0	0	so	0	0	0	0	0	0	0	0	os	os	S	A
	LPN6	S	S	S	0	0	0	so	0	0	0	0	so	0	os	0	0	os	S	S
	LP1N1	A	0	os	0	A	A	A	0	S	A	S	os	so	S	S	S	A	S	0
	LP1N2	A	0	0	0	A	sa	sa	0	sa	as	so	0	0	os	so	S	sa	so	os
	LP1N3	S	0	0	0	so	0	sa	0	0	sa	S	0	0	so	0	S	S	so	0

Fig. 2. (Top) The dorsal aspect of the cerebral-pleural-pedal ganglia of *Tritonia gilberti*. The approximate sizes and locations of several cells are indicated. The nerves are labeled thus: *RCN1*, right cerebral nerve 1; *P*, pedal; *Pl*, pleural. (Bottom) A tabulation of the sources of synaptic input (*S*) and paths of axons (*A*) for nineteen cells, selected on the basis of their relatively large size. A pair of lower-case letters indicates that both observations are made frequently although the first mentioned is more likely. Cells numbered 36–53 are located on ventral surfaces.

synaptic input that were synchronized in time and were of the same relative amplitudes. Cells that appeared, on the bases of location and pigmentation, to be members of a bilaterally symmetrical pair, such as numbers 1 and 29, often received such common inputs. This feature of the cellular organization was found also to be constant from animal to animal.

Opisthobranch mollusks are particularly useful in studies of the physiology of individual nerve cells since their nerve cell somata are usually quite large, pigmented, and located on the surfaces of the ganglia (*4, 6*). But attempts to record the electrical events taking place in the ganglia during normal behavior have so far failed owing to the continuous erratic movements of the ganglia as a result of the muscular activity in adjacent structures. This mobility makes successful penetrations of specific cell bodies exceedingly difficult. A further problem is attributable to the anatomy of many opisthobranchs, notably *Aplysia*. The ganglia in which the interesting events transpire tend to be dispersed through the body, and radical dissection is required to gain access to any number of them simultaneously. Most of *Tritonia's* nerve cell bodies are aggregated in the cerebral, pleural, and pedal ganglia, located together on the dorsal side of the esophagus. Immobilization of the ganglion complex on a rigid suspension apparatus permits routine penetration of selected cells with intracellular microelectrodes. The activities of these cells during spontaneously occurring behavior was monitored, and the responses of the whole animal to stimulation of a number of specific cells could be observed. A range of motor responses, from local contractions of body muscles to relatively complex actions engaging most of the animal for periods up to 30 seconds, could be evoked, and a notable constancy of function in specific cells was observed from animal to animal.

The relatively superficial, dorsal location of the ganglia permitted ready access to them. A short incision was made in the skin and muscles of the body walls, and the animal was suspended, in a small tank filled with seawater, from an array of threads which also served to hold open the incision (Fig. 3). A circular assembly of hooks was placed directly over the ganglia and rigidly attached to the aquarium walls. The thin muscular sheath surrounding the brain was impaled on the hooks, and a pair of fine silver wires was passed beneath the ganglia and rigidly fixed to the hook-ring assembly on both ends. Finally, to further increase mechanical stability, the whole hook-ring assembly was raised slightly, putting the connecting muscle sheaths under a slight stress. The nerve trunks and ganglia themselves were nowhere touched or strained by the apparatus. The epineural sheath was softened locally by brief enzymic digestion (5 percent Pronase). Two microelectrodes which could either stimulate or record were used to monitor cellular activities or to elicit impulses in selected nerve cell bodies.

Initially, a survey was made of the background electrical activity in 35 recognizable cells in the undisturbed animal. Unlike cells observed in isolated ganglia from this animal and in the isolated ganglia of other opisthobranchs (*7*), none of the cells in the intact animal fired spontaneously for long periods. Instead, most cells apparently received random synaptic input with an occasional spike occurring some distance away in one or another of the axonal branches. Only rarely did a spike invade the soma under these conditions. On this account, it is suggested that the long-term, "spontaneous" spiking activity often seen in this and other isolated molluscan ganglion preparations is the result of injuries associated with dissection and cannot be assumed to participate in the central nervous function of the intact animal.

The motor activities evoked by shocks applied directly to specific cell bodies fell into five different categories.

