

high concentration in many of them, has been found (like homoarginine, which it accompanies) to be without toxic effects in the mouse and chick¹⁷. The toxicity of γ -hydroxyhomoarginine¹⁸⁻²⁰, which also occurs in varying concentrations in certain of these species, has not yet been determined, however, nor have the compounds designated A_4 , N_2 and B_4 been isolated in the pure state.

To illustrate the sub-division of the genus and the resolution of most of the compounds discussed, extracts of the seeds of two species from each of the first four groups of Table 1 have been subjected to ionophoresis (5 kV for 15 min) on paper at pH 3.6 (Fig. 1). β -(γ -Glutamylamino)propionitrile being uncharged at pH 3.6 is not resolved from the neutral amino-acids by this method, but its presence may be readily detected chromatographically on paper using lutidine-water (65:35 by vol.) which separates it from the other ninhydrin-reacting compounds found in comparable concentrations in the extracts examined (R_F 31).

It is appreciated that much work on the biosynthesis of these compounds, their phylogenetic significance and in some instances their toxicity remains to be done. Nevertheless, it is hoped that this account may explain some of the unexpected and apparently contradictory results reported in the literature on lathyrism and lead to a clearer understanding of the problems involved.

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CONTINGENT NEGATIVE VARIATION: AN ELECTRIC SIGN OF SENSORI-MOTOR ASSOCIATION AND EXPECTANCY IN THE HUMAN BRAIN

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THE amplitude of the electric responses evoked in the human brain by sensory stimulation is usually rather small compared with that of the intrinsic background activity. However, recently developed methods of electronic averaging and cross-correlation¹ have disclosed consistent patterns of response, particularly in the extensive non-specific regions of the frontal lobes². Records from scalp electrodes (which inevitably introduce considerable spatial diffusion) have been compared with those from multiple electrodes implanted in frontal cortex for therapeutic purposes³; these latter can be left in position for several months and demonstrate convergent responses to auditory, visual and tactile stimuli in most regions of medial, lateral and orbital frontal cortex. The scalp responses are seen as compounded of these widespread synchronized discharges which, however, involve only a very small proportion of the neural tissue in any one zone.

The non-specific responses (in contrast with those in specific primary sensory areas) showed marked 'habituation' when stimuli in any modality are repeated frequently and monotonously, but their amplitude is restored when stimuli are associated. The rule is that the electro-negative components of the responses to the first (that is conditional or warning) of two stimuli are progressively augmented while those to the second stimuli (considered as unconditional or 'indicative') are progressively attenuated. These effects are generally emphasized and accelerated if the subject is instructed to perform an operant response to the indicative stimuli, which thereby become 'imperative'.

A series of experiments has been performed to investigate the effects on these components of varying the probability of association between the conditional and imperative stimuli, and also the influence of varia-

tions in effector participation and mental attitude. The effects of direct suggestion under hypnosis have already been described⁴.

The non-specific responses to conditional stimuli usually consist of three main components: a brief surface positive wave, a brief surface negative wave superimposed on this, and a much more prolonged surface negative component which may last several seconds, particularly in children. The details of the first two components depend somewhat on the modality, the responses to auditory stimuli being usually larger, simpler and more consistent than those to visual or tactile stimuli. The features which seem most closely related to the contingency of the situation and to the attitude of the subject are the prolonged secondary negative waves and their interaction with the subsequent responses to imperative stimuli.

Because of its dependence on the statistical relationship between the conditional and imperative stimuli this protracted component of the conditional response is referred to here as the 'contingent negative variation' (CNV). This effect can be recorded accurately only with equipotential non-polarizable electrodes and long time-constants or directly coupled amplifiers. For these experiments, specially prepared and selected silver-silver chloride electrodes were used, connected to an Offner type TC 16-channel recorder, fitted with a two-channel automatic wave analyser and a two-channel barrier-grid averager⁵. In order to permit comparison of responses in a variety of situations during a session of reasonable duration with intervals of several seconds between stimuli, the results from sets of only six or twelve presentations were stored and averaged, and the outputs from the averagers were written out directly on the recorder with increased gain and paper speed at the end of each set of presentations, which lasted about 90 sec.

