Effects of Anesthetics on Neural Mechanisms

Selective Actions of Volatile Anesthetics on Synaptic Transmission and Autorhythmicity in Single Identifiable Neurons

N. Chalazonitis *

THE STUDY of volatile anesthetic action on the giant neurons of *Helix* and *Aplysia* was motivated by the operational facilities offered by these preparations: the possibility of working on isolated centers, thus avoiding the often marked changes of blood pressure caused by the drugs; large sized neurons permitting impalement with two independent microelectrodes, then remaining entirely stable over prolonged periods; and, rapid equilibration of the anesthetic partial pressure in the atmosphere with the saline surrounding the almost "naked" neuron. The depressive action of volatile anesthetics on the electrical parameters of the neuronal membrane was investigated early in single cells.[1, 2] The studies were brought about by recording with extracellular electrodes on isolated giant axons of *Sepia*, as well as on nerve cells of *Aplysia*. Later, a more extensive analysis was undertaken by intracellular recording of the neuronal activity.[3, 10, 11, 14, 22]

Experimentation on neurons of *Aplysia* and *Helix* was undertaken not only for the operational facilities of their large size, but mainly because in a given ganglion, the neurons are directly identifiable [4] and one can study simultaneously the activity of the main types: synaptically activated or inhibited neurons; autorhythmic neurons.

Our purpose here, is first to examine volatile anesthetic (VA) action on the bioelectrical parameters of the neuronal membrane (membrane potential, excitability, resistance). Such an analysis requires actually "deafferented" neurons, that is, neurons in a state of "synaptic isolation" from other units. Some giant neurons of the visceral ganglion of *Helix* can be adapted to fulfill such conditions. Next, we shall consider the main types of identifiable neurons namely, the synaptically activated and the autorhythmic. Finally, the question of selective action, will be examined by simultaneous recordings in two different neurons, submitted to the same conditions of external partial pressures. In addition, for a given VA, comparison of sensitivities will be made among different membrane sites of a given neuron: the somatic and the axonic; or among two post- and/or peri-synaptic areas of a given neuron, activated through two independent synaptic inputs during VA action.

Material and Methods

The identifiable neurons of the *Aplysia* visceral ganglion displaying the characteristic activities [4] already described were explored by microphysiological methods. Similar procedures were applied to the nerve cells (pleural and visceral ganglion) of *Helix*. By a careful narrow excision of the connective sheath, a few "quasi naked" neurons were prepared for microelectrode impalement under direct vision. The desheathed cells were thus more directly exposed to the anesthetic vapors, than other cells in the ganglion which were kept relatively protected. Partial pressures of different volatile anesthetics, such as halothane, ether, chloroform, were established using calibrated flowmeters and calibrated air flows. In all experiments, anesthetic action was studied at a

* Departement de Neurophysiologie Cellulaire, Institut de Neurophysiologie, Centre National de la Recherche Scientifique, Marseille, France.

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constant external P_{O_2} (about 100 mm. of mercury) and P_{CO_2}. The mean temperature, normal for such preparations, was 22° C. ± 2. The preparation was placed and VA diluted in the air, was admitted to a thermostated gas chamber, as previously described.[7, 10, 11] The isolated ganglion was carefully introduced; one or two of the afferent fibers were laid on pairs of stimulating electrodes. Bent micropipettes were introduced through small openings. Impalement of the cells was conducted under microscopic visual control. Two independent microelectrodes were inserted into the same cell, whenever a current input was needed.

Effects of Anesthetics on Bioelectrical Parameters of the Membrane

Membrane Potential. In general, the immediate effect of the VA was a membrane depolarization with concomitant increase of excitability; this is the "prenarcotic hyperexcitability phase,"[2, 3] extremely transitory (seconds). As VA action continued, in most cases membrane potential (MP) increased again. The membrane hyperpolarized and the cell become hypoexcitable after 2 to 3 more minutes, provided that the partial pressure of VA was adequate (approximately 1.5 to 2 times the partial pressure used for anesthesia in man and depending upon the neuron examined) (fig. 2, *B;* fig. 4, *C*). However, the level of the membrane potential during the narcotic phase was not determined solely by progressive adsorption of the anesthetic molecules in the somatic membrane. Frequently, the firing of other neurons, either excitatory or inhibitory, or both, contributed to the evolution of MP and to the excitability of the explored neuron. Changes in MP during VA action may thus be grossly summarized as an early transitory depolarization followed by hyperpolarization.

