EFFERENT AND OTHER EFFECTS ON ARTERIAL CHEMORECEPTORS

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DECLARATION

Some of the work in this thesis was done in collaboration with others. The division of labour was as follows:

1. The studies using microelectrodes were done with Dr D. I. McCloskey. N.W.G. usually prepared the microelectrodes while D.I.McC. dissected out the carotid bodies, although the roles were reversed on some occasions. The actual experimental procedure was shared.

2. In the studies of oscillations all the computing was handled by B. S. Nail, who wrote the programmes. N.W.G. was present at all computing sessions and was responsible for feeding the raw data into the Interface. The blood gas analyses were done by B.S.N. or Mrs Janet Blyth. N.W.G. wrote the first draft of Paper II, and collaborated with R.W.T. in writing the final version.

3. The analysis for Paper III was done entirely by N.W.G. from computer records prepared in collaboration with B.S.N. N.W.G. wrote the paper.

4. Papers I, II and III were all modified following the comments of the referees of the journals concerned.

Signed:

R W Torrance

Dr R. W. Torrance
(Supervisor)

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ABSTRACT

This thesis asks two questions. First, is there an efferent control system of the arterial chemoreceptors other than by control of the vasculature? Secondly, do single chemoreceptors show an oscillation in their discharge with respiration and, if so, what is the natural history of the oscillations?

The general methods for the recording of the discharge of single chemoreceptor fibres of the sinus nerve of the cat are described. Specific experimental details are described in the appropriate chapters.

Efferent control

Microelectrodes were used to record intracellular potentials from the carotid body *in vitro* and *in vivo* in an attempt to link efferent inhibition arising from electrical stimulation of the sinus nerve with changes in the membrane potentials of the cells. 176 cells were recorded from *in vitro* and 24 cells *in vivo*. These were probably Type I cells. Membrane potentials ranged from 15-60 mV with a mean of about 30 mV. No 'physiological' or pharmacological stimulus that was used affected these potentials.

Potassium chloride solution depolarised the cells reversibly. Stimulation of the sinus nerve at intensities sufficient for maximal excitation of the 'C' fibres within it had no effect on the recorded potentials. Neither transient junctional-type potentials nor slow drifts of potential were seen.

In parallel experiments the sinus nerve was stimulated electrically whilst recording the discharge in single chemoreceptor fibres peeled from the nerve distal to the stimulating electrodes. Stimulation could cause adventitious excitation of the recorded afferent resulting in antidromic depression, which mimicked a true physiological inhibition of the discharge. Some of the features of this adventitious excitation and the ease with which it occurred were investigated using an isolated segment of vagus, baroreceptor fibres, and chemoreceptor fibres. Low concentrations of local anaesthetic could raise the threshold for adventitious excitation whilst not affecting the normal passage of impulses. When looking for true inhibition, a continuous check for adventitious excitation was kept, utilizing the fact that chemoreceptors show a minimum interspike interval of about 10 msec. With this check, stimulation of the sinus nerve still caused a reduction in discharge in afferent fibres, to about 70% of control, though this was rather

variable. In four out of five fibres tested, the inhibition was reduced or abolished by close intra-arterial injection of atropine.

In conclusion: the true inhibition of discharge would seem not to be synaptic but vasomotor in origin.

Oscillations in discharge

Most single fibres of the cat and dog showed oscillations in discharge in phase with respiration when summed by computer over many respiratory cycles. The trough in the oscillation followed inspiration with a delay equal to the lung to carotid body circulation time. The amplitude of these oscillations was increased by decreasing respiratory frequencies or by increasing metabolism by poisoning with dinitrophenol. Hypercapnia or hypoxia decreased the relative amplitude of the oscillations but the absolute amplitude remained approximately constant. Oscillations from pairs of fibres recorded simultaneously were similar in phase and relative amplitude. The oscillations were not affected by sectioning or stimulating the cervical sympathetic trunk, nor by sectioning the sinus nerve whilst recording from an afferent from the otherwise intact nerve. The most likely source of the oscillation is thought to be the respiratory oscillation of P_{a,CO_2} in the carotid blood. It is

proposed that the responses can be explained in terms of families of parallel transient P_{a,CO_2}-response curves that are steeper than the fans of response curves described for the steady state by other workers and cut these curves at the mean P_{a,CO_2}.

A non-sympathetic efferent fibre that was recorded from showed no oscillation in the discharge with respiration, and interval distribution histograms of its discharge did not have the simple exponential form shown by afferent discharge.

Earlier workers had described the discharge of single chemoreceptors as random and this was seemingly at variance with an oscillating discharge. In an attempt to resolve this it was assumed that the mean frequency of the random process varied over the respiratory cycle, and statistical tests showed that, with this assumption, the impulses were random with respect to each other although they were not random with respect to the respiratory cycle. That is, the probability of the occurrence of an impulse varies with a respiratory periodicity but not with the time since the occurrence of the previous impulse.

The technique of recording from two fibres simultaneously on separate electrodes was originally used to see if it would be

justifiable to apply the results of summing the discharge of one fibre over many cycles to the response of the whole nerve, i.e. many fibres over one cycle. This recording method allowed further comparisons between fibres, when the arterial stimulus must have been the same for both receptors, by going back over the experimental records and comparing the frequencies of discharge of the pair of fibres at any time. In some experiments specific manoeuvres were performed to obtain further information. The frequencies of discharge of fibres were compared: at steady mean rates; during the responses to various ventilatory stimuli; at the intra-arterial injection of 100% CO_2-saline; as well as in their oscillatory behaviour over the respiratory cycle. If allowances were made for differences in characteristic mean frequencies of discharge, the sensitivities to any change of arterial stimulus proved remarkably similar, that is the discharge of one receptor could give as much information as that of the whole population. There were indications that receptors might differ in threshold, peak response, and adaptation. The most striking finding was that the sensitivity of a receptor could change with time, although, at any one moment, the chemoreceptors are a surprisingly homogeneous population. The results suggest that there is a single mechanism responsible for the sensitivity to hypoxia and hypercapnia.

Chapter 1

General Introduction

1.1 The shape of the thesis

The work presented is in two main parts and, although there are links between them, they can be regarded as two separate topics: studies relating to the efferent pathway to the carotid body that runs in the glossopharyngeal nerve; and studies on the oscillation in chemoreceptor discharge that occurs with respiration.

When I started the experimental work in September 1969 there was the newly discovered possibility of a specific inhibitory neural pathway that could influence directly the response of the chemoreceptor endings (Neil & O'Regan, 1969a, b) to their adequate stimuli, the P_{a,O_2} and P_{a,CO_2}-pH_a of the carotid blood. If there was such a pathway, all the previous work on the activity of chemoreceptor afferents would have to be repeated as it had all been performed on the cut nerves and so with the efferent pathway

interrupted. Also, if the efferent pathway acted by influencing the actual generation of impulses by the chemoreceptor terminals, then perhaps studying its influence could give a clue to this process and help us to open the 'black box' of the carotid body. With the latter point particularly in mind the main lines of approach to the efferent pathway were to attempt to confirm the stimulation experiments of Neil & O'Regan (1969a) under a variety of circumstances, particularly in vitro, and, in parallel experiments, look with microelectrodes for any changes of membrane potential within the carotid body that might be associated with the inhibition.

In the spring of 1970 I began to realise that the technique of stimulating the sinus nerve close to the recording electrode, as used by Neil & O'Regan (1969a), introduces an experimental artefact and that much of their work was therefore suspect. To add to this, the microelectrode work was proving difficult and entirely negative. The appearance of a paper on the artefact (Fidone & Sato, 1970) confirmed my interim conclusion that the efferent pathway was probably not the lever with which to open the black box.

However Fidone & Sato (1970) did not seem fully to realise the significance of their own results to the conclusions of Neil &

O'Regan (1969a, b), and the full publication of the work using electrical stimulation (Neil & O'Regan, 1971a) left some unsatisfactory loose ends. Further experiments were done to investigate the mechanism of the artefact and a few loose ends tied to add some constructive comment to what was otherwise a destructive statement.

The second main topic of the thesis is the natural history of the oscillations in the discharge of chemoreceptors that occur with respiration. This investigation was a sequel to the work of Black & Torrance (1971) who obtained evidence that imposed oscillations in the activity of chemoreceptor afferents could influence ventilation. Previous workers had seen respiratory oscillations in the integrated activity of chemoreceptors of the sinus nerve, but little work had been done on single fibres. The investigation was purely of the response of the receptor considered, for the most part, as an isolated unit; no further work was done on the significance of these oscillations in the control of respiration.

The technique of recording simultaneously from two single fibres on separate electrodes, used originally as a test of whether it was justifiable to apply the results obtained from single fibres to the oscillations of the integrated activity of the whole nerve,

led to the additional topic presented as the final section of the thesis. This approach has raised some possibly disquieting problems for the experimenter wishing to quantify the response of the chemoreceptors.

1.2 The carotid body

Our ideas of the workings of the arterial chemoreceptors are in a state of flux. In recent years many of the older, supposedly well-established, facts have been doubted, refuted, and some even subsequently re-established. The newer evidence on many points is frankly contradictory and in other areas confusing. Therefore to review the whole subject would be time-consuming and not particularly helpful, and so I shall concern myself mainly with those topics that are relevant to a discussion of the subject of the first part of the thesis, the efferent pathway. Because this must touch on many facets of the physiology of the chemoreceptors this Section will serve also as an introduction to the second part of the thesis on the oscillations in discharge.

Roots

Chemoreceptor activity is grouped in the carotid body, situated at the carotid bifurcation, and the aortic bodies, a group of islands of chemoreceptor tissue in the aortic arch. They are sensitive to the P_{O_2}, P_{CO_2} and pH of the arterial blood: increasing hypoxia, hypercapnia or acidaemia will increase the mean rate of discharge. They are the afferent limb of a reflex to increase the ventilation. The basic structure is two types of cell arranged in groups, or glomeruli, in which one type, on which the afferent nerves terminate, is enclosed by the other type. They are highly vascular, with a high oxygen consumption, and the high blood flow is influenced by efferent autonomic nerves.

The early work, anatomical (de Castro, 1926; 1928) and physiological (Heymans & Rijlant, 1933; Bogue & Stella, 1935), was done around 1930, and de Castro (1928) in fact inferred the correct physiological role of the carotid body from anatomical studies. The bulk of the research on chemoreceptors has been done on the carotid body because of ease of access, and in the literature 'chemoreceptors' and 'carotid body' tend to be used synonymously. This is reasonable as a generalisation, and will hold here, but the

carotid and aortic bodies do appear to differ in some respects.

Recent reviews of arterial chemoreceptors include Torrance (1969, 1974), Mitchell (1970), Biscoe (1971) and Howe & Neil (1972).

The responses of chemoreceptors

The stimulus-response curve to hypoxia at constant P_{a,CO_2} is hyperbolic (see e.g. Eyzaguirre & Lewin, 1961a, and Hornbein, Griffo & Roos, 1961 - integrated whole nerve; Biscoe, Purves & Sampson, 1970 - single fibres). The receptors fire, albeit at a very low frequency, even at oxygen tensions of 600 torr (Biscoe et al., 1970) and the discharge increases little until the P_{a,O_2} falls below 90-100 torr when the discharge begins to increase and does so ever more rapidly as the P_{a,O_2} falls further. At very low oxygen tensions the receptors are depressed and their discharge falls again (Black, McCloskey & Torrance, 1971).

The response to P_{a,CO_2} at constant P_{a,O_2} is approximately linear over the normal range of P_{a,CO_2}. The slope lessens at high and low P_{a,CO_2} making the overall curve sigmoid (Eyzaguirre & Lewin, 1961a).

Hypoxia and hypercapnia interact: the mean frequency of discharge at a given P_{a,O_2} or P_{a,CO_2} is higher if the other stimulus is

greater. A full quantitative study using single fibres has not yet been published and would be a very valuable addition to the literature, but work on the integrated response shows that the interaction is more than additive (Hornbein, 1968; Fitzgerald & Parks, 1971). Thus the resulting change in the frequency of discharge seen on changing both the mean P_{a,O_2} and mean P_{a,CO_2} to new mean levels is greater than the sum of the changes in frequency if the P_{a,O_2} and P_{a,CO_2} are changed individually. It is important to realise that this refers only to steady-state levels of P_{a,O_2} and P_{a,CO_2} and not to the increase in discharge at the change of gas tension (see Section 6.3).

The speed of response of afferents from the cut sinus nerve to a large step change of P_{a,CO_2} is rapid and adapts. The response is slower, and there is no adaptation, to a similar change of P_{a,O_2} (Black, McCloskey & Torrance, 1971). Inhibition of carbonic anhydrase, an enxyme known to be present in the carotid body (Lee & Mattenheimer, 1964), prevents the fast CO_2 response, and the response to large steps of P_{a,O_2} or P_{a,CO_2} are then similar (Black, McCloskey & Torrance, 1971; Travis, 1971). This suggests that the rapid response, to a large change in CO_2 at least, arises via the subsequent change in pH at the receptor. The evidence

from steady-state experiments is controversial with the sides equally divided into those who believe that CO_2 does not exert an effect over and above its effect through pH_a (Gray, 1968; Hornbein, 1968) and those who believe that CO_2 has an effect per se (Neil & Joels, 1963; Biscoe, Purves & Sampson, 1970). Work on the aortic chemoreceptors does not help. Sampson & Hainsworth (1972) reported that lowering the pH_a at constant P_{a,CO_2} could even cause a fall in the frequency of discharge.

Hornbein, Griffo & Roos (1961) reported oscillations in the discharge of the integrated response that occurred with respiration, suggesting that the speed of response of chemoreceptors was fast enough for them to follow the oscillations in the blood gases. This is dealt with in more detail in Section 6.3

Blood vessels and blood flow

The organ is well supplied with blood. There is an undoubted efferent nerve supply to the carotid body to regulate the blood flow (see below) and the main question in the problem of efferent control is whether there is any non-vascular efferent supply.

The pioneer work of de Castro is summarized in the Wates symposium (de Castro & Rubio, 1968). The blood vessels make up

25-33% of the total volume of the carotid body. The tissue is arranged in lobules each supplied by an arteriole. Tortuous sinusoids wind around the glomeruli of cells within a lobule. Arteriovenous anastomoses enable blood to by-pass the sinusoidal system. They are mostly situated peripherally and occur less often in the deeper parts. This pattern was seen in all arterial chemoreceptor tissue, including aberrant tissue. The artery to the carotid body, its arterial and some of its arteriolar branches, have baroreceptor endings in their walls.

Daly et al. (1954) measured the total blood flow as 40 µl per minute (2000 ml/min/100 g) and subsequent investigators agree with this (Purves, 1970a; McCloskey & Torrance, 1971). These studies used inflow or outflow techniques, Keller & Lübbers (1972) used a hydrogen clearance method and arrived at the same figure. Daly et al. (1954) found that the flow was proportional to the blood pressure, as did Biscoe, Bradley & Purves (1970) and Purves (1970a). McCloskey & Torrance (1971) saw autoregulation of the blood flow in some experiments on the denervated carotid body of rabbits and cats. In the cats barbiturate anaesthesia prevented autoregulation, which might be why the earlier investigators, who used barbiturates, did

not see the phenomenon. However, McCloskey & Torrance (1971) point out that one of the published records of Purves' (1970a) does in fact show autoregulation.

Sympathetic fibres reach the glomus directly from the superior cervical ganglion and also by a circuitous route entering the carotid body with the sinus nerve (Biscoe & Sampson, 1968). Daly et al. (1954) stimulated the cervical sympathetic and reduced the blood flow. Purves (1970b) confirmed this and also implied a tonic sympathetic outflow to the carotid body by showing that cutting the pre- or post-ganglionic cervical sympathetic ipsilaterally increased the blood flow by about 25%. There are vasodilator fibres in the glossopharyngeal nerve (Neil & O'Regan, 1971a).

Thus the carotid body has a high blood flow and the capability exists to control local and overall flow in a variety of ways. De Castro & Rubio (1968) reported changes in the size of the carotid body, reflecting the volume of blood in the sinusoidal system, when the cat breathed different gas mixtures. There were concomitant changes in the speed of flow through the surface veins so they could not make any statement on total blood flow. They supposed that the

control system acted through the baroreceptor endings and arterio-venous shunts that they had seen. No one else has reported these changes in the size of the carotid body, though Ungar reported having looked for them (see the discussion of De Castro & Rubio, 1968). Purves (1970a) showed that the total blood flow through the carotid body increased by about a third in severe hypoxia or hypercapnia whether the sympathetic supply was intact or not. The sinus nerve was intact so perhaps this hyperaemia was mediated by the vasomotor efferents that run in it. Hypercapnia may have a further local role: autoregulation was sometimes produced by hypercapnia, and Purves (1970b) found that stimulation of the cervical sympathetic had a greater effect in hypercapnia.

As Purves (1970b) himself points out, there is an apparent inconsistency in that the blood flow <u>increases</u> in conditions of heightened sympathetic activity although stimulating the sympathetic electrically decreases the flow. He sees the role of the sympathetic as that of redistribution of blood within the glomus. Keller & Lübbers' (1972) hydrogen clearance method, which uses microelectrodes inserted into the glomus, has enabled local flows to be measured. The peripheral flow was apparently less than the flow through the

centre of the carotid body, but this may be because the volume increases towards the periphery, as the method measures values of flow related to unit weight rather than as absolute values. They claim in their Discussion that they were unable to show any consistent effect of hypoxia or hyperoxia on local flow, though they do not publish the observations on which they base this. The authors also seem rather confused by the previous literature, saying that Daly et al. (1954) saw autoregulation when in fact Daly et al. (1954) found that flow was linearly related to blood pressure.

The hydrogen clearance method may one day prove more useful, but how 'local' are the flows measured? De Castro & Rubio (1968) give the impression of gross changes in blood flow through large local volumes, but Purves (1970a, b) thinks of many parallel vascular beds within the glomus. One could suppose that these are the lobules of De Castro & Rubio (1968) and that the hydrogen clearance method is not local enough to measure the difference in flow between adjacent vascular beds. The same group (Acker, Lübbers & Purves, 1971) had previously concluded that the blood flow was not homogenous from work on local P_{O_2}, but this too is controversial.

Oxygen consumption

I have to discuss $\dot{V}_{O_2}$ because if efferent nerves can affect it they may well alter the discharge. Also it may be of importance in the process of chemoreception.

The very high blood flow means that the arterio-venous oxygen difference is very small. Comroe & Schmidt (1938) found that the reflex hyperpnoea to carbon monoxide poisoning in dogs was abolished by bubbling oxygen through the poisoned blood and concluded that the carotid body could extract all the oxygen it needed from the plasma. Duke, Green & Neil (1952) saw no increase in the discharge of the whole sinus nerve during carbon monoxide poisoning. Apparently the arteriovenous difference was negligible and the chemoreceptors were responding to the oxygen tension of the blood rather than to the oxygen content. When Daly et al. (1954) reduced the blood flow to one quarter by lowering the blood pressure, they were able to measure an A-V difference of 2 vols %, equivalent to 0.5 vols % at normal flow. This gave an oxygen usage of 9 ml/min/100 g, but, as they themselves admitted, they were at the very limits of the capability of their method. All the more credit to them that their figure has been corroborated. Mills & Edwards (1968) used more

refined recording techniques than Duke et al. (1952) were able to use and showed that carbon monoxide poisoning would excite the chemoreceptors. Using these results they came up with an A-V difference of 0.35 - 0.5 vols % (Edwards & Mills, 1969). Purves (1970a) used gas chromatography and this gave an A-V difference of 0.35 vols % and a $\dot{V}_{O_2}$ of 7.5 ml/min/100 g.

The carotid body thus apparently has the highest oxygen consumption in the body and it is an obvious conclusion that this is intimately connected with the process of chemoreception. Fay (1970) found that the $\dot{V}_{O_2}$ of the saline perfused carotid body was much lower, 1.5 ml/min/100 g; Joels & Neil (1968) remarked that the carotid body did not sustain responses as effectively when perfused with saline as it did when perfused with blood.

Daly et al. (1954) assumed that the $\dot{V}_{O_2}$ of the carotid body was constant and that the A-V oxygen difference therefore varied inversely with the blood flow. According to Purves (1970b) this is so in the sympathectomized carotid body, but if the sympathetic supply is intact the A-V difference does not change, i.e. the $\dot{V}_{O_2}$ decreases with a decrease in flow as the perfusion pressure is

reduced. Hypoxia and hypercapnia also reduced the $\dot{V}_{O_2}$ provided that the sympathetic was intact.

There are some aspects of this work that are rather disquieting. First, the method of measuring oxygen content, although more accurate than that used by Daly et al. (1954), is still only accurate to 0.15 vols % (see Purves, 1970a; table 1) in measuring an A-V difference of 0.35 vols %. It was even used for an A-V difference of 0.05 vols % (see Purves, 1970a; table 3, line 2).

Secondly, Biscoe, Bradley & Purves (1969) confirmed Neil & O'Regan's (1969a) observation that stimulating the distal cut end of the sinus nerve increased the blood flow through the glomus and added that the A-V oxygen difference was increased by stimulation, i.e. there was a rise in the $\dot{V}_{O_2}$. The later full paper (Biscoe, Bradley & Purves, 1970) makes no mention of this, and nor does Purves (1970a, b) although the implications are important to his interpretation. Thus in hypoxia the sympathetic discharge increases and acts to reduce the $\dot{V}_{O_2}$ while the glossopharyngeal efferent discharge acts to increase the $\dot{V}_{O_2}$. (As an aside at this point: blood flow increases if efferent discharge is increased by electrical

stimulation of the sinus nerve or by systemic hypoxia with the sinus nerve intact, but does hypoxia cause hyperaemia in the totally denervated carotid body?).

Thirdly, the most important conclusion that Purves (1970b) reaches is that the oxygen consumption may be the single most important factor in the mediation of the chemoreceptor responses. This is giving the sympathetic nervous system, which seemingly profoundly affects $\dot{V}_{O_2}$, a very important role. Surely, then, there should be obvious differences between the the responses of the chemoreceptors to natural stimuli before and after the sympathetic is cut? In fact the responses of the chemoreceptors are qualitatively the same even in the superfused preparation (Eyzaguirre & Koyano, 1965a).

The effects on the discharge of the blood flow and sympathetic innervation

The confused state of affairs concerning the blood flow and oxygen consumption is not helped by, and certainly does not help, interpretation of the effects of blood flow and the sympathetic nervous system on the discharge of chemoreceptors. Floyd & Neil (1952) showed that stimulating the cervical sympathetic enhanced the discharge of the whole sinus nerve and this has been attributed

to the decrease in blood flow (Daly et al., 1954). The effects of stimulation are not apparent in vitro (Eyzaguirre & Lewin, 1961c) which lends further credance to this view. The discharge of the aortic chemoreceptors is increased by stimulation of the stellate ganglion (Mills, 1968) or a fall in blood pressure (Lee, Mayou & Torrance, 1964). However Biscoe, Bradley & Purves (1970) found that the discharge of afferents of the sinus nerve was little different from control in the steady-state following a change in blood pressure (over the range 60-160 mm Hg) and venous flow (10-60 µl/min). Furthermore the initial transient change in chemoreceptor activity at a change of blood pressure was not mirrored by any autoregulation of the blood flow. The activity of all the fibres studied showed an increase in their frequency of discharge when the blood pressure fell below 50-60 mm Hg. This contrasts with the rabbit which has a very low threshold pressure, 7-18 mm Hg, for excitation of the chemoreceptors (Ott, Kiwull & Wiemer, 1971), even though the blood flow is of the same order (McCloskey & Torrance, 1971; Weigelt et al., 1973).

One is brought back to local blood flows being important by the observations of Mitchell & McCloskey (in preparation) that

stimulation of the cervical sympathetic always increased the discharge in multifibre strands of the sinus nerve but single fibre preparations were not necessarily affected, and those single fibres that were affected by stimulation of the sympathetic were not necessarily those affected by a change in overall blood flow.

Recording from the sinus nerve usually involves first cutting the nerve and, as we have seen, there are both vasoconstrictor and vasodilator fibres in the nerve. Recording from an afferent whilst leaving the rest of the nerve intact (Neil & O'Regan, 1971b) is technically difficult and one cannot be certain that the efferent fibres are not damaged. The investigation of the effect of blood flow and $\dot{V}_{O_2}$ on chemoreception requires a method of recording from absolutely intact nerves, for instance extracellular recording with microelectrodes of single units within the glossopharyngeal ganglion, and a more accurate method of measuring oxygen usage. We need some definitive experiments not to add to previous evidence but to supersede it.

Structure

The components of the carotid body are two specific types of cell, nerve endings, and blood vessels (Fig. 1) in a tough connective

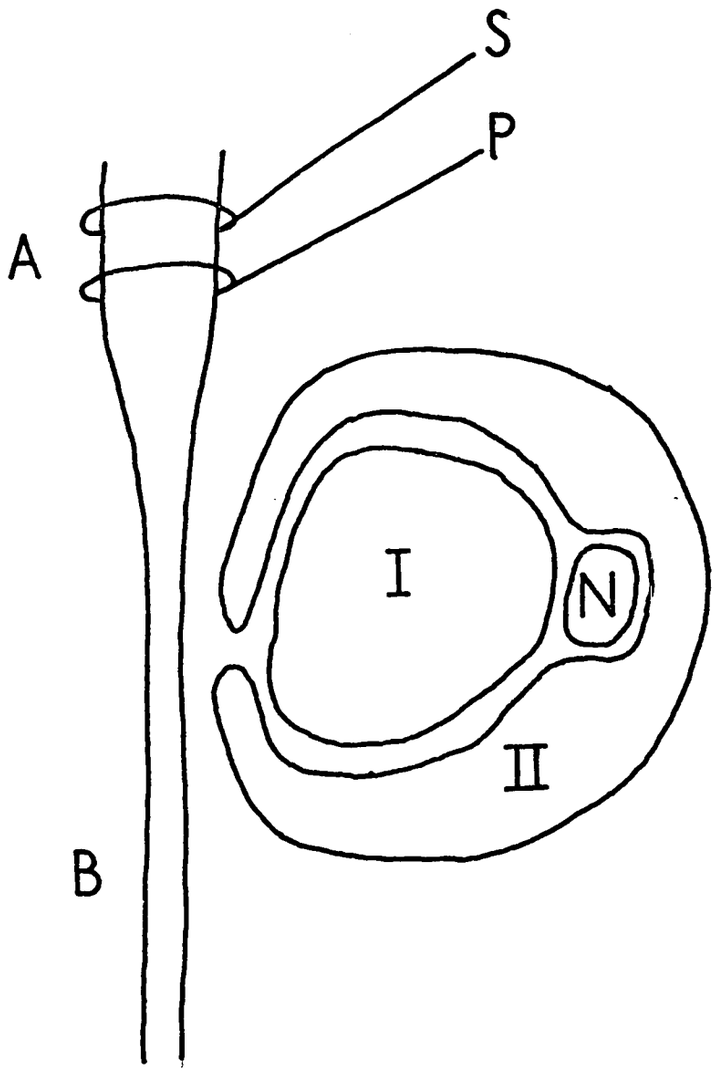

Fig. 1: A diagram to show the essential structure of the carotid body. A Type I cell (I) is surrounded by a Type II cell (II). Between these is a nerve ending (N). A sinusoid (B) is supplied by an arteriole (A) which has sympathetic (S) and parasympathetic (P) efferent nerves running to it.

tissue capsule. The cell types have been variously labelled but Type I and Type II (de Kock, 1954) is probably the best nomenclature because it ascribes no function. Type I cells are roughly spherical and tend to be grouped together, the groups being invested by the smaller Type II cells through which the nerve fibres run to terminate in expansions in contact with the Type I cells. The relation of the nerve fibres to the Type II cells resembles that of nerves and Schwann cells hence the term 'sustenacular cells' for Type II cells (Ross, 1959). There are also nerve fibres supplying the vasculature that degenerate if the sympathetic innervation is cut (Biscoe & Stehbens, 1967) and parasympathetic fibres supplying the micro-ganglia that can be found in the fibrous sheath of the carotid body or at the surface of the organ (de Castro & Rubio, 1968).

The blood flows through sinusoids and is not in direct contact with the nerve endings. Hess (1968) reported that there was at least one layer of Type II cell cytoplasm between a Type I cell and the blood; Eyzaguirre et al. (1972) saw some processes of Type I cells separated from the blood by only a basement membrane.

De Castro (1928) concluded that the expanded nerve endings were afferent because they degenerated after the sinus nerve had

been cut but not after the glossopharyngeal nerve was cut intracranially. The cell bodies of these endings were therefore in the glossopharyngeal ganglion and, as this was a sensory ganglion, the endings were afferent. De Castro saw the Type I cell as the transducer - with one pole pointed at the blood and the other at the nerve ending.

This is the classical view of the arterial chemoreceptor but in 1966, at the Wates symposium, de Kock & Dunn described some very fine nerve endings that ended without contacting Type I cells at all (de Kock & Dunn, 1964; 1966). Perhaps these fine nerve endings were the chemoreceptor afferent endings and the fat nerve endings were doing something else.

The possibility of efferent control

In 1959 Lever, Lewis & Boyd had spoken of microvesicles in the fat nerve endings. Biscoe & Stehbens (1965) confirmed this and suggested that the fat nerve endings might be efferent, releasing the contents of the vesicles to act synaptically on the Type I cell. When Biscoe, Lall & Sampson (1970) repeated de Castro's (1928) original experiment they found that intracranial section did cause degeneration of the fat nerve endings, but with a longer time

course than the degeneration following section of the sinus nerve so that de Castro (1928), who only waited 10-12 days before histology, would not have seen any degeneration.

By this time Neil & O'Regan had produced their first electrophysiological evidence of a non-vascular efferent pathway (Neil & O'Regan, 1969a, b). Biscoe & Sampson (1968) had recorded two types of efferent activity in the cut central end of the sinus nerve. One type was of sympathetic origin. The other type was a sparse non-rhythmical discharge that showed an increase in its frequency after an intravenous injection of adrenaline, or in systemic hypoxia or hypercapnia even when the chemoreceptor nerves were cut. Neil & O'Regan used two experimental approaches. Stimulating the trunk of the cut sinus or aortic nerve whilst recording from an afferent strand peeled from the nerve distal to the stimulating electrodes inhibited the discharge in the afferent strand (Neil & O'Regan, 1969a). They also recorded from afferent and efferent slips of the sinus nerve with the remainder of the nerve in continuity hoping to maintain its efferent innervation intact. The efferent discharge increased when the afferents of the ipsilateral carotid body were excited (Neil & O'Regan, 1969b).

The efferent innervation described by Neil & O'Regan (1969a, b) apparently reduced the discharge of the afferents in hypoxia. Sampson & Biscoe (1970) described how the P_{a,O_2}-response curve was shifted upwards after section of the remainder of the sinus nerve, the effect being most pronounced at low P_{a,O_2}. Neil & O'Regan (1971b) reported their earlier results more fully: if, whilst recording from an afferent slip, the main trunk of the nerve was cut in steady-state hypoxia the frequency of discharge in the efferent slip increased, though there was little change in steady-state normoxia. They envisaged a negative feedback loop acting directly at the level of the afferent endings to suppress the discharge. One surprising observation was that the response to severe hypoxia had an earlier onset if recorded from the cut rather than the otherwise intact sinus nerve. In a true negative feedback loop the speed of response will not be affected as, unless the time in the loop is very short, a response has to occur before the feed-back can cut it down. One would expect a reduced peak response, as they report, provided the response takes some time to build up to allow the feedback to act. It would be interesting to know if, with the otherwise intact nerve, the peak discharge was followed by a reduction as the system

'hunted' around a null point as a negative feedback system can do. They make no mention of this, but they were causing very severe hypoxia (the inspired mixture was 5% O_2 in N_2) and could not have reached a steady state.

Neil & O'Regan (1971b) do not make any observation in hypercapnia but there is no reason to suppose that the effects on efferent activity, and its effect on afferent activity, could be any different from those in hypoxia. Hypercapnia would be a more useful tool to investigate the speed of response of the chemoreceptors with and without the efferent supply intact as the response to hypercapnia is more rapid than that to hypoxia (Black, McCloskey & Torrance, 1971).

So, in a few years, the mode of function of the carotid body had been turned on its head. Now the fat nerve endings were efferent, modifying the response of the Type I cells to the levels of the blood gases, and a search began for the afferent terminals, which could be the fine nerve endings of de Kock & Dunn (1964; 1966).

It is here that the results from electron microscopy become confusing. The contact between the fat nerve ending and the Type I

cell acquired the term 'synapse', which was unfortunate as this implies the function of the transmission of nervous information. The microvesicles of Lever et al. (1959) became 'synaptic vesicles'. Authors described sub-types of Type I cells, different species of fat nerve ending, and specialised membrane thickenings at all sorts of cell contacts (see Howe & Neil, 1972). There was no longer a single story and the electrophysiologist could pick a histology to suit any theory of function. While Biscoe (1971) and others were championing the Type I cell-nerve ending contact as efferent, Kobayashi & Uehara (1970) described 'afferent synaptic complexes' in the mouse. Kobayashi (1971b) saw both afferent-type and efferent-type 'synapses' in a number of species in the course of a comparative study, and this paper includes a useful list of previous ultra-structural studies of the carotid body (see his Table 1).