The stimuli, singly or in pairs, were presented automatically at irregular intervals of 3–10 sec. When paired, the interval between associated stimuli was usually one-half or one second, but longer intervals have also been used. The conditional stimuli were either single flashes (*F*) or single clicks (*C*); the indicative or imperative stimuli were repetitive clicks or repetitive flashes. The operant response was provided by a button which the subject was asked to press when the repetitive imperative stimuli were given; this could be connected so that it terminated the imperative stimuli, but the circuit was so arranged that it was ineffective if pressed before the onset of the imperative stimuli.

Observations have also been made during the establishment of involuntary conditioned blink responses to clicks preceding puffs of air to the cornea. Records were obtained from electrodes on several parts of the head, but those illustrated here were all from a vertico-mastoid pair, an upward deflexion indicating negativity of the vertical electrode with respect to the mastoid. Simultaneous records were also obtained of the electro-myogram of the operant muscles, the stimuli, variations in palmar skin resistance, pulse rate and respiration.

The development of the CNV and its interaction with the imperative responses are illustrated in Fig. 1, which is from a normal adult subject. The averages of 12 responses to isolated clicks (Fig. 1*A*) consist of a small positive wave almost obscured by a much larger negative component. The responses to isolated trains of flashes (Fig. 1*B*) show several brief negative components, also superimposed on a small positive deflexion. When the clicks are paired with the flicker about 1 sec later (Fig. 1*C*) both responses are reproduced with little alteration. In these conditions both responses dwindle to noise-level after about 50 presentations. The addition of an operant response by the subject (Fig. 1*D*) is accompanied by a pronounced change in the conditional response to the clicks; a large CNV appears, rising to the peak amplitude of the imperative response during the interval between them. The imperative response itself is now seen merely as a sharp drop in potential difference

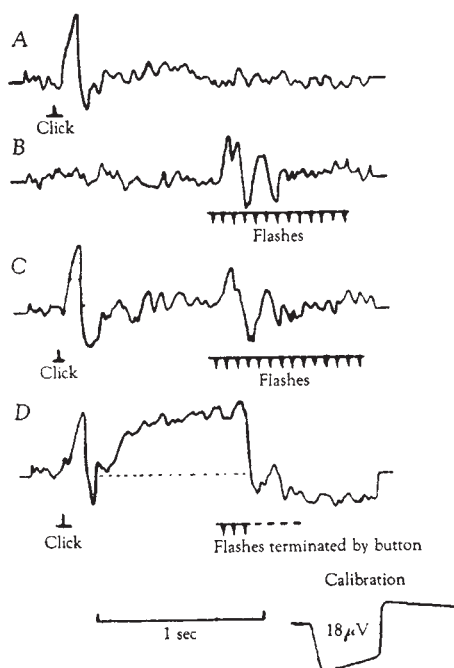


Fig. 1. Averages of responses to 12 presentations. *A*, response in fronto-vertical region to clicks; *B*, flicker; *C*, clicks followed by flicker; *D*, clicks followed by flicker terminated by the subject pressing a button as instructed. The contingent negative variation (CNV) appears following the conditional response and submerges the negative component of the imperative response