"Direct" Membrane Excitability. Measurement of changes in "direct" excitability is a better criterion to gauge the progressive action of VA. The action depends upon the continuously increasing concentration of VA in the membrane: low concentrations elicited hyperexcitability, increased concentrations provoked hypoexcitability (fig. 3, *A*). However, in neurons of *Aplysia,* the hyperexcitability phase was not always observed during the action of halothane. In some cases, the membrane became hyperpolarized, *d'emblée,* entering the narcotic phase without initial hyperexcitability. In neurons of *Helix,* hyperexcitability was determined with 2 per cent halothane. If the anesthetic partial pressure did not exceed 2 per cent, the narcotic phase was never reached in many neurons of *Helix.* For hypoexcitability, 3 to 5 per cent of halothane was required (fig. 1). Narcotic hypoexcitability of the membrane in response to direct mechanical stimulus was shown in the *Aplysia* neuron by extracellular recording [2] and to the electrical stimulus by intracellular recording in the *Aplysia* neuron,[10, 14] and in the motoneuron.[19] Narcotic hypoexcitability was reversible: using the same current intensity, the spike reappeared after admission of air (fig. 1, *C;* fig. 2, *D*). Recovery time was always relatively long, 5 to 6 times the narcotic time. A long recovery time of cortical electrogenesis from halothane narcosis has also been reported.[18]

The question arose, to what extent is narcotic hypoexcitability ascribable to the membrane hyperpolarization. Actually, the spike failure could be overcome simply by increasing the intensity of the injected depolarizing current (fig. 2, *C*). As narcosis is a gradually progressing process, and if the intensity of the depolarizing current is increasingly adjusted, a spike may be elicited at different levels of narcosis. The greater the depth of narcosis, the higher the intensity of outward current required merely to elicit a spike. This intensity may then be taken as a measure of the instantaneous value of the narcotic level. The means of describing quantitatively the time course of the membrane narcosis is thus provided. However, in deeper narcosis, the spike generation failed, in spite of increasing the intensity of "compensating" outward current (fig. 6, *E* and *F*). Obviously, at this step, membrane hypoexcitability is not solely ascribable to the narcotic hyperpolarization.

Membrane Resistance. Changes of membrane resistance (MR) during VA action were studied by injecting transmembrane current pulses of a constant intensity (Δi) and recording the MP diplacement (Δv). The ratio

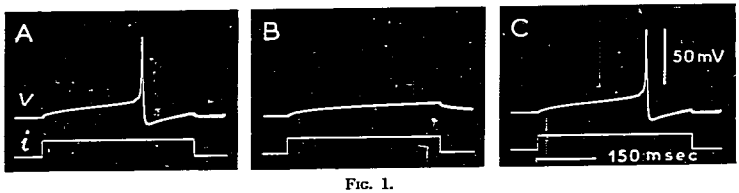


FIG. 1.

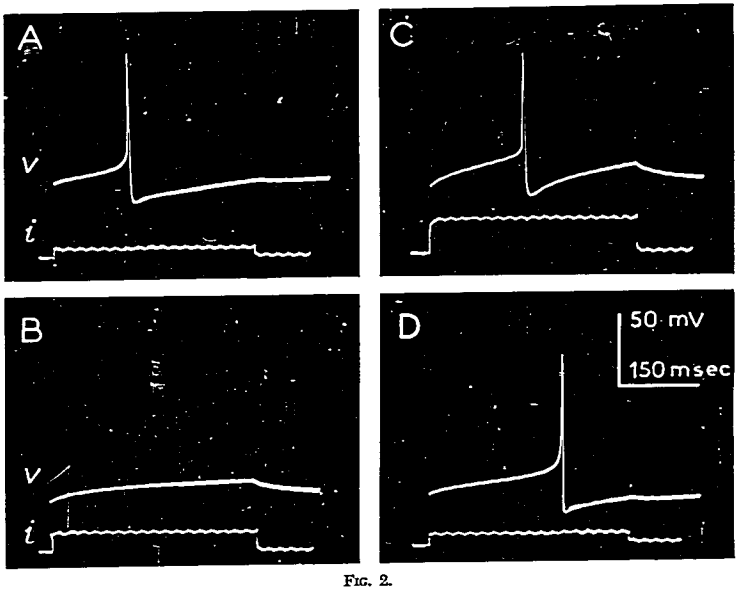
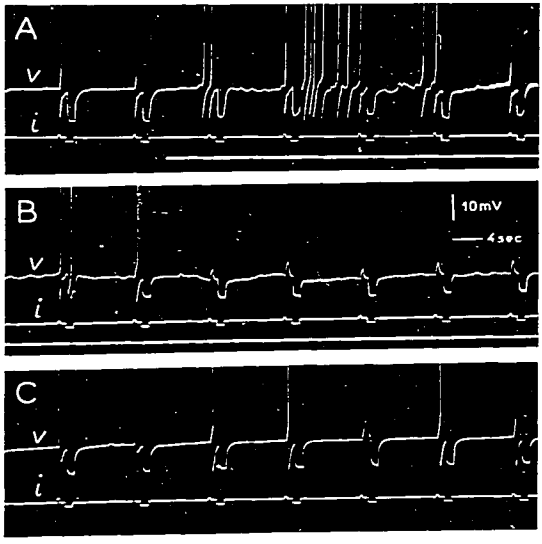


FIG. 2.