But there was no confirmation of the existence of any fine nerve endings. At the Wates symposium Hess (1968) had suggested that de Kock & Dunn (1968) had been looking at pre-terminal fibres and this was becoming more and more likely. When Hess & Zapata (1971) repeated de Castro's (1928) degeneration experiments yet again, they reaffirmed his story: the fat nerve endings degenerated

only after extracranial section of the sinus nerve and not after intracranial section of the glossopharyngeal nerve. Presumably, in the experiments of Biscoe, Lall & Sampson (1970) some operative trauma during the difficult intracranial section must have caused the development of fibrous constrictions that strangled the cell bodies in the ganglion or the axons in the nerve and there was late degeneration of the endings. Neither did Neil & O'Regan's (1969a; 1971a) electrophysiological evidence of an efferent innervation go unchallenged (see Chapter 4).

The initiation of response

An efferent pathway could directly affect the process of the initiation of impulses at the receptor terminals so I shall consider some aspects of this.

The discharge of an afferent can be related to the gas tensions of the carotid blood but the actual stimulus is the availability of oxygen and CO_2/H^+ to the receptor. Histologists give 15-30 μ as the distance between the blood and the most remote parts of the carotid body (Ross, 1959; de Castro, 1951). The calculated partial pressure of oxygen in the tissue (P_{T,O_2}) is about 10 torr less than the capillary P_{O_2} (Forster, 1968), assuming a spheroidal arrangement

of the tissue with groups of cells surrounded by blood. Schur (unpublished) describes a cylindrical arrangement of the tissue around the blood vessels and this would give a somewhat larger drop from the capillaries. The predicted P_{T,O_2} in the centre of the glomus is therefore high, provided that a substantial volume of arterial blood is not shunted. Keller & Lübbers' (1972) finding that the blood flow through the centre of the carotid body is of the same order as the overall flow suggests that if shunts exist they must be very local. The sinusoidal A-V oxygen difference is also of the same order as the overall A-V difference; Mills & Edwards showed this in their experiments on the discharge of the chemoreceptors in carbon monoxide poisoning (Mills & Edwards, 1968; Edwards & Mills, 1969). Thus, even local shunts are not significant enough for one to need to alter predictions of P_{T,O_2}. Direct measurement of the P_{T,O_2} in the centre of the carotid body gives a figure of about 50 torr (Acker et al., 1971; Whalen & Nair, 1973).

Prediction and measurement both indicate that a part of the transduction process is sensitive to changes of oxygen tension well above the critical P_{O_2} for oxygen uptake by the enzymes of the respiratory chain. Yet the evidence from experiments on the

discharge of chemoreceptors suggests that metabolic poisons excite the chemoreceptors by interfering with the production of ATP (Joels & Neil, 1964; 1968), and that hypoxia excites in the same way. Mills & Jöbsis (1972) obtained spectrophotometric evidence of two types of cytochrome a_3 (cyt. a_3) in the carotid body. One had the normal high affinity for oxygen that cyt.a_3 has in other tissues, but the other had an unusually low affinity for oxygen and was 50% reduced at a P_{O_2} in the perfusate of 90 $\pm$35 torr. There was a positive correlation between the reduction of this cyt.a_3 and the frequency of impulses recorded from the sinus nerve. The experiments had to be done on perfused tissue to remove the haemoglobin and the authors admit that they were not able to wash it all out. There is no risk of confusing the fluorescence produced by the oxidation state of haemoglobin with that produced by the cyt.a_3 as the wavelengths are different, but it could be that the incomplete perfusion had created long diffusion paths for oxygen so that the remote cyt.a_3 needed a high P_{O_2} in the perfusate for its oxidation.

Acker et al. (1971) saw a gradient of P_{T,O_2} from high values in the centre of the carotid body to values of 0-10 torr at the periphery and raising the P_{a,O_2} affected the central P_{T,O_2} far more

than the peripheral P_{T,O_2}. They maintain that there is no need to postulate an end-oxidase of low affinity for oxygen as there will always be low P_{T,O_2}'s in the glomus. However, Whalen & Nair (1973) report that the peripheral P_{T,O_2} is higher than the central P_{T,O_2} and that there are few places where the P_{T,O_2} is less than 10 torr. Both sets of observations cannot be correct.

It is not known if the glossopharyngeal efferents alter the P_{T,O_2}, but the first priority in this field must be to solve the disagreement on the peripheral P_{T,O_2}. Until this is done any experimental observations must be open to doubt.

Transmitters in the carotid body

If there is indeed an efferent inhibition which is not vascular a transmitter is presumably involved at the nerve ending which mediates this inhibition. I have, therefore, to discuss the subject of transmitters in the carotid body: their presence and possible significance.

The early physiological work on the chemoreceptors coincided with the emergence of acetylcholine as the transmitter at the neuromuscular junction and other synapses. Schweitzer & Wright

(1938) first suggested that acetylcholine might be a sensory transmitter in the carotid body, being released by the Type I cells to act on the nerve endings.

Since then many substances have been proposed as the sensory transmitter. Probably the most favoured and most tested candidate has been acetylcholine, and Eyzaguirre has been its most staunch supporter. He has done a large quantity of work with the whole gamut of cholinergic pharmacological agents, mostly on the superfused carotid body as first described by Eyzaguirre & Lewin (1961b). His most celebrated experiments are the Loewi-type preparations in which two carotid bodies were suspended in the stream of superfusate and stimulation of the upstream carotid body caused excitation of the one downstream (Eyzaguirre, Koyano & Taylor, 1965). This excitation was enhanced by eserine, depressed by hexamethonium, and blocked by mecamylamine or acetylcholinesterase. These drugs also had the predicted effect on the response of the superfused carotid body to applied acetylcholine (Eyzaguirre & Koyano, 1965b; Eyzaguirre & Zapata, 1968a). However, Biscoe (1963) found that hexamethonium only blocked the effect of acetylcholine on some occasions and did not alter the response to hypoxia. Douglas (1952) had demonstrated

this earlier on the reflex response to acetylcholine. Sampson (1971) showed that, in vivo, mecamylamine would block the action of applied acetylcholine but did not alter responses to 'natural' stimuli in contradiction to the earlier results of Eyzaguirre & Zapata (1968a). Sampson (1971) also pointed out that Eyzaguirre & Zapata (1968a,b) had used concentrations of mecamylamine far in excess of that required to block transmission in ganglia, and concluded that it had been acting as a local anaesthetic. Eyzaguirre (Eyzaguirre et al., 1972) had already needed to postulate that applied acetylcholine was also acting at extra-synaptic sites to explain the dichotomy between the pharmacological and natural stimuli, and the picture was getting rather hazy. It was rather odd that enough acetylcholine was diffusing from the upstream glomus to excite the downstream glomus in the Loewi-type experiments if the true post-synaptic surface was inaccessible to administered acetylcholine and blocking agents. Paintal (1969) calculated that the carotid body would have had to release virtually 50% of its own weight in acetylcholine if the responses of the downstream glomus were to be attributed to acetylcholine diffusing from the upstream glomus. Metz (1969) had reported the release of acetylcholine from

the isolated carotid body *in vivo*, but only in hypoxia, or hypoxia plus hypercapnia. No acetylcholine was recovered in normoxia or in hypercapnia alone. These results are not convincing evidence that acetylcholine is the prime mover in the natural responses of the organ and, as Biscoe (1971) points out, he collected the perfusate in a pool on skeletal muscle.

There are other lines of evidence that suggest it is unlikely that acetylcholine is the transmitter. Hebb (1968) reported a far lower concentration of choline acetylase in the carotid body than in the superior cervical ganglion although they both contained approximately the same concentration of acetylcholine. She also drew attention to the high concentration of the enzyme in the sinus nerve and suggested that 30% of the fibres could be small cholinergic efferent fibres. These fibres may be the parasympathetic fibres mediating the hyperaemia seen on stimulating the sinus nerve electrically (Neil & O'Regan, 1971a) and which, I believe, are responsible for the inhibition of discharge during electrical stimulation (see Section 4.3).

At the other end of the cholinergic path there is surprisingly little cholinesterase in the carotid body (Biscoe & Silver, 1966). It is mostly pseudocholinesterase and occurs mostly in relation to blood vessels and a few cells which Biscoe & Silver took to be possible aberrant sympathetic ganglion cells. Ballard & Jones (1970) claimed that the butylcholinesterase was localised uniformly on the cell membranes of the Type II cells although their pictures are not good and they admit that the granularity of the reaction product did not make it possible to localize the enzyme to any particular cell membrane.

Much of the work on acetylcholine has been done with the de Castro picture in mind (de Castro & Rubio, 1968). The microvesicles in the fat nerve endings look cholinergic but this does not fit with the de Castro picture of sensory transmission from the Type I cell. Although clear-cored vesicles do occur in the Type I cell, they never appear in the vast agglomerations seen in the pre-synaptic terminals of proven cholinergic pathways. It must also be said that the fat nerve endings do not contain such vast numbers either. It is possible that acetylcholine is released by the fat nerve endings and excites some other structure in the carotid body but this

suggestion is open to all the criticisms mentioned above. Also, if the fat nerve ending releases a transmitter in response to hypoxia and hypercapnia, why could an action potential not be generated without the intermediate step?

Catecholamines have been proposed as transmitters. The Type I cells have a highly granular cytoplasm and the electron microscope reveals vesicles with an electron dense core (e.g. Dearnaley, Fillenz & Woods, 1968) similar to the vesicles in the adrenal medulla. The carotid body is known to contain catecholamines, although the type and amount present are in dispute. Kobayashi (1971a) tabulates the conclusions of previous investigations: dopamine emerges as the catecholamine found most frequently in the largest amounts. Lishajko (1970) reported that human chemodectomas contained dopamine and that it could be released from, or taken up by, isolated vesicles by a process inhibited by reserpine and competitively reduced by noradrenaline or adrenaline. Blümcke, Rode & Niedorf (1967) saw a decrease in the number of dense-cored vesicles in the Type I cells of the rat carotid body after a period of anoxia but Al-Lami & Murray (1968) saw no evidence of this in the cat and thought that there might even have been an increase.

Reserpine reduces the catecholamine content of the carotid body, as judged by fluorescence techniques, without apparently affecting the dense-cored vesicles (Niedorf, Rode & Blümcke, 1970), although Chen & Yates (1968) claimed that the content of the vesicles - i.e. the visual density of individual vesicles - was reduced.

The vesicles seem to be sensitive to impulses in the sinus nerve. Stimulation of the nerve causes a decrease in vesicle content which is atropine sensitive (Yates, Chen & Duncan, 1970), and the effect of reserpine is apparently dependent on the continuity of the sinus nerve. One cannot be certain which fibres are required intact. Stimulating the nerve will excite all the afferents as well as any efferents. Yates et al. (1970) administered reserpine for two days after they had sectioned the sinus nerve but did not know if the afferents were still conducting at this time.

I feel that one must be very cautious in interpreting results based on such a subjective criterion as the content of a vesicle which can vary so much from section to section because of such things as uneven staining.

Zapata, Hess, Bliss & Eyzaguirre (1969) did a full investigation of the role of catecholamines in the carotid body. The amount

present and the appearance of the granules was unaffected by hypoxia. Chronic denervation by sectioning the sinus nerve or the sympathetic supply caused no changes. There was no change in the frequency of discharge on applying catecholamines to the superfused glomus, although Biscoe (1963) had seen some potentiation of discharge. Zapata et al. (1969) concluded that catecholamines were not excitatory transmitters in the carotid body, but they did not rule out a role as mediators of mild inhibitory effects. Sampson (1972) claims that, in vivo, catecholamines cause a decrease in the frequency of discharge before the development of an increase due to vasoconstriction. Dopamine caused only inhibition without the subsequent facilitation and he believes electrical stimulation of sinus nerve efferents is mediated by dopamine because both are blocked by α-blocking drugs. However, he did not check for adventitious excitation and antidromic depression (see Chapter 4).

Nobody has yet looked for dopamine in the venous effluent of the carotid body.

A pause

This has provided a background against which I can present my own results. Having done so, I shall return to more general discussion in Chapter 5 which deals with the relevance of these results to the various theories of chemoreceptor function.

Chapter 2

General Methods

Preparation of animals

Most of the experiments were done on cats (1.5 - 3.5 kg). (Three dogs were used when studying respiratory oscillations: see Section 6.3). The cats were anaesthetized with pentobarbitone sodium (Nembutal, Abbott), 42 mg/kg injected intraperitoneally. The trachea was cannulated low in the neck and the right femoral vein cannulated for topping-up doses of anaesthetic. Anaesthesia was deep enough to abolish reflex movement to pinching between the claw pads with the leg extended. An artery was always cannulated to record the blood pressure. The right femoral artery was used unless an experiment required close intra-arterial injections when an artery close to the carotid body was used, usually the lingual, occasionally the brachial.

Dissection

For experiments in which the vagi were used, or both sinus nerves were wanted, or the carotid bodies were excised, the approach was medial with the cat on its back. The muscles of the midline plus the trachea and oesophagus were removed between the hyoid bone and the tracheal cannula.

Only one sinus nerve was wanted in most experiments and then the approach was lateral. This approach enables the dissection to be done remote from the carotid body and minimises the risk of damaging its blood supply. It also yields a longer length of nerve than does the medial approach.

The animal was laid on its right side and an incision made from the angle of the jaw, over the masseter muscle, curving round over the transverse process of the atlas, and then for an inch or so caudally. The parotid gland was removed and the sternomastoid and digastric muscles separated from their insertions on the mastoid process. The transverse process of the atlas was cleared of muscle and nibbled down and the overlying muscles removed; this allowed a wide working area. The tympanic bulla was nibbled away, and finally a large lymph node lying over the carotid sinus removed, revealing

the glossopharyngeal and carotid sinus nerves lying horizontally in the plane of focus of the microscope.

At this stage the cat was moved from the preparation room to the experimental room. The cat's head was fixed with a jaw clamp, the skin flaps retracted and liquid paraffin (at 37 - 38°C and equilibrated with 95% O_2 + 5% CO_2)poured in to form a pool. The area was further cleared under a dissecting microscope and, if required, the cervical sympathetic trunk and/or vagus were cut together with all the ganglio-glomerular nerves that could be identified. The dissecting plate (stainless steel, coated with blackened epoxy resin on its upper surface) was slid under the intact sinus-glossopharyngeal nerves and rested on the edge of the tympanic bulla.

The sheath was removed from the nerve using sharpened watch-maker's forceps and a small piece of razor blade held in a pin-vice. With the lateral approach it was possible to dissect the sinus nerve out from within the sheath of the glossopharyngeal nerve and then it was easy to strip the sheath down the sinus nerve like taking off a sock. Sometimes the sinus nerve left the glossopharyngeal very early and then the sheath had to be removed more painstakingly

to avoid damaging the fibres. Few-fibre strands were peeled from the nerve and further divided until a strand contained only one fibre.

Recording

The electrodes were stainless steel. The single fibre strand was laid over one electrode and a cut length of nerve connected the second, indifferent electrode to the dissecting plate, through which the cat was earthed. The signals were amplified (Tektronix type 122 preamplifier), displayed on an oscilloscope (Tektronix type 502 or 502A) and fed to a loudspeaker. Impulses could be recorded on an ink-jet recorder (Elema-Schönander Mingograf type 24B) and a tape recorder (Thermionic T3000). A storage oscilloscope (Tektronix type 549) was useful for checking inter-impulse intervals (see below) and for later photographing stored results.

Tests for single chemoreceptor fibres

A fibre was accepted as a chemoreceptor if it showed a profound reduction of discharge on a sudden change to breathing 100% O_2, an increase in mean frequency when breathing hypoxic gas mixtures, and, in some experiments, a brisk response to a close intra-arterial

injection of saline equilibrated with 100% CO_2. The easiest test that a fibre was single was that no intervals between impulses were less than 7 msec (Biscoe & Taylor, 1963).

Blood pressure was measured with an Elema-Schönander EMT 490A transducer head connected to a type EMT 460 electro-manometer. Leads and amplifier were available for recording the ECG. Respiratory air-flow was measured with a Fleisch pneumotachograph head and electromanometer (Furness Controls Ltd). An electronic integrator gave a signal of tidal volume from the flow signal. A time-trace was available for 1, 10 and 100 msec pulses. Any of these signals could be recorded on available recording channels.

Artificial ventilation

Some animals were paralysed with gallamine triethiodide (4 mg/kg i.v.) and ventilated with a Starling Ideal pump modified to give a continuous range of frequencies. A careful note of the anaesthetic needs of the cat were made before the gallamine was given. Gas mixtures were made up by proportional timing of flow at constant pressure using a water manometer, and were stored in Douglas bags. They could be administered by pump, or via respiratory valves to the spontaneously breathing cat.

Temperature was maintained at 36-38°C with an electric blanket.

Chapter 3

Efferent Control: Studies with Microelectrodes

3.1 Methods

Dissection

The area was exposed by medial dissection (see Chapter 2) and one carotid body, as free from overlying connective tissue as possible, removed complete with a long length of the carotid sinus nerve. The cat was maintained anaesthetized and at approximately 37.5°C with the second carotid body in situ until required.

Superfusion apparatus

The carotid body was transferred to a perspex trough[+] (Fig. 2) and fixed in position. In early experiments a small entomological pin was used but it was difficult to position the pin and the carotid body was either distorted or half destroyed. Later the Ag-AgCl

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[+]The trough was built by Chris Hurst

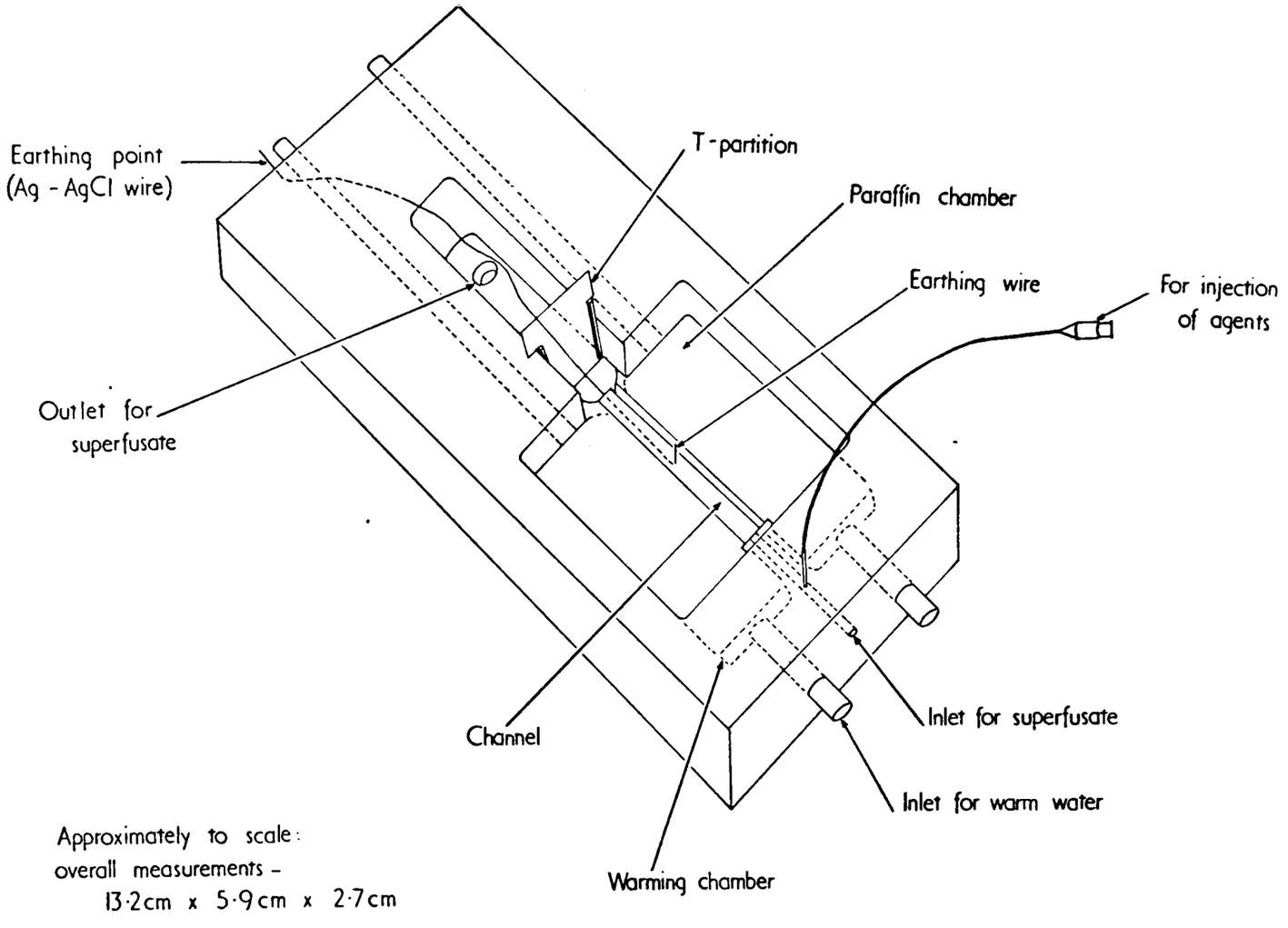


Fig. 2: The perspex perfusion chamber viewed obliquely from above. Approximately to scale.

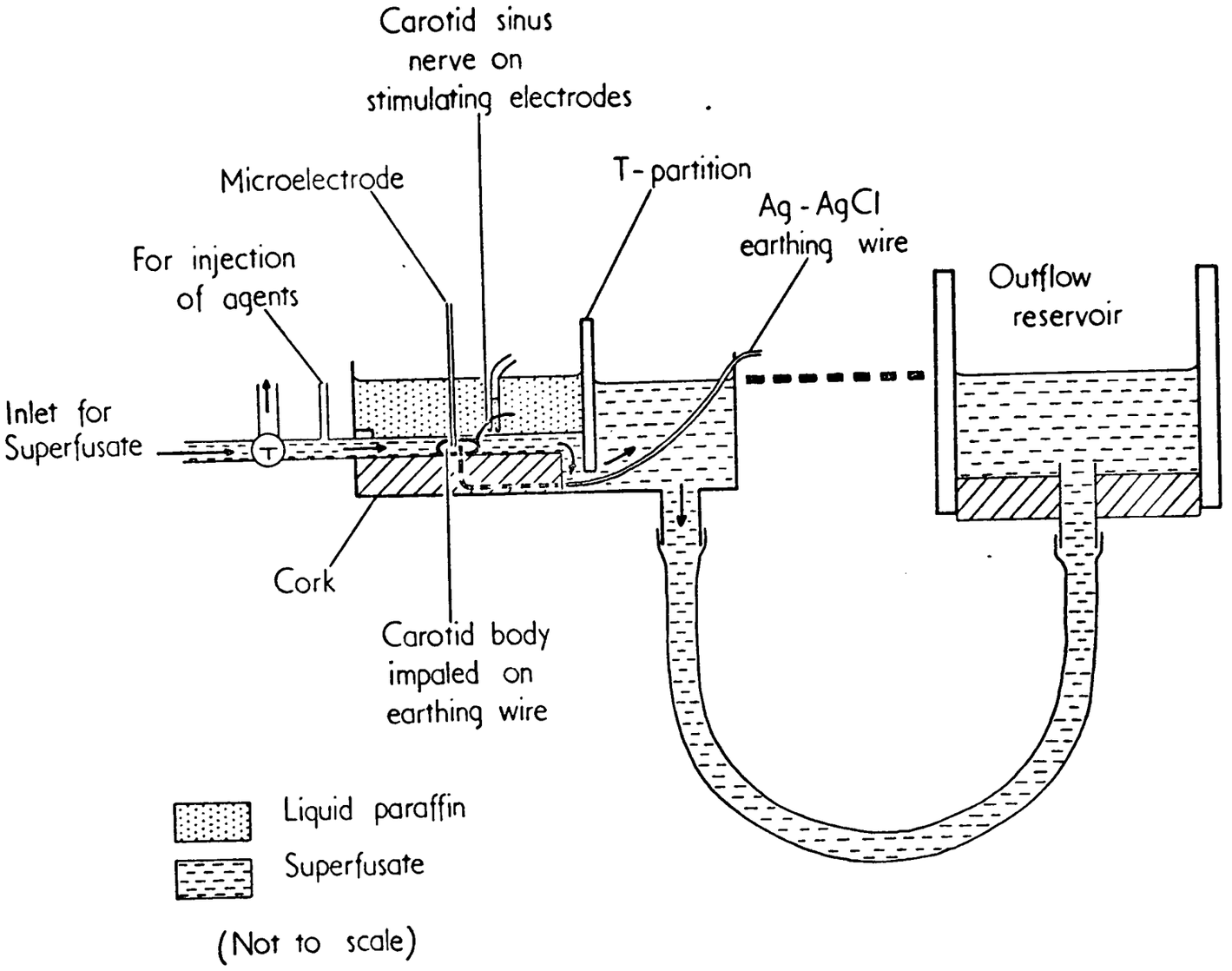
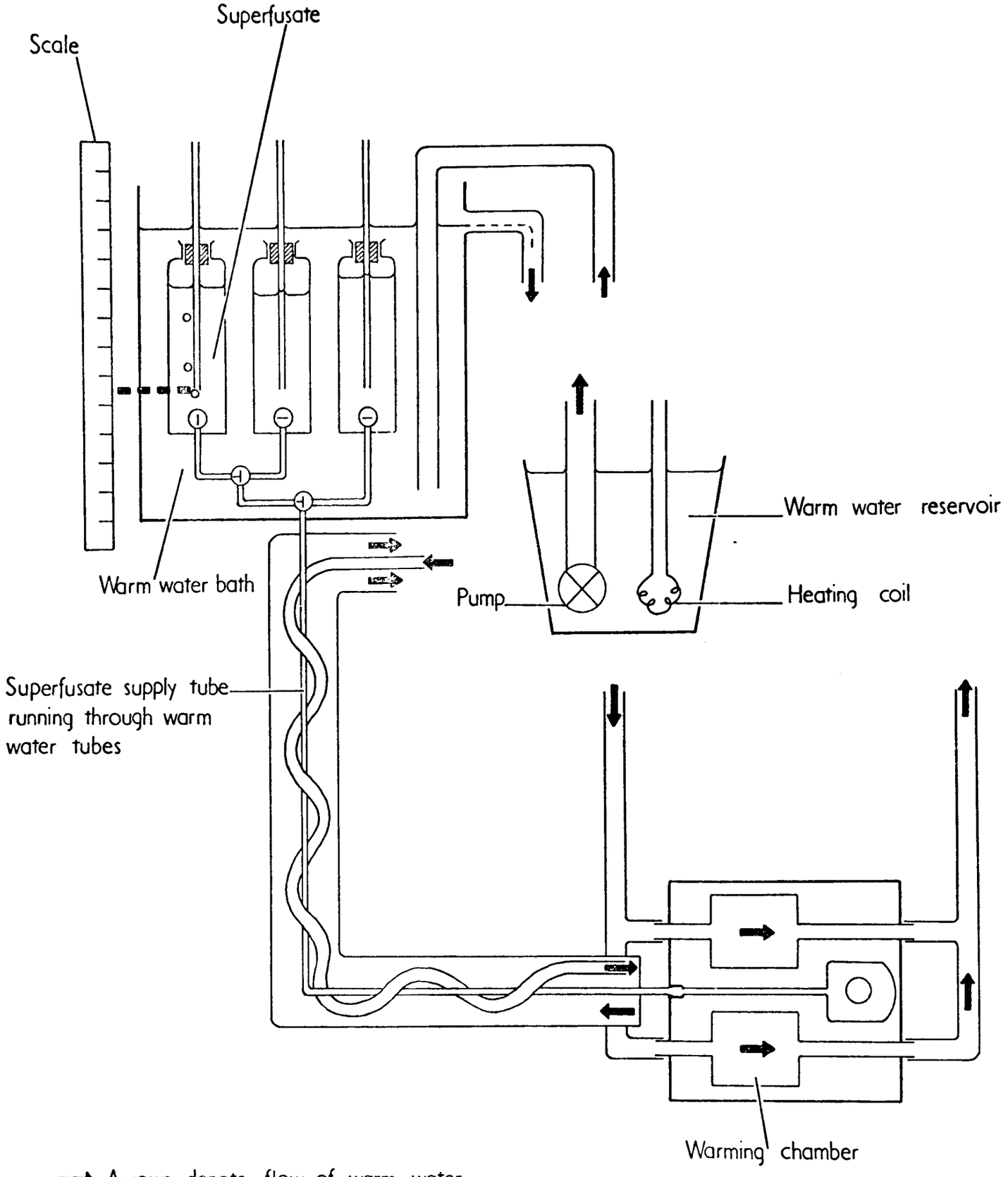


Fig. 3: A diagrammatic representation of the arrangement of the carotid body and electrodes and the flow of superfusate. The height of the outflow reservoir was adjustable but needed adjusting only rarely as its volume was very large in comparison with that of the perfusion channel.



➡ Arrows denote flow of warm water.
The tubes connecting the different parts of the system are omitted for clarity.

(Not to scale)

Fig. 4: The warm water heating system. The water was heated in a reservoir and then circulated to the warm water bath, along a counter-current system for keeping the piped superfusate warm, and into the warming chambers of the perspex block. The temperature of the superfusate passing over the carotid body was 36-37°C.

earth wire was led beneath the cork and the carotid body impaled on it (Fig. 3).

The carotid body lay in a stream of superfusate, Ringer-Locke solution equilibrated with 95% O_2 + 5% CO_2 (pH adjusted with Tris buffer to about 7.45), delivered from constant pressure-head bottles. Above this stream was a layer of paraffin, also equilibrated with 95% O_2 + 5% CO_2, into which the sinus nerve could be lifted for recording or electrical stimulation (Fig. 3).

The superfusate and chamber were kept at 36-37°C by a warm water heating system (Fig. 4).

This type of <u>in vitro</u> preparation had been described by Eyzaguirre & Lewin (1961b), but there were problems with the outflow of superfusate. At first the superfusate was allowed to overflow a perspex barrier as Eyzaguirre & Lewin (1961b) described, but this made the level of fluid in the perfusion channel oscillate and caused shifts in the D.C. level of the record. Leading the fluid away with pieces of cotton did not help, and sucking the fluid away with a hypodermic needle connected to a vacuum tap caused artefactual spikes to appear on the record. The eventual solution was as in Fig. 3, the perfusion channel led to a wide, deep outlet and away

to a large outflow reservoir. The superfusate flowed out under a T-partition that held the paraffin back in its chamber.

The warm water bath stood on a table, the height of which could be adjusted to allow the rate of flow of superfusate to be altered. The maximum rate was 10-12 ml/min. The volume of the channel was 1.5 ml. Superfusate could be drawn through the connecting tubing rapidly, or a sample removed for testing of pH etc., by means of the tap on the inlet line (Fig. 3).

Drugs and test solutions were injected through fine tubing connected to a hypodermic needle set into the perspex block.

Microelectrodes and micromanipulator

The microelectrodes were made from hand-pulled glass capillary tubing (from the University Glass-Blowing unit) cut into lengths and pulled in pairs in an electronic puller (lent by Miss R. J. Banister). They were filled with 3M KCl by boiling cautiously under reduced pressure in a conical flask until bubbles had ceased coming from their shanks. All were inspected under a microscope for damage or the presence of air bubbles and any that contained air bubbles were heated once more in the KCl. If that did not work they were

discarded. Microelectrodes of resistance 15-100 MΩ were used. They were clamped in the end of an insulated threaded rod and moved using a mechanical micromanipulator of the Huxley (1961) type that allowed movements of 0.2μ.

Electrical apparatus

Ag-AgCl wires connected the microelectrode and the earthing wire to the input stages of a cathode follower. The earthing wire was connected via a calibrator unit providing back-off potentials in 10, 1 and 0.1 mV units. A battery-resistor circuit could be connected to the microelectrode and its resistance measured from the voltage drop across it. The micromanipulator and perfusion bath were bolted to a solid table and enclosed in a shielded metal box having the front open for the experimenter. The only electronic equipment inside the box was the input valve of the cathode follower, enclosed in its own metal box together with the switches for putting the battery-resistor in circuit, plus the electrodes for recording from and stimulating the sinus nerve.

The recorded voltages and nerve impulses were displayed on an oscilloscope (Tektronix type 502A) and photographed (Cossor

Oscillograph Camera Model 1458) on 35 mm paper (Kodak Linagraph RP30).

The stimulator was a constant current device (see also Section 4.1). Stimuli were always supramaximal for excitation of all the C fibres of the sinus nerve (Fig. 6, Section 4.1). The current dialled could be converted approximately to a voltage by referring to a graph of current versus voltage constructed from observations on isolated segments of sinus nerves. In some experiments the voltage was read directly by connecting an oscilloscope trace across the stimulating electrodes.

3.2 Experimental Procedure

The major part of the study was done *in vitro* using 26 carotid bodies from 14 cats. Further observations were made *in vivo* (5 cats).