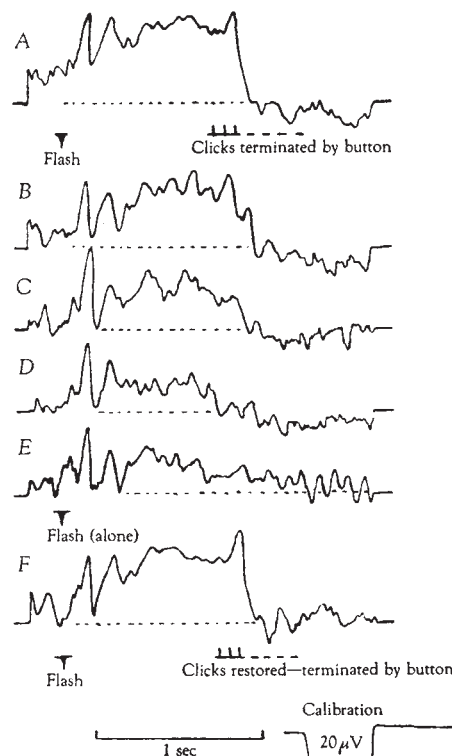


Fig. 2. Averages of 6 presentations. *A*, responses to flicker followed by clicks terminated by subject's operant response. The CNV is fully developed; *B*, average of first six presentations after withdrawal of the imperative stimuli; the CNV is slightly attenuated and there is a 'conditional' negative wave at the phase where the clicks would have been expected; *C*, *D*, *E*: progressive diminution of the CNV as the expectancy of reinforcement subsides. *F*, restoration of the imperative stimulus quickly re-establishes the CNV

to the base line. This pattern is maintained indefinitely as long as the subject is attentive and presses the button promptly. It is the same whatever the modalities of the first and second stimuli (Fig. 2*A*). However, when the imperative stimulus is withdrawn, the responses to the unreinforced conditional stimuli show a progressive subsidence of the CNV which disappears almost entirely after 30 unreinforced trials (Fig. 2*A-E*). Restoration of the imperative auditory stimulus immediately re-established the CNV (Fig. 2*F*). The dependence of the CNV on the contingency of the association between the two stimuli is shown most clearly when their relation is made equivocal. After a long series of regular associations of flashes with clicks (Fig. 3*A*) the probability of association was 'diluted' by presentation of 27 unreinforced flashes at random between 21 associated pairs, providing only 21 reinforcements out of 48 presentations. Averages taken during this series of equivocal presentations show that the CNV is progressively reduced to about half its previous amplitude, while the imperative response reappears as a negative wave (Fig. 3*B, C, D*). In most normal adult subjects the CNV shows signs of attenuation when the probability of occurrence of the association drops below 0.7 during 12 or more trials; but the total number of trials, their distribution in time and the critical proportion of reinforcements needed for suppression of the CNV vary considerably from subject to subject. Unequivocal restoration of the conditional stimulus once again brings about a rapid re-establishment of the CNV with submergence of the imperative negative wave (Fig. 3*E*).

Another factor which influences the form of the CNV is the attitude of the subject. During one experiment the subject was told that during a long series of unequivocal presentations of clicks followed by flashes he could decide

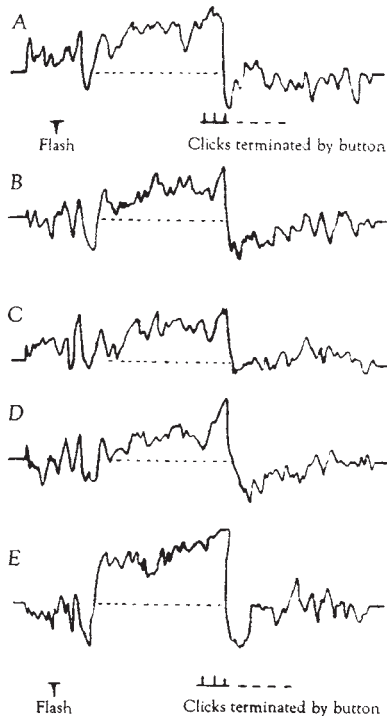


Fig. 3. Averages of six presentations. *A*, responses evoked by regular, repeated association of flashes with clicks terminated by subject; *B*, eight unreinforced flashes interspersed at random between the six associated stimuli reduce the CNV by equivocation; *C*, *D*, continued equivocation progressively attenuates the CNV and the negative component of the imperative response emerges; *E*, unequivocal association again restores the CNV.

