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THE BASIS OF SENSATION*

SOME RECENT STUDIES OF OLFACTION

BY

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It is probably inevitable that there should be a period when successive Banting lecturers will find themselves with little to say about their proper theme. We commemorate a man whose work was directly responsible for the prolongation of many million lives, whose biography should be far more interesting than that of most successful scientists. But those best qualified to give a picture of his unusual talents have already spoken, and he did not live so long ago that the lecturer can deal with the historical setting of his career. Successive Harveian Lectures add to our knowledge of Harvey's discoveries by describing the theories and politics of the Jacobean age: it is so far away and the thought of that period is so different from ours that the more we hear about it the better we shall understand Harvey. But the period of the first world war is too close to many of us to be treated objectively. A century hence historians may have plausible theories to show why we thought and acted as we did, and may try to explain why the sustained emotional pressure of the first war seemed different in quality from that of the second; but these lectures will be much more convincing when there is no one left alive to say that we did not feel like that.

Impressions from a Wartime Meeting

I am, in fact, too much a contemporary of Banting to pass historical judgment, and I had too little contact with him for personal reminiscence. Indeed, I met him only once—at the beginning of the second world war, when he was on a mission in England. Naturally it was difficult not to colour one's impression of him to match the almost legendary background of the insulin period, but even if one had known nothing about it I think one would have set him down as a man capable of great achievement. He was gentle and friendly, but one could feel his energy and determination. I had the impression that he would grasp at the essentials and be impatient of the details, and that he would face the simple issues which never occur to subtle but more conventional minds; and, of course, like all of us, he wanted very badly to be doing something. I think my strongest impression is of this impelling desire for action: since he had once scored so notably by taking the bull by the horns, he might have been even more impatient than he was. But he certainly gave me the impression of someone who had

it in him to take the bull by the horns again and would put through what he wanted to do by convincing everyone not so much that it was the best and the only thing to do, but that he would not rest till he had done it.

Soon after that he was killed, but, whatever he might have done, it is difficult to believe that the outcome could have satisfied his vision of what science might accomplish. His laurels came early, and he was not the kind of man to think he had done all that he ought to do. That was how he seemed at a brief meeting in the last war, and I have mentioned that impression because it is still curiously vivid. But wartime memories are notoriously coloured by the mood we were in; at that time the prevailing mood of biologists and medical scientists led them to believe that their science could have great practical uses in war if people in high office could only be persuaded to grant facilities and if the scientist could make up his mind what he wanted them for. The medical history of the war shows that they were not far wrong. But these were prodromal symptoms; after the temperature rose in the summer of 1940 we should probably have shown a different complexion. In this place where Banting lived and worked you know far better what sort of man he was, and it is enough for me to set down my feeling of the unusual kind of ability and courage which made him still a leader, not at all weighed down by the thought that no one could score again as he had done twenty years before.

Banting and Best's work has saved innumerable lives, but it has another reason to make it memorable—the fact that it was conceived by a young medical practitioner, able and ambitious, but with little training in research. It stands, therefore, as a constant beacon to young people who enter on the study of physiology or medicine and are not content to think as they are told. It should encourage them to believe that the first sight of the wood may give the clue that cannot be seen by all the experienced professors who are lost among the trees. Most of us are more or less lost and would be grateful for fresh light, though the older we get the more grudgingly we might accept it. At all events, most of us could point to chapters in the textbooks which seem to need the fresh outlook of a mind not weighed down by too much knowledge of details. Advertising our difficulties may not be the best way of getting out of the wood, for if our hearers paid much attention to what we told them we should

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drag them into the forest to share our obscurity. But it is better to speak of the mess we are in ourselves than to enlarge on that of our colleagues; and so the best tribute I can pay to Banting's memory must be to tell you how much we want new light in the field I know best, that of the physiology of sensation.