The microelectrode was advanced until it could be seen under the dissecting microscope to be dimpling the surface of the carotid body. The electrode was then moved in small increments whilst watching the recorded voltage on the oscilloscope. The connective tissue sheath of the carotid body is very tough and many electrodes broke. Eyzaguirre & Lewin (1961c) used chymotrypsin to partially digest the connective tissue, but enough successful penetrations

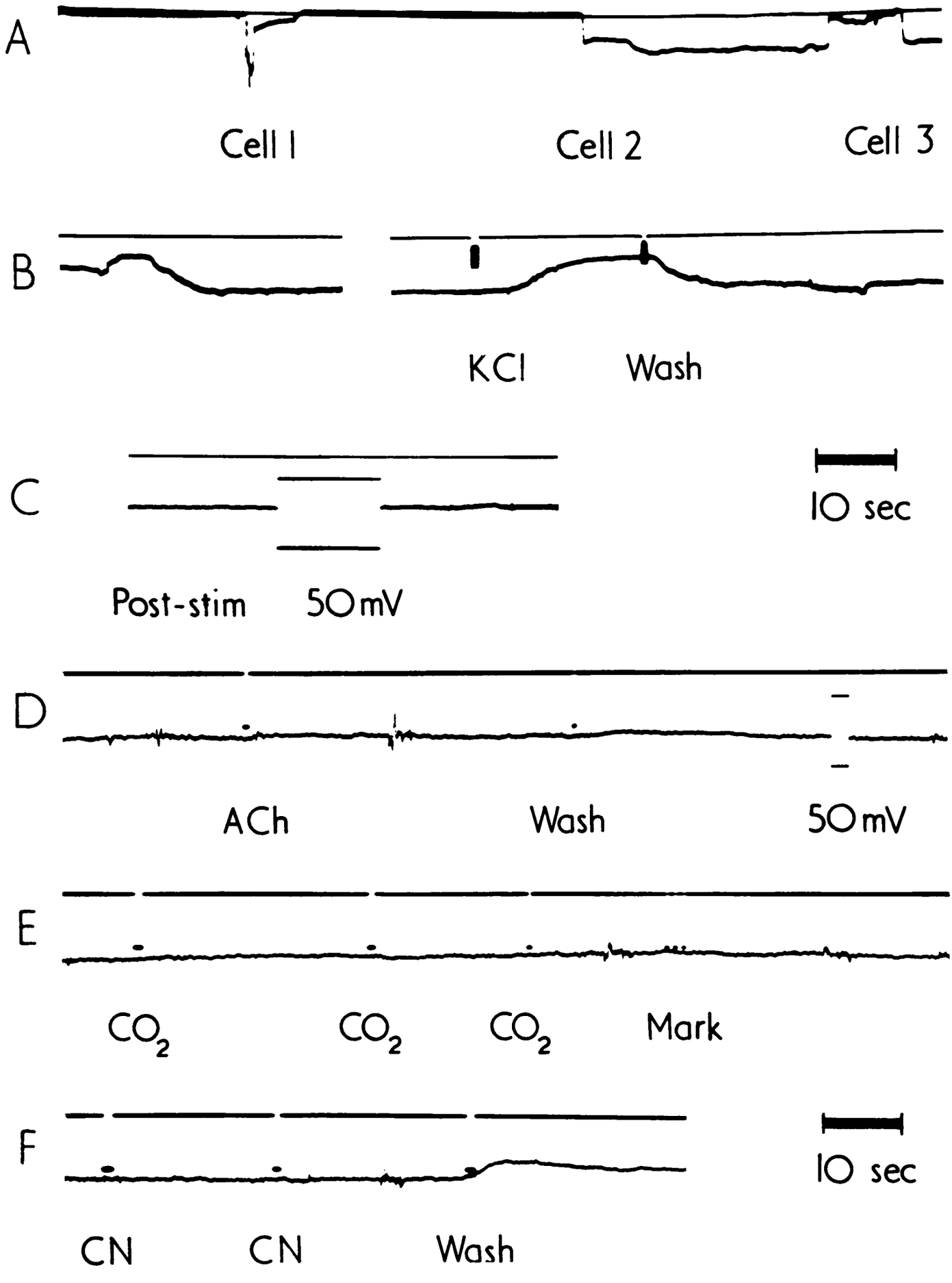

A
Cell 1
Cell 2
Cell 3
B
KCl
Wash
C
10 sec
Post-stim
50 mV
D
ACh
Wash
50 mV
E
CO_2
CO_2
CO_2
Mark
F
10 sec
CN
CN
Wash

Fig. 5: Intracellular recordings made with KCl-filled glass microelectrodes in the carotid body. Upper traces: zero baseline and signal markers; lower traces: voltages recorded by the microelectrode.

A-C: a discontinuous record during one drive into the carotid body. A: cell 1 was only held for a few seconds. On driving further cell 2 was found and the potential maximised, but it was lost also. Cell 3 was found shortly afterwards and (B, cont.) maximised. Note how the potential wanders around zero during the drive. After the break in B an injection of KCl (0.2 ml, 150 mEq/l.) into the flow caused depolarization readily reversed by speeding up the flow and washing the KCl clear. Only cell 3 was counted as a recorded cell. Between B and C the sinus nerve was stimulated at 6V, 1.5 msec, 20 Hz for one minute.

D-F: a continuous record of the application of various agents. Doses: 100 µg ACh, 20 µg NaCN, in 0.1 ml Ringer-Locke solution; CO_2 as 0.1 ml of Ringer-Locke equilibrated with 100% CO_2. The first wash (D) was by increasing the flow, the second (F) by an injection of 1 ml fresh superfusate. The apparent response to the latter is probably a consequence of a sudden change of fluid level - the potential did not return to its pre-injection level (cf. KCl above).

Voltage and time calibrations as indicated.

were made in the first few experiments to make it preferable to break some electrodes rather than risk further insult to the carotid body. Probably only the most superficial cells were penetrated but those nearer the centre of the organ were very likely dead as in vitro insufficient oxygen is supplied by diffusion in from the surface (Forster, 1968).

There was no way of avoiding artefactual tip potentials as the electrode drove into the connective tissue. Small tip potentials could be backed off with the calibrator unit. If the tip potentials became very large (above 20-30 mV) the electrode was withdrawn and its resistance checked. The electrode was also withdrawn if it was bending visibly. During a drive the base-line would wander $\pm$ 5 mV around zero, the criterion for penetration of a cell was that the potential changed suddenly on advancing the electrode. Further fine manipulations would usually maximise the potential (Fig, 5A - C) and it sometimes helped if, after a penetration that registered 10-20 mV, the table was given a sharp tap.

0.1 - 1.0 ml of KCl solution (150 mEq/l.) was injected into the flow and this caused a reversible depolarisation (Fig. 5B) only if the microelectrode had previously penetrated a cell by the

criterion of a sudden change of potential. All potentials recorded *in vitro* were tested in this way. If the KCl had no effect even though there had been a sudden potential change it was assumed that the electrode had been damaged and it was withdrawn and checked. KCl had no effect on the base-line potential if the electrode was free in the superfusate above the carotid body. The KCl test was difficult *in vivo* because muscular fasciculations dislodged the microelectrode, and also the blood flow through the carotid body stopped (as judged by simple inspection of the veins) presumably because of spasm of the vasculature. Nevertheless, on two occasions injections of KCl into the common carotid artery did cause a reversible depolarisation and, once this had been demonstrated, a sudden potential change was the sole criterion used that a potential was recorded intracellularly provided that, on withdrawing the electrode, there was a sudden change of equal and opposite polarity.

The carotid body remained responsive throughout the experiments as judged by recordings of afferent activity from single, few or multi-fibre strands of the sinus nerve. Activity increased in response to stoppage of the flow of superfusate or to injection of 100 μg NaCN into the flow. Stimulation of the main trunk of the

nerve whilst recording from a fine afferent slip occasionally caused depression of chemoreceptor activity (Neil & O'Regan, 1969a), but when this occurred it was profound and could well have been due to antidromic depression (Fidone & Sato, 1970; see also Section 4.3).

(On one particular occasion one or two stimulus pulses were enough to silence the discharge for a number of seconds. This observation was the first indication of a possible artefact in the stimulation technique although, with hindsight, I think it is probable that here the mechanism of the silence was block of conduction in the recorded fibre rather than antidromic depression (see Section 4.3, Figs. 3∝).)

3.3 Results

Membrane potentials of 15-60 mV (mean 27 mV) were recorded from 176 cells *in vitro*. The values *in vivo* were similar, 15-60 mV (mean 31 mV) from 24 cells. It is not known which cell types were penetrated but the Type I cells tend to occur in groups and are larger and rounder than the Type II cells. It is probable that the majority of observations were made on Type I cells. A cell could usually be held for over 10 minutes *in vitro* although the potential sometimes decayed rapidly after penetration. *In vivo* cells could

only be held for up to 3 minutes, the electrode was usually dislodged by the pulsations of the underlying carotid artery. Intracellular potentials were usually lost abruptly after an advance of 10-20 μ but occasionally the microelectrode was advanced 100 μ whilst holding a steady potential. This may have been due to physical displacement of the carotid body by the electrode, or else may have marked the passage of the electrode through a glomerulus of closely packed Type I cells.

Apparently normal resting potentials were seen on two occasions when the carotid body was cut across in vitro and the microelectrode inserted at the cut surface.

Agents were all dissolved in Ringer-Locke and injected into the flow in 0.1 ml of solution. The agents used were NaCN (20 μg or 100 μg), ACh (20 μg or 100 μg) and saline equilibrated with 100% CO_2. The carotid body was made asphyxic by stopping the flow. All these procedures excited chemoreceptor afferents but failed to affect the membrane potentials (Fig. 5 D-F).

Stimulation of the sinus nerve at intensities far in excess of the threshold for excitation of the C fibres had no effect. Transient potential changes were looked for by triggering an

oscilloscope from the stimulus pulse, but none were seen. Neither were any D.C. changes seen after prolonged stimulation, one minute at 10-20 pulses per second (Fig. 5 B-C).

3.4 Discussion

These findings confirm the results of Eyzaguirre and co-workers (Eyzaguirre & Lewin, 1961c; Eyzaguirre & Koyano, 1965c; Eyzaguirre, Leitner et al., 1970) on the size of the membrane potentials of carotid body cells and on the absence of effects of NaCN, ACh and asphyxia on these potentials. Yet all these stimuli increase the rate of chemoreceptor discharge and also cause a slow potential attributed by Eyzaguirre, Leitner et al. (1970) to the generator potential of the afferent terminals. Eyzaguirre's results are extended to show that hypercapnia and, in particular, that stimulation of the sinus nerve are without effect.

The arguments for there being an efferent pathway to the carotid body other than the sympathetic supply are presented in more detail elsewhere. The conclusion from the work reported here is that, if there is an inhibitory pathway from efferent nerves to the Type I cells and thence to the chemoreceptor afferent terminals, then its mechanism is unlike the inhibitions seen in other sensory

systems in that it does not involve any form of electrical transmission, whether synaptic, i.e. involving a transmitter, or ephaptic. Now of course it is feasible that a transmitter might work via a change in, say, Cl^- conductance and this would not be seen if the membrane potential of a given cell were approximately at the equilibrium potential of the Cl^- ion. However, the range of membrane potentials was large enough to invalidate this criticism. There is no answer to the criticism that a transmitter might operate without causing an alteration in a membrane potential, except that this has not been demonstrated anywhere else.

If an effect had been seen on stimulation of the sinus nerve, that might have been good evidence of the existence of a specific synaptic inhibitory mechanism; the negative results that were obtained are unfortunately only circumstantial evidence that there is no such inhibition. However it is interesting that Flock & Russell (1973) have demonstrated hyperpolarising potentials of 2-7 mV arising in hair cells during analogous stimulation of efferent fibres in the lateral line organ of burbot, and this stimulation causes inhibition of the afferents at the receptor

level (Russell, 1971a, b). Detweiler & Alkon (1973) showed inhibitory coupling between pairs of hair cells in the statocyst of a mollusc. The inhibition has characteristics of chemical transmission and occurs on mechanical stimulation of the hair cells. Some of the cells are also electrically coupled and both types of coupling occurred within a statocyst or between contralateral statocysts. Coupling was abolished by section of the nerve supplying the statocyst recorded from. Fillenz (personal communication: see Fillenz, 1973) has mentioned the similarities between the SIF cell and the Type I cell. This is a catecholamine-containing cell that was known by the earlier histologists as the small granular cell: SIF stands for "small, intensely fluorescent" cell (Eränko & Härkönen, 1965). The SIF cell is intermediate in many respects between the adrenal medullary cell and the post-ganglionic sympathetic neurone. They have been given a role as interneurones in ganglionic transmission but, unlike the analogous situation in the carotid body, there is electrophysiological evidence of this inhibition at the cellular level (Libet, 1970) and this is not mediated by changes in ionic conductances but is of longer latency and probably involves active transport and metabolic processes.

There is a second conclusion from the work with micro-electrodes. All the afferent fibres of the sinus nerve must have been fired antidromically during electrical stimulation as the stimuli were supramaximal for the 'C' fibres. Therefore the mechanism of antidromic depression seen in chemoreceptors (see Fidone & Sato, 1970; and Section 4.3) is not due to any known type of electrical or chemical coupling between the afferent nerve ending and the Type I cell.

(Some of the results and conclusions of this work have been published, see Goodman & McCloskey (1972)).

Chapter 4

Efferent Control and Stimulus Artefacts

4.1 Methods

Dissection and recording etc. were as in General Methods (see Chapter 2).

Electrical stimulation

Ag-AgCl stimulating electrodes were used. The set of electrodes consisted of 3 wires, the extra one could be earthed if this helped to reduce the size of the stimulus artefact in the recordings.

A constant voltage stimulator was used in most of the experiments in which afferent activity of single fibres was under study. The device delivered square pulses 0.06 - 10 msec in duration and up to 42 V in amplitude. The stimuli were isolated from earth. The stimulator could be triggered internally by an oscillator or externally from the time-base ramp of an oscilloscope (Tektronix type 502).

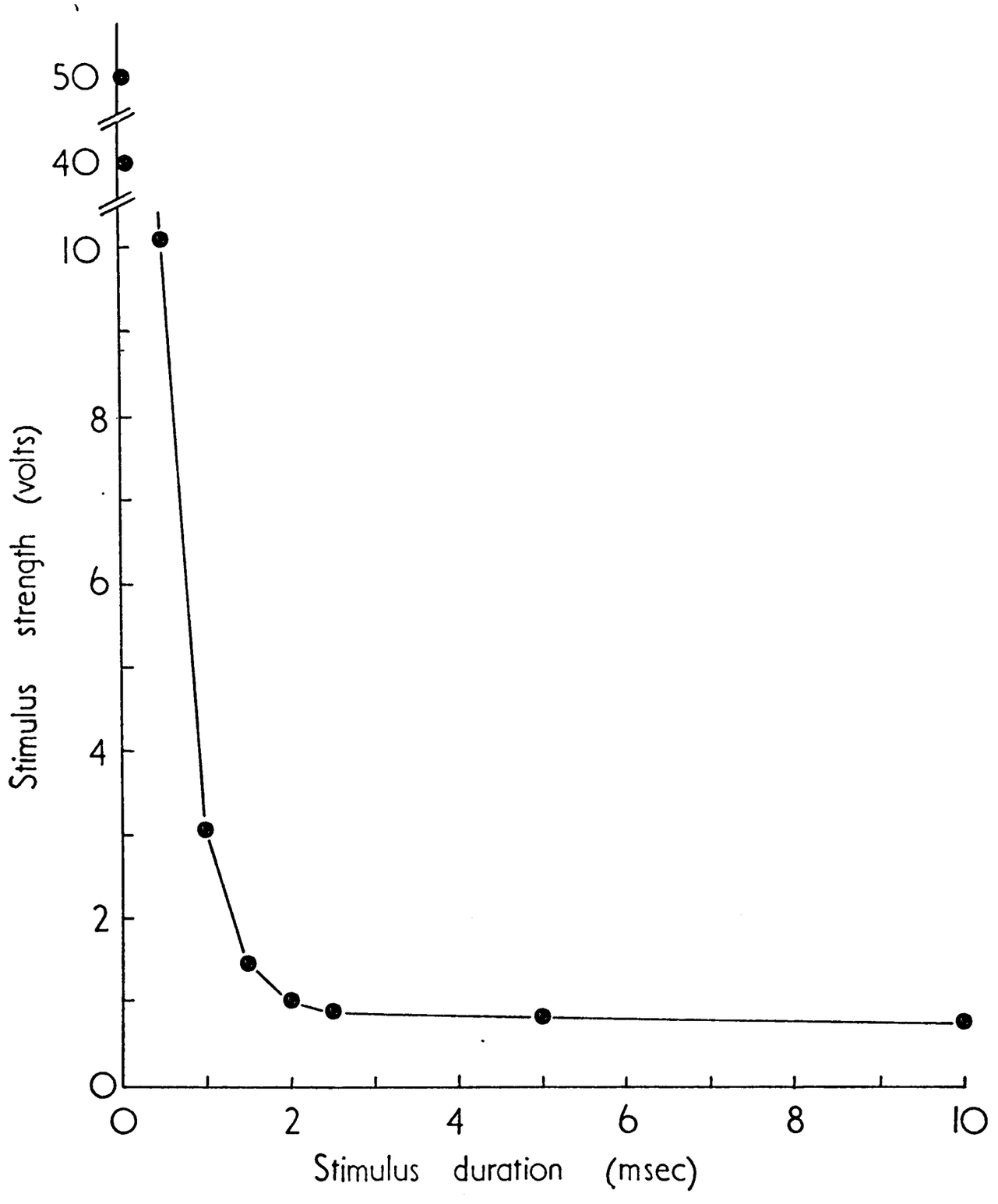


Fig. 6: The strength-duration curve for maximal excitation of the 'C' fibres of the carotid sinus nerve of the cat determined in vitro.

A constant-current stimulator was used in some of the later experiments on afferent activity and in the experiments with micro-electrodes. This was a more flexible device with more facilities for triggering and for delivering paired stimuli than the constant-voltage stimulator.

The actual voltage applied between the stimulating electrodes could be read off an oscilloscope trace connected across them. Fig. 6 shows the strength-duration relation of the 'C' fibres of the carotid sinus nerve constructed using the constant-current stimulator, with the current converted to voltage.

4.2 Investigation of stimulus spread

4.2.1 The model system

Results

If an electrical stimulus is applied to a nerve fibre a driven action potential is recorded from that fibre provided that the conduction distance from the point of stimulation to the recording electrode is long enough. If that distance is short the stimulus artefact will be large, and the conduction time may be so short

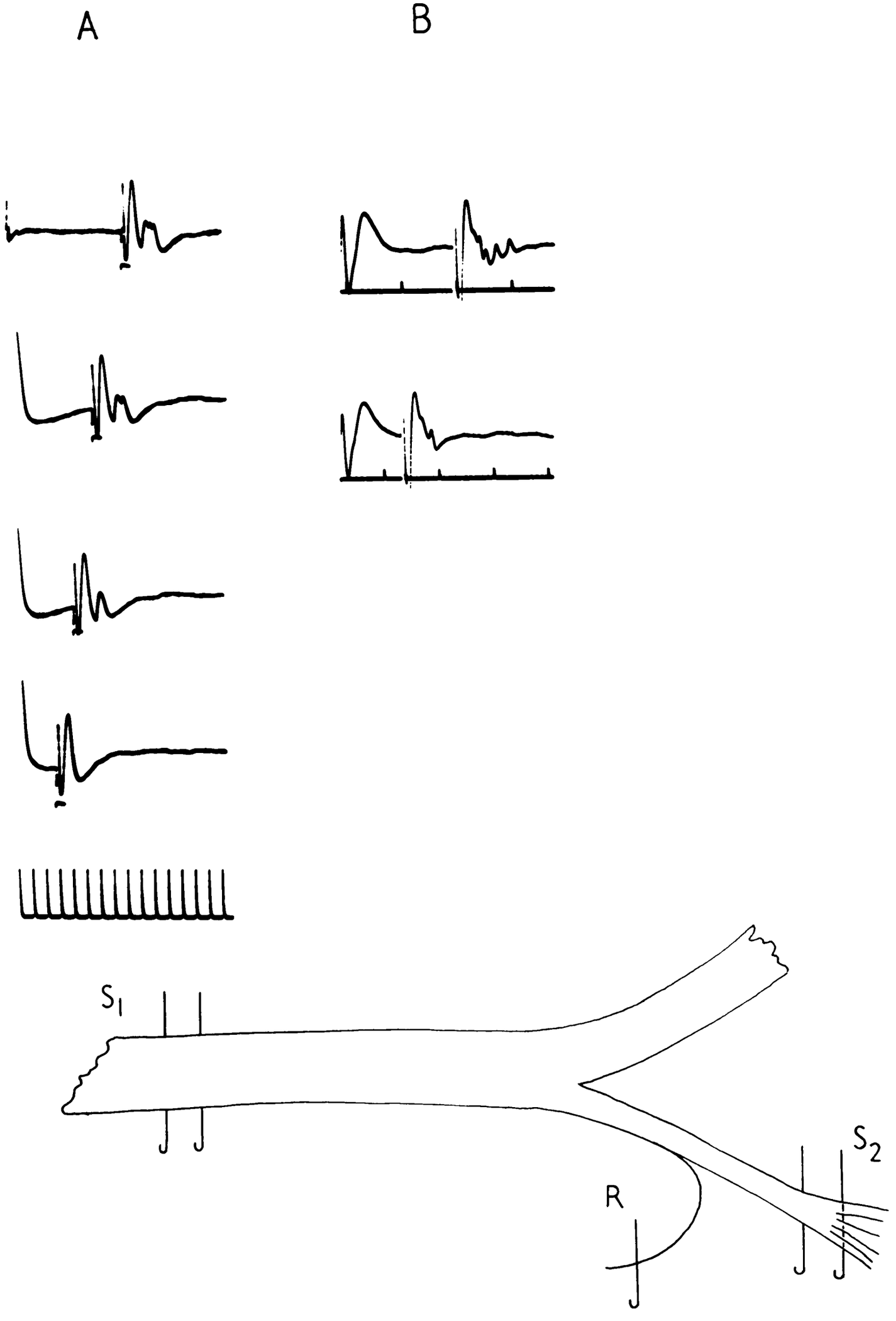

A
B
S1
S2
R

Fig. 7: The Model System, using an isolated segment of vagus in the neck. A few-fibre strand was peeled from a separated bundle of the vagus and laid over the recording electrode, R. The remainder of the bundle was laid over the stimulating electrodes, S_2. A second set of stimulating electrodes (S_1) was placed under the main trunk of the vagus low in the neck at the other end of the isolated segment. Stimulating at S_1 caused action potentials at R. Stimulating at S_2 had no effect unless the stimulus was large enough to cause excitation by stimulus escape of the fibre from which recordings were made when, with the appropriate interval between S_2 and S_1, the impulses driven by S_1 were abolished by collision.

A: each trace triggered from S_2; S_1 at bar under each trace. Reducing the interval between the stimuli abolished the action potentials driven by S_1. Time = 1 msec pulses.

B: another few-fibre strand. S_1 indicated by a break in the time trace (10 msec pulses).

Note that the impulses driven by S_2 are lost in the stimulus artefact.

Stimulus parameters: A: S_1 = 3 V, 1 msec; S_2 = 14 V, 1 msec

B: S_1 = 5 V, 0.5 msec; S_2 = 20 V, 0.5 msec.

that the driven action potential is lost in the stimulus artefact. The model system enabled the detection of excitation of fine strands of nerve in this type of situation by a variation of the well-known collision technique of Douglas & Ritchie (1957). The situation differs from that just described in that the point of excitation is remote from the point of application of the electrical stimulus.

The vagus was sectioned just caudal to the nodose ganglion and also low in the neck. The sheath of the vagus was opened high in the neck and a bundle of fibres of about the same size as the carotid sinus nerve was separated from the vagus. A fine slip was separated from this bundle and the electrodes arranged as in Fig. 7.

Stimulating at S_1 (1 - 5 V, 0.25 - 1.0 msec) caused driven action potentials to appear at the recording electrode. Stimulating at S_2 had no effect on these action potentials unless the stimulus was large enough to cause excitation by stimulus spread of the fibre from which the record was being made. In Fig. 7A, no action potentials can be seen to follow S_2 but a number follow S_1 at constant latencies. As the delay between S_2 and S_1 is decreased

these action potentials disappear. There are three humps visible in the top trace of Fig. 7A and by the bottom trace they have gone. Fig. 7B is another example of the same phenomenon.

This implies that S_2 was exciting or blocking the fibres in the fine slip of nerve even though the stimulating electrodes were not directly applied to them. The action potentials were lost in the stimulus artefact but, if the fibres were being excited by S_2 rather than blocked by it, the occurrence of these action potentials can be deduced: impulses driven by S_2 collided with the impulses driven by S_1 and stopped their propagation to the recording electrode. If this analysis is correct then, for each fibre in a slip, the delay from S_2 to S_1 over which extinction occurs should be equal to $(t + r)$ msec, where t is the conduction time from S_1 to the recording electrode and r is the refractory period of the fibre to S_1. The conduction times from S_1 were measured and the refractory periods of each fibre measured by supplying conditioning and test shocks at S_1. Values obtained in this standard way agreed with those obtained by collision with an impulse set up by stimulus spread from S_2.

A strength-duration relation was obtained for the fibre of lowest threshold in one of the studied slips to each of the stimuli S_1 and S_2. S_1 produced the classical inverse relation (Table 1). To determine the S_2 relation, S_1 was set above threshold and the S_2 - S_1 delay set so that collision would occur when S_2 excited the fibre. S_2 was increased until the impulse driven by S_1 failed to be recorded. The voltage threshold for S_2 was independent of the duration of the stimulus pulse for the range of durations 0.06 - 1 msec (Table 1).

Table 1: Voltages at threshold with varying stimulus duration to direct (S_1) and indirect (S_2) stimuli. Determined with the Model System.

Stimulus duration (msec)	S_1 voltage at threshold	S_2 voltage at threshold
0.06	2.0	14
0.25	0.6	14
0.5	0.3	14
1.0	0.2	14

Slight changes in recording conditions could alter the threshold to indirect excitation. If tissue fluid accumulated on the stainless steel plate below the paraffin pool the voltage threshold to S_2 increased. This was tested by placing drops of saline from a hypodermic syringe onto the steel plate while S_2 was just above threshold. This caused an increase in the threshold to S_2, i.e. the action potential driven by S_1 was seen at R, and the threshold reverted to its control value when the saline was removed with a moistened cotton wool wick.

A cotton wool pledget soaked in 2% lignocaine (cf. Neil & O'Regan, 1971a) was placed on the stimulated bundle of nerve between S_2 and the recorded slip. It was difficult to restrict its effect and the anaesthetic blocked the driven impulses in the recorded slip. A drop of lignocaine at a much greater dilution (0.01%), applied to the region where the slip left the stimulated bundle, raised the threshold to excitation by S_2 whilst not blocking the impulse driven by S_1. (Excess saline had not been removed).

Discussion

The indirect stimulus could excite the fibres in the recorded slip of nerve:

1. because they were branching fibres;

2. by ephaptic transmission at the cut S_1 end of the nerve (Granit, Leksell & Skoglund, 1944);

3. by evoking a synchronous volley in the nerve fibres surrounding them;

4. by some form of stimulus spread from S_2 to the recorded slip.

Branching fibres are unlikely. Unless all the fibres branched very near to the junction of the fine slip with the stimulated bundle of nerve, one would expect to see action potentials from at least some of the fibres driven by S_2 with a latency approaching, or even up to twice as great as, the latency between S_1 and the action potentials driven by it. This is because an impulse would have to travel from S_2 to the branching point and then back to R. This also rules out ephaptic transmission.

Myelinated axons can be facilitated or depressed by surrounding synchronous activity but this alone will not evoke an action

potential in an otherwise inactive axon (Rosenblueth, 1941; Marazzi & Lorente de No, 1944).

This makes stimulus spread to the fine strand the most likely possibility and this is compatible with the analysis of collision presented above. The strength-duration relation of the indirect stimulus (Table 1) means that it is not simply current spread as the voltage threshold is independent of the duration of the stimulus pulse. I suggest that the exciting stimulus is the capacitive current flowing in the recording system as the capacity between the signal leads and earth charges up or discharges. If the shortest pulse used, 0.06 msec, was far greater than the time constant of this system then it would not be possible to reduce this current except by reducing the capacity to earth.

The most probable site of excitation is at the boundary between the paraffin and the saline.

4.2.2 Baroreceptors

In the Model System an 'antidromic' impulse can be timed to abolish the 'orthodromic' one either by collision or by leaving the fibre refractory to the stimulus S_1. The effect of simple collision

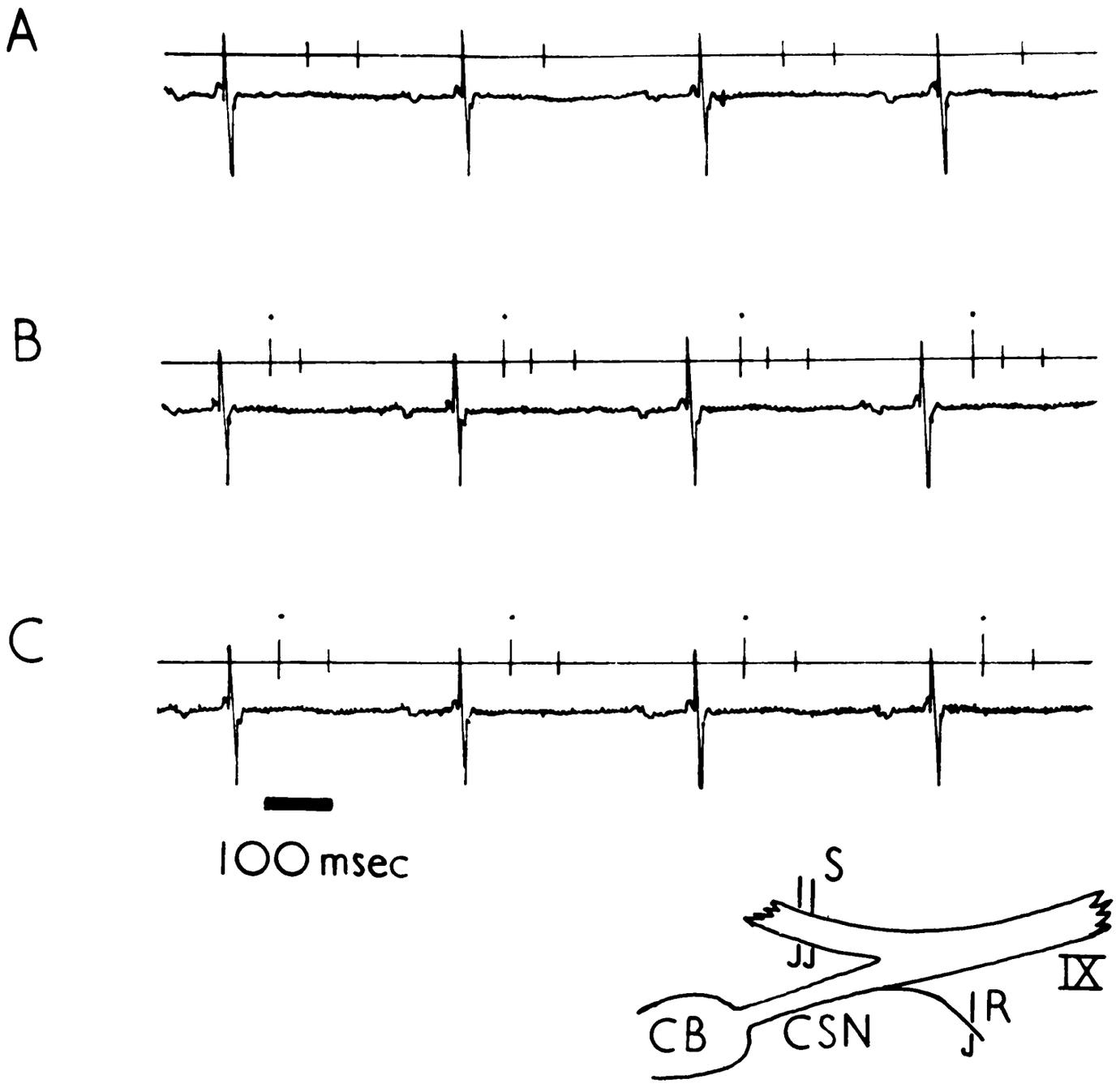

A
B
C
100 msec
S
JJ
IX
CB
CSN
JR

Fig. 8: Cat: nembutal. Recording from a single baroreceptor fibre (upper trace of each pair). CB = carotid body and bifurcation; CSN = carotid sinus nerve; IX = glossopharyngeal nerve; R = recording electrode; S = stimulating electrodes. Note that IX is cut both centrally and peripherally and S is applied to the cut distal end so that the stimulus is applied to a portion of nerve with an electrical, but not a neural, connection with the baroreceptor at R.

The stimulus is triggered with a constant delay from the ECG (the lower trace of each pair).

A: no stimulus. The first baroreceptor impulse occurs approximately 120 msec after the ECG.

B: stimulus on, 5 V 0.25 msec. The stimulus artefact is denoted by the mark above the trace. The first impulse still occurs at 120 msec.

C: stimulus now 10 V 0.25 msec. The first impulse is delayed and occurs 145 msec after the ECG. There has been adventitious excitation of the recorded slip of nerve and the resulting antidromic impulse has 're-set' the baroreceptor nerve ending.

(Records retouched).

with an antidromic impulse would be the same in a fibre excited naturally by a receptor, but, if the antidromic impulse reached the end of the fibre without colliding, there could be more complex effects on the receptor. Matthews (1933) showed that an antidromic impulse would reset the receptors in the mammalian muscle spindle. Antidromic and orthodromic impulses are equivalent in their effects on the excitability of mechanoreceptors (Lindblom, 1958; Burgess & Perl, 1973).