not to press the button for the operant response. The average of six of these trials showed almost complete absence of a CNV (Fig. 4*A*), but this reappeared immediately when the subject decided to resume the operant response. The CNV is also susceptible to social stimuli in the form of verbal statements. When the imperative stimulus was withdrawn without notice or warning (Fig. 2*B-E*) the change in the pattern of physiological stimuli ultimately suppressed the CNV, but only after about 30 trials. In contrast, when the subject was told: 'There will be no more flashes' the CNV disappeared immediately (Fig. 4*B*). In this objective sense, therefore, progressive changes in subjective expectancy produced by repetitive physiological experience can be matched against those established by a single phrase. There may be a qualitative as well as a quantitative difference between the effects of physiological and social suppression. In Fig. 2*B*, the first average after unexpected withdrawal of the visual imperative stimuli, the CNV is slightly smaller than during reinforcement, but there is a small negative wave followed by an abrupt fall in potential difference exactly 1 sec after the conditional stimulus, that is at the instant when the imperative stimuli would have been expected. Bearing in mind that this is the average of six trials and that the second, third and fourth sets of unreinforced trials show steadily diminishing signs of this effect it may be regarded as a true conditioned brain response induced by the long experience of unequivocal presentations but extinguished as the probability of association declined when the imperative stimuli were withheld. There was no sign of this conditioned response when the subject was told beforehand that there would be no reinforcement (Fig. 4*B*); in fact, with this 'social extinction' there was a slight slow positive variation following the conditional response. Before the next series the subject was told that the flashes would be restored, but this was not done; the first three trials showed an appreciable CNV but the second three showed none,

and the average consequently contained only a small CNV (Fig. 4*C*). These features reflected accurately the subjective reports of fluctuations in expectancy induced by the conflict between the verbal and physiological experiences.

When a subject has become accustomed to the presentation of conditional and imperative stimuli separated by 1 or 2 sec the CNV can be sustained even when the imperative stimulus is omitted if he is instructed to estimate the time-interval and perform some action at the moment when the imperative stimulus would have occurred (Fig. 4*D* and *E*). The termination of the CNV is therefore not an 'off-effect'; furthermore, a purely mental judgment of a time-interval, without operant response, is often accompanied by a prominent CNV in trained subjects.

In most adult subjects the development of a consistent CNV with an abrupt termination is associated with a marked reduction in reaction-time to the imperative stimulus. This is not due to the establishment of a time-reflex, as was at first supposed, since the subjects seldom make a single false response when the imperative stimulus is withdrawn, as in the extinction or equivocation trials. Electromyograms of the operant muscles sometimes show a slight change in their tonic balance following the first few unreinforced conditional stimuli, suggesting a persistent anticipatory bias.

The close correlation between attentiveness, expectancy, contingent significance, operant response and the ampli-