FIGS. 1 and 2. Depression by halothane of the direct excitability of somatic membrane. *Upper traces* (v): intracellularly recorded somatic potentials. *Lower traces* (i): injected current pulses (threshold intensity: 0.5 to 1×10^{-9} A, in normal conditions, in air). (*Helix* neuron.) FIG. 1. (*A*) Threshold response to current pulse. (*B*) Only local depolarization, after 5 per cent halothane administration for 3 min. (*C*) Recovery after 2.5 minutes in air. FIG. 2. Compensation of the narcotic hyperpolarization, by injection of outward current. (*A*) Normal neuron, in air; response to d.c. pulse (current intensity: 0.5×10^{-9} A). (*B*) Halothane 10 per cent for 6 minutes: hyperpolarization and failure of spike elicitation despite doubling current intensity (1×10^{-9} A). (*C*) Halothane 10 per cent for 6 more minutes; elicitation of an action potential, but with a 3×10^{-9} A depolarizing pulse, compensating for narcotic hyperpolarization. (*D*) Recovery: 11 minutes in air. Current intensity 0.5×10^{-9} A.

$\Delta v/\Delta i$ began to decrease with the membrane depolarization, during the phase of prenarcotic hyperexcitability (fig. 3, *A*). MR was then reduced only to approximately 90 per cent of its normal value. The decrement became greater and greater during the consecutive membrane hyperpolarization (hypoexcitability phase). The MR could then be reduced to 30



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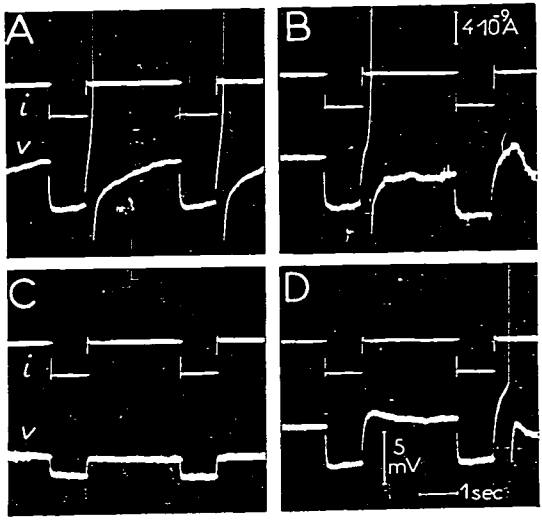


FIG. 4.

FIGS. 3 and 4. Changes in the membrane potential, conductance and excitability by anesthetic vapors. FIG. 3. *Upper trace* (*v*): somatic responses and membrane potential displacements. *Helix* neuron. *Middle trace* (*i*): current pulses in two consecutive steps, brief depolarizing pulse (excitatory) followed by a hyperpolarizing one (monitoring changes in membrane resistance). *Lower heavy traces* record time of exposure to halothane, 10 per cent. (*A*) Pre-narcotic hyperexcitability phase, reached in seconds (spontaneous spiking), with a concomitant slight increase in conductance. (*B*) After 4 minutes, decrease in excitability, increase in membrane potential and increase in conductance (≈ 30 per cent). (*C*) Recovery after 12 minutes in air. FIG. 4. Changes in membrane parameters of *Aplysia* neuron produced by chloroform. *Upper traces* (*i*): hyperpolarizing current pulses. *Lower traces* (*v*): displacement of the membrane potential and "off" spike. (*A*) Initial, unanesthetized state. (*B*) Exposure to CHCl₃ (54 mm. Hg): hypoexcitability, failure of the "off" spike. (*C*) At the 50 second of action: increase of membrane conductance: increase of MP; inexcitability. (*D*) Recovery after 40 seconds in air.

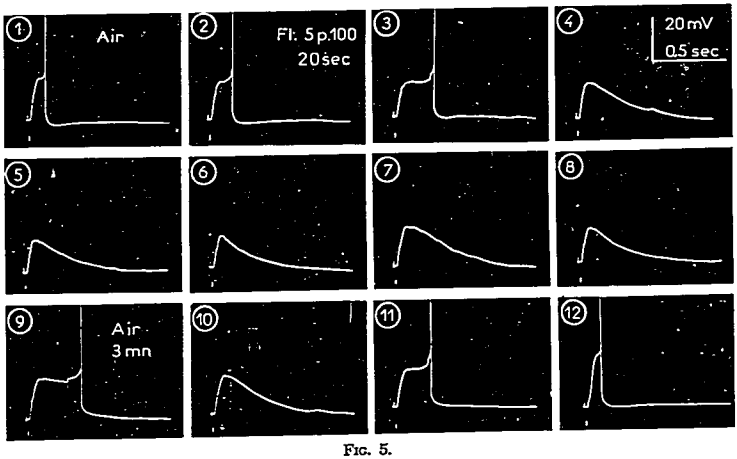


FIG. 5.