The close stimulation technique was set up using single baroreceptor fibres of the sinus nerve. The stimulus was a single pulse triggered from the ECG with a variable delay. The baroreceptor shown in Figs. 8 & 9 fired once or twice per systole: once at about 120 msec after the start of the QRS complex and, if there was a second impulse, again at about 190 msec. The stimulus that was used to excite the baroreceptor by stimulus escape was, in the experiment illustrated, applied to the cut peripheral end of the glossopharyngeal nerve distal to its junction with the sinus nerve (Fig. 8). It is highly improbable that there could have been a neural connection between the stimulated segment of nerve and a

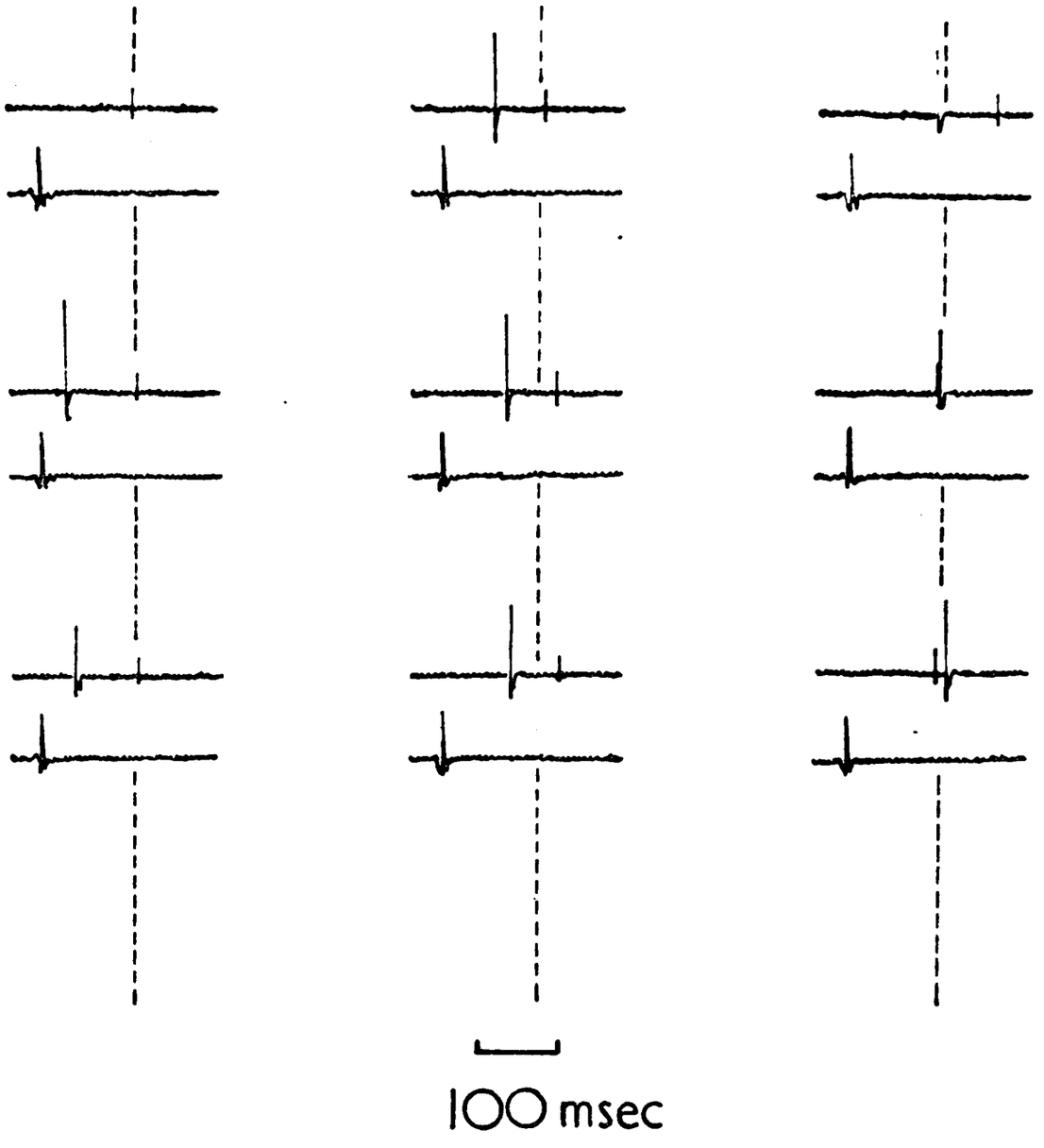


Fig. 9: Cat: nembutal. Activity from a single baroreceptor fibre of the sinus nerve. Stimulation of the cut distal end of the glossopharyngeal nerve (as in Fig. 8). The stimulus is triggered from the ECG (lower trace of each pair) at a varying delay. The dotted vertical lines indicate the approximate normal position of the baroreceptor impulse at about 120 msec after the ECG. Stimulus held constant at 14 V, 0.5 msec. Column 1 top: no stimulus. By second record of 2nd column stimulus causes resetting but in last two records of 3rd column impulse precedes stimulus and so is not affected by it. (Records retouched).

known baroreceptor fibre coursing in the sinus nerve to the recording electrode at R.

As the stimulus intensity was increased a threshold voltage was found at which the first baroreceptor impulse was delayed (Fig. 8C). Using a suprathreshold stimulus the first impulse was unaffected until the ECG - stimulus delay was more than 50-60 msec, after which the impulse became progressively more delayed as the ECG-stimulus delay was increased (Fig. 9). The time between the stimulus and the first impulse was never less than about 70 msec - the time between the first and second baroreceptor impulses. If the ECG-stimulus delay was more than about 120 msec then the first impulse was not affected. Thus, in this situation too, a stimulus can cause adventitious excitation of a recorded fibre. The resulting anitdromic impulse resets the baroreceptor ending and, like other mechanoreceptors (see above), antidromic and orthodromic impulses are equivalent. This particular baroreceptor had an 'excitation cycle' of about 70 msec which started about 50-60 msec after the ECG. An antidromic impulse reaching the receptor later than this time would reset the excitation cycle in the same way as does the first, orthodromic, impulse under normal circumstances.

4.2.3 Conclusion

A fine recorded slip of nerve can be excited by stimulus escape from a pair of stimulating electrodes applied to a nearby nerve trunk. This can be readily demonstrated in a Model System using an isolated segment of nerve and with afferent fibres whose receptors discharge predictably, and it is a reasonable assumption that the danger of adventitious excitation exists while experimenting with chemoreceptor fibres when, owing to the random nature of their discharge, one must use indirect methods to show it.

The implications of this work, and the means of monitoring for antidromic impulses in chemoreceptor fibres, are discussed in Section 4.3 (Paper I).

4.3 Paper I

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EFFERENT CONTROL OF ARTERIAL CHEMORECEPTORS MEDIATED BY GLOSSOPHARYNGEAL FIBRES AND ARTIFACTS INTRODUCED BY STIMULATION TECHNIQUES

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SUMMARY

1. In anaesthetized cats, stimulation of the cut carotid sinus nerve generally caused a reduction in discharge in afferent fibres peeled from the nerve distal to the stimulating electrodes, though this was somewhat variable. In four out of five fibres the inhibition was reduced or abolished by close intra-arterial injection of atropine. Only single fibres were used.

2. There was a risk of adventitious excitation of the afferent fibre by stimulus escape. Some of the features of this excitation and the ease with which it occurred were investigated using an isolated length of vagus, baroreceptor fibres and chemoreceptor fibres. Low concentrations of local anaesthetic could raise the threshold for adventitious excitation whilst not affecting the normal passage of impulses.

3. During stimulation of the efferent components of the sinus nerve, a continuous check for adventitious excitation was kept, utilizing the fact that chemoreceptor fibres show a minimum inter-spike interval of about 10 msec.

4. The true inhibition of discharge would seem to be vasomotor in origin.

INTRODUCTION

The chemoreceptors of the carotid sinus nerve respond to changes in P_{O_2}, P_{CO_2} and pH of the arterial blood but their responses to these stimuli can be modified by efferent nerve impulses to the carotid body. Floyd & Neil (1952) showed that sympathetic nerves have a facilitatory effect, probably by a vasoconstrictor mechanism (Daly, Lambertsen & Schweitzer, 1954). More recently a second, glossopharyngeal inhibitory effect on the chemoreceptors has been shown (Neil & O'Regan, 1971*b*)

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but it has been suggested that this effect is not of vasomotor origin (Neil & O'Regan, 1971*a*; Fidone & Sato, 1970; Biscoe, 1971). The present study suggests that this is a vasomotor inhibitory influence.

Biscoe & Sampson (1968) recorded centrifugal glossopharyngeal impulses from the carotid sinus nerve and Neil & O'Regan (1971*b*) showed that they are most easily evoked by excitation of the ipsilateral carotid body. These impulses appear to reduce chemoreceptor discharge, for the discharge in hypoxia of a chemoreceptor afferent dissected from the side of an uncut sinus nerve is increased if the sinus nerve is then cut proximally to the slip recorded from. Sampson & Biscoe (1970) reported similar findings.

To analyse this further, Neil & O'Regan (1971*a*) stimulated the sinus nerve electrically and showed two effects. At 2–7 V (without stimulus isolation) there was a rise in blood flow through the carotid body and this was abolished by an injection of atropine close intra-arterially. With the stimulus at 20–35 V (isolated) there was an inhibition of discharge in chemoreceptor fibres peeled from the nerve distal to the stimulating electrodes. This inhibition was not abolished by atropine but was abolished by placing a cotton-wool pledget soaked in 2 % procaine on the sinus nerve between the stimulating electrodes and the point at which the recorded afferent left the nerve. They concluded that they were stimulating efferent inhibitory fibres and that these were distinct from the vasomotor fibres.

Fidone & Sato (1970) repeated the stimulation experiments and showed that, at the intensities used by Neil & O'Regan (1971*a*) to depress discharge, it was possible to excite the afferent fibre by stimulus spread, and so cause a cumulative antidromic depression of the chemoreceptor ending. At lower intensities there was a real inhibitory effect but they ruled out the possibility of its being vasomotor on Neil & O'Regan's (1971*a*) evidence on the use of atropine.

Here, I extend Fidone & Sato's (1970) observations and draw attention to the likelihood of the occurrence of antidromic depression in Neil & O'Regan's (1971*a*) experiments. The findings have been briefly reported (Goodman, 1972).

METHODS

Twenty-two cats (1·8–2·7 g) were anaesthetized with pentobarbitone sodium (Nembutal, Abbott), 42 mg/kg I.P. and further doses of 2 mg/kg I.V. as needed. The trachea was cannulated, as were the femoral vein and either the femoral or lingual artery. Temperature was maintained at 37–38° C.

Dissection. For experiments on the vagus, the muscles of the mid line with the trachea, pharynx and oesophagus were retracted caudally. The carotid sinus nerve was approached laterally. This approach has the advantage that all dissection can be done remote from the carotid body and the risk of damaging its blood supply is minimized. It also yields a longer length of nerve than the medial approach. The

parotid gland was removed and the sternomastoid and digastric muscles separated from their insertions on the mastoid process. The transverse process of the atlas was cleared and nibbled down, and the overlying muscles removed; this allowed a wide working area. The tympanic bulla was nibbled away and finally a large lymph node lying over the carotid sinus region was removed, revealing the glossopharyngeal and carotid sinus nerves lying horizontally in the plane of focus of the microscope. The cervical sympathetic trunk was sectioned above and below the superior cervical ganglion, as were all the ganglio-glomerular nerves that could be identified.

Recording. The nerve to be used was covered in warm mineral oil and dissected on a stainless-steel plate by conventional methods. A pair of stainless-steel recording electrodes fed into a Tektronix type 122 preamplifier. The fine slip of nerve lay on the plate for a short distance after leaving the carotid sinus nerve and then lifted to reach the electrode. A cut length of nerve connected the second electrode to the steel plate through which the animal was earthed. Impulses were displayed on an oscilloscope (Tektronix type 502A) and fed to a loudspeaker, tape recorder (Thermionic T 3000) and ink-jet recorder (Elema-Schönander Mingograf type 24B). The tape recorded records could later be fed into a storage oscilloscope (Tektronix type 549) and photographs taken of stored sweeps.

Tests for chemoreceptor activity. These included reduction of discharge on a sudden change to breathing 100 % O_2, increase in mean frequency when breathing hypoxic gas mixtures, and a brisk response to a close intra-arterial injection of saline equilibrated with 100 % CO_2 (Black & Torrance, 1971).

Blood pressure was measured with an Elema-Schönander EMT 490A Transducer head connected to a type EMT 460 electro-manometer and recorded on a channel of a Mingograf.

Stimulation. The stimulating electrodes (Ag–AgCl) were applied to the cut carotid sinus nerve proximal to the point at which the recorded slip left the main trunk of the nerve (Fig. 1, top). The stimuli were square pulses 0·06–10 msec in duration, up to 42 V in amplitude. All stimuli were isolated from earth and the voltages reported are those actually applied to the nerve. Frequency of stimulation varied from single shocks to brief bursts of 90 Hz.

A Model System was used to study some of the characteristics of stimulus escape. The vagus was cut high and low in the neck and a stimulus (the 'direct' stimulus) applied through Ag–AgCl electrodes to the distal end of this isolated segment. A slip of nerve of similar diameter to the carotid sinus nerve was dissected away from the side of the vagus at the proximal end of the segment and a fine slip separated from this. The fine slip was placed on recording electrodes and the coarse slip, representing the main trunk of the sinus nerve, was placed on a second pair of stimulating electrodes which delivered the 'indirect' stimulus. The parameters of stimulation at the two sites could be varied independently. 'Direct' stimulation would set up driven impulses in the fine slip of nerve, i.e. equivalent to orthodromic impulses in the sinus nerve set up by receptors. The parameters of the 'indirect' stimulus could be altered to investigate how impulses set up by stimulus escape might affect these driven impulses by collision with them or by causing refractoriness at the distal electrodes.

Drugs used. Lignocaine hydrochloride (Xylocaine, Astra-Hewlett Limited), and atropine sulphate (British Drug Houses) dissolved in Ringer solution.

RESULTS

Throughout this study the positioning of the electrodes was as described in Methods and illustrated in Fig. 1.

Excitation of the recorded fibre

At relatively high stimulus intensities and with the stimulating electrodes close to the recording electrodes it is possible to cause excitation of the recorded fibre of the carotid sinus nerve (Fidone & Sato, 1970). It is not usually possible to see the impulses caused by this excitation because, owing to the short conduction distance in the recorded fibre, they are lost in the large stimulus artifact. Occasionally driven action potentials

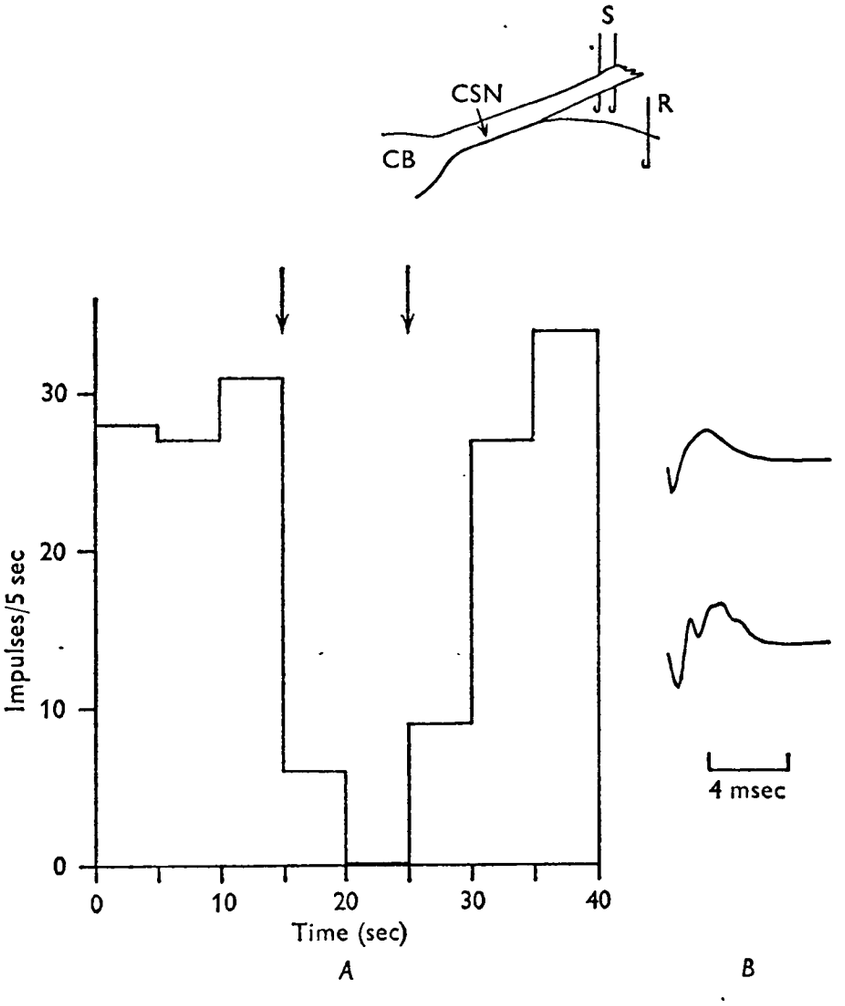

Fig. 1. Cat: Nembutal. Afferent activity recorded from a single chemoreceptor fibre of the sinus nerve. Stimulation of the sinus nerve as shown in the diagram (top). CB = carotid body, CSN = carotid sinus nerve, S = stimulating electrodes, R = recording electrode. (*A*) Histogram of afferent activity. At the first arrow the stimulus was increased from 5 V to 14 V (0·06 msec, 5 Hz). At the second arrow the stimulus was turned off. (*B*) Photographs of the tail of the stimulus artifact. Upper record: during stimulation at 5 V; lower record: during stimulation at 14 V. Note the appearance of ripples on the artifact given by impulses driven by the stimulus.

could be seen and correlated with a decrease in the frequency of firing of the afferent fibre (Fig. 1).

One needs to differentiate between the effects of antidromic impulses and of impulses arising in efferent fibres of the sinus nerve. Fidone & Sato (1970) showed that if a stimulus caused an antidromic impulse then there was a silent period following each stimulus before the next spontaneous impulse, and they used this silent period as a monitor of the occurrence of antidromic impulses. This method has been used throughout the work reported here. If excitation by stimulus spread were to occur, sweeps

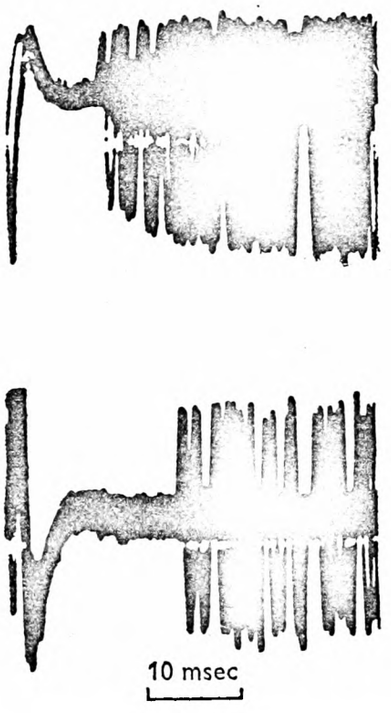

Fig. 2. Cat: Nembutal. Photographs of superimposed sweeps on the face of a storage oscilloscope. In the upper record the sweeps were triggered by spontaneously occurring action potentials and show that the shortest intervals are about 10 msec. Lower record: different fibre during stimulation (15 V, 0·06 msec, 5 Hz) of the sinus nerve with the electrodes arranged as in Fig. 1. Sweep triggered by the stimulus. The presence of a silent period after the stimulus indicates that the stimulus was causing excitation of the afferent fibre.

triggered from the stimulus and stored on a storage oscilloscope would gradually build up a picture of a silent period immediately following the stimulus (Fig. 2). This was so with both the carotid sinus and aortic depressor nerves. The over-all fall in the frequency of discharge seen during a train of stimuli causing a silent period was so large that it could not be accounted for simply by the time 'lost' after each stimulus. However, it was not possible by altering the intensity of the stimulus to dissociate the

phenomenon of the silent period from that of the precipitous fall in discharge and so presumably this fall was due to an effect of the antidromic impulses on the nerve ending over and above simple collision and refractoriness. This is termed antidromic depression (Fidone & Sato, 1970).

In general I found that the threshold for adventitious excitation of the afferent was of the order of 14–20 V, although in some preparations excitation and consequent depression was observed at 6–7 V (Fig. 4*e*). In some experiments no excitation was obtained using the maximal stimulus intensity of 42 V. Occasionally a fibre would be affected during one period of stimulation but not during another apparently similar one a few minutes later. As Fidone & Sato (1970) point out, the variation is very likely due to changes in the current paths to earth. These changes were not thoroughly investigated here, although in some experiments earthing an electrode between the stimulating electrodes and the carotid body would prevent excitation of the afferent fibre when previously excitation had been occurring. The occurrence of adventitious excitation was unaffected by cutting the sinus nerve at a point between the stimulating electrodes and where the recorded fibre left it. At this point the nerve lay on the dissecting plate and so there was a conduction path through the thin layer of saline on the plate providing an electrical connexion between the stimulating electrode and the fibre slip. However, all neural connexions between the stimulating electrodes and the carotid body had been severed by cutting the nerve.

It was noticed when investigating the thresholds for adventitious excitation of fibres of the carotid sinus nerve that the pulse duration of the stimulus did not seem to have any effect. The Model System (see Methods) was used to define this more clearly. The strength–duration relation for excitation of a vagal fibre by stimulus escape with the apparatus I used showed that the voltage was independent of the duration over the range 0·06–1·0 msec in contrast to the classical strength–duration curve for excitation by direct stimulation through electrodes applied to the same fibre within the vagal trunk. This implies that adventitious excitation is due to the flow of a capacitative current and is interesting in view of Neil & O'Regan's (1971*a*) comment that 'alteration of the pulse duration from 0·5–5·0 msec did not modify the depression caused by stimulation' even though these authors claimed to be studying the effect of direct stimulation of efferent fibres of the sinus nerve.

The Model System was also useful for investigating the effect of local anaesthetic on adventitious excitation. A cotton-wool pledget soaked in 2 % lignocaine was placed on the stimulated portion of the nerve between the stimulating electrodes and the recorded slip (cf. Neil & O'Regan, 1971*a*). It was difficult to restrict its effect and the anaesthetic blocked

the driven impulses in the recorded slip. A drop, at the greater dilution of 0·01 %, applied to the region where the slip left the stimulated nerve raised the threshold to adventitious excitation whilst not blocking con-

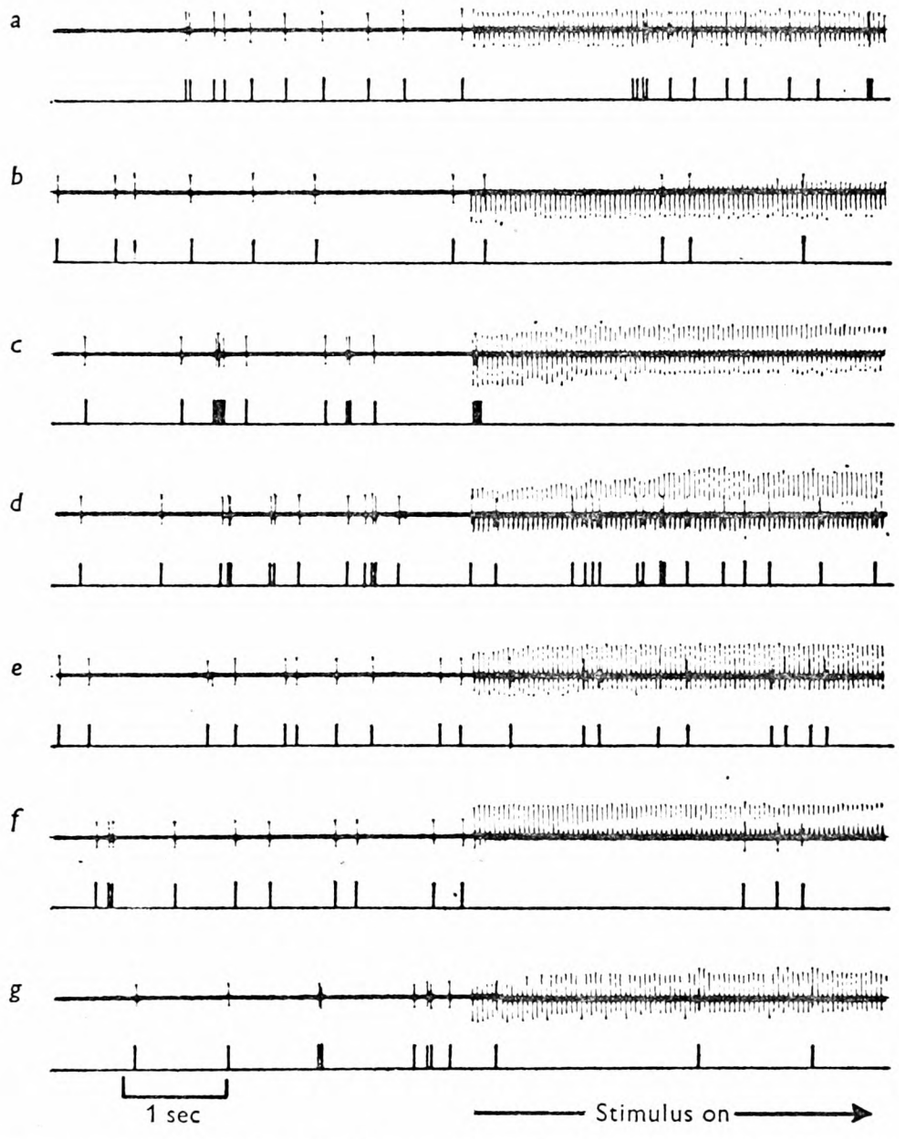

Fig. 3. Cat: Nembutal. Afferent activity recorded from a single chemoreceptor fibre of the sinus nerve. Stimulation of the same nerve, as in Fig. 1. Each record shows the 4 sec immediately before and after the start of stimulation at 21 Hz. The occurrence of an impulse is indicated by the key below each experimental record. The large deflexions on the experimental records are the stimulus artifacts. (*a–c*) Before lignocaine. Discharge is unaffected in (*a*) (5 V) when there was no silent period following each stimulus. (*b*) 15 V, threshold for an effect on discharge. (*c*) 20 V, there was now a silent period, and it is correlated with a precipitous fall in discharge. (*d–g*) After the application of 0·001 % lignocaine. The discharge is now unaffected by 15 V (*d*) or 20 V (*e*) but is affected by 25 V (*f*), which caused silent periods. (*g*) During recovery from the lignocaine, 20 V.

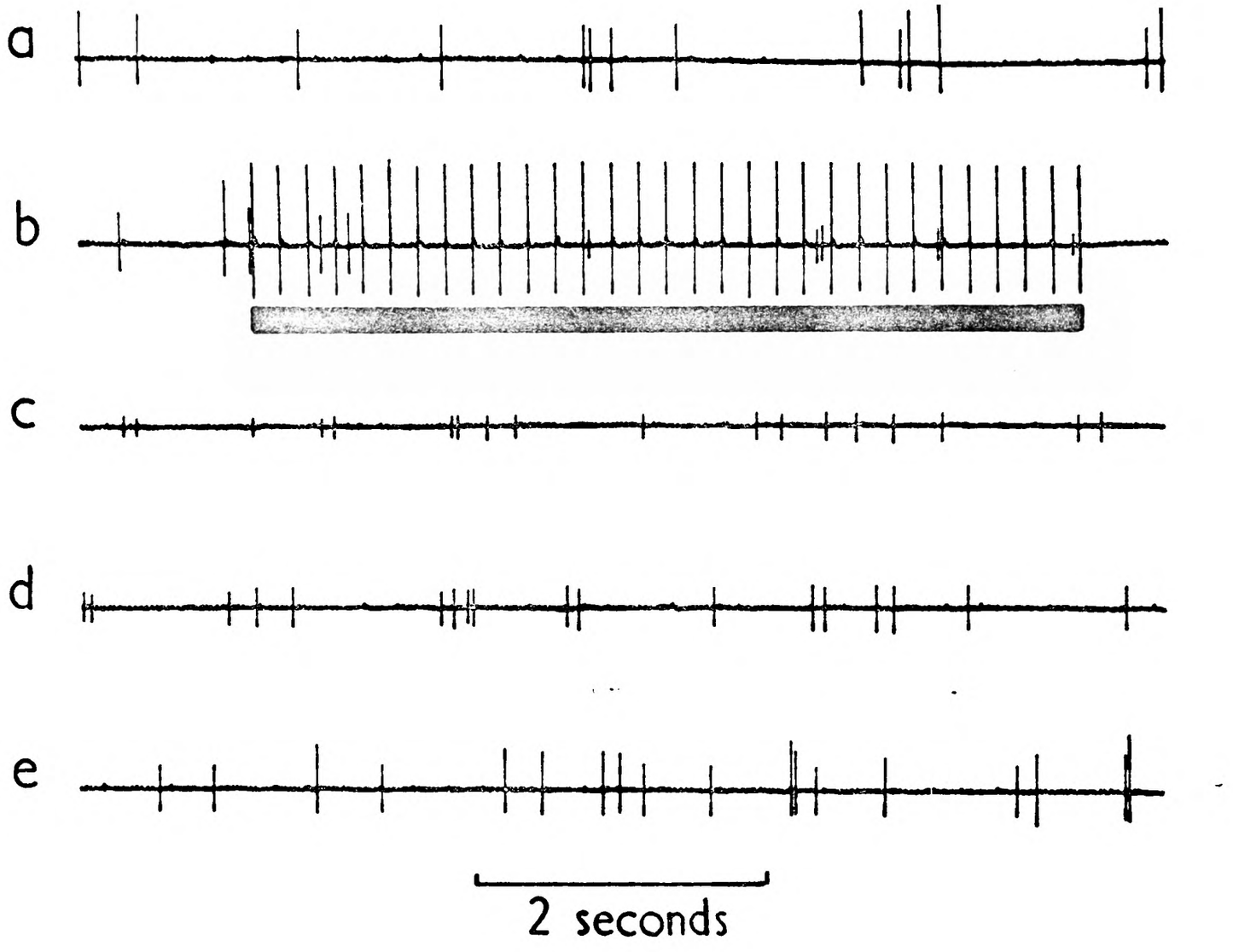

Fig. 3α : Cat: nembutal. a-e: continuous recording from a few-fibre preparation of chemoreceptors from the sinus nerve, probably containing 3 fibres. Stimulation of the same nerve, as in Fig. 1, at 14 V 0.06 msec 5.2 Hz as indicated by the bar under b.

Note the reduction in amplitude of the recorded nerve impulses caused by the stimulus and the gradual recovery over c-e. The apparent frequency of discharge is reduced over the period of stimulation but it is not possible to say whether the real frequency has been reduced as conduction may be completely blocked in one of the fibres of the preparation. Records retouched.

duction of impulses driven by the direct stimulus (excess saline had been removed).

The use of local anaesthetic on the carotid sinus nerve gave similar results. In Fig. 3 a drop of lignocaine in a concentration of only 0·001 % raised the threshold to excitation by stimulus spread whilst orthodromic impulses were not affected.

Neil & O'Regan (1971*a*) could not show any disturbance of the firing of baroreceptors of the sinus nerve when recording from baroreceptor fibres and stimulating the main trunk of the sinus nerve. I, in contrast, found that, if the stimulus to the main nerve was set to be triggered from the e.c.g. at a variable delay, it could reset the timing of the baroreceptor impulses if it was applied shortly before the first impulse of a burst would normally occur. A longer-term effect of antidromic depression of baroreceptors could only be produced by stimulation at high frequency; a brief burst at 90 Hz would usually depress baroreceptor firing for the next few heart cycles.

Impairment of conduction in the recorded fibre

In some fibres, large stimuli caused the amplitude of recorded impulses to fall, gradually recovering after cessation of the stimulus. Sometimes stimulation caused complete block of conduction. That this was not due to nerves moving on the recording electrodes was checked visually. Neither was it due to an alteration in recording conditions as the noise level remained the same. (See Extra Fig - Fig. 3a).

Conduction failure probably occurred in fibres in which the safety factor for conduction was already low. A break-down of conduction in fibres subjected to large stimuli has been reported by Laporte & Bessou (1959) and attributed to reversible dielectric break-down.

True effects on chemoreceptors of stimulating the sinus nerve

True efferent inhibition was studied in fourteen single chemoreceptor fibres of the carotid sinus nerve and one of the aortic depressor nerve. The stimulus was set below the threshold for exciting the afferent fibre and a strict watch was kept to check that there was no silent period after each stimulus as mentioned above. This was necessary *throughout the observations* because in some experiments some unnoticed change would lead to excitation of the afferent fibre when a few minutes previously there had been no such excitation (Fig. 4*e*).

All stimuli were at 20 Hz, the frequency at which Fidone & Sato (1970) reported that inhibition was maximal. The discharge frequency of the fibres used was in the range 2–5 impulses/sec. Fibres firing at a higher frequency than this were not used as the real inhibition is overcome by

an increase in discharge (Fidone & Sato, 1970). Occasionally the firing of a fibre was reduced to this level by the administration of 30 % O_2 in N_2.