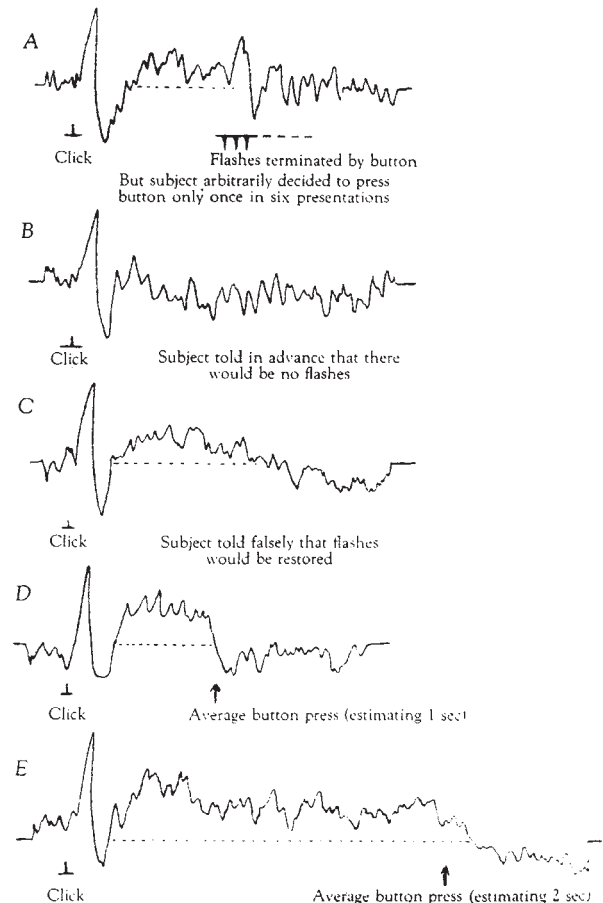


Fig. 4. Averages of six presentations. *A*, the subject decided arbitrarily not to press the button in response to the clicks; the CNV disappears (compare Fig. 1*D*); *B*, when told in advance that the imperative stimuli would be withdrawn the CNV vanishes at once (compare Fig. 2*B-E*); *C*, subject told falsely that flashes would be restored; CNV reappears for first three presentations and then subsides; *D*, without imperative stimulus, subject is asked to press button at estimated one second after click; CNV develops as for associated stimuli; *E*, the same, with subject estimating 2 sec for button press.

tude of the CNV suggests that this may be the electric sign of cortical 'priming' whereby responses to associated stimuli are economically accelerated and synchronized. The final discharge, whether in voluntary action or mental decision, is indicated by the abrupt decline in the CNV about 120 msec after the imperative stimuli, corresponding with the positive phase of the non-specific response to isolated stimuli.

The mechanisms underlying this remarkable effect can be inferred from other sources. Caspers⁶ has described similar variations in cortical potential in rats during sensory stimulation and arousal which are attributable to depolarization of the apical dendritic feltwork of the cortex. Rowland and Goldstone⁷ observed slow shifts of voltage in cats with implanted cortical electrodes during conditioning; they interpret these as "extension of an electrical arousal effect in terms of activation of cortical structures based on relation to drive". Rusinov⁸ has also described slow negative waves recorded by Shvets⁹, which appeared in the cortex of rabbits with the first appearance of conditioned movements; these were more generalized in the early stages than when the responses were 'concentrated' and vanished during extinction.

In patients with chronic electrodes implanted over frontal cortex the CNV can be recorded from widely separated areas and remains generalized indefinitely during

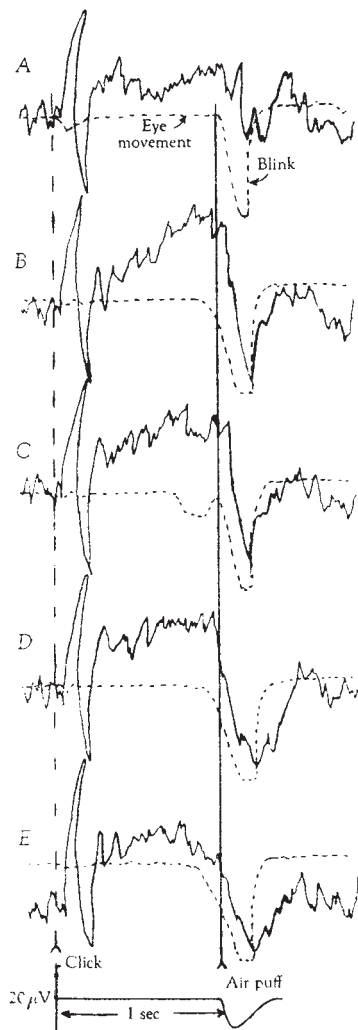


Fig. 5. Averages of 12 presentations; 'classical' conditioning of involuntary blink. Conditional stimulus click, unconditional stimulus air-puff to eye. A, first 12 trials show brain responses to both clicks and puffs; blink starts just after puff; B, second 12 trials: CNV appears, with conditioned blink just before puff which evokes no brain responses since eyelid is shut before corneal stimulus; C, D, E, progressive decline in CNV as conditioned response is consolidated