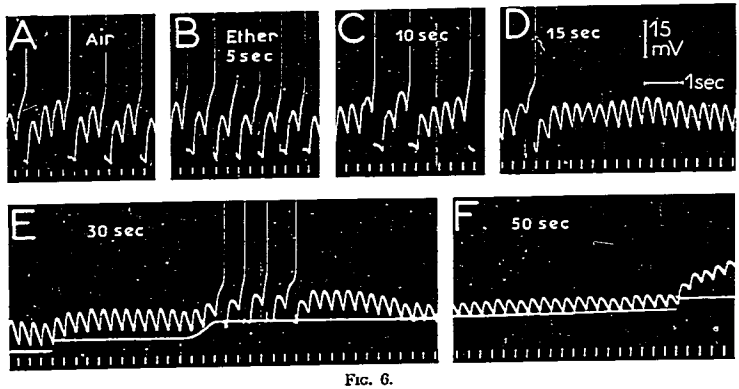
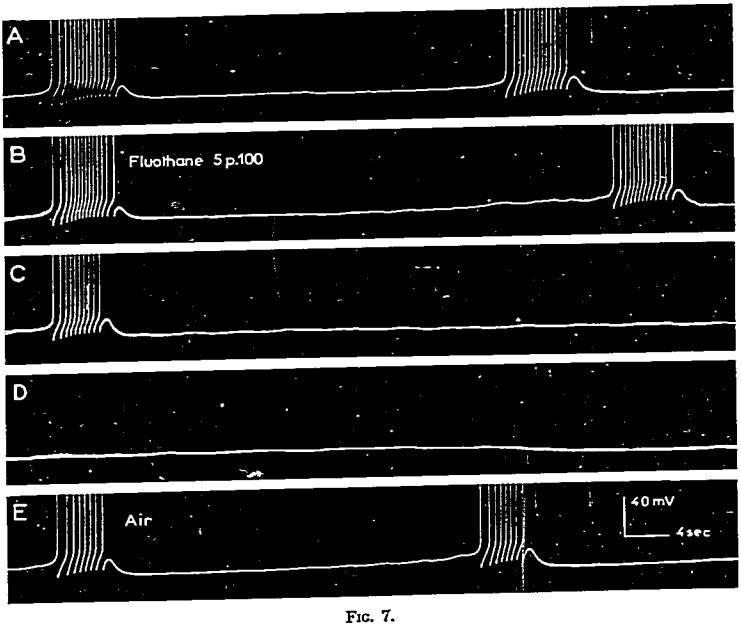


FIG. 6.

FIGS. 5 and 6. Depression of the synaptic activability by volatile anesthetics. FIG. 5. Action of halothane. *Aplysia* neuron under standard synaptic activation at 0.3 $sec.^{-1}$ frequency. (1) Normal state; (2 to 4) After 20 seconds of halothane, 5 per cent; spike generation delays and finally disappears; (5 to 8) halothane prolonged for another minute, the EPSP's amplitude varies periodically; (9 to 12) After 3 minutes in the air, progressive recovery of spike generation. FIG. 6. Depressed by ether action, the synaptic activation is transiently restored by DC depolarization. *Aplysia* neuron, under standard synaptic activation at 3 $sec.^{-1}$ frequency, as indicated by the underlying vertical signals. (*A*) Normal state, EPSP's summation and intermittent spiking. (*B*) Ether (100 mm. Hg partial pressure) for 5 seconds. Prenarcotic hyperexcitability: spiking increased by 50 per cent. (*C* and *D*) Ether continued; hypoexcitability. (*E*) Marked hyperpolarization; failure of spiking; transitory restoration by gradual inputs of depolarizing current, compensating for anesthetic hyperpolarization and hypoexcitability. (*F*) At 50 seconds of ether, decrease in EPSPs amplitude; failure of restoration by depolarizing current.



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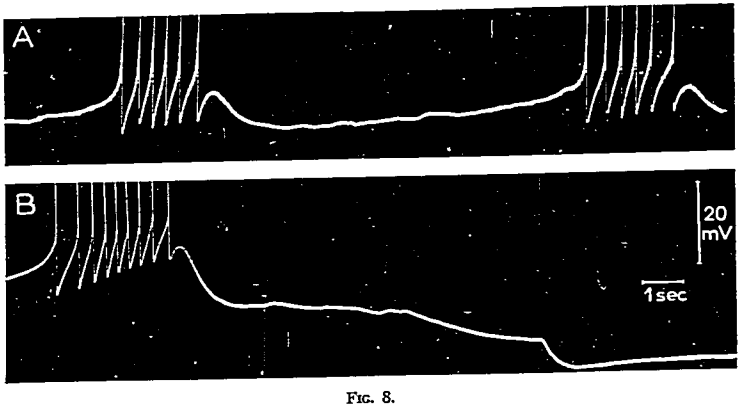


FIG. 8.

per cent of normal value, in air (fig. 3, *B* and fig. 4, *C*). Such changes in MR were entirely reversible in air (fig. 3, *C* and fig. 4, *D*).

Thus it appears that the high membrane conductance converges with the concomitant hyperpolarization to determine the marked hypoexcitability of deep narcosis. The reversible actions of VA on the MR may be interpreted as a reversible adsorption of the narcotic molecules, perhaps in some lipoid structures of the somatic membrane.

Effects of Volatile Anesthetics on Synaptic Processes in "Stable" Membrane Potential of Neurons

Early data were reported on the spinal cord using ventral root recording or focal leading,[6, 12] and on autonomic ganglia.[16] In single neurons, synaptically activated, data were reported by extracellular microelectrode recording in the *Aplysia* neuron,[2] more recently by intracellular recording,[3, 10, 14] and in the motoneuron.[19, 20, 21]

Here, the study of VA action on the synaptic processes of *Aplysia*, and *Helix* identifiable neurons will be considered in the light of changes in the concomitant membrane potential, excitability and resistance and changes of the "stable" membrane potential, inactive under normal conditions in air.