1) Many cells, when stimulated either by single shocks or by bursts of frequencies from 1 to 100 per second caused no apparent muscle response (for example, cells 5, 6, 11, 12, 22, 31, 32). Some of these same cells responded only weakly even to the most violent sensory stimulation. These two characteristics, coupled with the observation that such cells often had a distinctive white pigmentation, suggest the possibility of a neurosecretory function (*8*). There were, on the other hand, cells with no obvious motor function which normally received a high background level of synaptic bombardment and had the usual orange cytoplasmic pigments (for example, cells 8 and 19).

2) Contractions were evoked in localized regions of the body-wall musculature by direct stimulation of a num-

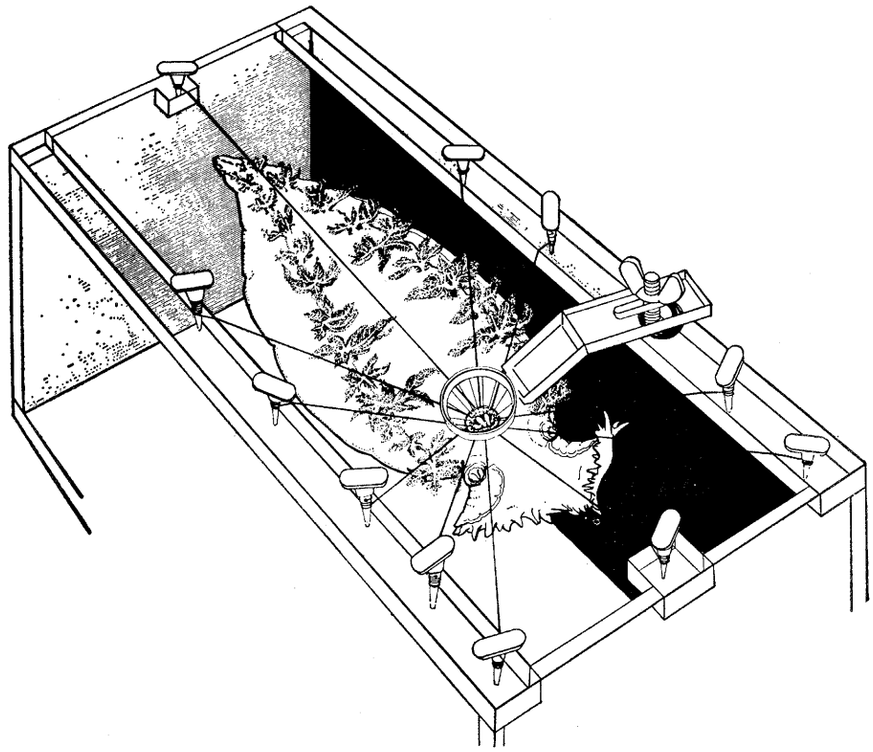
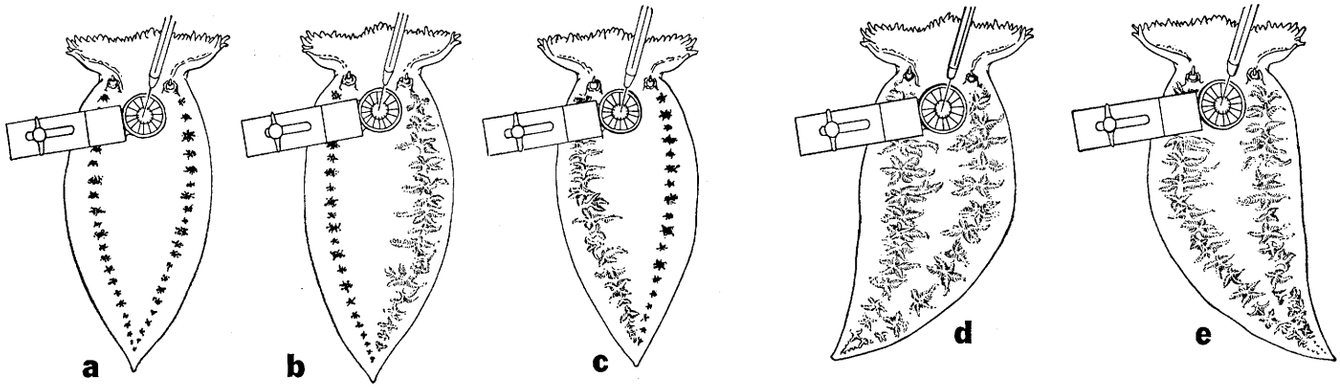


Fig. 3. The nudibranch, *Tritonia,* suspended in a tank filled with seawater by threads which retract the edges of the incision. The animal is relatively free to engage in normal motor activities.