A Fundamental Difficulty

There is, first of all, a fundamental difficulty which does not seem to me to be soluble at all by experimental science—the difficulty of connecting physical and mental events, of bringing sensations and thoughts into the framework that we use so effectively for describing our external world. I cannot myself see how further knowledge of brain physiology will help us to reconcile mind and matter, but I am prepared to believe that a hundred years hence it will be difficult to understand why we ever puzzled about it. Even now there are many scientists and many philosophers who themselves find no difficulties and think they arise from faulty premises and outworn habits of thought. They may well be right. I should have more courage in thinking them wrong if I could forget the curious chapter in the history of biology when the scientific world was divided between the Ovists, who held that the embryo was a development of the ovum alone, and the Animalculists, who held that it was essentially a development of the spermatozoon. The notion that it might come from both together seemed too absurd to be considered—until something happened to break the spell and the joint origin became a reasonable idea.

No doubt the Renaissance produced many examples of the same sort, difficulties which vanished when ideas which had seemed inherent and unchangeable were found to be out of date. And so I am ready to believe that future generations will not find it as difficult as I do to include my mind in the scientific picture of the world. I am content to think that it is a puzzle for me, but that it may be no puzzle at all to someone without my particular preoccupations.

Cellular Mechanism of the Nervous System

If we discuss sensation we can scarcely leave the mind out of it, but I want to do so in what follows, for it will be mainly concerned with the cellular mechanism of the nervous system. I want to discuss the general organization of the nervous system which makes us react differently to different external events. We have a large number of receptor cells sensitive to different material changes, and we have a large number of motor neurones coupled to the muscles which produce our reactions. The environment and our reactions to it are changing all the time, and much of what we do cannot well be related to any one sensory stimulus, but it is a convenient simplification to think of a particular pattern of receptor stimulation as the cause of a particular pattern of response, and I make no apology for doing so. Admit, then, that the stimulation of certain receptors will produce a particular sequence of movement and the stimulation of others will produce a different sequence. What sort of organization is there to account for all the differential reactions which our nervous system will produce?

Obviously a great deal depends on the course of the pathways from the receptors to the motor nerve cells. We turn right or left when the stimulus is one-sided, because the signals enter the central nervous system from that side and are not evenly distributed; and we react differently to a sound or a light because the signals come by the auditory and the optic nerves and follow a different route. In less elaborately organized animals—sea anemones, for

instance—each group of receptors may serve a particular group of muscle fibres; the connexions are fixed and there is no central integration. But in the vertebrates there is a great deal of integration, and that means that the connexions and the route travelled by the signals can be varied considerably. The responses are different movements, and each involves a particular spatial and temporal distribution of activity in the motor nerve cells—a particular changing pattern of excitations and inhibitions. In the same way the signals from the receptors have particular spatial and temporal distribution for each event. The afferent and the efferent pattern are partly related by anatomical connexions, but there is an intermediate region where the connexions are sometimes open and sometimes closed and where a great variety of transmission is possible.

Sensory Excitation

The gross distribution of the sensory excitation is obviously of the first importance. We can recognize and react appropriately to a spoken word because it produces a particular sequence of excitation patterns in the cochlea, and we can recognize a face because the retinal excitation has the right shape. But the receptors of the eye show us colours as well as shapes, and the receptors of the skin give sensations of hot and cold and of various sorts of pain as well as of contact. We are aware of (or, if the mind is to be kept out of it, we react to) particular qualities of the stimulus as well as to its location and its intensity. Differences in the colour of adjacent patches in the visual field may perhaps be signalled by differences in the temporal evolution of the signals from them. This would avoid the need to credit the retina with special nerve fibres for colour from each point, but we know that the skin does use a different set of fibres to signal contacts, temperature, and pain, fibres of different diameter which lead to different pathways in the spinal cord. So we must be prepared to find that a sensory surface uses a variety of fibres to signal differences in the quality of the stimulus and that the result depends both on the general distribution of the excitation and on the particular varieties of nerve fibre which convey it.

How do these different sorts of fibre produce different effects in the central nervous system, different kinds of sensation, and a different reaction of the body? Though the fibres may be intimately mixed in the nerve trunks, there is often an anatomical sorting out when they enter the central nervous system; for instance, the fibres from the skin which signal touch extend up to the brain stem, whereas the non-medullated C fibres end in the cord. Even where there is no detectable sorting out it remains an obvious possibility that fibres from different kinds of receptor may have different kinds of dendritic connexion. But we are still faced with the need for a process which balances one excitation against another, and I believe we may be mistaken in thinking of the central nervous system as though it were made up of neurones which differ only in their structural features. I think we ought to consider the possibility of some variation in their chemistry as well.