The impaled neuron was submitted to standard synaptic activation at a constant frequency. Figure 5 illustrates a routine processing: the nerve fiber afferent to the impaled *Aplysia* neuron was stimulated at 1 $sec.^{-1}$ frequency with a constant, minimal threshold intensity. After a 20 second action of halothane at 5 per cent concentration (partial pressure ≈ 12 mm. of mercury, in air) the signs of narcotic hypoexcitability were already evident: increase in the delay of spike generation (fig 5 (2) and (3)), then rapid failure (fig. 5 (4)). However, excitatory postsynaptic potentials (EPSPs) were continuously evoked without a measurable delay in their onset. A slight decrease in their amplitude and in the time constant of their decay developed during the narcotic action (fig. 5 (5) to (8)). The effects were completely reversible after 3 min. in air: spike generation was re-established with a gradually decreasing latency (fig. 5 (9) to (12)).

In most cases, a pre-narcotic enhancement of the synaptic activability preceded the phase of narcotic depression. As shown in figure 6, *B* in an *Aplysia* neuron, after 5 seconds of ether action, transmission was effective once every 2 EPSP instead of once per 3 or 4 EPSP, in air. During the narcotic phase, the membrane hyperpolarized, synaptic transmission failed, and amplitude of the EPSPs' decreased, with no delay in their onset (fig. 6, *E* and *F*).

In conclusion, pre-narcotic enhancement of synaptic transmission and its consecutive failure during the narcotic phase are probably ascribable to the postjunctional and/or perisynaptic membrane potential and changes in excitability as noted previously. A failure of the pre-synaptic processes, decrease in the rate of propagation or conduction block in the preterminal afferent branches is ruled out as there was no detectable delay in the onset of the EPSPs. On the other hand, increase in membrane conductance during the narcotic hyperpolarization phase could well be responsible, at least in part, for the reduction in EPSP amplitude and the increase in their decay rate.

Action of Anesthetics on Autoactive Neurons

Neurons firing at fairly reproducible frequencies, at normal P_{O_2} and temperature, in the absence of any apparent extrinsic triggering, are "autoactive neurons." Two classes of autoactive neurons are considered:

Neurons Autoactive at a Constant Frequency (Regularly Autoactive). Changes produced by chloroform, diethyl ether or halothane were entirely determined by concomitant changes in the membrane potential, excitability

FIGS. 7 and 8. Depressive action of volatile anesthetics on the *Br* type autorhythmic cell of *Aplysia*. FIG. 7. (*A*) Normal activity in air. (*B*) After 30 seconds of halothane, decrease of slow wave pattern frequency. (*C* to *D*) Failure of pattern generation after 2 minutes of halothane. (*E*) Recovery in air, 20 minutes. FIG. 8. Indirect inhibition of the slow wave pattern under chloroform. (*A*) Normal, in air. (*B*) Sudden suppression of slow wave and deep hyperpolarization through firing of an inhibitory interneuron (6 minutes chloroform, 10 mm. Hg partial pressure).

and resistance. An increase in spike frequency was effected during the pre-narcotic depolarization of the membrane. Later, during the narcotic hyperpolarization phase and increasing conductance, the spike frequency decreased with a further decrease in the amplitude, all effects being reversible.

The Second Type of Firing Neuron Displays Rhythmic Changes in MP, with Trains of Spikes.[7] Pre-narcotic hyperexcitability, transient increase in slow wave frequency, was rarely observed. Under the influence of chloroform, narcotic hypoexcitability often started abruptly, *d'emblée*, the membrane hyperpolarized to an extreme and periodic wave formation was abolished (fig. 8). This process implies that there is a potent synaptic inhibitory invasion.[14]

The effect of halothane on the same neurons was in general analogous but more reproducible. The slow wave frequency started to decrease concomitantly with the gradual increase of the MP (fig. 7, *B*). Amplitude and duration of the slow waves gradually changed. Almost complete extinction of the pattern was observed much later (fig. 7, *D*).

Selectivity of Volatile Anesthetics Action Among Neurons and Among Membrane Sites

In *Aplysia* nerve centers, topographically and morphologically identifiable nerve cells display specific activity patterns and react differentially to various agents, suggesting genuine differentiations in the structural organization of their membranes. Hence differential effects of the VA among various cells and in a same cell between distinct membrane sites were expected. Studies on selective actions entailed simultaneous recordings in many different superficial neurons identically exposed to the VA. Occasionally, the behavior of other, non-impaled neurons was deduced via the synaptic inputs they conveyed to the explored neurons (fig. 10 (3) and (4)).

Differential Time Courses of Changes in MP Under VA Action. The simplest and most immediate basis for comparison was provided by the changes in MP. In this respect due care was taken to explore neurons superficially situated, those offering equal accessibility to the VA, because in deeply situated neurons, interpretation of chronological differences would be complicated by diffusibility.

In equally exposed neurons at a given partial pressure of VA, the time course of the changes membrane potential seldom were parallel. Most often, one neuron exhibited a prenarcotic depolarization phase while others had already reached narcotic hyperpolarization and depression (fig. 9).