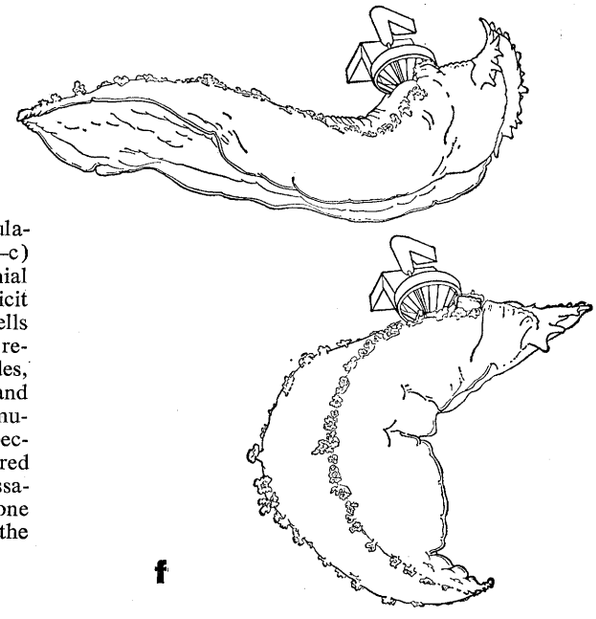


Fig. 4. Responses to stimulation of identifiable cells. (a–c) Withdrawal of the branchial plumes. Cells 15 and 24 elicit withdrawal of all plumes. Cells 1 and 29 produce selective retraction on left and right sides, respectively. (d–e) Left and right turning caused by stimulation of cells 3 and 33, respectively. (f) Swimming triggered by a single spike or the cessation of a spike train in any one of a number of cells on the pleural ganglia.

ber of cells. These contractions, in isolation, appeared not to be part of any coordinated actions.

3) Coordinated contraction of muscles, including such acts as turning (cells 3, 33) and retraction of branchial tufts (cells 1, 2, 15, 24, 29, 30), was elicited by stimulation of certain other cells (Fig. 4). That these are motor cells with extensive fields of innervation, as opposed to premotor cells synapsing on a number of primary motor neurons, seems probable since their axons pass out to the periphery in the nerve trunks that innervate the appropriate motor regions (*6*). It is possible, however, that the final motor elements are in the peripheral nerve net.

4) Unlike these discrete muscular contractions, a general withdrawal of peripheral body regions (tips of the oral veil, some branchial tufts, and the rhinophore sheaths) and a slight stiffening of the body walls occurred in response to stimulation of any one of a group of cells located mainly on the posterior regions of the pleural ganglia (cells 15–18 and 23–28).

The amplitudes and speeds of the responses described in 2–4 were qualitatively proportional to the frequency of stimulation in the range of 2 to 25 per second.

5) Perhaps the most interesting response was the triggering of a relatively complex, fixed pattern of action involving a large number of muscles in coordinated sequence. This mollusk, like others, has evolved a vigorous escape response to the touch or proximity of any of a number of echinoderms. The voracious starfish *Pycnopodia helianthoides* is among the most effective releasers of this behavior. The escape consists of a general withdrawal followed by elongation, stiffening, and then a strong contraction of the longitudinal foot muscles, causing the animal to push off the substrate and fold into an upside-down U. Roughly 2 seconds later, an equally swift contraction of the longitudinal dorsal muscles bends the animal into a U-shape in the opposite direction, and a swimming motion is accomplished (Fig. 4). The dorsal-ventral foldings repeat until the animal resettles on the substrate after approximately six cycles. Such swimming may separate the nudibranch from its adversary by as much as a meter.

A single shock applied to one of several cell bodies will, at certain times, initiate this entire behavior. The strength or duration of the escape movements do not appear to depend upon the number or frequency of shocks applied. Surprisingly, this patterned behavior is also occasionally triggered at the cessation of a train of shocks, although triggering has never been observed during an extended burst of stimulation. It is of some interest that the cells effective as triggers include those in the previous category, namely, the cells that cause a general withdrawal and stiffening. Also, the triggering cells are only effective occasionally and erratically, an indication that there are certain factors, unspecifiable at present, involved in creating a "readiness to occur" or a "set." This phenomenon, observed in the normal behavior of animals, is widely accepted (*9*). My evidence indicates that "readiness to occur" is reflected in a small number of anatomically specified cellular units which cause the initiation of certain behavioral acts.