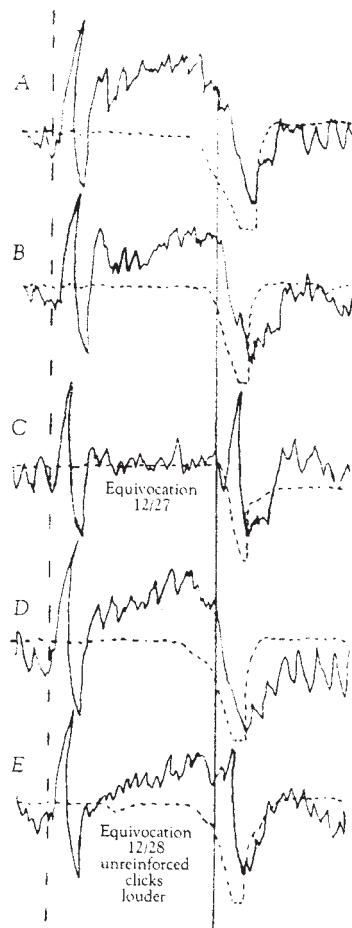


Fig. 6. Averages of 12 presentations of click and puff to eye (same series as Fig. 5). A, first 12 presentations after interval; CNV is larger than in Fig. 5E but conditional response is still present; B, second 12, CNV is smaller; C, equivocal association of clicks (only 12 of 27 reinforced) abolishes CNV and conditioned blink; brain response reappears since eye is open to corneal stimulus; D, regular association of clicks and puffs restores CNV and conditioned blink; E, equivocation as in C, but subject detected slight difference in loudness of reinforced and unreinforced clicks

operant conditioning as in scalp records. During the establishment of a 'classical' defensive reflex, however, the CNV is more prominent in the early stages. This is shown in Fig. 5, taken during the acquisition of a conditioned corneal blink reflex elicited by clicks followed by puffs of air to the eye. In Fig. 5A the blink follows the air puff, but in Fig 5B, the average of the second dozen presentations, there is a prominent CNV, the blink precedes the air puff and the brain response to the corneal stimulus, which was prominent at first, has disappeared. In subsequent trials (Fig. 5C, D, E) the CNV shrinks progressively, until after about 60 trials it is scarcely above the base-line and the eye is closed well before the air-puff. The effects of 'probability dilution' or equivocation are particularly clear in this situation (Fig. 6). When the significance of the association between the auditory and corneal stimuli is diminished by partial reinforcement both the CNV and the conditioned response vanish entirely and the brain response reappears (Fig. 6C). However, when the subject discovered a slight difference between the reinforced and unreinforced conditional stimuli the CNV and conditioned response return, Fig. 6E.

Although the electric field of the CNV is generally limited to the anterior regions, there is some indication that variations in its spatial extent may be related to the degree and pattern of contingent response interaction, particularly in children. The application of these methods to the investigation of neuropsychiatric disorders has

already shown a surprisingly close correspondence between the objective signs of cerebral expectancy and the mental state of disturbed patients.

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RECEPTORS IN THE SUCKER OF THE CUTTLEFISH

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CERTAIN proprioceptors of crustaceans have proved to be useful in experimental investigations because both the cell body and dendrites lie at the periphery^{1,2}. This situation has been found in the mantle of cephalopods^{3,4}. The work recorded here shows that such multipolar nerve cells with the characteristics of receptors are also found in the suckers of the cuttlefish.

The general arrangement of the tentacular arm of *Sepia officinalis* has been described by Guérin⁵ and my previous papers⁶⁻⁸. The sucker is cup-like, joined to the arm by means of a short peduncle (Figs. 1 and 2). The cup contains three principal zones where primary receptors can be demonstrated. These are: (1) the external side of the infundibulum (*A*, Fig. 1); (2) the flat rim of the cup (*B*, Fig. 1); (3) the bottom of the acetabulum (*C*, Fig. 1). These three zones contain several sorts of sensory cells, differing in shape and presumably in their capacity of recording information. Zone *A* is covered with columnar epithelium 30–40 μ thick (all the numerical data given in this paper are related to suckers of approximately 3 mm

diameter, from adult animals, 10 cm mantle-length). There are two types of receptors, probably touch- and chemo-receptors, in zone *A*. The touch-receptors have round or flask-like cell bodies lying in the inferior third of the epithelial layer and their axons go to one of the twelve nerve bundles that radiate from the peduncle into the wall of the sucker (Figs. 1 and 2). From the intra-epithelial course of the axon may arise some fine collaterals branching between the epithelial cells and intermingling with each other in a fine plexus at the base of the epithelial layer. The other type of cell is probably a chemo-receptor, because similar cells have been found in the lip of *Sepia officinalis*⁹. These cells are tapered and end at the free surface of the epithelium with a tuft of short cilia (2–3 μ long). It is very difficult to state the number of these receptors because they stain only sporadically with the methods used (Bielschowsky, Cajal), but an approximate estimate is not less (probably much more) than 600 and 100 for touch- and chemo-receptors respectively in each sucker.