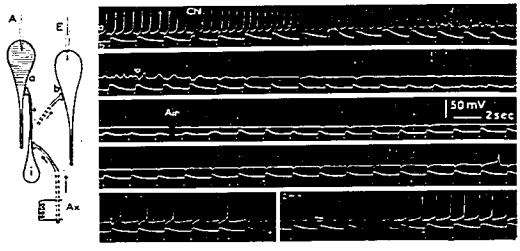
Differential VA Actions Among Neurons Synaptically Controlled. In two cells receiving the same afferent input, and similarly exposed to a given partial pressure of VA, the narcotic abolition of synaptic transmission was rarely established simultaneously. Often, while synaptic transmission was abolished in some neurons, in others it was still effective. Depression was more or less delayed. Such differences in narcotic latencies might be attributed to differences in affinity for the VA, that is, differences in saturability of the reacting membranes.

Of particular interest were the cells under "reciprocal" or opposite synaptic control: in paired cells explored simultaneously with intracellular electrodes, a minimal threshold stimulus to the appropriate nerve pathway elicited simultaneously an EPSP in one cell and an IPSP in the other.[5, 8, 9] In such "reciprocal" cells, the respective EPSPs and IPSPs generally reach narcotic depression simultaneously provided that the two explored cells were equally exposed to the narcotic action. In figure 9, narcotic hyperpolarization was reached simultaneously in the two reciprocally activated nerve cells. The opposite or "antergic"[8] post-synaptic potentials vanished in parallel and recovered simultaneously in the presence of air. In figure 11, however, narcotic hyperpolarization supervened earlier in the (*b*) soma than in the (*a*) soma. Accordingly, the amplitude of the (*b*) EPSP rapidly decreased while that of the (*a*) IPSPs remained unchanged.

Differential Action of VA in Synaptically Stimulated Soma and Antidromically Conducting Axon of the Same Neuron. In *Aplysia* and in *Helix* cells, the antidromically conducted potentials did not necessarily invade the soma. In general, axonic conduction stopped at the

junction of axon hillock. In all cases in *Aplysia* as well as in *Helix* cells, when exposed to chloroform or to halothane, the antidromically conducted potentials persisted when the spike elicited by EPSPs were already abolished by the VA (fig. 11).

It follows, then, that the sensitivity to the action is less in the axonic membrane, than for the somatic membrane.[10] However, despite persistance of axonic conduction when somatic excitability was already decreased, some axons conducting with a greater sensitivity could be narcotized earlier than the explored soma: with 5 per cent halothane, with external re-



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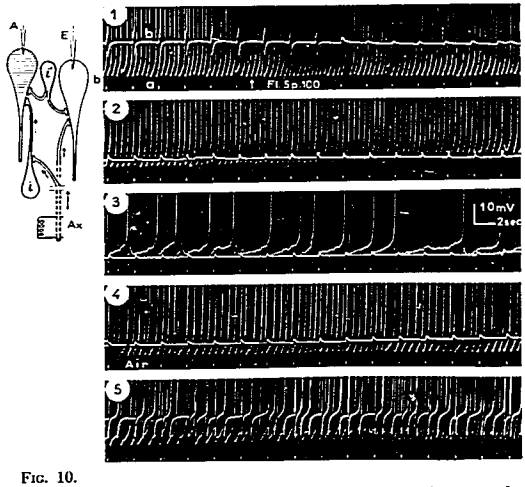

FIG. 10.

FIG. 9 and 10. Varying effects of volatile anesthetics in different identifiable neurons. FIG. 9. Continuous recording of chloroform action on two *Aplysia* neurons simultaneously explored, in the same ganglion. Standard stimulation of appropriate nerve (0.5 sec.$^{-1}$) results in small IPSPs on autoactive (*a*) neuron (upper trace) and EPSPs on the resting neuron (*b*, lower trace). In first double recording, chloroform (100 mm. Hg) readily decreases spike amplitude of (*a*) neuron, but not that of IPSPs. Simultaneously, (*b*) neuron depolarizes transiently and amplitude of the local somatic potential (superimposed on decaying phase of the EPSP) increases. At that stage, (*b*) neuron is in the pre-narcotic hyperexcitability phase, while (*a*) neuron is already in hypoexcitability state. At end of the second double recording, membranes of both cells are hyperpolarized, EPSPs and IPSPs vanish almost simultaneously. With admission of air (third double recording) all signals progressively recovering. At fourth recording, 2 minutes later, recovery improved. FIG. 10. Differential action of halothane (5 per cent) on two different neurons (*Aplysia*) simultaneously explored in same ganglion. (1) Standard stimulation given to the nerve, determines EPSPs and threshold transmission in (*b*) neuron (upper trace), but IPSPs on autoactive (*a*) neuron (lower trace). Administration of halothane abolishes synaptic spike on (*b*) cell, but does not modify (*a*) spontaneous activity. (2) One minute later, halothane, hyperpolarization of (*b*) membrane, but no change in (*a*) autoactivity. (3) One minute later, decrease in (*a*) autoactivity because of inhibitory bombardment from firing interneuron (*i'*) entering the picture (in its pre-narcotic hyperexcitability phase). Note simultaneous EPSPs invasion of (*b*) neuron, originating probably from same firing interneuron (*i'*). (4) Admission of air: fast recovery for (*a*) neuron (interneuron *i'* stops firing), slow redepolarization for (*b*) membrane. (5) Later, (*b*) neuron crosses metanarcotic hyperexcitability phase (compare *b* in (1)).