So I am going to discuss the suggestion that the neurones from different varieties of receptor may liberate somewhat different material when an impulse reaches their dendritic endings and that the precise composition of the molecular output decides which of the succeeding neurones will be stimulated. It is true that this merely substitutes differences in humoral excitants for differences in dendritic structure, and there is more evidence for the latter, but I make the suggestion because I have been forced to realize how nervous elements which look much the same may differ greatly in their chemical sensitivity.

Olfactory Receptors

I am thinking, of course, of the olfactory receptors. The nose can distinguish an immense variety of molecules brought to it in the air drawn in at each inspiration. When we smell something we are aware of the intensity of the

smell, and we can usually tell where it comes from, but our chief awareness is of pure quality without any spatial or temporal attributes. Now the olfactory receptors are nerve cells furnished with hairs, which project into the mucous layer on the olfactory surface, and at the opposite pole with a non-medullated axon, which runs through the cribriform plate to end in the olfactory bulb. The number of these cells runs into millions even in a relatively small olfactory organ like that of man, and they are so closely packed that the position of the bulge formed by the nucleus varies from cell to cell. In some the nucleus is nearer the hairs and in others nearer the axon, but this is the only histological difference visible in a stained preparation. Two kinds of olfactory hair have been described in fresh preparations from the frog, and it is possible that they are present in the mammal too, but there is no indication of any sharp division into two or more kinds of cell. In fact, the organ seems to be composed of a large surface packed with receptors which differ from one another only in the position of the nucleus in the cell and the position of the cell in the olfactory surface.

It is not possible to record the signals which pass to the olfactory bulb in the non-medullated axons, but we can record axon spikes in the neurones to which they lead. These are the mitral cells: large nerve cells with medullated axons which can be reached by a wire electrode thrust into the olfactory bulb. Since there are only 70,000 of them in the rabbit's bulb for many million receptors, the axon discharge must be a condensed version of the receptor signals, and a great deal of interaction may have taken place in the synaptic regions. But the receptors in touch with one mitral cell seem to be confined more or less to one part of the olfactory epithelium, though the extent of the receptive field is unknown.

Not only are the mitral-cell signals at one remove from the receptor signals, but they can be recorded profitably only in highly abnormal conditions, under anaesthesia deep enough to suppress all the spontaneous activity in the bulb. But if in such conditions we find that different smells give distinctive discharges they would scarcely fail to do so in the normal animal.

In the early stages, before I had learnt how to record from single units, I could get little satisfactory evidence of differential sensitivity in the receptors leading to any one point in the bulb. Smells strong enough to produce a mitral discharge would usually cause one in many units; sometimes the axon spikes seemed larger or smaller according to the nature of the stimulus, but that was all. On the other hand, there was no doubt that the different regions of the olfactory organ were not equally sensitive or equally accessible to all the molecules which could stimulate some part of it. Amyl acetate, ethyl acetate, and ether would always evoke a discharge in the anterior part of the bulb—corresponding to the anterior part of the olfactory surface—and the less volatile oils would rarely stimulate there, though they would give a large discharge at the back of the bulb. I still think that this large-scale difference in the distribution of the excitation is probably an important factor in the discrimination of smells, for in many animals the olfactory surface is so large and is folded into such complex channels that molecules of different properties carried in by the air stream would be unlikely to be deposited in an identical pattern. Another distinguishing feature may be the undoubted difference in the temporal evolution of the excitation with different substances. Volatility and solubility in water both favour a rapid rise and decline of the discharge, with little persistence between one inspiration and another. Thus the spatial and temporal pattern of the excitation will vary with the molecules which enter the nose, in much the same way as the pattern varies for the eye with the object in view and for the ear with the sound.

But these differences in timing and distribution cannot be shown to give more than a rough sorting out into a few categories, possibly dependent on the behaviour of the

material in water and in lipoids. Some further discriminating mechanism is needed to account for the great variety of smell which can be recognized even by the human nose, and it is supplied by the selective sensitivity of the individual receptors.