Clearly defined patterns of size, pigmentation, and location are seen in the appearance of the cells in the rind of these ganglia from animal to animal, and there is uniformity in the detailed structure of the dendritic fields. A number of identifiable nerve cell bodies can be stimulated, and their activity can be recorded. Some of these cells elicit muscular contractions. These activities are produced reliably from ani-

mal to animal and are proportional in speed and extent to the frequency of stimulation. However, a single shock or the cessation of a train of shocks in certain other cells occasionally releases a complex escape pattern comprising 30 seconds of coordinated activities. The execution of this pattern is not appreciably modified by the frequency of stimulation.

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Effects of Thiopental Sedation on Learning and Memory

Abstract. *Subjects who were administered thiopental showed a loss of memory for events discussed while they were under sedation. We tested the subjects for recognition memory of pictures and recall of associated pairs of letters and words, and found that the subsequent memory loss was correlated with the concentration of thiopental in the venous blood at the time the material was learned. Retention did not appear to be state-dependent because the subject, while under sedation, could recall material learned prior to sedation, and because recall was not facilitated by reinstatement of the sedation.*

We have observed that thiopental sedation may be accompanied by loss of memory for events discussed by patients while under the influence of the drug. In this experiment we examined the questions of whether the memory decrements produced by barbiturate sedation are caused by failure of initial fixation, by interruption of the so-called memory-consolidation process (*1*), or whether the difficulty is one of not being able to remember when the physiological state at retrieval differs from that during learning (state-dependent retrieval) (*2*).

All subjects were paid volunteers (control, $n = 2$; group 1, $n = 12$; group 2, $n = 10$). While the learning and recall procedures were essentially alike throughout, for group 2 thiopental was administered by intermittent injection (thiopental, 2.5 percent) and the subject's state of alertness was monitored through response to random digits presented by a prerecorded tape, one digit occurring every 3 seconds for 5 minutes (*3*). The subject was required to press a telegraph key every time he heard three consecutive odd numbers; an increase in errors was taken as an index of loss of vigilance owing to sedation. For group 2 the drug was continuously infused (thiopental, 0.3 percent) and an operant-response measure was used as an index of loss of vigilance (*4*). A loud high tone was presented in randon alternation with a loud tone of lower pitch. A tone occurred every 10 seconds and remained on until the subject terminated it by pressing the correct one of two buttons. The slope of the subject's cumulative response curve was taken as an index of his level of alertness. We tried to keep the subjects of both group 1 and group 2 at a level of sedation just short of unconsciousness.

Two sets of learning and memory materials were used. One consisted of ten simple line drawings of familiar objects which the subject was asked to describe when they were first presented, to insure that the pictures were adequately perceived while the subject was under sedation. In a later test the subject selected the familiar pictures from a set containing half familiar and half novel ones. The second kind of material consisted of six easily associated pairs of letters and words (for example, W-water, F-flower, C-camel). These were learned by the anticipation method to the point of one perfect recitation (*5*). In a later test for recall, the cue letters alone were presented. Both materials were so easy that two control subjects, who were always given a placebo instead of thiopental, made perfect scores throughout. Further evidence of the ease of the learning materials was obtained from the ten subjects of group 2 who learned a list of associated pairs before they were placed under sedation. Of these, eight learned in a single trial, and two learned in two trials.

Before the drug was administered, a base-line blood sample was taken and vigilance tests were given. The subjects of group 2 learned a control set (set A) of the associated pairs. In order to test for possible retrograde amnesia (or possible state-dependent recall), the subjects were tested for retention immediately after the drug took full effect. For both groups, the first set of pictures (set 1, recognition test) was now presented and the subject described each one. After the presentation of the pictures, six associated pairs (set B) were learned to one perfect recitation. After a lapse of 30 minutes (filled with incidental conversation to maintain consciousness; blood samples were also taken and vigilance tests administered), the subject was tested for recognition of the set 1 pictures and recall of the set B associated pairs. This method determined the extent of memory loss under sedation. Then, another blood sample was drawn, vigilance tested, and another set of materials learned: set 2 of recognition pictures and set C of associated pairs. After a final blood sample and vigilance test, the experiment was terminated for the day. The subject commonly slept for 2 or 3 hours, was sent home, and returned to the laboratory the following day for a 24-hour retention test.

Electroencephalograms recorded periodically throughout the session showed clear modifications, indicating that sedation was adequate.

Groups 1 and 2 represent essentially two experiments, because some of the