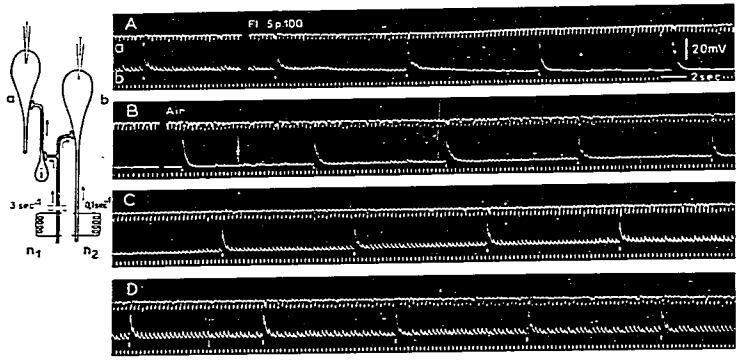

FIG. 11. Two different *Aplysia* neurons simultaneously explored under halothane (5 per cent). Stimulation at 3 sec.$^{-1}$ of the n_1 nerve determines at 3 sec.$^{-1}$ frequency IPSPs in the (*a*) soma and EPSPs in the (*b*) soma. Stimulation at ≈ 0.1 sec.$^{-1}$ of the n_2 nerve elicits remote antidromic potential in (*b*) soma. All recordings continuous. (*A*) Halothane, (*a*) membrane depolarizes slightly while (*b*) hyperpolarizes. Extinction of the EPSPs for (*b*), but persistence of IPSPs with increased amplitude, for (*a*). (*B* to *D*) Air, recovery for both signals, earlier for EPSPs. Note that remote axonic potential in the (*b*) soma did not undergo significant change during halothane.

cording of the response of the stimulated nerve afferent to the *Helix* ganglion, it occasionally happened that amplitude decreased before the somatic response, synaptically elicited, has disappeared (Chalazonitis and Takeuchi, unpublished work).

Differential Depression by VA Among Distinct Synaptic Membrane Areas in the Same Neuron. When two different pathways were alternately stimulated to elicit EPSPs and spikes, the VA often depressed transmission via one pathway (increase of spike delay, then abolition of transmission) more rapidly than by the other. The delay between the two indicates the differences in times required for attainment of a critical concentration in the respective post- and/or peri-synaptic membranes, thus revealing the differential properties of two areas of membrane in the same neuron.

Discussion

Selectivity of VA action on different membranes are interpretable on the hypothesis of differences in the "critical narcotic concentration" in these membranes, in addition to differences in kinetics of adsorption of the VA molecules, hence to differential latencies. Whenever a critical concentration of molecules of VA is reached in a neuron, that neuron undergoes narcotic depression, while in others the critical concentration may have not yet been attained. The same hypothesis may be adapted to deal with the differential narcosis of the somatic membrane, as with respect to the axonic site. Earlier narcotic abolition of transmission in one synaptic pathway, while others are still operative would, likewise imply differences in critical concentrations in the synaptic areas at different synaptic inputs. Selective narcotic action would then be evidence of basic cytostructural and cytochemical differences among the neuronal membranes studied, and would provide further support to the concept of functionally differentiated patches, that is, "mosaic" like membranes.[1]

The concept noted above of critical concentrations of a narcotic, in each nerve cell membrane, is an adaptation at the molecular level of a previously held general theory of narcosis;[12, 15, 17] namely, that whatever the nature of the narcotic the same critical concentration is sufficient to narcotize a given organism. Other effects deserving consideration

at the molecular scale are the well known narcotic actions on enzymatic processes.[2] Should redox enzymes be shown to exist in the electrogenic membranes themselves, such effects would acquire added interest.

From the neurophysiological point of view, hyperpolarization and increase in the membrane conductance are changes in bioelectrical parameters sufficient to account for the narcotic depression of direct excitability and of synaptic activation. The narcotic actions interfere with and oppose the membrane mechanisms of normal activation, but activation continues to be displayed through the genesis of intrinsic membrane currents, able to create local depolarization or even to promote firing. However, narcotic action cannot simply be ascribed to the formation of "narcotic inward currents" counteracting "generator currents." As a matter of fact, the narcotic effect may be compensated only to a very limited extent by supplementing a narcotized area with an appropriate outward current.

Summary

Narcotic action on the higher nerve centers by volatile anesthetics is substantiated at the neuronal level, by a depression in membrane excitability. The increase of the threshold current required for spike elicitation may be taken as a measure of the narcotic depression. Such increase of the threshold is ascribable to membrane hyperpolarization and to the increase in the membrane conductance both of which develop during narcotic depression.