Impulses were counted over the minute or half-minute before (control), during and after stimulation. For any change in chemoreceptor activity to be termed as inhibition or facilitation there had to be a change of at least 10 % in the mean impulse frequency over that period relative to the control period. The irregularity of the discharge of chemoreceptor afferents did not allow of any greater certainty.

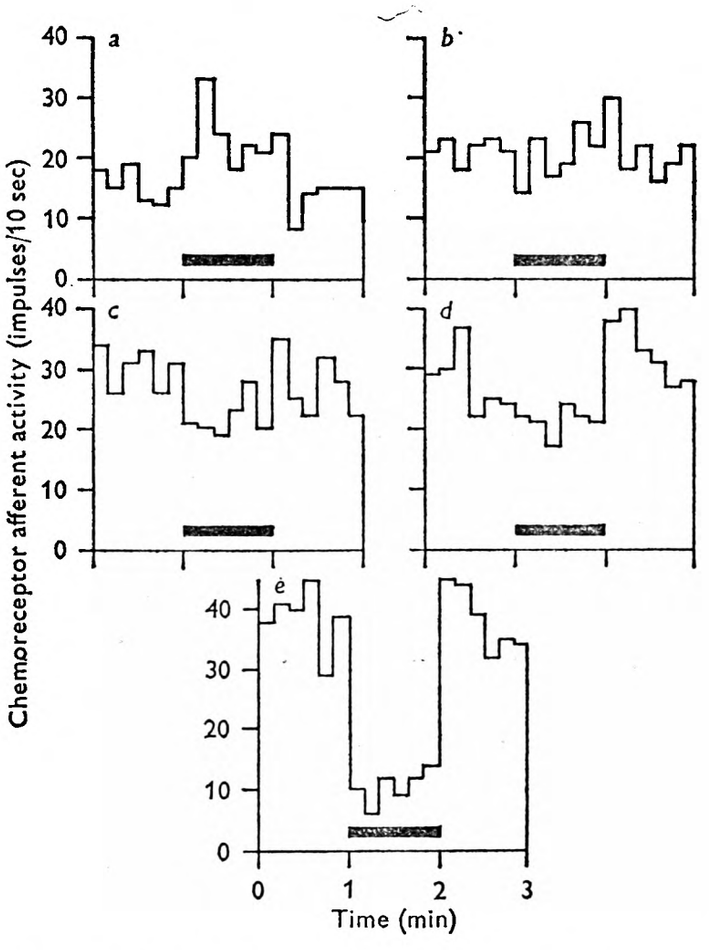

Fig. 4. Cat: Nembutal. Histograms of afferent activity recorded from a single chemoreceptor fibre of the sinus nerve. Stimulation of the same sinus nerve as in Fig. 1. All histograms refer to the same fibre showing changes in response on raising the stimulus intensity. Stimulus pulses 1 msec duration and delivered at 20 Hz. Periods of stimulation indicated by bars under middle portions of the histograms. (*a*) Stimulus = 5 V; number of impulses during stimulation = 150 % of control, after stimulation = 99 % of control. (*b*) 5 V; during = 95 %, after = 107 %. (*c*) 7 V; during = 72 %, after = 91 %. (*d*) 7 V; during = 76 %, after 118 %. (*e*) 7 V; during = 27 %, after = 99 %. There was adventitious excitation of the afferent during stimulation, as shown by the silent period, in (*e*) only.

Seven of the fourteen fibres of the carotid sinus nerve showed an inhibition of discharge on each occasion that a suitable stimulus, subthreshold for adventitious excitation of the afferent, was applied. One such fibre is illustrated in Fig. 4(*c–d*). The mean value for this inhibition was 28 %, i.e. to 72 % of control value. The largest genuine inhibition seen was 51 %, i.e. to 49 % of control, but this was only seen in one fibre. The mean frequency of discharge in the period after cessation of stimulation was 98 % of control. The effects of the real inhibition rarely gave very obvious changes in discharge as can be seen from Figs. 4–6.

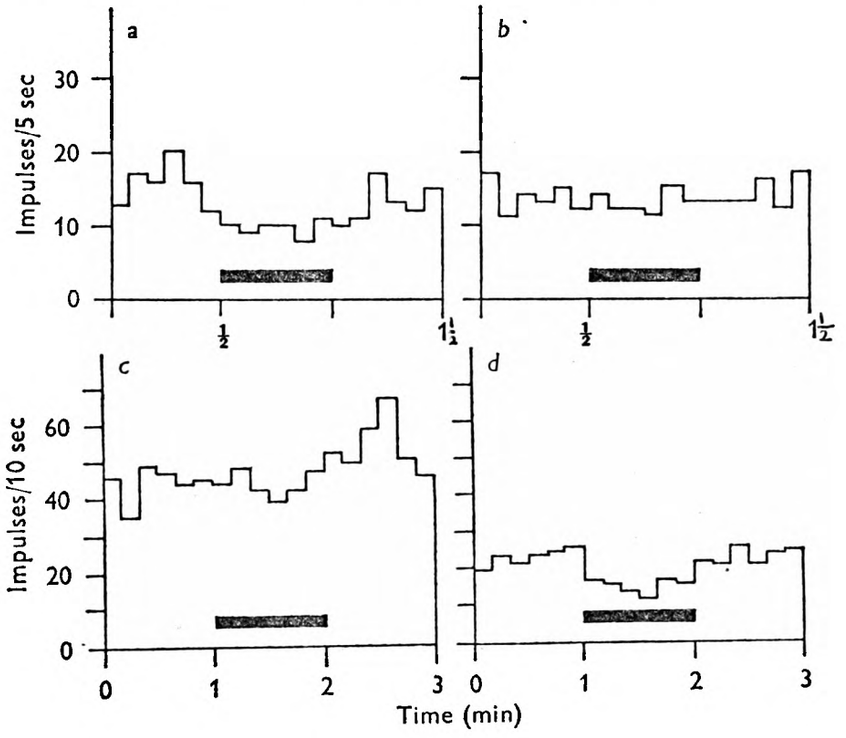

Fig. 5. Cat: Nembutal. Histograms of afferent activity recorded from single chemoreceptor fibres of the sinus nerve. Stimulation of the same nerve, as in Fig. 1. Each histogram refers to a different fibre showing the varying responses to stimulation. Stimulus pulses 1 msec duration and delivered at 20 Hz. Periods of stimulation indicated by bars under middle portions of the histograms. (*a*) Stimulus = 6 V; number of impulses during stimulation = 62 % of control, after stimulation = 83 % of control. (*b*) Stimulus = 7 V; during = 94 %, after = 102 %. (*c*) 5 V; during = 99 %, after = 121 %. (*d*) 6·5 V; during = 64 %, after = 98·5 %.

Four others of the fourteen fibres gave variable responses even though, for each fibre, the same stimulus was used on each occasion. These four fibres were stimulated a total of fourteen times: on six occasions there was inhibition, on seven no change greater than 10 %, and on one occasion a facilitation of discharge. The mean rate of discharge during stimulation was 92 %, and in the period after stimulation 106 %, of control. The

remaining three fibres showed no effect of stimulation (during: 97 % of control; after: 104 % of control).

Fig. 5 shows responses to stimulation of four different fibres. The fibre in Fig. 5(*a*) was stimulated only once and showed a fall in discharge; that in Fig. 5(*b*) showed no change in three periods of stimulation; that in Fig. 5(*d*) was stimulated four times, always showing a fall in discharge. The fibre in Fig. 5(*c*) usually showed an inhibition but on the occasion illustrated gave no response during stimulation and then the frequency of discharge increased after the stimulus had been turned off.

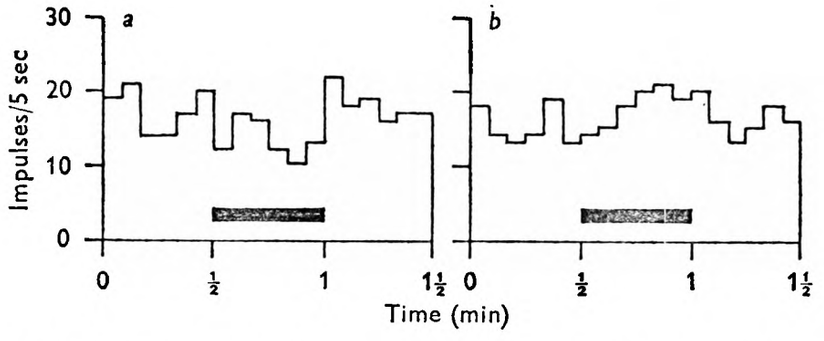

Fig. 6. Cat: Nembutal. Histograms of afferent activity recorded from a single chemoreceptor fibre of the sinus nerve. Stimulation of the same nerve, as in Fig. 1. Both histograms refer to the same fibre and show the effect of a close-arterial injection of 1 mg atropine on the response to stimulation. Stimulus parameters: 6·5 V, 1 msec, 20 Hz. Periods of stimulation indicated by bars under middle portions of the histograms. (*a*) Before atropine; number of impulses during stimulation = 76 % of control, after = 104 %. (*b*) After atropine; during = 118 %, after = 108 %. There was no adventitious excitation during either of these periods.

Five of the seven fibres that consistently showed inhibition were tested after a close intra-arterial injection of 1 mg atropine. With four of the fibres atropine reduced or abolished the inhibition. Before the administration of atropine, stimulation reduced the discharge of these fibres to an average frequency that was 70 % of that in the control period, the frequency recovering to an average of 95 % of control in the period following stimulation. After atropine, the average frequency was 96 % of control during stimulation, and 104 % of control in the period following. Fig. 6 shows a fibre in which atropine reversed the effect of stimulation so that it became a facilitation of discharge. The effect of atropine on the inhibition started to wear off quite quickly and a second dose would be needed to maintain the effect during a subsequent stimulation 10 min later.

A feature of these experiments was the variability of changes in impulse frequency. Two fibres showed an increase in frequency if stimulated at 4–5 V and a decrease if stimulated at 7 V (Fig. 4). One fibre showed no

change during stimulation but a slight increase in impulse frequency after (Fig. 5*c*). It was as if there were two effects of stimulation, one inhibitory and the other excitatory, and atropine brought out the excitatory effect.

The time course of the response to stimulation varied. The most common was a fall in frequency in the first 15 sec of stimulation followed either by a levelling off or a gradual rise in frequency over the rest of the period of stimulation.

Only one fibre from the aortic depressor nerve was studied and the main depressor nerve was stimulated only once, causing a fall to about 80 % of control in the discharge of the fibre. Atropine was not used.

It was apparent that fibres used in these experiments did not survive very well. The size of the potentials recorded from fibres fell and the greatest number of periods of stimulation that a fibre survived was 5 (three fibres) before the signal to noise ratio fell and made further study impractical. This happened over about half-an-hour, but without stimulation chemoreceptor fibres can easily survive for 1–2 hr or even longer. Thus it seems that even when frank excitation of the recorded fibre does not occur the stimuli do nevertheless affect the nerve fibre in some detrimental way.

DISCUSSION

Fidone & Sato (1970) showed, as it is here confirmed, that stimulating the carotid sinus nerve can exert two effects on the afferent activity in a fine slip of nerve peeled off the main nerve distal to the stimulating electrodes: a physiological inhibition and, at higher intensities, an antidromic depression. These two phenomena are entirely separate and there are two points at issue.

(*a*) What is the mechanism of the inhibition of chemoreceptors mediated by efferent fibres of the glossopharyngeal nerve?

(*b*) What is the likelihood of excitation of the afferent fibre by stimulus spread in this situation and what controls should one use to check for its occurrence?

Mechanism of the inhibition

Biscoe & Sampson (1968) showed that the carotid sinus nerve carries sympathetic and glossopharyngeal efferents. Neil & O'Regan (1971*b*) showed that the latter could inhibit chemoreceptor discharge.

Fidone & Sato (1970) took Neil & O'Regan's (1971*a*) evidence on the use of atropine to show that the inhibition which they had found and which was mediated by C-fibres was not vasomotor. However, it is possible that Neil & O'Regan (1971*a*) were studying the effect of atropine on antidromic depression rather than on the true inhibitory effect (see below).

In the present work, atropine was administered whilst checking that adventitious excitation of the afferent, and hence antidromic depression, was not occurring. Atropine blocked the inhibition. This reinstates the possibility that inhibition is vasomotor.

The variability of the chemoreceptor response to stimulation of the efferents in the present study is suggestive of vasomotor effects. The change from a facilitation to an inhibition of discharge on raising the stimulus, the reversal of the effect of stimulation by the administration of atropine, and the time course of the effects, all correspond to the effects reported by Neil & O'Regan (1971*a*) on blood flow through the carotid body. For, as Neil & O'Regan (1971*a*) point out, stimulation of the sinus nerve will excite the sympathetic and the glossopharyngeal efferent fibres running within it (Biscoe & Sampson, 1968) so that the resulting change in blood flow and hence impulse frequency will depend on a balance between the facilitatory vasoconstriction and the inhibitory vasodilatation.

If the nerve endings are affected by the local changes in blood flow one could explain why some afferent fibres showed no effect on stimulation. This is akin to the effect of stimulating the cervical sympathetic on the discharge of chemoreceptors. Whereas multifibre preparations show an increase in impulse traffic, single fibres do not give so consistent a picture. Some fibres may double their impulse frequency whilst others may show no change at all (D. I. McCloskey &. J. H. Mitchell, unpublished). Fidone & Sato (1970) do not say whether they found any fibres whose discharge was unaffected by stimulation of the sinus nerve.

If the sympathetic facilitatory effects on chemoreceptors are through a vasoconstrictor mechanism it would be surprising if a set of vasodilator fibres did not have the opposite effect. It could be argued that both vasomotions on stimulation of efferent nerves to the carotid body are the result of metabolic changes within it produced by non vasomotor efferents. Such an explanation is consistent with Purves' (1970) claim that the sympathetic reduces the oxygen uptake of the carotid body, and with Mills & Jöbsis' (1972) suggestion from the study of the state of NAD in the carotid body that sinus nerve efferents increase metabolism. It should be borne in mind, however, that the A:V difference of the carotid body is tiny and the control of its blood flow is thus unlikely to be dictated by changes in its metabolism. Also, changes in P_{a,O_2} do not affect $\dot{V}_{O_2}$ in the denervated carotid body.

The stimulus parameters used to elicit the real inhibition are similar to those used by Fidone & Sato (1970) – about 6 V, 1 msec. Neil & O'Regan (1971*a*) found such a stimulus gave vasodilation. Their stimulus was not isolated, but for stimulating the length of nerve on the stimulating electrode an isolated stimulus will be more effective than a non-isolated one

of nominally equal voltage. They imply in the Methods section of their report on the stimulation experiments (Neil & O'Regan, 1971*a*) that a smaller stimulus will be necessary in the non-isolated situation 'due to the increase in the field of excitation'. This would only be so if the structures they were actually stimulating were some distance from the stimulating electrodes and so needed current spread to excite them. However, they specifically state that the stimulated portion of the nerve did conduct impulses even after prolonged bouts of stimulation. It seems reasonable to say, then, that their stimulus was similar to that which causes the fall in the frequency of discharge due to the real inhibition.

Likelihood of adventitious excitation

Neil & O'Regan (1971*a*) maintained that they had not caused adventitious excitation, and hence that they were not looking at antidromic depression because of their observations with local anaesthetic, because they showed baroreceptors to be unaffected by stimulation (their Fig. 8) and because when using the aortic depressor nerve the stimulating electrodes were 2–3 cm from the recorded afferent.

The examples of inhibition that they publish are far more profound than those reported by Fidone & Sato (1970) and here for the true inhibition. Local anaesthetic correctly applied and suitably restricted should certainly block conduction in the efferent fibres, but Neil & O'Regan (1971*a*) show procaine blocking a deep inhibition, as if the situation were like that reported here in which lignocaine raised the threshold to adventitious excitation.

To affect baroreceptors it is necessary to put in single stimuli triggered from the e.c.g. and thus show re-setting of discharge, for baroreceptors seem not to be as prone to cumulative antidromic depression as are chemoreceptors. Neil & O'Regan's (1971*a*) published record shows only the application of a train of stimuli unrelated to the e.c.g. and applied at 5 Hz. The time scale is not large enough to detect re-setting.

Although increasing the distance between the sets of electrodes will reduce the chance of excitation of the afferent fibre, I have seen instances of such excitation when working on vagal afferent fibres and aortic depressor fibres with the stimulating electrodes at a distance of 2–3 cm from the recording electrodes.

To be sure of the validity of results obtained in these stimulation experiments it is absolutely necessary to check *all the time* that excitation of the afferent fibre is not occurring by looking for the silent period following each stimulus. It is likely that in some of Neil & O'Regan's (1971*a*) experiments there was no adventitious excitation, but for the reasons given above I suspect that excitation was occurring in the majority of the

published records. The important point is that unless one has checked for excitation one cannot say that it has not occurred. Also single fibres must be used as it is possible for only one fibre in a multifibre preparation to be affected by either antidromic depression or conduction block whilst the others are unaffected.

It could be argued that the silent period seen when excitation has occurred was nevertheless due to a short-lasting inhibition mediated by slowly conducting fibres in the sinus nerve. This is unlikely for the following reasons. (1) Fidone & Sato (1970) showed that the silent period could be elicited by stimulating the sinus nerve distal to the point at which the recorded slip left the nerve. Thus the stimulus was being applied directly to the recorded slip and in these circumstances the threshold for showing a silent period was far lower, corresponding to the threshold of the chemoreceptor A-fibres to direct stimulation. (2) When one uses the aortic depressor nerve the silent period begins immediately after the stimulus. If it were an inhibition mediated by slow fibres, because of the longer conduction distance, one would expect some spontaneous impulses before the inhibition set in. (3) A silent period could still be produced when the stimulus was being applied to a cut length of the sinus nerve having no nervous connexion with the carotid body and, in this situation, the voltage threshold for antidromic depression was unchanged.

What is the reason for the silent period following adventitious stimulation? If one studies the pattern of discharge of fibres from the carotid sinus nerve, one never sees interspike intervals of less than 7 msec (Biscoe & Taylor, 1963: circulation of the carotid body intact). Thus if a chemoreceptor fibre is excited electrically to conduct antidromically, and the antidromic impulse, on reaching the terminal, leaves it in the same state as does an orthodromic impulse, no spontaneous impulse should be recorded after this stimulus for at least 7 msec plus the time for conduction of the antidromic impulse down to the ending and conduction of the first spontaneous orthodromic impulse back to the recording electrode, i.e. $(2t+\mathrm{r})$ msec. If there is collision the silent period should be at least r msec, the minimum interval between impulses, but this should be rare.

This analysis of the silent period differs from that of Fidone & Sato (1970), who explained it in terms of the relative refractory period of the chemoreceptor *fibres* which they determined away from the nerve endings by the classical double-shock technique.

The site of adventitious excitation was probably somewhere near to the junction between the recorded fibre and the rest of the nerve, most probably at the saline-paraffin boundary.

If Neil & O'Regan (1971*a*) were not studying antidromic depression, and they were not looking at the inhibition studied by Fidone & Sato

(1970) and myself, then the alternative explanation is that a third mechanism was involved in their experiments.

Neil & O'Regan's (1971*a*) suggestion that the efferent control of the carotid body is not a vascular one fitted well with the suggestion (Biscoe, Lall & Sampson, 1970) that the large nerve endings on the type I cells of the carotid body are efferent. De Castro (1928) had originally claimed that they were afferent. However, the present work questions whether there is any but a vasomotor efferent control of the carotid body. Furthermore, Goodman & McCloskey (1972) failed to find evidence that the synapses on the cells of the carotid body altered the membrane potentials of the cells, and Hess & Zapata (1972) have recently confirmed De Castro's (1928) original suggestion that the nerve endings on the cells are afferent and so contradict Biscoe *et al.* (1970). Thus De Castro's (De Castro & Rubio, 1968) view of the carotid body would appear to be sustained, except for his as yet untested suggestion that the glossopharyngeal efferents to it hold its blood flow constant in the face of a change of blood pressure (De Castro, 1951).

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REFERENCES

BISCOE, T. J. (1971). Carotid body: structure and function. *Physiol. Rev.* **51**, 437–495.

BISCOE, T. J., LALL, A. & SAMPSON, S. R. (1970). Electron microscopic and electrophysiological studies on the carotid body following intracranial section of the glossopharyngeal nerve. *J. Physiol.* **208**, 133–152.

BISCOE, T. J. & SAMPSON, S. R. (1968). Rhythmical and non-rhythmical spontaneous activity recorded from the cut central end of the sinus nerve. *J. Physiol.* **196**, 327–338.

BISCOE, T. J. & TAYLOR, A. (1963). The discharge pattern recorded in chemoreceptor afferent fibres of the cat carotid body with normal circulation and during perfusion. *J. Physiol.* **168**, 332–344.

BLACK, A. M. S. & TORRANCE, R. W. (1971). Respiratory oscillations in chemoreceptor discharge in the control of breathing. *Resp. Physiol.* **13**, 221–237.

DALY, M. DE B., LAMBERTSEN, C. J. & SCHWEITZER, A. (1954). Observations on the volume of blood flow and oxygen utilization of the carotid body of the cat. *J. Physiol.* **125**, 67–89.

DE CASTRO, F. (1928). Sur la structure et l'innervation du sinus carotidien de l'homme et des mammifères. Nouveaux faits sur l'innervation et la fonction du glomus caroticum. *Trab. Lab. Invest. biol. Univ. Madr.* **25**, 331–384.

DE CASTRO, F. (1951). Sur la structure de la synapse dans les chemocepteurs: leur mécanisme d'excitation et rôle dans la circulation sanguine locale. *Acta physiol. scand.* **22**, 14–43.

DE CASTRO, F. & RUBIO, M. (1968). The anatomy and innervation of the blood vessels of the carotid body and the role of chemoreceptive reactions in the autoregulation of the blood flow. In *Arterial Chemoreceptors*, ed. TORRANCE, R. W., pp. 267–276. Oxford: Blackwell.

FIDONE, S. J. & SATO, A. (1970). Efferent inhibition and antidromic depression of chemoreceptor A-fibres from the cat carotid body. *Brain Res.* **22**, 181–193.

FLOYD, W. F. & NEIL, E. (1952). The influence of the sympathetic innervation of the carotid bifurcation on chemoreceptor and baroreceptor activity in the cat. *Archs int. Pharmacodyn. Thér.* **91**, 230–239.

GOODMAN, N. W. (1972). Efferent control of arterial chemoreceptors. *J. Physiol.* **225**, 31–32*P*.

GOODMAN, N. W. & MCCLOSKEY, D. I. (1972). Intracellular potentials in the carotid body. *Brain Res.* **39**, 501–504.

HESS, A. & ZAPATA, P. (1972). Innervation of the cat carotid body: normal and experimental studies. *Fedn Proc.* **31**, 1365–1382.

LAPORTE, Y. & BESSOU, P. (1959). Modifications d'excitabilité de motoneurones homonymes provoquées par l'activation physiologie de fibres afférentes d'origine musculaire du groupe II. *J. Physiol., Paris* **51**, 897–908.

MILLS, E. & JÖBSIS, F. F. (1972). Mitochondrial respiratory chain of carotid body and chemoreceptor response to changes in oxygen tension. *J. Neurophysiol.* **35**, 405–428.

NEIL, E. & O'REGAN, R. G. (1971*a*). The effects of electrical stimulation of the distal end of the cut sinus and aortic nerves on peripheral chemoreceptor activity in the cat. *J. Physiol.* **215**, 15–32.

NEIL, E. & O'REGAN, R. G. (1971*b*). Efferent and afferent activity recorded from few-fibre preparations of otherwise intact sinus and aortic nerves. *J. Physiol.* **215**, 33–47.

PURVES, M. J. (1970). The role of the cervical sympathetic nerve in the regulation of oxygen consumption of the carotid body of the cat. *J. Physiol.* **209**, 417–431.

SAMPSON, S. R. & BISCOE, T. J. (1970). Efferent control of the carotid body chemoreceptor. *Experientia* **26**, 261–262.

Chapter 5

Postscript to the Studies on Efferent Control

Inside the black box: what is the chemoreceptor?

The interpretations that physiologists have made of their results have always been influenced, not surprisingly, by the current morphological thoughts. Eyzaguirre, in his papers published between 1961 and 1965, held that the Type I cell was the sensor releasing acetylcholine to excite the nerve endings. This conformed with de Castro's histology (see de Castro & Rubio, 1968). Mills & Jöbsis (1972) did their spectrophotometry at a time when apparently de Castro had been disproved by Biscoe, Lall & Sampson (1970). Stimulating the sinus nerve electrically at a perfusate P_{O_2} of 7-9 torr caused oxidation of NADH, i.e. stimulation was affecting the high affinity cyt.a_3. As the large nerve endings, which they took as efferent, were in 'synaptic' contact only with the Type I

cells, they assumed that these were the cells containing the high affinity cyt.a_3. By exclusion, the Type II cells contained the low affinity cyt.a_3. In their view the Type I cells were metabolic cells in a pathway controlling the environment of the sensors, which were the Type II cells. They proposed that the chemical signal could be converted to an electrical one by the release of K^+ ions from the Type II cell to excite the afferent nerve fibres. Paintal (1967) had also suggested the idea of a metabolic cell and a sensor cell, and saw the Type II cell exciting the afferent fibres by a mechanoreceptive process (Paintal, 1971).

The re-establishment of the fat nerve endings as afferent (Hess & Zapata, 1971), plus our evidence that stimulation of the sinus nerve does not affect the membrane potentials recordable from the carotid body (see Chapter 3), means that Mills & Jöbsis (1972) will have to propose that the same nerve ending is both afferent and efferent. The same is true for the picture given by Kobayashi (1971b) of 'afferent-type' and 'efferent-type' synapses, as there appears to be only one type of nerve ending on the Type I cell (Biscoe & Pallot, 1972). Hess suggested that the sensory

terminals might also be motor to the Type I cells in the discussion of Eyzaguirre & Zapata's (1968c) paper at the Wates symposium.

Some form of dual function of the sensory endings with the possible release of a substance from them could explain the sensitivity of chemoreceptor afferents to antidromic stimulation, though there is still the problem that antidromic impulses are apparently more potent in producing depression of chemoreceptor endings than are orthodromic impulses. This is only my subjective impression: surprisingly low rates of antidromic stimulation frequently silenced chemoreceptor discharge, and the silence could outlast the stimulation by a number of seconds. However, I was stimulating the recorded afferent within the main trunk of the sinus nerve, which means that I was stimulating many of the other afferents and possibly those vasomotor efferents with lowish thresholds. It would be better to study the effects of antidromic depression in chemoreceptor afferents by using a single electrode that could function as either a stimulating or recording electrode to stimulate only the fibre from which one is recording.

I saw re-setting in baroreceptors as did Matthews (1933) in the muscle spindle. Although Lindblom (1958) showed that orthodromic

and antidromic impulses were equivalent in producing depression of the slowly-adapting mechanoreceptor of the skin that he was studying, he also showed a more long-term depression than simple re-setting following an impulse, so a more long-term depression in chemoreceptors would not be a unique phenomenon.

Biscoe (1971), following the idea that the large nerve endings were efferent, proposed that the high $\dot{V}_{O_2}$ of the carotid body was due to the high metabolic rate of the fine afferent nerve endings in maintaining their resting potential. Biscoe & Taylor (1963) had suggested that the random discharge of chemoreceptors could be due to thermal noise, and this needed very fine endings. On present evidence this would mean that the chemoreceptor afferent fibres became very fine before widening out to form the terminals on the Type I cells. If this fine length of fibre were the area where impulses were generated, then the fat nerve ending could act to modify the discharge of the generator region, or again it could be an 'efferent' terminal because of bidirectional propagation of impulses.

Eyzaguirre, Leitner et al. (1970) recorded a graded 'generator potential' from the carotid body, but intracellular work has not

revealed what structure is responsible. Perhaps the technique of inserting microelectrodes into tissue slices will be more helpful (Fidone, O'Leary & Eyzaguirre, 1971).

Mitchell, Sinha & McDonald (1972) regenerated the cut carotid sinus nerve into connective tissue remote from the carotid sinus region and recorded chemoreceptor activity from fibres of the regenerated nerve although no Type I cells were seen in the resulting neuroma. It seems, then, that the nerve fibres themselves are able to respond to the adequate stimuli of chemoreception. This phenomenon is not unknown (Chalazonitis & Arvanitaki, 1970). O_2 and CO_2 could act directly in competitive interaction as ligands at sites on the cell membrane. Lahiri & Delaney (1973) suggest a conformational change at a receptor site to explain the O_2-CO_2 interaction, but this is only a brief statement and they do not indicate if this is an independent conclusion or whether they were aware of the type of work reviewed by Chalazonitis.

However, Zapata, Hess & Eyzaguirre (1969) regenerated other sensory fibres into the denervated carotid body, and they too claimed to have recorded responses typical of chemoreceptors. The regenerated nerve terminals contained vesicles. They only approached

but were not applied to, the Type I cells. The Type I cells looked normal.

If the results of Mitchell's and Eyzaguirre's groups are both valid one must suppose that the nerves are specific, but that there is also a trophic influence exerted by a structure in the carotid body on the regenerating terminals. Although a chemoreceptor discharge is recordable in either circumstance it is not entirely normal unless both nerve and carotid body tissue are present. Further experiments are needed.

Yates et al. (1970) found that the decrease in density of the vesicles in the Type I cells seen on stimulating the sinus nerve was blocked by atropine, i.e. that there was a cholinergic link between the large nerve endings and the Type I cells. Mitchell & McDonald (1973) take this even further. They envisage a complex situation in which the afferent fibres form excitatory-inhibitory reciprocal synapses with the Type I cells, which themselves form synapses with one another. 50% of the Type I cells are 'interneurones' that are not innervated by glossopharyngeal fibres but some of these are innervated by sympathetic fibres.

I have some objections to Mitchell's model. He postulates a rapidly developing inhibition transmitted between structures in the glomus, but no electrical activity can be detected (Chapter 3). He supposes that transmission is cholinergic between the Type I cell and the afferent ending and dopaminergic in the reverse direction, and the whole process results in a profound inhibition; but acetylcholine excites the chemoreceptors. My major objection is that he bases much of his theory on experiments involving electrical stimulation of the sinus nerve and could well be studying antidromic depression.

If it is accepted that the large nerve endings are the afferents, and yet the Type I cells have no direct role in the generation of impulses, one is faced with the question of their function.

Christie (1933) suggested an endocrine role for the carotid body and this theory has surfaced again in recent years. The vesicles in the Type I cells may not contain catecholamines but a peptide. Pearse has claimed the Type I cell as one of his APUD series (Pearse, 1969 - Table 1). Helpap (1970) followed the uptake of labelled phenylalanine by the Type I cells and found them more like endocrine cells than vegetative or spinal ganglion cells.

Tramezzani et al. (1971) suggested that erythropoietin was produced by the carotid body but their results have not been confirmed (Hansen et al., 1973; Gillis & Mitchell, 1972; Sampson et al., 1972).

It is not possible to give a definite answer to the question that heads this chapter - what is the chemoreceptor? To construct a view of the carotid body must mean rejecting some of the evidence because so much of it is directly contradictory. That being said, I can give my personal view of what would be the most useful working hypothesis for the moment. It is at the fat nerve endings that the electrical part of the process of chemoreception starts and the Type I cells, whilst they may exert some influence on the endings, are primarily something else, for example part of a neuroendocrine system. Perhaps it is their influence that makes the nerve fibres into chemoreceptors during embryological development. It is possible that the afferent nerve endings release something in the course of their excitation, and this may affect the Type I cells. The Type II cells enclose the whole Type I cell-nerve ending complex and they could, like the glia they represent, be cells regulating the environment of the chemoreceptors. There is no

direct efferent synaptic control of the process of chemoreception, but local blood flows, controlled by sympathetic and parasympathetic nerves, are important at least in experiments in the anaesthetized cat.

Foreword to the study of oscillations

O False and treacherous Probability,
Enemy of truth, and friend to wickednesse;
With whose bleare eyes opinion learnes to see,
Truth's feeble party here, and barrennesse.

First verse of Sonnet ciii by
Fulk Greville, Lord Brooke.

Chapter 6

Oscillations in Chemoreceptor Discharge

6.1 Introduction

The purpose of the study was to look for and characterise oscillations in the probability of discharge of single chemoreceptor fibres that might occur as the gas tensions change over the respiratory cycle.

The discharge of the single fibres was recorded on tape together with a record of the ventilatory airflow and the raw data analysed at a later date using a digital computer.

Section 6.2 deals with the methods not described already.

Section 6.3 is a paper accepted for publication by Respiration Physiology. It is retyped here complete in its accepted form but the references are listed in the main list at the end of the thesis. There are three additional figures, and the

numbering of the figures has been altered to accommodate them (see the note on the title page). There is a link between the two topics of the thesis in this paper. One section deals briefly with the effect of efferent fibres on the oscillation, and, in another, the pattern of discharge of an efferent fibre is reported.

Section 6.4 is a reprint of a paper already published in Brain Research.

Section 6.5 is a short note of the relevance of the results to the discussion at present going on amongst respiratory physiologists on the role of oscillations in discharge in the control of ventilation.