Selective Sensitivity

This was not evident until a method was devised for exposing the rabbit's nose to a concentration of the stimulating molecules which could be increased gradually from zero, the relative strength being signalled at each moment on the record of the impulse discharges. The method is simply to send two streams of air into the facepiece which covers the snout. Both are purified by filtration through charcoal (a necessary precaution to make them inodorous), and one is passed through a small bottle containing the substance to be tested, usually in liquid form. The rate of flow of this stream can be gradually increased by opening a screw valve, and the extent of the opening is signalled on the recording film.

With this arrangement and with a suitable position of the electrode and a good cranial circulation it is nearly always possible to show that different substances in threshold concentration will employ different mitral cells for signalling the stimulus. Usually a particular substance can be found that will evoke a discharge which is confined to a single unit near enough to the electrode to give large axon spikes. Another substance may give a threshold discharge confined to another single unit, though there will often be several units in action, and none of them close enough to the electrode to give distinctive axon spikes.

A discharge confined to one mitral unit is presumably due to an excitation confined to the particular group of receptors which lead to it, and since adjacent mitral units react to different substances it follows that adjacent groups of receptors have some degree of selective sensitivity. The degree of specificity varies greatly from one unit to another. The electrode may be close to a mitral cell which is in such unstable equilibrium that a very slight lightening of the anaesthesia will make it discharge spontaneously. Such a cell will usually start a discharge when the nose is tested with a wide variety of smells, and it may be impossible to pick out one which seems to have any specific relation to that cell alone. And the specific relation is a matter of degree; when one substance gives a threshold discharge confined to a single unit, related substances will usually stimulate that unit in low concentration, though they will stimulate other units as well. For instance, if benzene vapour gives a single series of large axon spikes, toluene and xylene will evoke similar spikes, though even at the threshold they will be mixed with smaller spikes from other units.

Many substances have been used in searching for these specific effects, but no attempt has been made to cover the whole range of odours. Yet enough experiments have been made to show that the substances tested fall into distinct groups, each tending to pick out particular mitral units, and that within each group one substance usually shows the greatest specificity—that is, one substance is most often found to give a discharge limited to a single one of the units within range of the electrode. Such substances are acetone in a group which includes esters like amyl acetate and ethyl acetate, benzene in the group of aromatic hydrocarbons, octane in the group of paraffin hydrocarbons, heavy oils, etc. Dipentene, cedar-wood oil, and eucalyptus belong to the large group of terpenes and related compounds, and all three have often given single unit discharges. Probably there are many other groups—for example, mercaptans. At least ten different substances have been found to pick out a single unit, but sometimes the same unit is picked out by several substances from different groups—for example, dipentene and acetone, benzaldehyde and amyl acetate, etc. In fact, the evidence suggests that particular types of molecular configuration have an affinity for particular varieties of receptor, but that the physical properties of the substance are also important.

In view of the regional differences in sensitivity found in the earlier experiments one would expect that the units responding, for example, to acetone would be found more often in the front part of the bulb and those responding to heavy oil at the back. There is undoubtedly a segregation of this kind, although, in the middle region, unit discharges have been obtained with substances from all the groups. It is true that most of these units can be made to discharge to a wide range of stimuli if the concentration is raised, but there will always be outlying parts of the olfactory organ where it has the threshold value needed to bring out the differential effects.

Assessment of the Different Factors

A great deal remains to be done before we can assess the different factors concerned in olfactory discrimination, but the important point is that neighbouring groups of olfactory receptors have marked differences in their sensitivity to different smells. I have no doubt, therefore, that olfactory discrimination is not due solely to the complex structure of the organ—that is, to the deposition of the material in different patterns on its surface owing to differences in the rate of air flow, size of molecules, etc.

In fact the olfactory epithelium proves to be a large collection of nerve cells which look alike, apart from the position of the nucleus, but differ so much in their properties that some will be excited by a trace of acetone vapour and others by a trace of benzene. This, of course, is no more than would be expected by anyone who contemplates the remarkable discriminative power of the nose. But it is an important point to have established, and I do not regret the time I have spent in trying to develop a different hypothesis.