The depression of excitability, the narcotic phase, is often preceded by a transitory hyperexcitability, the prenarcotic hyperexcitability phase, due to an early brief depolarization of the neuronal membrane.

Abolition of the somatic spike elicited by a threshold EPSP, without any delay in the onset of the latter, is the primary synaptic sign during narcotic hypoexcitability. Decrease amplitude of EPSP and increase in its decay rate are subsequent effects, ascribable to the narcotic increase of the membrane conductance. The data submitted here point to the prime role of the subsynaptic membrane in controlling narcotic abolition of synaptic transmission.

In autorhythmic neurons, decreases in frequency of both spikes and waves, in the case of neurons of low frequency waving, are the first signs of the narcotic phase, both related to the membrane narcotic hyperpolarization. Complete arrest of autoactivity is a more delayed effect, often hastened by inhibitory interneuronal firings.

Simultaneous recordings in different neurons show that the narcotic phase is often reached at extremely different latencies. Therefore, some neurons are selectively more sensitive than others.

In comparing the somatic and the axonic sensitivities to volatile anesthetics in the same neuron, it was shown that the somatic sensitivity is always higher.

In a given neuron submitted to distinct synaptic inputs, narcotic abolition of transmission occurs earlier in some synaptic pathways and is delayed in others. Narcotic selectivity provides evidence of differentiation among different membrane areas, in a given neuron.

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Discussion

Dr. Fink: I would like to add a word about the point that Dr. Chalazonitis made concerning the possibility that some nerve fibers might be even more easily narcotized than their somata. My evidence is indirect. I made some experiments with Sepia giant axons, bathing them with anesthetic vapors of various known supranarcotic strengths, *i.e.*, sufficient to block conduction. I assumed that during recovery, the anesthetic was being eliminated by simple diffusion. By narcotizing the fiber with two different supranarcotic partial pressures, and timing the recovery, one could calculate the exact partial pressure present at the instant of the return of the action potential. I found that the partial pressures which narcotized the squid axon at the ambient temperature, about 20° C., were extraordinarily similar to the partial pressures used in clinical narcosis. Despite the large difference in temperature, the sensitivity of these giant axons to the vapors was of the same order as the sensitivity of the organized nervous system of higher vertebrates.

Dr. Krnjević: Were you testing isolated axons, or axons *in situ*? Isolated axons probably have a rather low safety factor.

Dr. Fink: They were isolated axons, after peeling off most of the small fibers.

Dr. Somjen: Dr. Fink, your criterion of recovery was restoration of conduction of the spike?

Dr. Fink: Restoration of the propagated action potential. May I remark that this action potential, when it reappeared, was not immediately of maximum amplitude, that, particularly with chloroform, there was a slow recovery of the spike to the control amplitude.

Dr. Butler: Where you measured the membrane resistance and it altered with the induction of anesthesia, can you give us some idea of the degree of increase in membrane resistance?

Dr. Chalazonitis: The membrane resistance decreased continuously under the action of anesthetic vapors. During the pre-narcotic hyperexcitability phase, the decrease was very low (about 10 per cent). During the narcotic phase (corresponding to a decreased excitability) the decrease in membrane resistance amounted to 40 to 60 per cent of the normal MR value.

Dr. Krnjević: Do you get any change in membrane resistance when there was no evidence of background synaptic activity?

Dr. Chalazonitis: Yes, even on the very quiet neurons.

Dr. Krnjević: In completely quiet cells?

Dr. Chalazonitis: Yes. The narcotic effect of increase in membrane conductance could not be ascribed to synaptic processes.

Dr. Fink: One of your records was from an *Aplysia* soma with an intra-cellular electrode for recording and another one for injecting current. With orthodromic stimulations in the presence of ether vapor, this cell became inexcitable; then by injecting a small current, excitability was restored. With continued exposure to anesthetic, the cell again became inexcitable, but responsiveness was again temporarily restored by the injection of a stronger current. This was repeated several times, although eventually the cell became refractory. The suggestion I wish to make is that, if one had equilibrium conditions, one might be able to use the amplitude of the current needed to restore excitability, as a measure of the intensity of narcosis.

Questioner: Did you explain the context in which you used the word "narcosis" as distinct from anesthesia?

Dr. Chalazonitis: Well, anesthesia is the loss of perception of pain, the Greek meaning of the word. One cannot "anesthetize" a neuron because the neuron does not feel pain.

Dr. Ngai: In this sense narcosis is the correct term, meaning inexcitability.

Dr. Van Bergen: I have no experience with electrical stimulation of individual nerve fibers; but in stimulating whole nerves, temperature plays a major role. Regarding Dr. Fink's proposal that the *amount* of stimulating current required to restore an action potential is useful as an index of narcosis, I wonder if the current were sufficient to increase the temperature of the preparation?

Dr. Chalazontis: Cooling generally depolarizes and activates the somatic membrane; conversely, warming repolarizes and decreases the membrane excitability. Therefore, if by passing current through the membrane, an elevation of temperature, even extremely small, could be expected to be operative, it should determine repolarization and reinforce the narcotic effect. Warming could not be responsible for the abolition of narcosis. The effects of depolarizing current counteracts narcotic action by interfering with the narcotic hyperpolarization.