6.2 Methods

6.2.1 Blood gases

In some experiments arterial P_{O_2}, P_{CO_2} and pH were measured from 1-2 ml samples taken from the arterial cannula. The gas electrodes were mounted in thermostated cells and all measurements made at 38°C. All the equipment was Radiometer (O_2: type E 5044; CO_2: type E 5030).

In the oxygen electrode oxygen diffuses from the blood sample across a membrane and is reduced at the platinum O_2-sensitive cathode generating a current that is measured. The CO_2 electrode measures the pH of a bicarbonate solution into which the CO_2 diffuses. The electrodes were calibrated with gas mixtures the tensions of which had been accurately measured with a Lloyd-Haldane apparatus.

The equipment was switched on for a number of hours before use to ensure stability. It was always obvious if an electrode was damaged because it would not give constant readings on calibration runs. The membranes were replaced at the first suspicion of malfunction.

The equipment was in a different part of the laboratory. Samples were transferred in heparinised syringes immersed in a beaker of ice. There was usually another group using the equipment at the same time and the samples were left in the ice until use: this was never longer than 5 minutes.

6.2.2 Computing

The tape recorder (Thermionic T3000) allowed 3 channels of data to be recorded, with the fourth channel used for vocal

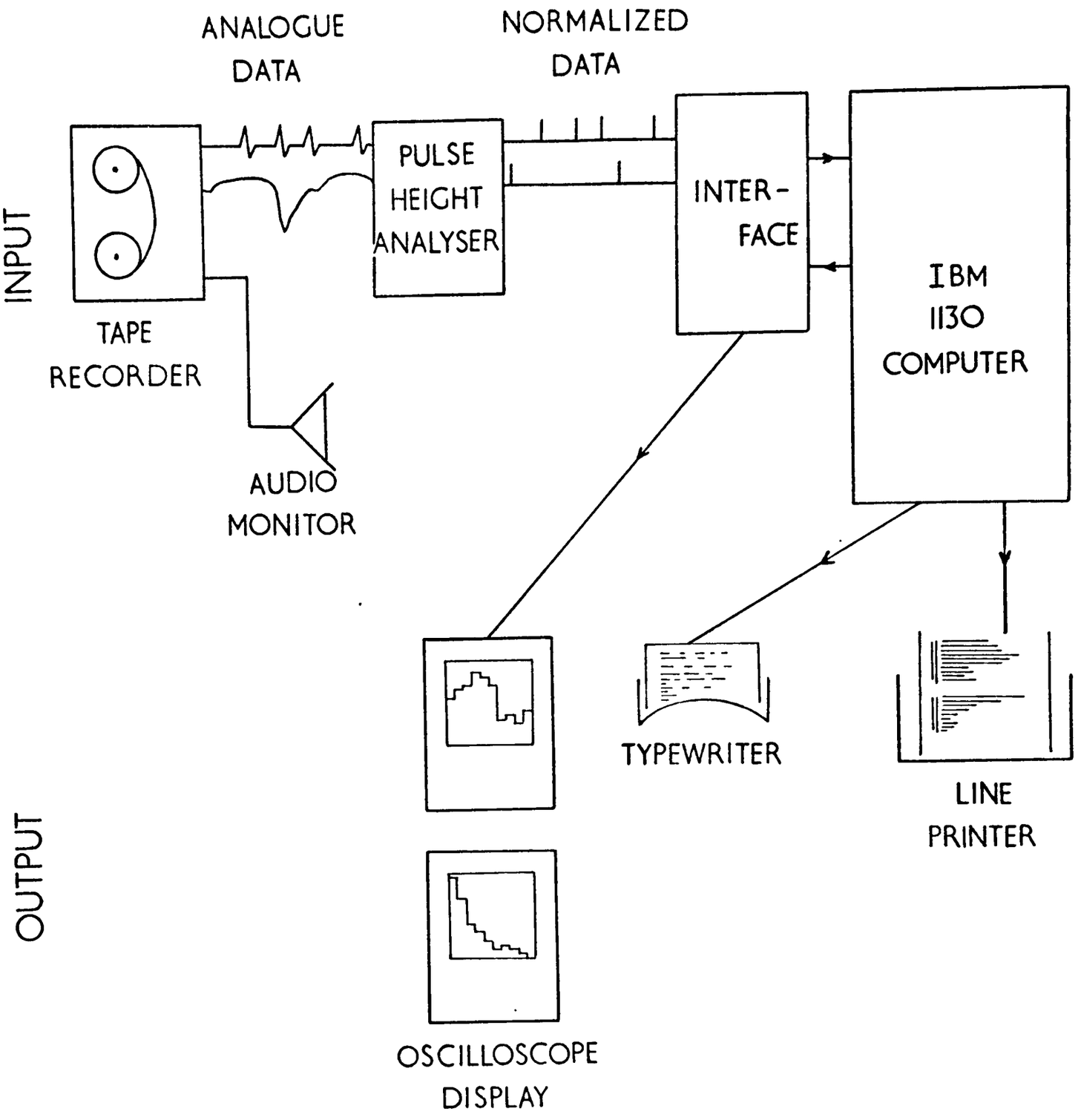


Fig. 10: A simplified block diagram of the set-up for computer analysis of data.

identification of records. Tape speed was $3\frac{3}{4}$ inches per second throughout.

The recorder was taken to the Computing Laboratory and the data processed with a system incorporating an IBM 1130 digital computer. The computer was interfaced to accept and distinguish between signals from the event (nerve impulses) and stimulus (inspiratory airflow) channels. The programmes were written by Bruce Nail (1972).

The basic elements of the system are shown in Fig. 10. The recorded action potentials were replayed into a pulse height analyser (PHA) and standardized pulses passed on to the interface. The signal-to-noise ratio was always high enough for there to be no danger of triggering the PHA erroneously from base-line noise (see Figs. 11, II/5 & II/9) but there was sporadic trouble with spurious clicks produced by the tape recorder which was satisfactorily gated out.

A stimulus marker was needed from as near to the start of inspiration as possible. The base-line of the signal of air-flow was not smooth and the rise-time of the inspiratory airflow was slow compared to that of an action potential (Fig. 11). The PHA was not sensitive enough to trigger reproducibly from this and so

the signal of airflow was amplified and filtered to reduce the higher frequency noise and this modified signal triggered an oscilloscope (Tektronix type 565) from which the timebase trigger output fed the PHA.

In artificial ventilation a simple switch and battery circuit provided a signal at the start of the pump cycle.

The outputs of the tape recorder and the two PHAs were monitored throughout computing input runs.

The computer was programmed to sense the source of a pulse, to measure the inter-pulse intervals and to store them in core.

The output via the line-printer consisted of listings of inter-event and inter-stimulus intervals and graphical representations of histograms. Additional information and the results of statistical tests could be output via the typewriter. The histograms could be viewed on an oscilloscope (Tektronix type 502) (Fig. 10).

Programmes were available for post-stimulus time histograms (PST), interspike interval distribution histograms (IH), and autocorrelelograms.

Sources of error

1. The computer measures time in terms of a counting loop that takes 58 μsec to perform and, although an external pulse is sensed almost instantaneously (within 1 μsec), it cannot be acted upon until the current programme instruction is completed. The longest instruction in the counting loop was 16.6 μsec, so that the precision of the computer in measuring intervals is of the order of 80 μsec.

2. Constant differences in the tape speed when recording and replaying.

3. Random errors due to moment-to-moment irregularities in tape speed when recording and/or replaying.

4. Errors in the measured length of the respiratory cycle because of the noisy base-line and slowly-rising signal.

The effects of 1-3 above were tested by recording 100 msec time pulses on tape and analysing them as a train of events. In 740 inter-event intervals the range was 99.000 msec - 99.983 msec and the mean 99.432 msec, showing that there was a constant error in tape speed of about 0.6% and random errors of $\pm$0.5 msec in the measurement of an interval. The bin width in a PST was never less

PST 98
NO.INTFRVALS =1164 1238 NO. EVENTS DETECTED = 72 1

1,1250,200,0,1
NO. STIM.DETECTED= 72
454584.750 1164 2.550
72 6313.676 167.374 0.158
415.407 23

Fig. 11: The raw data and computer output. From above: spikes, air-flow, lines of temporal equivalence, PST.

The figures below the PST are from the typewriter and have been mounted below the lineprinter output. The figures represent:

First line: the boundaries of the interval sequence called from store, bin width, and codes for computer operations.

Second line: the number of stimulus signals detected.

Third line: sampling interval (msec), number of events, and event frequency (impulses per sec).

Fourth line: number of inspiratory signals, mean length of the respiratory cycle (msec), standard deviation, and respiratory frequency (per sec).

Fifth line: χ^2(E = mean number of impulses per bin), and number of bins used.

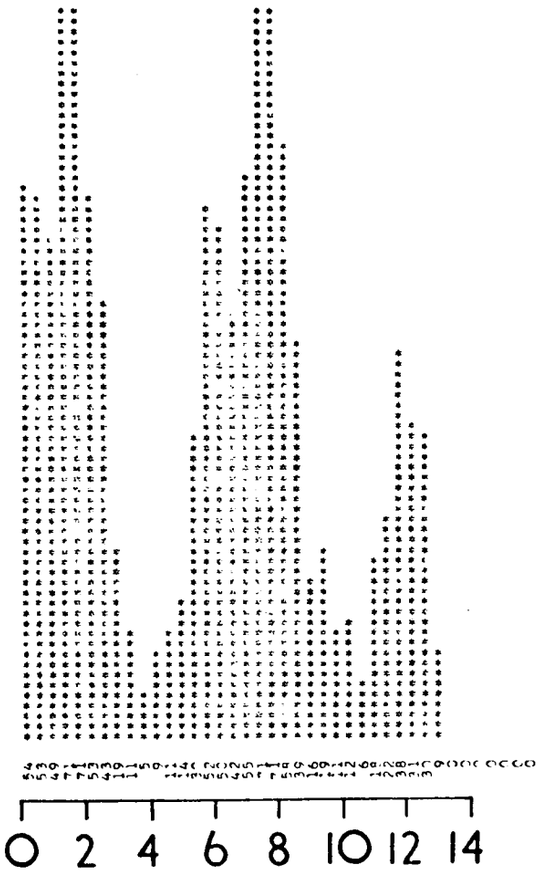
than 100 msec but a bin width of 10 msec was used in some IHs.

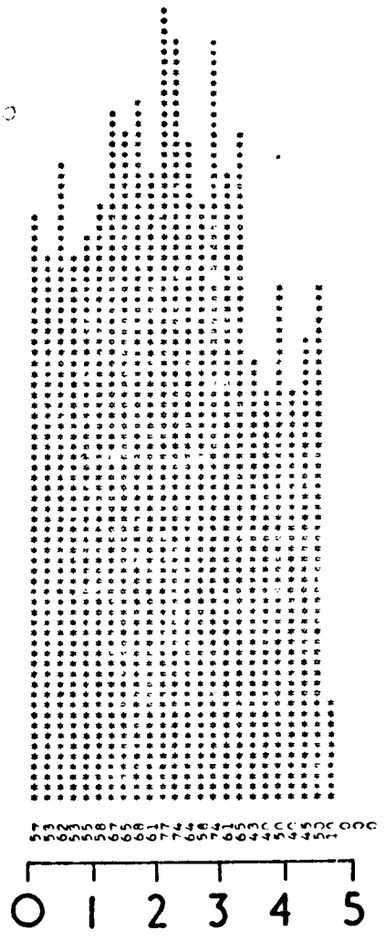
Triggering from the air-flow produced the largest errors. A record of air-flow from an artificially ventilated cat was analysed as a train of events. The mean length of 40 cycles was 3.85 sec and the range was 3.79 - 3.90 sec. The usual bin width for a PST was 200 msec, so the maximum jitter was of the order of half a bin. This error includes irregularities in the pump cycle. When the electrical signal off the pump was used as a stimulus marker this error was shown to be small (see Fig. 12, top right).

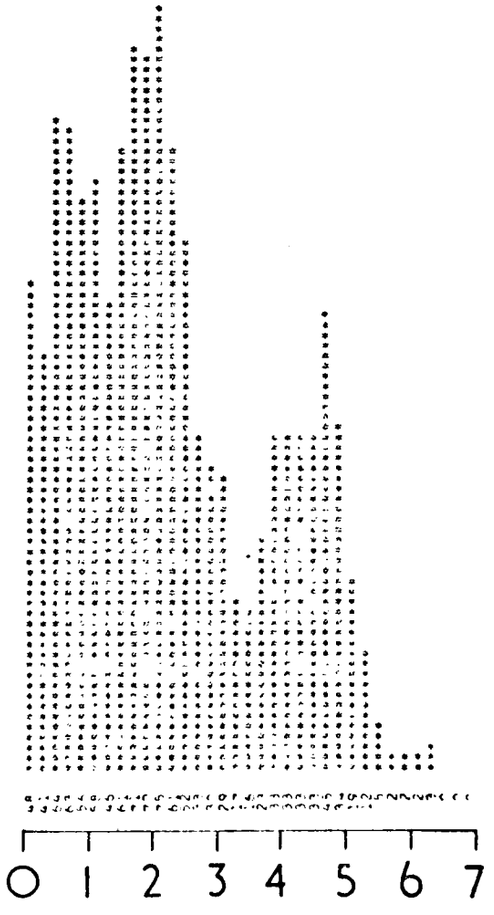
Taped data were frequently analysed months after the experiment was recorded. Repeated analysis of the same segment of tape showed that the system remained reliable over a long period, within the limits outlined above.

Presentation of results (see also Section 6.3)

A specimen print-out of a PST by the line-printer and the corresponding statistical information from the typewriter are shown in Fig. 11 together with a record of the raw data (from the tape). The raw and processed data are joined by lines of temporal equivalence to illustrate the principle of time bins. Fig. 12 shows four specimen PSTs.


0 2 4 6 8 10 12 14


0 1 2 3 4 5


0 1 2 3 4 5 6 7

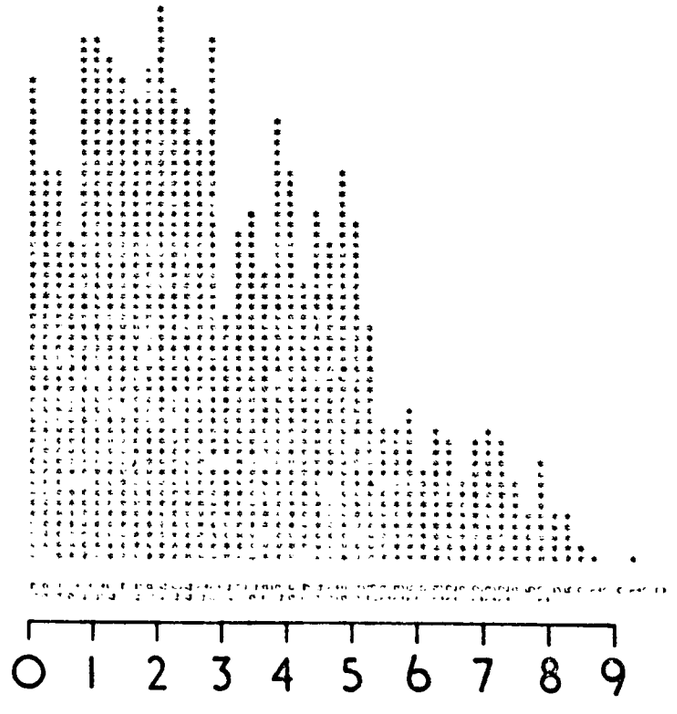

0 1 2 3 4 5 6 7 8 9

Fig. 12: Four sample PST's (lineprinter output).

Top left: summation over 36 double cycles during spontaneous ventilation. Length of double cycle = 12.45 sec ±1.07 sec (S.D.).

Top right: 38 single cycles during artificial ventilation, 4.633 ±0.018 sec. Because the cycle is not an exact multiple of the bin width the last bin (4600-4800 msec) will only have been collecting impulses for 0.033 sec. per cycle, i.e. one-sixth of the normal time. The bin contains 10 impulses, about one-sixth of the expected total for a bin of normal width at that time in the cycle.

Bottom left: 33 single cycles during spontaneous ventilation, 5.17 ±0.53 sec. The impulses collected after 5.6 sec were probably because of a small number of slightly longer breaths not coming within the normal distribution of breath length.

Bottom right: a summation during very irregular spontaneous ventilation, this PST is useless for analysis.

(Time in seconds).

In Section 6.3, i.e. Paper II, PST is referred to as PDH - probability density histogram. The terms are virtually synonymous as used here: strictly a PST relates events to one point in time, the stimulus, whilst a PDH relates events to the time continuum between stimuli.

6.3 Paper II: Oscillations in the discharge of single carotid chemoreceptor fibres of the cat. By Goodman, N. W., Nail, B. S. & Torrance, R. W.

N.B. All figures are numbered with a prefix II. Figures II 6, II 7 and II 10 are additional and so Figures 6 and 7 as they appear in the paper have been renumbered II 8 and II 9.

ABSTRACT

Most single chemoreceptors of the sinus nerve of the cat and dog show oscillations in discharge in phase with respiration when summed by computer over many respiratory cycles. The trough in the oscillation follows inspiration with a delay equal to the lung to carotid body circulation time. The amplitude of these oscillations is increased by decreasing respiratory frequencies or by increasing metabolism by poisoning with DNP. Hypercapnia or hypoxia decrease the relative amplitude of the oscillations but the absolute amplitude remains approximately constant. Oscillations from pairs of simultaneously recorded fibres are similar in phase and relative amplitude. The most likely source of the oscillation is thought to be the respiratory oscillation of P_{a,CO_2} in the carotid blood. It is proposed that the responses can be explained in terms of families of parallel transient P_{a,CO_2}-response curves that are steeper than the fans of response curves described for the steady state by other workers, cutting these curves at the mean P_{a,CO_2}.

INTRODUCTION

We have studied the oscillations that occur in the discharge of chemoreceptor fibres at the frequency of respiration by summing the discharge of single fibres over many respiratory cycles and suggest that they are principally to be attributed to oscillations in P_{a,CO_2}.

Hornbein, Griffo & Roos (1961) noticed that the discharge of chemoreceptors may oscillate at the frequency of respiration. It has been supposed that the oscillation in discharge is a response to an oscillation in the chemical stimulus provided to them by the blood and that this immediate stimulus is an attenuation of the oscillation of gas tensions that occurs in the alveoli with respiration (Yokota, Hoofd & Kreuzer, 1973; Yokota & Kreuzer, 1973).

The first work on the oscillation in discharge was done by summing the discharge of many chemoreceptor fibres over single respiratory cycles (Hornbein et al., 1961; Biscoe & Purves, 1967; Leitner & Dejours, 1968). Some form of summing is made necessary by the nearly random occurrence of impulses from single chemoreceptor

fibres (Biscoe & Taylor, 1963), but doubts exist about the validity of integrating the discharge of groups of fibres. We have preferred to sum the activity of undoubted single units over many respiratory cycles.

Biscoe & Purves (1967) and Leitner & Dejours (1968) did occasionally sum few-fibre discharges over many respiratory cycles, and Band, Saunders & Wolff (1971) and Band & Wolff (1973) have also given preliminary reports of such observations. Preliminary reports of some of our observations have also appeared (Black et al., 1973).

METHODS

Cats were anaesthetized with pentobarbitone (Nembutal, Abbott) 42 mg/kg i.p. and further doses of 2 mg/kg i.v. as needed. Dogs were premedicated with 1 mg/kg acetylpromazine (Boots: acepromazine maleate, BPC) and anaesthetized with pentobarbitone i.v. The animals were laid on their right sides and the carotid sinus nerve was approached laterally (Goodman, 1973). In experiments in which the carotid body was sympathectomized the ipsilateral cervical sympathetic trunk was sectioned above and below the superior cervical ganglion and all the ganglio-glomerular nerves that could be

identified were cut.

Single units were obtained from the sinus nerve by conventional methods and lifted on to one of a pair of stainless steel electrodes. The other, indifferent, electrode made contact with the animal via a dummy length of nerve on to the stainless steel dissecting plate through which the animal was earthed. The whole was covered with warm mineral oil. When the discharges of two single units were recorded simultaneously from two separate slips of nerve the two signal electrodes were referred to a single indifferent electrode to minimize difficulties arising from earth loops. Impulses were pre-amplified, monitored on an oscilloscope, and recorded on a tape recorder (Thermionic T 3000) and ink-jet recorder (Elema-Schönander Mingograf type 24B). Tests for chemoreceptor activity included: reduction of discharge on a sudden change to breathing 100% O_2, increase in mean frequency when breathing hypoxic gas mixtures, and a brisk response to a close intra-arterial injection of saline equilibrated with 100% CO_2. To check that a unit was single a storage oscilloscope was used to show that no intervals between recorded impulses were shorter than the refractory period of the chemoreceptor endings, about 7 msec (Biscoe & Taylor, 1963).

Blood pressure was monitored from either the lingual or femoral artery and recorded on a channel of the Mingograf. Respiratory air flow was measured with a Fleisch pneumotachograph head and electromanometer. The signal was recorded on a channel of the tape recorder and the Mingograf. Animals paralysed with gallamine (Flaxedil, May & Baker) were artificially ventilated by intermittent positive pressure with a Starling ideal pump modified to give an electrical signal at the start of inspiration which could be recorded on a third channel of the tape recorder. In some experiments the P_{O_2}, P_{CO_2} and pH of arterial blood samples were measured with Radiometer equipment.

Electrical stimulation of the cervical sympathetic trunk was through Ag-AgCl electrodes at a stimulus intensity supra-maximal for dilatation of the ipsilateral pupil.

Computing

The data were later fed into an IBM 1130 digital computer programmed to produce a post-stimulus time histogram (PST) using a signal triggered from the inspiratory air-flow, or by the electrical signal from the ventilation pump, as the stimulus for the start of the computer cycle. The analysis depends on the computer's being

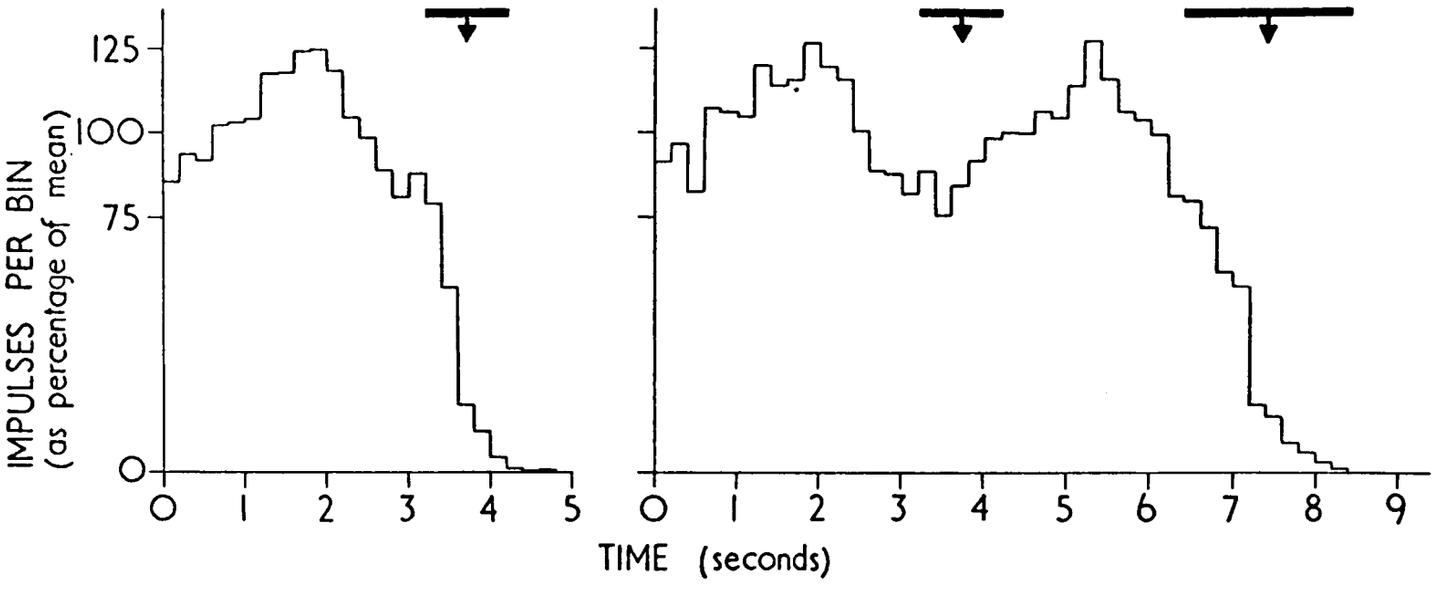


Fig. II 1: Cat: nembutal, spontaneous ventilation. Probability density histograms (PDH's) of the discharge of a single chemoreceptor fibre of the sinus nerve. Ordinate: the number of impulses per bin expressed as a percentage of the mean number per bin. Abscissa: time in seconds from the inspiration that triggered the computer cycle. Left: PDH summed over 252 single respiratory cycles. Total number of impulses about 7400, mean frequency of discharge 8.0 imp/sec. Most of the respiratory cycles were within the range 3.2 - 4.2 seconds as shown by the bar above the histogram: the arrow denotes the average length. Right: using the same series of impulses but constructed over 126 double respiratory cycles. First bar and arrow as Left; the second bar indicates the range (6.4 - 8.4 seconds) of most double respiratory cycles and the arrow denotes the average length of a double cycle.

triggered at the same point in each respiratory cycle. We tested the accuracy of triggering from the record of air flow using an air flow obtained in artificial ventilation. The computer measured the respiratory cycle with a range of 100 msec around the actual length: the slurring of the pattern in spontaneous ventilation would be negligible.

In these experiments the final result is more correctly described as a probability density histogram (PDH), for it relates the likelihood of an impulse's occurrence to the time from the start of inspiration and over the respiratory cycle. Because the inherent discharge of chemoreceptors is random (Biscoe & Taylor, 1963) it was necessary to collect a large number of impulses to bring out clearly any oscillation. The programme could handle 1400 intervals. With tape recorded runs having more than this number, two or more computing runs could be made and the resulting histograms summed. If 6000-8000 intervals were collected in this way the PDH became a tolerably smooth curve (Fig. II.1). The number of respiratory cycles which had to be summed depended on the rate of firing of the chemoreceptor unit.

If the respiratory cycle was of irregular length the PDH would tail off in the later bins of the cycle. A clearer picture of an

oscillation in the discharge could be produced by programming the computer to ignore every alternate inspiratory signal, thus showing the fluctuations in the probability of firing of a unit summed over double respiratory cycles (Fig. II.1). This also allowed for respiratory cycle lengths which were not exact multiples of the bin length.

Programmes for interval histograms and autocorrelelograms were also available (Nail, 1972).

The amplitude of an oscillation

To make the oscillations easier to compare one with another a simple averaging technique was used. To the contents of each bin were added the bins preceding and following it, and then an average value was obtained by dividing by three - a 3-point running average (Band, Saunders & Wolff, 1971). The amplitude of an oscillation was the difference between the highest and lowest points obtained in this way. In this paper absolute amplitude is given as impulses per second, and relative amplitude as a percentage of the mean number of impulses per bin, i.e. of the mean rate of discharge. In the figures of this paper results are usually presented as variations of discharge around a mean of 100% for ease of comparison of discharges in

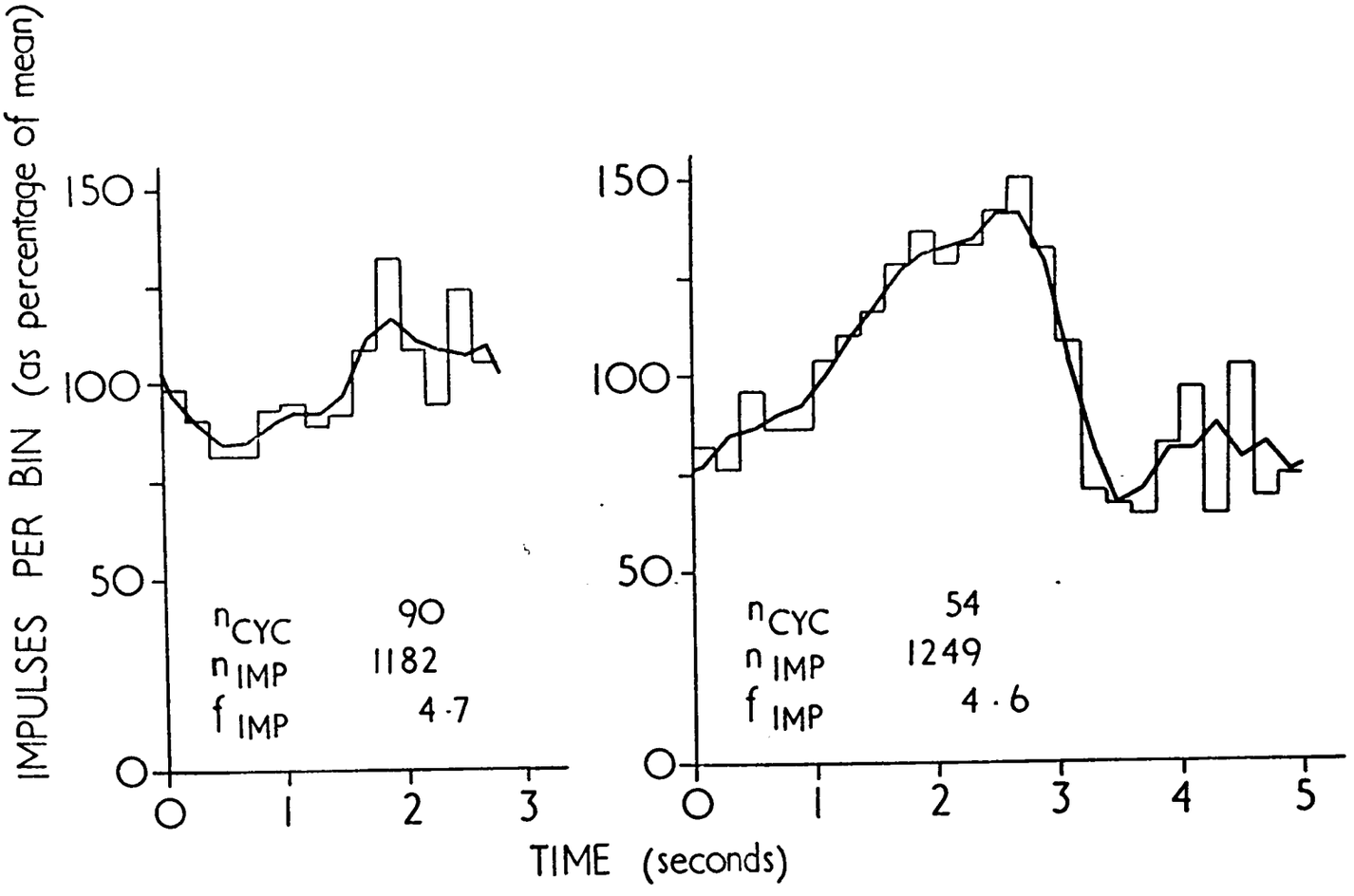


Fig. II 2: Cat: nembutal. PDH's: general layout as in Fig. II 1. Number of respiratory cycles (n_{CYC}), total number of impulses (n_{IMP}), and mean frequency of discharge (f_{IMP}) as stated. Left: spontaneous ventilation. Right: the same single fibre, the cat now paralysed with gallamine and artificially ventilated at a lower frequency. The continuous lines drawn through the histograms are the three-point running averages (see text).

different units and in different conditions. This has particularly been done when presenting the activity of two fibres that were recorded from simultaneously.

RESULTS

We have studied oscillations with respiration in the discharge of single chemoreceptor fibres dissected from the carotid sinus nerve of 29 cats and two large dogs. The animals breathed spontaneously or else were artificially ventilated. We were concerned with the factors which determine the appearance of oscillations in discharge and so selected respiratory frequencies at which they were likely to be prominent (Fig. II.2). They can usually be seen at a respiratory frequency of 25 per minute but are much attenuated at 30 per minute. As the respiratory cycle was decreased from 4 to 2 seconds the amplitude of the oscillations in discharge declined much more rapidly than did the duration of the respiratory cycle. Oscillations in discharge were prominent at respiratory frequencies of 10-15 per minute, and most of our observations were made on cats which breathed at such low frequencies, either after vagotomy or else during artificial ventilation. When gas transport was increased by

poisoning with dinitrophenol (DNP) oscillations in discharge could easily be detected at respiratory frequencies much higher than those at which they disappeared at a normal oxygen uptake.

In considering whether an oscillation in discharge had been abolished by some procedure it was useful to compare the shape of the histogram during that procedure with its shape when earlier there had very clearly been an oscillation in discharge. For example, in Fig. II 4A and C there is clearly an oscillation in discharge, but 4E, taken on its own, does not show an oscillation so clearly. It does, however, have peaks and troughs in it which correspond quite well with those present in II 4C and so one can say that the oscillation present in II 4C is attenuated in II 4E but still persists. A further useful test came from summing over pairs of respiratory cycles. If the histogram showed two similar cycles of a wave one could confidently say that the fibre oscillated.

Administration of gas mixtures

If the percentage of oxygen or carbon dioxide in the gas mixture breathed by the animal was altered, the mean rate of discharge of a chemoreceptor unit altered and there could be changes in the oscillation also.