It may be that the point is important only in connexion with the working of the olfactory organ; the receptors there may be the only cells which have developed such a high degree of chemical discrimination. But nerve cells in other places may show traces of the same discriminative power. Let us suppose, then, that there are sheets of such cells in the central nervous system with dendrites ending freely amongst the dendrites of afferent neurones, so that material liberated from these might come into contact with the dendrites from more than one cell. It cannot be expected that the terminations of the afferent neurones would liberate molecules as different as acetone and benzene, but if some neurones liberate acetylcholine and some noradrenaline it is not impossible that the molecules liberated by the fibre from a temperature receptor might differ considerably from those liberated by a pain fibre. The ultimate destination of the different signals reaching the dendritic network would then be determined by the different chemical sensitivities of the neurones whose dendrites are stimulated.

It is perhaps worth pointing out that the excitation of an olfactory receptor is a rapid process which ceases usually at the end of inspiration when the delivery of vapour is suspended, that the number of molecules needed to excite must be very small, and that they must be removed or neutralized rapidly. The same could be said of the excitation of a nerve cell by a humoral transmitter. I am bound to admit the difficulty of believing that all the different groups of molecules which stimulate the olfactory receptors are dealt with by different specific enzymes, as cholinesterase deals with acetylcholine, but they are certainly inactivated rapidly and completely.

Speculations which cannot be put to experimental test are seldom worth elaborating, and I cannot pretend that we should be in a much better position if this speculation turned out to be correct. A selective transmission determined by the nature of the material set free in the dendritic network might allow more variation than one determined by the detailed connexion of dendrites, but that is all that can be claimed for it.

Yet I think the olfactory organ may have something to teach us about the organization of the nervous system.

Since its nerve cells are found to have such selective properties we must be ready to look for similar properties in nerve cells elsewhere.

Conclusion

That, I am afraid, is a poor conclusion to a lecture which commemorates a man whose researches were of such immediate practical benefit to so many people. I wish I had something better to offer than these incomplete results on a sense organ which has never roused much interest. I can still recall the intense interest which the work of Banting and Best aroused in all of us, in physiologists as well as in every doctor. But such triumphant victories come very rarely, and they are separated by the slow, plodding attack on a wide front. I have described a minor incident in that attack with a speculation attached to it; but I have done so because most of our research is like that, and because it is with such inconclusive results that you must contrast the spectacular advance which came with the finding of insulin.

PROTECTION AFFORDED BY SICKLE-CELL TRAIT AGAINST SUBTERTIAN MALARIAL INFECTION

BY

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The aetiology of sickle-cell anaemia presents an outstanding problem common to both genetics and medicine. It is now universally accepted that the sickle-cell anomaly is caused by a single mutant gene which is responsible for the production of a type of haemoglobin differing in several important respects from normal adult haemoglobin (Pauling *et al.*, 1949; Perutz and Mitchison, 1950). Carriers of the sickle-cell trait who are heterozygous for the sickle-cell gene have a mixture of this relatively insoluble haemoglobin and normal haemoglobin; hence their erythrocytes do not sickle *in vivo*, whereas some at least of the homozygotes, who have a much greater proportion of sickle-cell haemoglobin, have sickle cells in the circulating blood, with inevitable haemolysis and a severe, often fatal, haemolytic anaemia. There is also a much smaller group of sickle-cell anaemia patients who are heterozygous for the sickle-cell gene as well as for some other hereditary abnormality of haemoglobin synthesis (Neel, 1952).

It is thus possible to approach the problem from the clinical or the genetical side. From the clinical point of view it is important to distinguish between carriers of the sickle-cell trait who show no other haematological abnormalities and patients with sickle-cell anaemia, who have a haemolytic disease which can reasonably be attributed to sickling of the erythrocytes. From the genetical point of view the main distinction is to be drawn between those who are homozygous and those who are heterozygous for the sickle-cell gene. In the great majority of instances two classifications coincide—that is, most individuals with the sickle-cell trait are heterozygous and most cases of sickle-cell anaemia, in Africa at least, are homozygous for the sickle-cell gene.

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