The flat rim of the sucker (zone *B*) is covered by a single layer of epithelium, unusual in that each cell is covered by a tooth-like cuticular apparatus (Fig. 3). The size of the epithelial cells is 30–40 μ long and the cuticle is not less than 40–50 μ thick. The cuticle provides a ring-shaped surface by means of which the sucker holds the objects grasped. There are multipolar nerve cells between the epithelial cells (approximately 300 in each sucker) which send branching processes ('dendrites') to the base of the stratum (Figs. 4, 5 and 6). These cells are presumably tension receptors. The size of these irregular nerve cells varies from 20 to 60 μ (the size is taken as the average of the two largest diameters of the cell) and the processes of each cell spread over an area of 100 μ diameter, each dendritic field overlapping the neighbouring ones. The axon arises from the hillock of the cell but light microscopy shows no changes in structure (Nissl and Cajal methods). The diameter of the axon remains considerable (8–12 μ) along its entire course, and it may run without collaterals for more than 50 μ in the epithelium (Fig. 4), where it turns into the muscular wall of the sucker (Figs. 1 and 6). Here it intermingles with the fibres forming the nerves of the sucker running to and from the arm ganglion. In this epithelium some very fine fibres of unknown nature (probably coming from the sucker ganglion) branch and intermingle with the dendritic processes of the nerve cells previously described.

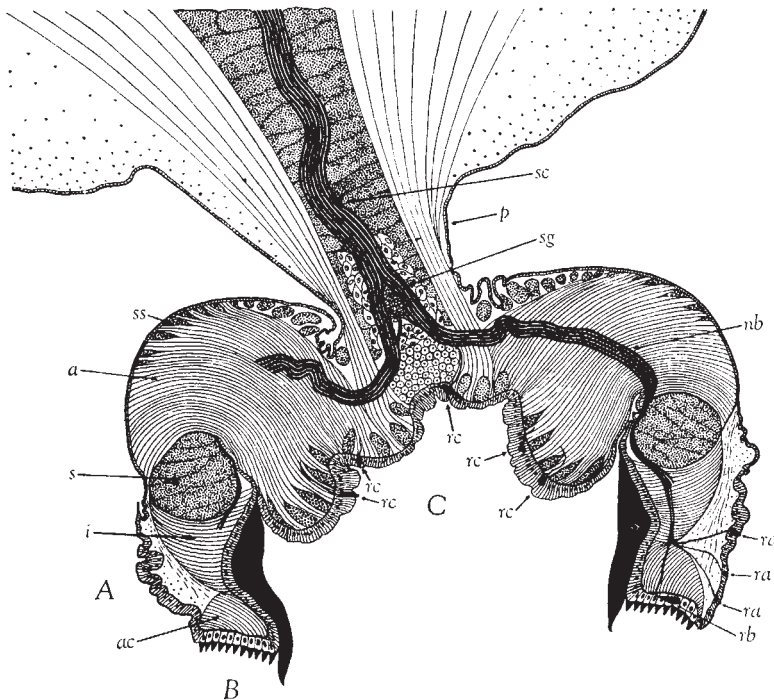


Fig. 1. Diagram of a sucker of *Sepia officinalis*, showing the general arrangement of the organ and its nerve supplies. *A*, *B*, *C*, zones of the sucker provided with different types of receptors. *a*, acetabular muscle; *ac*, acetabulo-cutaneous muscle; *i*, infundibular muscle; *nb*, nerve bundles radiating from the ganglion into the sucker; *p*, peduncle of the sucker; *ra*, receptors of the zone *A*; *rb*, receptors of the zone *B*; *rc*, receptors of the zone *C*; *s*, principal sphincter of the sucker; *sc*, sucker's nerve; *sg*, sucker ganglion; *ss*, secondary sphincters of the acetabulum