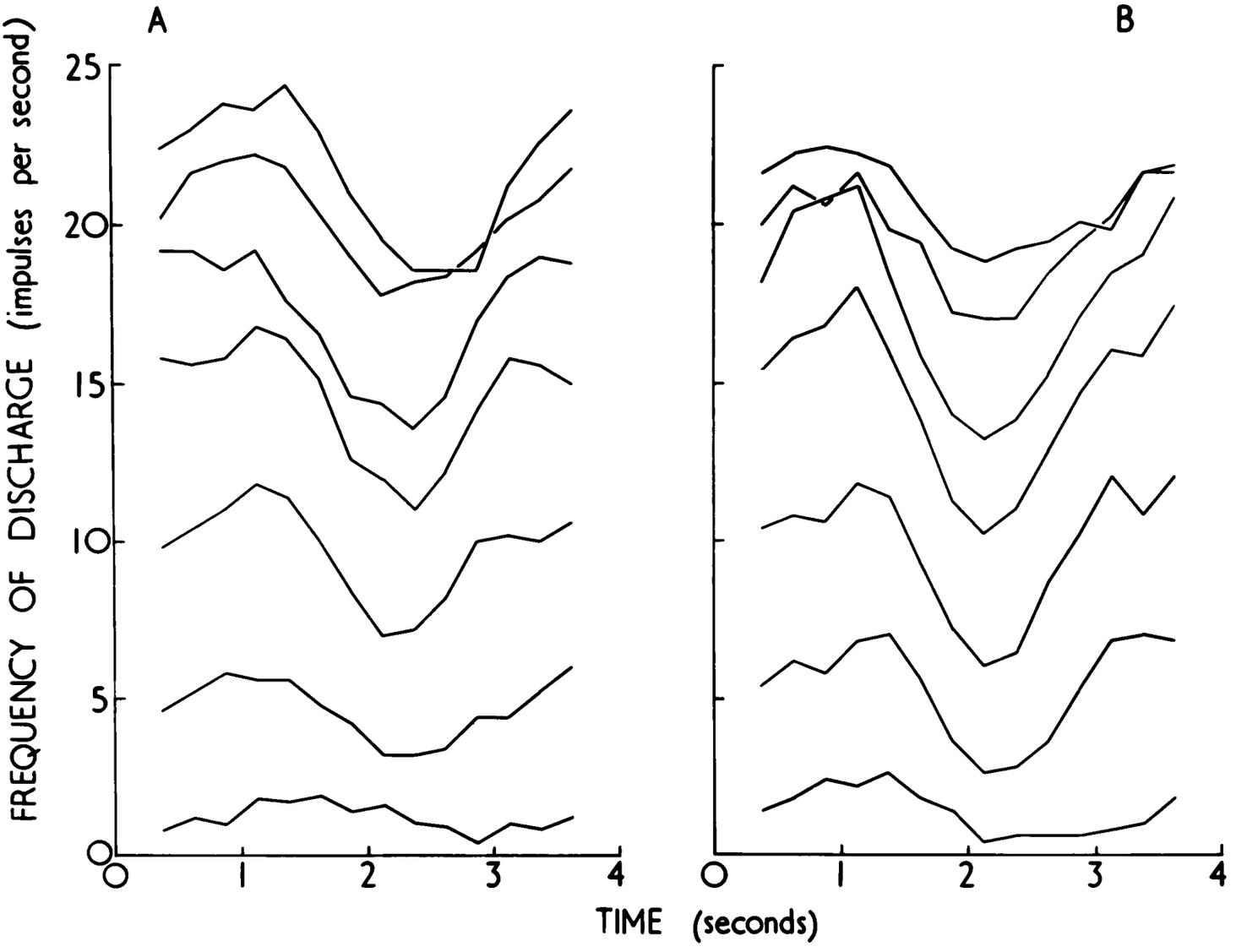

A
B
FREQUENCY OF DISCHARGE (impulses per second)
25
20
15
10
5
0
0
1
2
3
4
TIME (seconds)

Fig. II 3: Cat: nembutal, spontaneous ventilation. PDH's, shown as the three-point running averages only, from two simultaneous fibres during the development of hypoxia. One fibre is shown in A and the other in B. General layout as before, showing the absolute amplitude of the oscillations, the ordinate being the frequency of discharge in impulses per second. The original histograms were constructed by hand from a series of 130 consecutive breaths of a Mingograf record. Each line represents the sum of 20 consecutive respiratory cycles including the first few bins of each subsequent cycle, some impulses have thus been counted twice. The respiratory cycle was approximately 3 sec, and there was little ventilatory response to the hypoxia.

At breath number 30 in the series the animal was switched from breathing 100% O_2 to a mixture of 10% O_2 in N_2. Starting at the bottom of each of A and B, the running averages represent breath numbers 11-30, 31-50, 41-60, 51-70, 61-80, 91-110 and 111-130. The absolute amplitudes of the oscillations in discharge are low in hyperoxia (the lowest lines), steadily increasing as the mean rates of discharge rise. For one fibre (A) the absolute amplitude reaches a maximum, remaining fairly constant as the mean rate of discharge rises further. For the other fibre (B) the absolute amplitude falls off at the highest mean rates of discharge. The relative amplitudes of oscillation decrease progressively as the mean rates of discharge rise.

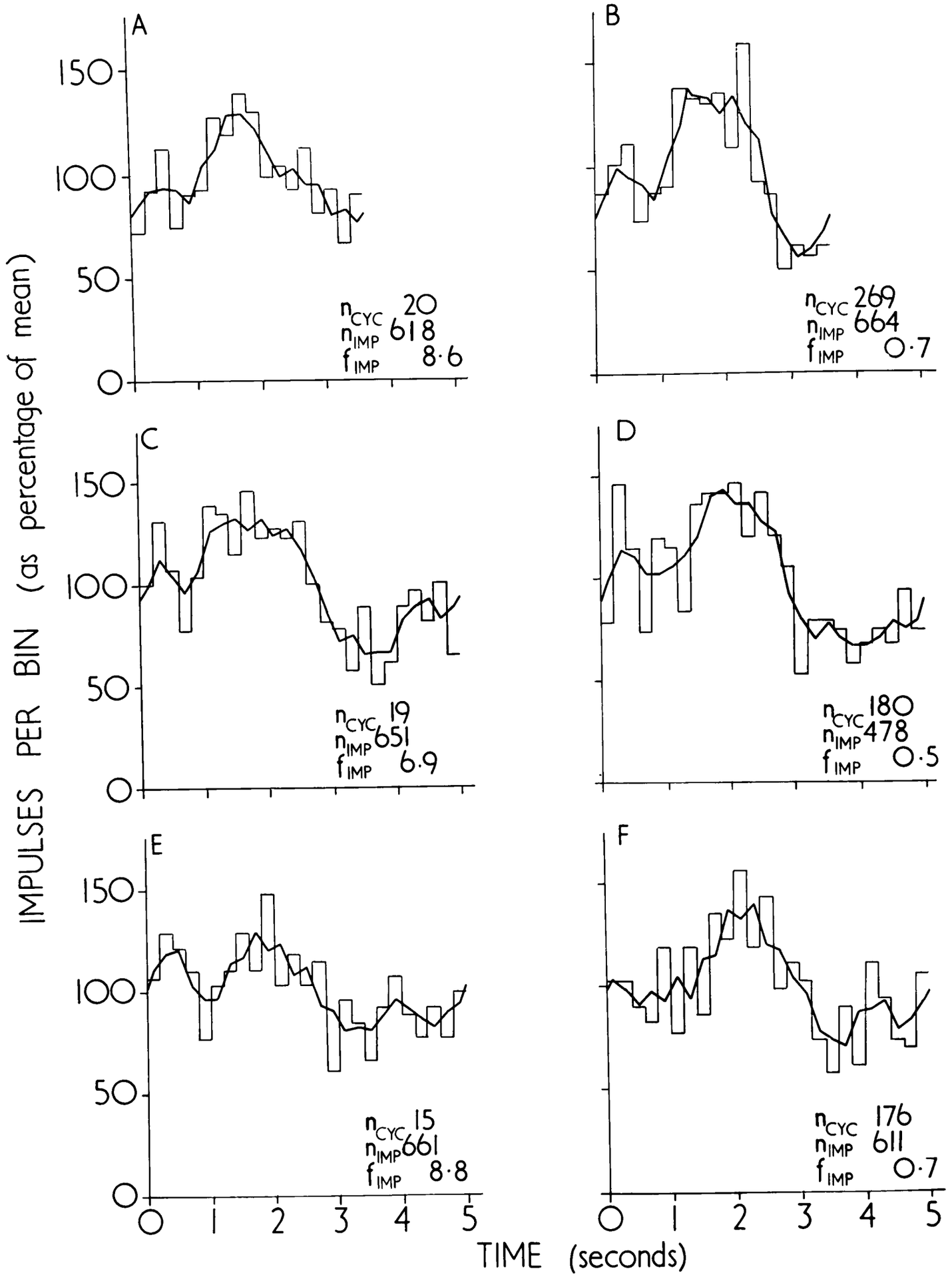

A
B
C
D
E
F
150
100
50
0
n_{CYC} 20
n_{IMP} 618
f_{IMP} 8·6
n_{CYC} 269
n_{IMP} 664
f_{IMP} 0·7
n_{CYC} 19
n_{IMP} 651
f_{IMP} 6·9
n_{CYC} 180
n_{IMP} 478
f_{IMP} 0·5
n_{CYC} 15
n_{IMP} 661
f_{IMP} 8·8
n_{CYC} 176
n_{IMP} 611
f_{IMP} 0·7
0 1 2 3 4 5
IMPULSES PER BIN (as percentage of mean)
TIME (seconds)

Fig. II 4: Cat: nembutal. PDH's from two single chemoreceptor fibres of the sinus nerve recorded simultaneously on separate electrodes. General layout as before. Histograms A, C and E from one fibre; B. D and F from the other fibre.

A and B: spontaneous ventilation, air

C and D: paralyzed with gallamine, artificially ventilated, air

E and F: artificially ventilated, 5% CO_2 + 21% O_2 in N_2 mixture.-

If the animal was given a hypoxic mixture the mean rate of discharge increased. The absolute amplitude of the oscillation in discharge might be little altered (Fig. II 3A), decreased (Fig. II 3B) or increased (not illustrated). The relative amplitude was always decreased. If the animal was given 100% O_2 to breathe, a sparse discharge usually persisted and this discharge had a respiratory oscillation of very marked relative amplitude (Fig. II 3). As the mean rate of discharge was then low the absolute amplitude of the oscillation was also low.

If an animal was given CO_2 to breathe the mean rate of discharge of a fibre usually increased modestly. Our method of summation did not allow us to follow breath-by-breath the transient changes in the oscillation in discharge as CO_2 breathing started, but when the mean rate of discharge had become steady in CO_2 breathing, the discharge still showed an oscillation with respiration and this was usually of the same absolute amplitude as in the control period, or else somewhat reduced. Its relative amplitude was always reduced (Fig. II 4E and F).

Acetazolamide (Diamox, Lederle) inhibits carbonic anhydrase and slows the response of chemoreceptors to a large step change of P_{CO_2}

(Black et al., 1971) so it might be expected to reduce an oscillation in discharge caused by an oscillation in P_{a,CO_2}. The drug also causes gross changes in the distribution of CO_2 because of CO_2-HCO_3^- disequilibrium (Maren, 1967). There is an increased gradient between a raised tissue P_{CO_2} due to failure of uptake of CO_2 from the tissues, and a lowered P_{A,CO_2} caused by hyperventilation probably due to a high P_{CO_2} in the environment of the central chemoreceptors. The P_{a,CO_2} measured instantaneously is low but when measured in a sample taken by syringe there is time for the uncatalyzed reaction to go to completion. We could not, therefore, know the mean P_{a,CO_2} at the carotid body, and even less did we know the tissue P_{CO_2} at the chemoreceptors. We would guess that it was not as high as in other tissues because the high blood flow through chemoreceptors probably enables most of the CO_2 to be carried away purely in solution as dissolved CO_2 without a marked rise in blood P_{CO_2}.

The response of the chemoreceptors to the drug was very variable. An intravenous injection was followed by a period of silence lasting 10-20 seconds, more likely due to the high pH of the solution than to any specific effect of the drug. The degree of recovery varied greatly: some fibres recovered to only 10-20% of their initial frequency of discharge, others regained their control frequency or

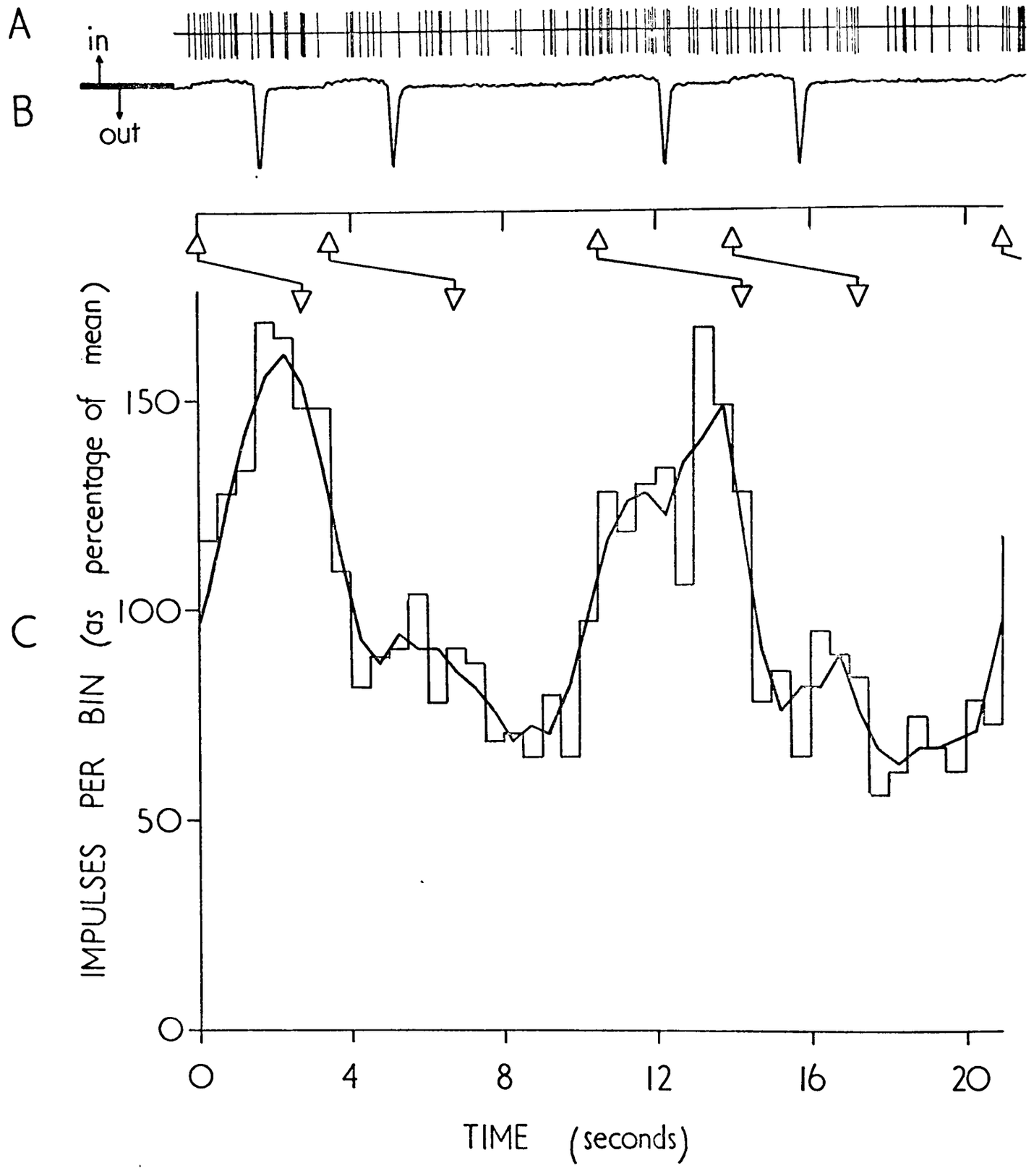

A
B
in
out
C
IMPULSES PER BIN (as percentage of mean)
150
100
50
0
0
4
8
12
16
20
TIME (seconds)

Fig. II 5: Cat: nembutal. Animal paralyzed with gallamine and artificially ventilated. The pump delivered no air to the cat on every third pump cycle.

A: the discharge of the single chemoreceptor fibre (retouched)

B: airflow

C: PDH. General layout as before with the computer triggered by every fourth inspiration. Total number of computer cycles = 27; total number of impulses = 2272; mean frequency of discharge $\simeq$ 4 imp/sec. The arrows between C and B link approximately corresponding points in the airflow and histogram.

even showed a steady rise in frequency over a few minutes to 150-200% of control. It made little difference whether the animal was breathing spontaneously or was artificially ventilated.

This made interpretation of the effects of inhibition of carbonic anhydrase on the oscillation in discharge very difficult. However, with those fibres whose discharge recovered sufficiently for us to collect enough impulses to make a summation, the oscillation in discharge of single chemoreceptor units persisted at relative amplitudes that were commonly smaller than those seen prior to the administration of the drug.

The relation of the discharge to ventilation

To determine which inspiration the trough in discharge is to be related to a cat was connected to the respiratory pump so that it delivered no air to the cat at every third pump cycle (Fig. II 5). The pattern of the oscillation in discharge showed that the dip in discharge in a histogram is to be related to the inspiration occurring 2-3 seconds before the dip takes place and this time corresponds to the lung to carotid body circulation time. Thus with a respiratory cycle of about four seconds or longer, the length we usually used, the first dip in the histogram should be related to the inspiration

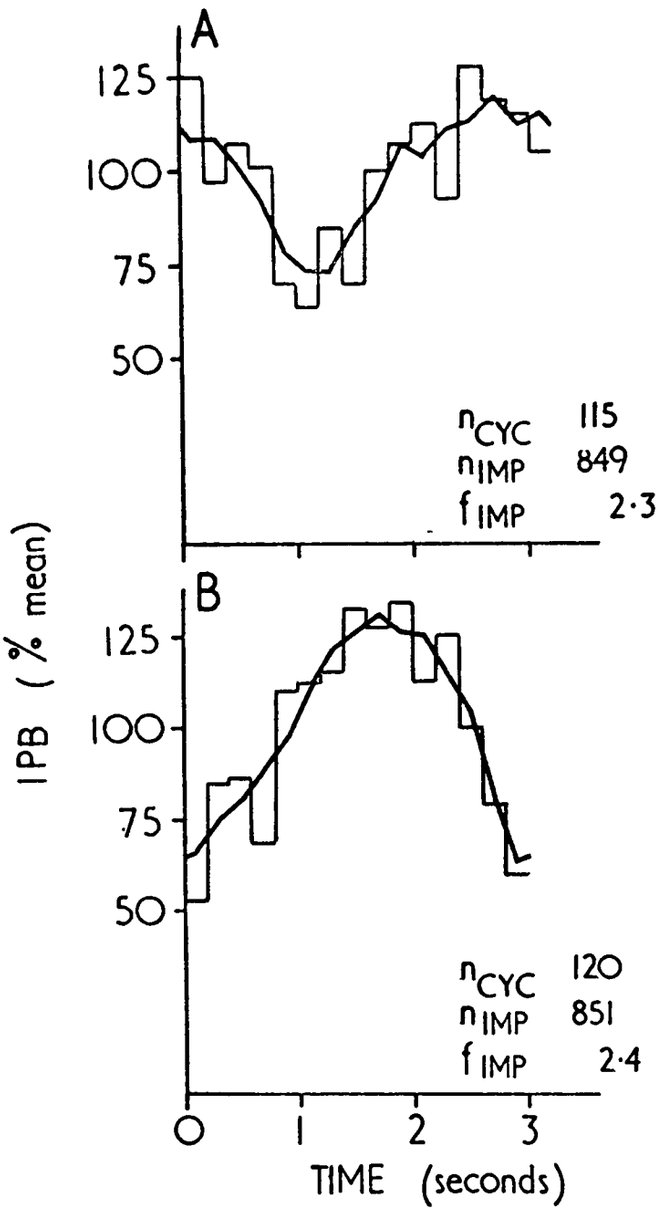


Fig. II 6: Cat: nembutal. Spontaneous ventilation, vagotomized. PDH's: general layout as before.

A: with a clip on the external carotid artery beyond the carotid body.

B: after the clip had been removed.

Note that in A the dip in discharge has been delayed and is correlated with the inspiration preceding the one that triggered the computer cycle.

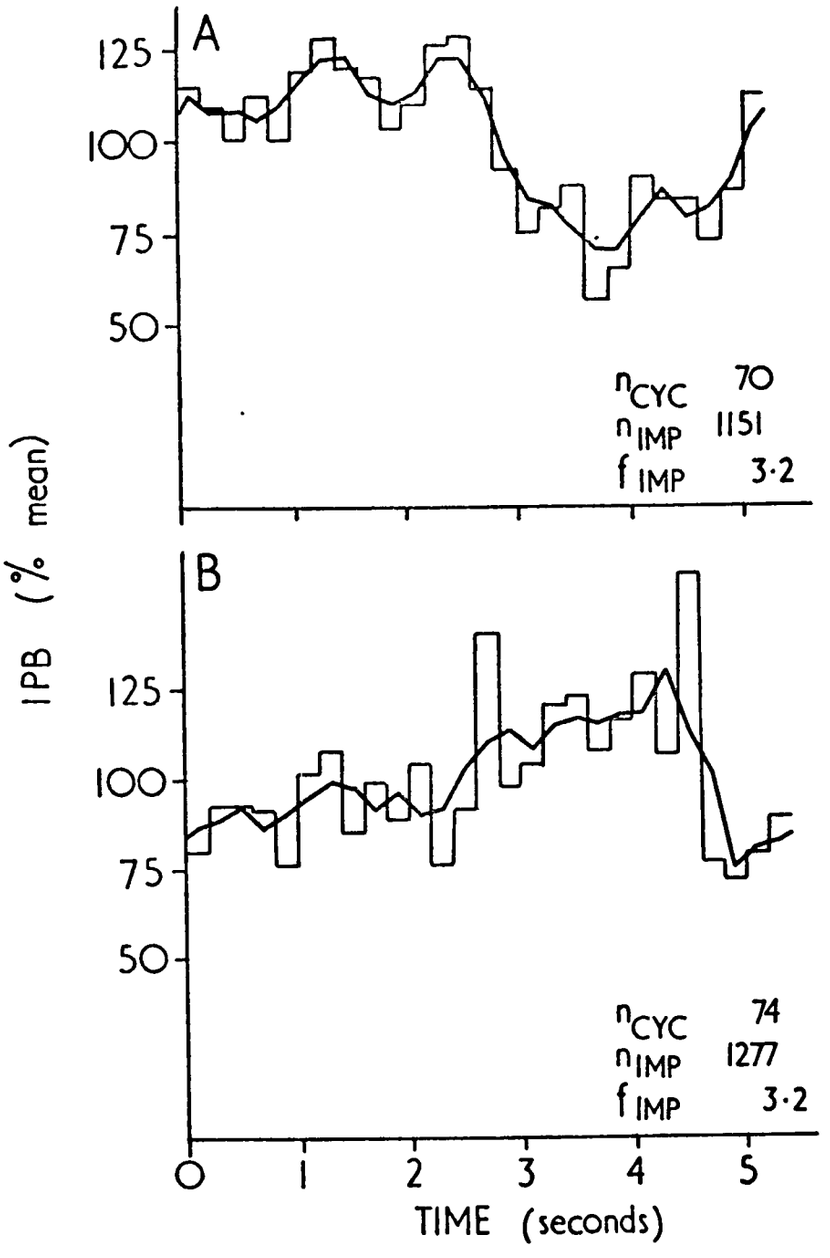


Fig. II 7: Cat: nembutal. Spontaneous ventilation, vagotomized.

PDH's: general layout as before.

A: control.

B: with a clip on the common carotid artery.

that triggered the computer cycle, but with a shorter respiratory cycle the first dip may be related to the inspiration preceding the one which triggered the computer cycle.

Clipping the external carotid artery reduced overall flow of blood along the common carotid artery and so delayed the arrival of blood at the carotid body by 1-2 seconds as judged by an injection of Evans Blue dye into the brachial artery. It also delayed the dip in the oscillation in discharge by a similar period relative to inspiration (Fig. II 6). Biscoe & Purves (1967) showed that oscillations in discharge persist after clipping the common carotid artery. We confirmed this and found that the dip in discharge here also came later, presumably because of the time taken for blood to traverse vertebro-occipital anastomoses (Fig. II 7).

Correlation of the discharge with the ECG

Band, Cameron & Semple (1969) reported oscillations in pH of the carotid blood at each heartbeat, and Gerhich & Moore (1970) reported oscillations in the discharge of chemoreceptors correlated with the ECG. We could not find any evidence of such a correlation when we used the ECG to trigger the computer cycle. The probability of occurrence of an impulse was constant over the heart cycle in

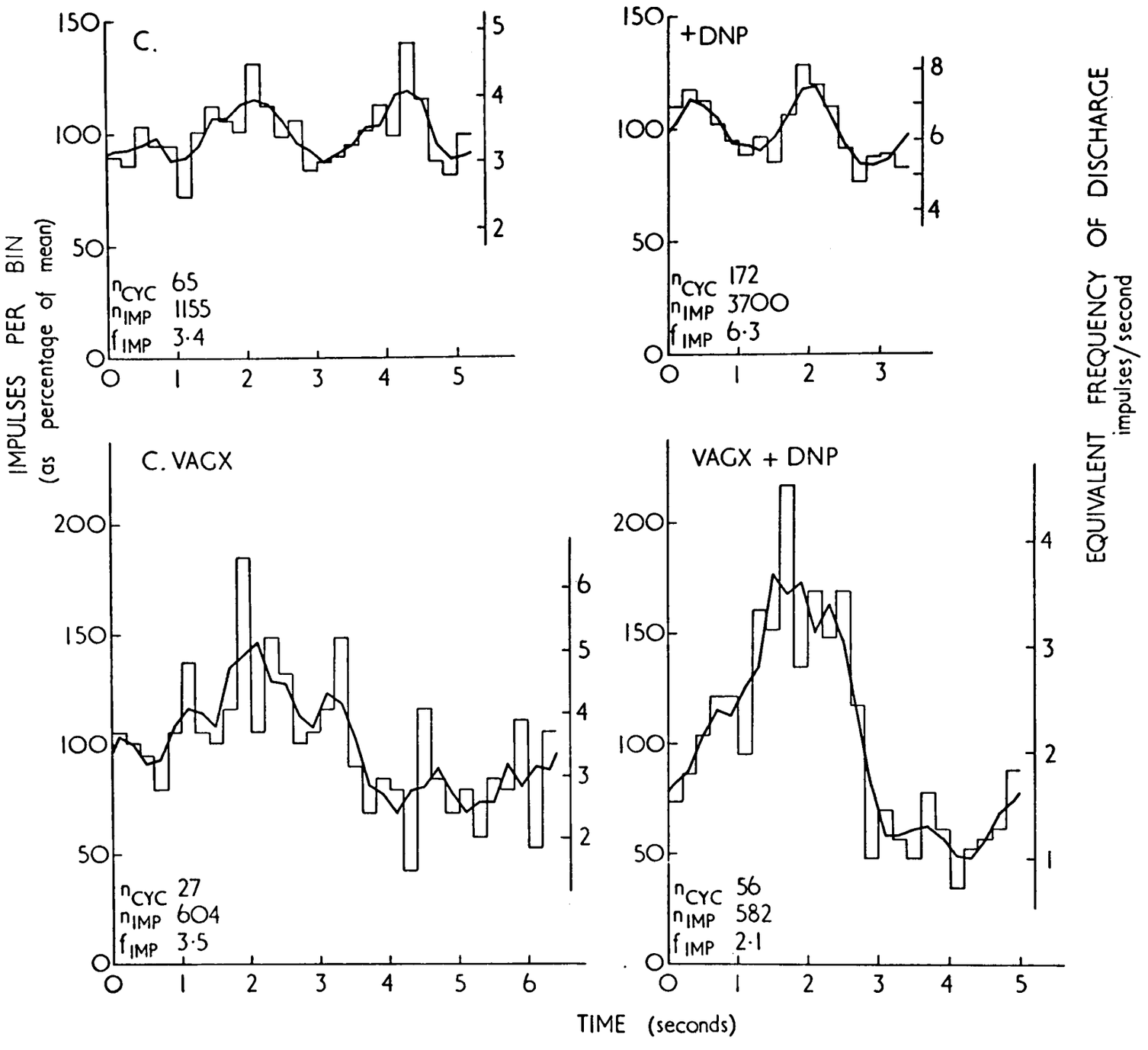


Fig. II 8: Cat: nembutal. Spontaneous ventilation. PDH's of the discharge of two single chemoreceptor fibres from different cats, layout as before.

Top: summations over double cycles. Vagi intact. C: control. + DNP: same fibre, after the administration of 7.2 mg/kg DNP.

Bottom: summations over single cycles. Vagi cut. C: VAGX: control. VAGX + DNP: same fibre, after the administration of 4.5 mg/kg DNP.

two fibres which both showed clear oscillations in their discharge in phase with the respiratory cycle. Nor was any evidence seen of an oscillation with the period of the pulse in any autocorrelelogram.

Administration of dinitrophenol

DNP (2:4-Dinitro-phenol, British Drug Houses) was administered to simulate the changes in gas transport that occur in exercise (Liang & Hood, 1973). This drug uncouples oxidative phosphorylation and so increases oxygen uptake several-fold. With small doses of DNP, CO_2 production is increased by a similar amount, but with large doses lactic acid is formed and drives off further CO_2 so that the RQ may become greater than unity. Cardiac output is increased and ventilation is stimulated.

DNP increased both the absolute and the relative amplitude of the oscillations in discharge. This was particularly striking in vagotomized cats in which the change in ventilation was principally an increase in depth with little change in frequency (Fig. II 8 bottom). After DNP the dip in discharge occurred sooner after the triggering inspiration and this can be attributed to DNP's increasing the cardiac output and so shortening the lung to carotid body circulation time.

In cats with the vagi intact, DNP increased the respiratory frequency but the chemoreceptors showed clear oscillations in their discharge at respiratory frequencies higher than those at which they would have been lost at a normal oxygen uptake (Fig. II 8 top).

One of the fibres studied did not show oscillations before DNP and none were brought out by DNP. In another cat, the effect of DNP was studied whilst the cat breathed 100% O_2. DNP only produced a minimal increase in ventilation. There was some increase in the mean rate of discharge of the chemoreceptor fibre. The absolute amplitude of the oscillation in discharge was unaltered and the relative amplitude was decreased.

It is well known that DNP excites chemoreceptors and injection of DNP intravenously did cause an immediate but transient increase in the rate of discharge which then settled to a somewhat higher rate than before the injection. This was presumably due to a high dose of the drug being delivered to the chemoreceptors initially because of the very high blood flow through the carotid body and then its partial removal as it was gradually distributed more evenly throughout other, less well perfused, regions. We always waited until this initial hyperactivity had passed off before starting to

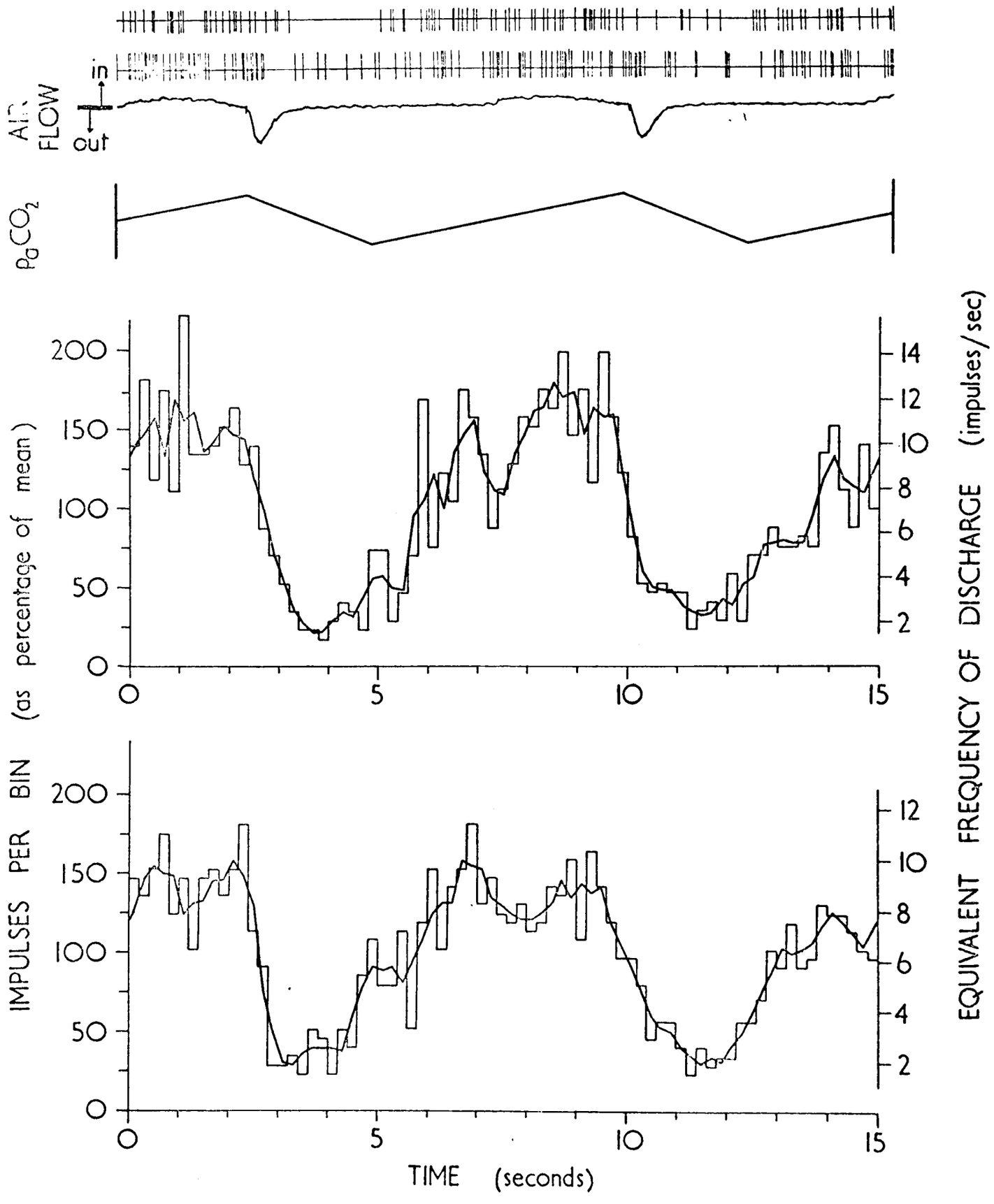

AIR FLOW
in
out
P_aCO_2
IMPULSES PER BIN (as percentage of mean)
EQUIVALENT FREQUENCY OF DISCHARGE (impulses/sec)
TIME (seconds)
0
5
10
15
50
100
150
200
2
4
6
8
12
14

Fig. II 9: Cat: nembutal. Spontaneous ventilation, animal vagotomized. From above downwards: discharge (retouched) of two single chemoreceptor fibres of the sinus nerve recorded simultaneously on separate electrodes; respiratory airflow; a representation of the P_{a,CO_2} in the carotid artery at the level of the carotid body; PDH's constructed over double cycles for each of the fibres, general layout as before.

record but, even so, we would not suggest that DNP had no direct effect on chemoreceptors.

The pattern of the oscillation

The shape of the oscillations in discharge changes if the respiratory frequency is changed. The highest respiratory frequencies at which an oscillation in discharge was clearly seen were about 40 per min after DNP, and the shape of the oscillation then looked like a sine wave (Fig. II 8 top). At lower respiratory frequencies, the sine wave becomes skewed and then saw-toothed (Fig. II 2) with the fall in discharge occurring over a shorter period than the subsequent rise. With very low respiratory frequencies, the fall is still rapid and the rise is slower but the rise may lead on into a plateau before the next fall (Fig. II 9).

In some oscillations there was a suggestion of some secondary structure. This became more obvious and less easily attributed to the random nature of chemoreceptor discharge when it was seen in both of a pair of fibres recorded simultaneously. For example, in Fig. II 4, the pairs of histograms A and B and also C and D have a small trough in the oscillation during the build-up to the peak. This trough is also present in histogram E but probably not in F.

The airflow was not biphasic and no explanation is offered for this phenomenon. It is interesting that it has the same short latency from the triggering inspiration in A and B as it has in C and D, even though the respiratory cycles are of very different lengths and in A and B the cat breathed spontaneously, whilst in C and D it was artificially ventilated.

The effect of the sympathetic supply to the carotid body

Cutting the sympathetic supply to the carotid body did not affect either the absolute amplitude, the shape, or the phase relation to ventilation of the oscillations in the discharge of chemoreceptors. Nor did brief bursts of stimuli (14V. 0.25 msec, 10 Hz for 0.7 sec) applied to the cervical sympathetic trunk and triggered at a constant, early or late, phase of the respiratory cycle, though such stimulation did raise the mean frequency of discharge of the chemoreceptor fibres by 10-30%, thus lowering the relative amplitude of the oscillations in discharge.

The effect of efferent fibres in the carotid sinus nerve

The carotid sinus nerve contains efferent fibres which are inhibitory to the chemoreceptors of the carotid body (Neil & O'Regan,

1971b; Sampson & Biscoe, 1970). In three experiments records were made from fibres peeled from the side of the sinus nerve whilst the rest of the nerve, and so hopefully its efferent fibres, were left in continuity. A run of discharge was first recorded with the nerve intact and the cat breathing air, and then with the cat breathing a hypoxic gas mixture. Whilst the cat still breathed the hypoxic mixture, the nerve was cut proximal to the recording electrode and a second run was recorded during hypoxia. The cat was returned to breathing air and a further run was recorded.

Cutting the nerve had no effect on the relative amplitude, form or timing of the oscillations, either during air breathing or during hypoxia. It must, however, be pointed out that cutting the nerve had no effect on the mean frequency of discharge (Neil & O'Regan, 1971b) and so it could be argued that the efferent inhibitory fibres to the afferent studied had been damaged whilst dissecting out the afferent fibre.

The timing of impulses in efferent fibres of the carotid sinus nerve

In a number of experiments we recorded efferent activity from the central end of the cut carotid sinus nerve. The activity was sparse and responded modestly to hypercapnia and hypoxia and more

briskly to adrenaline, as has already been reported by Biscoe & Sampson (1968). It also responded with a latency of about two seconds to the injection of 1 ml of saline bubbled with 100% CO_2 into the ipsilateral carotid artery via a cannula in the lingual artery. All known chemoreceptor nerves, both vagi and both carotid sinus nerves, had been cut.

We only managed to record for computer analysis the discharge of one unambiguous single fibre giving good-sized action potentials. This discharge did not show any correlation with the respiratory cycle. The discharge was certainly not regular but an interval histogram showed that it was not completely random (Biscoe & Taylor, 1963) in the way that the inherent discharge of chemoreceptors is. This supports a view that the centrifugal activity was not recorded from a branch of what is essentially a normal afferent chemoreceptor fibre, although, if this were so, the chemoreceptor would have had to be remote from the carotid or aortic bodies as their nerves had been cut.

The interval histogram declined exponentially for about 300 msec and then had a marked hump upon it with a peak at about 1000 msec

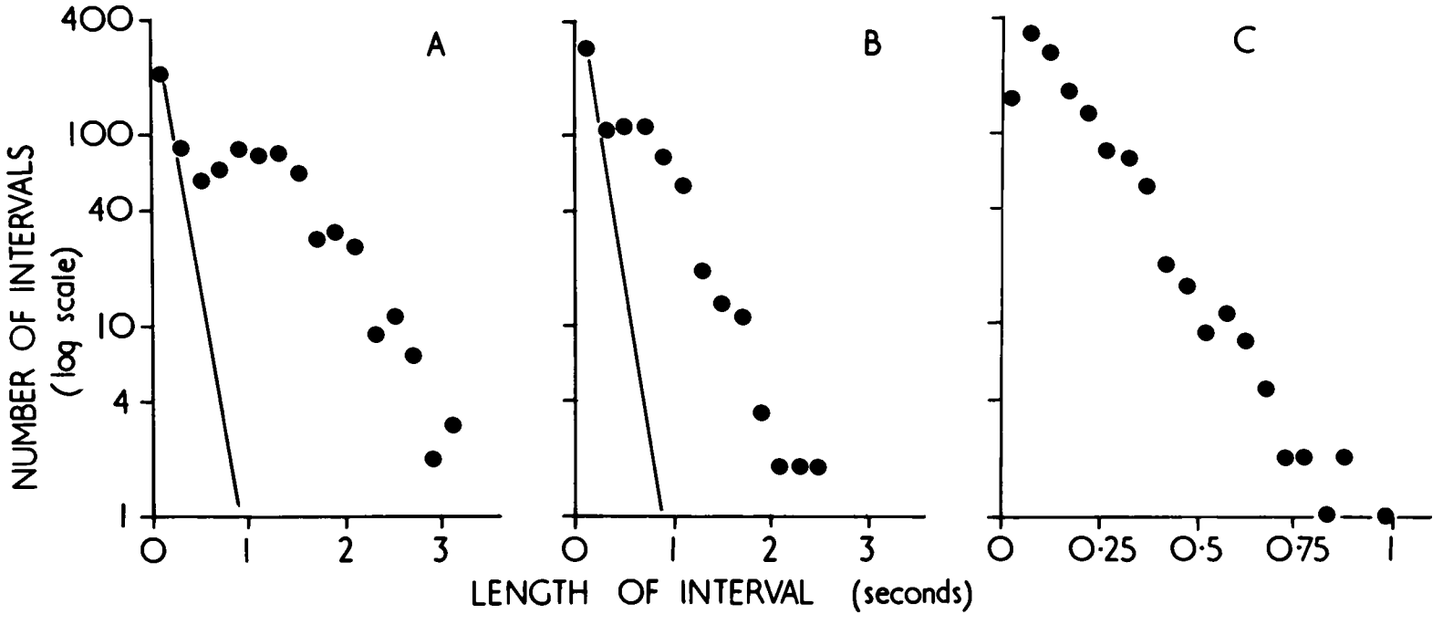

400
100
40
10
4
1
NUMBER OF INTERVALS
(log scale)
A
B
C
0
1
2
3
0·25
0·5
0·75
LENGTH OF INTERVAL (seconds)

Fig. II 10: Cat: nembutal. Interval distribution histograms constructed from the discharge of single fibre preparations of the sinus nerve. Ordinate: log. number of intervals. Abscissae: length of interval - note that the bin width is longer in A & B (200 msec) than in C (50 msec).

A: an efferent fibre. Animal breathing air. Total number of impulses (n_{IMP}) = 819, mean rate of discharge (f_{IMP}) = approx. 1/sec.

B: the same efferent fibre. Animal breathing 5% CO_2 + 21% O_2 in N_2. n_{IMP} = 780, f_{IMP} = approx. 2 per sec.

C: a chemoreceptor afferent fibre from another cat. n_{IMP} = 1297, f_{IMP} = 5.1 per sec.

The distribution of intervals from the afferent can be described as exponential. The efferent shows exponential form for the first 300 msec only. The lines drawn in on A and B are the exponentials constructed from interval distributions using the same intervals but a smaller bin width (20 msec) to show that the exponential process is seemingly unaffected by the increase in the mean rate of discharge of the fibre, the half-time being about 100 msec in both A and B.

(Fig. II 10). It was as if there was a basic, rather irregular, but certainly not random, discharge at a mean frequency of about 1 per second but after each impulse had occurred there was a transient but striking increase in the probability that another would occur. The behaviour of the fibre was reminiscent of that seen in somatic motoneurones when they show paired discharges (Calvin, 1972).

The occurrence of oscillations in the discharge of single chemoreceptor fibres of cats

Obviously if one ventilates an animal very slowly every fibre should show oscillatory behaviour (Heymans & Rijlant, 1933). In our experiments, if a fibre showed oscillations, these were readily detectable at respiratory frequencies of 20 per minute and the oscillations were quite large at 15 per minute. Yet of the 58 fibres studied 10 showed no evidence of oscillations whatsoever at these respiratory frequencies and another three showed only equivocal oscillations in discharge. These fibres were, in a way, frustrating in that we could test a range of conditions on a fibre and then when we later analysed the records it would emerge that the fibre was not oscillating anyway. Had we had on-line computing we would have been able to find how low the frequency of ventilation had to be for the discharge of these fibres to show clear oscillations.

Ten of the 13 fibres were from three cats. Although these fibres seemed to respond normally to changes in inspired gas tensions, the blood gases were normal, and the mean arterial pressure was within normal limits, there may have been some local circulatory disturbance which prevented the transmission of the oscillation of the stimulus. This was less likely to be the reason with the remaining three fibres that did not show oscillations in discharge as other fibres recorded from earlier or later in the same experiments did show oscillations. It is possible that there is a group of fibres which behave quite normally in the steady state but have a distinctly lower frequency of ventilation at which an oscillation in their discharge becomes apparent. Fidone & Sato (1969) have reported that 'C' fibre chemoreceptors respond less promptly to stimuli than do 'A' fibres. We did not measure conduction velocities.

Dogs

Single fibres dissected from the carotid sinus nerve of two large (25 kg; 30 kg) dogs showed oscillations in their discharge related to respiration. The time from inspiration to the dip in discharge was longer in the dogs, 4-6 sec, than in the cats, presumably reflecting a longer circulation time from the lung to the carotid body in the larger animals. The relation of the dip in discharge to the

the preceding inspiration was particularly clear, and was visible to the naked eye, in a record taken from one of the dogs when it was breathing slowly and irregularly.

DISCUSSION

The validity of the summing procedure

We have discussed in Methods the adequacy of the triggering of the computer cycle.

A problem is raised by the fact that spontaneous breathing is never absolutely regular, but this does not seriously blur the pattern of the histogram if the breathing is not very irregular. This point is illustrated by Fig. II 1, in which a variation of $\pm$0.5 sec in a respiratory cycle of about 4 seconds was tolerable. When summing was done over single respiratory cycles this variation caused the last few bins of the histogram from the 3400-3600 msec bin onwards to contain fewer impulses than they should have, erroneously indicating that the probability of an impulse's occurring had fallen. The problem was avoided if summing over double respiratory cycles was done, and this was our usual practice.

The discharge varies at the same frequency as respiration and the most obvious explanation of the variation is that it is caused by oscillations in the chemical stimulus in the blood. Thus the decline in discharge which starts with a delay of about 3 sec from the start of inspiration is to be attributed to the arrival at the carotid body of the first blood that left the pulmonary capillaries after inspiration had brought fresh air into the alveoli. The delay is made up of the time taken to draw back the dead space air into the alveoli, the circulation time from the pulmonary capillaries to the carotid body, and the response time of the chemoreceptors. The delay should be relatively independent of the duration of the respiratory cycle if the heart output remains constant.

On this interpretation, the build-up of discharge from the moment of triggering relates to the respiratory cycle preceding the inspiration that triggered the computer cycle. Because spontaneous respiration is not absolutely regular, there is some variation in when this inspiration occurred relative to the triggering inspiration and so the build-up of discharge may be subject to some blurring. But, proceeding from the dip in discharge caused by the triggering inspiration, the pattern of discharge is correlated with the

triggering inspiration and will be subject only to the variability imposed by the inherent randomness of chemoreceptor discharge. The second dip in discharge in a double cycle summation is the part of the pattern which is most susceptible to blurring, first because this dip in discharge relates to the inspiration following the triggering one, and secondly because the major part of the dip develops over about half a second and that is the order of variability of the duration of the respiratory cycle. The second dip in discharge of a double cycle summation was usually less steep and more prolonged than the dip in discharge related to the triggering inspiration. If, however, the respiratory cycle was short, as in Fig. II 8 top, the second dip in discharge in a two-cycle summation was the one to be related to the triggering inspiration and it was usually sharper than the first.

During artificial ventilation these problems do not arise, but it is still valuable to sum over double respiratory cycles because the respiratory cycle is usually not an exact multiple of the length of a bin.

It could be argued that the discharge of a single fibre, summed over many respiratory cycles, does not necessarily represent the discharge of many fibres summed over a single respiratory cycle.

This might be a valid criticism if our observations were used in a discussion of the significance of oscillations in discharge in the control of respiration. When, however, we recorded simultaneously the discharge of two fibres, the histograms for the two fibres were usually very similar in their shapes, the relative amplitude of the oscillations, and in their phase relation to respiration, even though there might be a ten-fold difference in their mean rates of firing. There was, however, a minority of fibres which did not show an oscillation in discharge and, if these are not an artefact of our experimental procedure, they would cause the overall amplitude of the oscillation in chemoreceptor discharge to be less than our summations would suggest.

Our summation procedure avoids some problems which arise when integration is used. Then zero discharge is defined as that present on 100% O_2, an unsatisfactory criterion: we find that 100% O_2 does not silence all fibres. Integrators have been used with time constants of one third of a second or longer, and such a time constant would tend to blur a rapid change in discharge. With an integrator and pulse height selector it is possible that some movement of the nerve on the electrodes will occur with respiration

and so give a false impression of an oscillation in discharge because the size of the potentials recorded changes. This problem can be easily dealt with when single fibres are used. Also one can then be confident that baroreceptor fibres are not present.

The source of the oscillation

With each respiratory cycle both the P_{a,CO_2} and P_{a,O_2} oscillate, and the oscillations in discharge of chemoreceptor fibres could be in response to oscillations in either or both of these oscillating stimuli. We suggest that the oscillatory discharge is due primarily to the oscillation of P_{a,CO_2} for the following reasons:

1. The chemoreceptors respond quickly and show adaptation to step changes of P_{a,CO_2} but respond less quickly and do not show adaptation to step changes of P_{a,O_2} (Black et al., 1971).
2. The speed of response to step changes of P_{a,O_2} is more variable from fibre to fibre (Black et al., 1971). Our studies on pairs of oscillating fibres recorded simultaneously showed little variability in the time of the dip in discharge from inspiration, and the change in discharge was rapid.
3. When the cat breathed 100% O_2 the discharge of chemoreceptors was greatly reduced but it still showed oscillations at the frequency

of respiration. The relative amplitude of the oscillations was increased but the absolute amplitude was reduced. In hypoxia, the mean rate of discharge increased. The absolute amplitude of the oscillation might be increased or decreased, but the relative amplitude was always reduced (Band & Wolff, 1973). The response curves of chemoreceptors to hypoxia would suggest a very different effect of hypoxia on oscillations. The curve is approximately a hyperbola and the absolute amplitude of the oscillations in P_{a,O_2} should be relatively independent of the mean P_{a,O_2}. Thus an oscillation in discharge due to an oscillation in P_{a,O_2} of constant amplitude should have an absolute amplitude given by the slope of the curve, and this curve, being a hyperbola, has a slope which is inversely proportional to the square of the P_{a,O_2}. It should have a relative amplitude which is inversely proportional to P_{a,O_2}. The effect of P_{a,O_2} on the relative amplitude of oscillations was the reverse of this prediction. Even if one accepts Purves' (1966) finding that oscillations in P_{a,O_2} are roughly directly proportional to mean P_{a,O_2}, the relative amplitude of the oscillations in discharge should be independent of P_{a,O_2}.

The oscillations could be due to the fluctuations in blood pressure that occur with respiration. However, the phase relation

of the dip in discharge to inspiration was unaffected by a change from spontaneous ventilation to positive pressure artificial ventilation, a manoeuvre which causes an inversion of the respiratory fluctuations in blood pressure, and it was delayed by clipping the external carotid artery.

If it is accepted that the oscillations are (1) due to P_{CO_2}, and (2) have an absolute amplitude that is independent of the mean rate of discharge when this is altered by hypoxia, this means that the chemoreceptors were following transient response curves to P_{a,CO_2} that are higher in hypoxia but are parallel to one another over any given range of P_{a,CO_2}. The steady state response curves to CO_2 reported by Fitzgerald & Parks (1971) are, however, strikingly different from this. They appear as a fan of lines radiating from a point on or below the P_{a,CO_2} axis at a P_{a,CO_2} of 10-20 torr. Thus they would predict an oscillation in discharge of relative amplitude not much affected by P_{a,O_2}, and also of a smaller absolute amplitude than we, and other workers (Band et al., 1971), have observed.

Black, McCloskey & Torrance (1971) observed that the response to a large step change of P_{CO_2} adapts with a half time comparable to, or longer than, the duration of a respiratory cycle. Thus one

would expect that the transient response curve would be steeper than the steady state one. If the response to a step change of P_{a,CO_2} adapts to 1/nth of its initial peak then the slope of the transient curve should be n times that of the steady state curve. Thus, if the transient response curves are indeed parallel whilst the steady state curves form a fan, adaptation to a change of P_{a,CO_2} is affected by P_{a,O_2}, being less in hypoxia and greater in hyperoxia.

In further support of the idea that adaptation is significant, the steady state response curves are not steep enough, at least at normal to high P_{a,O_2}, to predict oscillations in discharge of the amplitude we have seen in response to likely oscillations in P_{a,CO_2} in the arterial blood, which are about 1-3 torr at normal respiratory frequencies. The largest oscillations in pH that Band, Cameron & Semple (1969) saw were of 0.05 pH units in a cat breathing at a respiratory frequency of 10 per minute. In one animal breathing at this frequency the relative amplitude of the oscillation in discharge was 95%. The mean pH_a was 7.298, and the predicted relative amplitude of the oscillation in discharge to a pH oscillation of 0.05 pH units from Fitzgerald & Parks (1971) is only 60%. High relative amplitudes of oscillation (120-160%) were seen in hyperoxia,

although it is possible that this was in part due to a threshold effect of the very low mean frequency of discharge, but even in normoxia relative amplitudes of approaching 100% of the mean were not uncommon. The amplitudes in Fig. II 9 are 140% and 110% in an animal which was probably somewhat hypercapnic and hypoxic as it had been given a dose of anaesthetic not long before the runs were recorded. A fibre recorded from earlier in the same experiment, when the animal was breathing at 10 per minute, had shown oscillations of almost 100% during the administration of a 5% CO_2 mixture.

The pattern of the oscillation

If the receptor adapts, and its half time for adaptation is much longer than the respiratory cycle, its output should change in direct proportion to the level of the stimulus which affects it, but if it adapts greatly and very rapidly its output should more nearly represent the rate of change of the stimulus. Black, McCloskey & Torrance (1971) found a time constant of adaptation to CO_2 of a few seconds. Thus in slow breathing, with a marked expiratory pause, a changing degree of adaptation should not much affect the dip in discharge provoked by inspiration, but it should affect the

subsequent rise, tending to make it plateau just before the next dip. We saw such a plateau in discharge when the respiratory frequency was low (Fig. II 9), which would suggest that adaptation then contributed to the shape of this part of the oscillation. Also we sometimes found that the period over which discharge fell was distinctly shorter than inspiration, even if allowance is made for the time over which dead space air is being drawn back into the alveoli early in inspiration (Fig. II 9). This suggests that the off-effect, the reverse of adaptation at a fall of the stimulus, is here significant. Thus it would appear that the amplitude of the oscillation is greater than that predicted by the steady state response curve because adaptation is not very rapid, but that adaptation is rapid enough to have some part in determining the shape of the oscillation in discharge, at least at low respiratory frequencies.

If the respiratory frequency was increased but total ventilation was adjusted to keep the mean rate of discharge constant, the amplitude of the oscillation in discharge decreased. According to Yokota & Kreuzer (1973), increasing frequency of respiration reduces alveolar oscillations in gas tensions with respiration. Also the

amplitude of an oscillation in the blood leaving the pulmonary capillaries is more attenuated by the time it reaches the arteries if its frequency is greater. We seldom saw oscillations in discharge at respiratory frequencies of 30 and above when metabolism was not increased, but we saw clear oscillations at up to 40 per minute when metabolism was increased by DNP. This would suggest that the receptor can follow rapid oscillations in its stimulus but that at rest the oscillations are too small to give an oscillation in discharge that can be detected through the randomness of discharge. In exercise the reverse may well be the case. We have not obtained any evidence to suggest that slowness of response of the receptor contributes to the decline in the oscillations in discharge as the respiratory frequency is increased, though obviously there must be some limiting frequency beyond which the response of the receptor oscillates less. Fitzgerald et al. (1969) would suggest that this is near to normal respiratory frequencies.

We could not demonstrate any effect of the sympathetic supply to the carotid body, or of the efferent fibres running in the sinus nerve, on the amplitude or form of the oscillations. However these pathways exert their influence on the receptors, they do not affect the degree or time course of adaptation to an extent detectable in these experiments.

Mention has been made of the intrinsic randomness of the discharge of chemoreceptors (Biscoe & Taylor, 1963). If allowance is made for the fluctuation in the frequency of discharge with respiration, the discharge can be shown to retain the property of randomness, and there is no contradiction in describing it as both oscillatory and random (Goodman & Nail, 1973).+

+See Section 6.4

6.4: Paper III

Brain Research, 59 (1973) 379–383

Short Communications

Oscillatory behaviour and randomness of firing of chemoreceptor fibres in the cat

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Here an attempt is made to reconcile an observation that the discharge of chemoreceptors is random[4] with another observation that their discharge oscillates at the frequency of respiration[3].

The composition of the gas in the alveoli of the lungs changes cyclically with respiration[8]. On inspiration the alveolar partial pressure of oxygen rises and that of carbon dioxide falls. During expiration the opposite changes occur. These changes are reflected in the blood leaving the lungs and persist into the arterial blood[1,12]. The chemoreceptors of the carotid body respond to changes in P_{O_2}, P_{CO_2} and pH in the arterial blood and their response is fast enough for them to follow the oscillating signal in a cat breathing at 20–25 breaths/min (see ref. 3).

However, Biscoe and Taylor[4] had earlier reported that if one studies single chemoreceptor fibres their discharge is random, *i.e.* their generation is a Poisson process and there is no correlation between the time of occurrence of an impulse and the time of occurrence of subsequent impulses. Thus there is an apparent paradox in that a discharge which had been described as random can show oscillatory behaviour. The present analysis was undertaken to resolve this paradox.

Cats were anaesthetized with pentobarbitone sodium (Nembutal, Abbott, 42 mg/kg) and the activity of single chemoreceptor fibres of the carotid sinus nerve was recorded by conventional methods[6]. Impulses were monitored on an oscilloscope and recorded on one channel of a tape recorder (Thermionic T3000). Respiratory air flow was recorded on another channel. The recordings were later played through an interface system into a computer (IBM 1130 series) and, using the start of inspiration as the stimulus, a post-stimulus histogram (PST) of the occurrence of nerve impulses was constructed[10]. Because the cats did not breathe absolutely regularly a clearer picture of an oscillation was given if the computer was programmed to ignore every second inspiratory signal. This gave a PST summed over pairs of respiratory cycles. A programme for an interval histogram (IH) was also available.

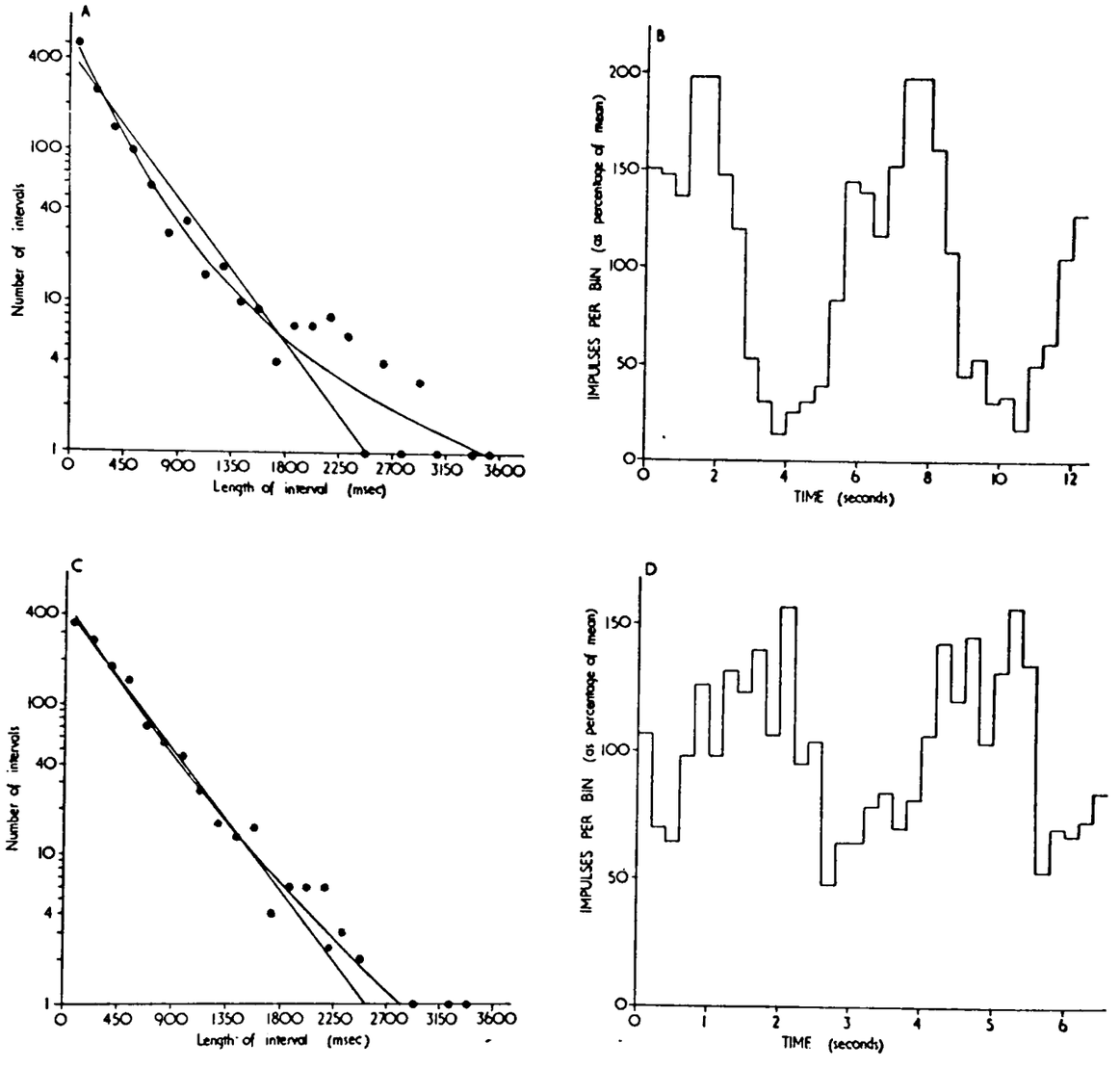

Fig. 1. Spontaneously breathing anaesthetized cats, recording from single chemoreceptor fibres of the carotid sinus nerve; A and B are from one animal, C and D from another. A and B: two different treatments of a series of approximately 1200 impulses. A: a histogram of the inter-pulse intervals, total number of intervals = 1197. ●, the observed values. The straight line is a prediction in a simple exponential form of the number of intervals in each class. The curved line is a prediction derived from summed exponentials based on the oscillatory discharge of the fibre. B: shows the oscillations in discharge over 36 double respiratory cycles, total number of impulses = 1119. Zero on the time scale represents the inspiration that triggered the computer cycle. C and D: a different series of approximately 1200 impulses. Layout as in A and B. Total number of intervals in C = 1199, total number of impulses in D = 1179. D was constructed over 78 double respiratory cycles. Overall mean frequency in both series was approximately 2.4 imp./sec.

The PSTs in Fig. 1B and 1D show the oscillations of the discharge of two fibres, from different cats, over double respiratory cycles. In both PSTs zero on the time scale corresponds to the start of inspiration that triggered the computer. In Fig. 1B the respiratory frequency was about 9.5 breaths/min, and in Fig. 1D it was about 18 breaths/min. The mean frequency of discharge of both fibres was about 2.4 imp./sec. The percentage amplitude of the oscillation in discharge at the lower respiratory frequency is about twice that at the higher respiratory frequency, although even at 18

breaths/min the oscillation is virtually 50% either side of the mean. Thus the discharge could not be described as random even on first inspection.

One of the properties of a Poisson process is that the lengths of the intervals between the events show an exponential distribution. Biscoe and Taylor[4] took a series of impulses from a number of single chemoreceptor fibres, constructed an IH for each series, and then compared the observed and predicted numbers of intervals in each class of interval in the histograms using the formula for a random series $n = N(1 - e^{-t/T})$ (see refs. 5 and 11). where n is the predicted number of intervals falling within the class width t, N is the total number of intervals in the series, and T is the mean interval. They showed that the IH did indeed have an exponential form, though the numbers of intervals in most of the series were less than 400.

A similar calculation was performed on the intervals between the impulses in the series used to construct the PST shown in Fig. 1B. The theoretical line, being exponential, is straight on a semi-log plot (Fig. 1A). The actual numbers of intervals that were observed in each class are plotted as discrete points on the same graph. The observed points fall below the theoretical line over the middle range of intervals and above at the extremes. This confirms what is obvious from the PST (Fig. 1B): that the discharge is not random.

However, the calculation was made assuming a single mean interval T. Yet with an oscillatory discharge the mean frequency is changing throughout the cycle. For example, with the fibre illustrated in Fig. 1B, the mean discharge calculated over the period 1.5–2 sec after inspiration started is double the overall mean frequency. One can therefore make a new prediction: if the impulses are random with respect to each other, then the overall IH will follow a line constructed by adding a series of exponentials, one for each of the time bins of the PST. Each of these exponentials is defined by $n = N_x(1 - e^{-t/T_x})$, where n is the number of intervals predicted within the class width t, and N_x is the number of intervals occurring at a mean frequency $1/T_x$, *i.e.*, having a mean interspike interval of T_x.

To make the calculation a PST was constructed over single respiratory cycles. The respiratory cycle was 6200 msec long and the width of each of the 31 bins of the PST was 200 msec. The PST was collected over 77 respiratory cycles and thus the total time represented by each bin of the PST was 77 × 200 msec, *i.e.*, 15.4 sec. The mean frequency for each bin, and hence T_x, could therefore be calculated. T_x varied between 203 msec and 2200 msec and the overall mean interval was 411 msec. Each of the 31 exponentials was calculated and then they were all summed. The line resulting from this summation is the curved line in Fig. 1A. This fits the observed points more closely than the simple exponential line.

However, an exponential distribution, although a consequence of a Poisson process, does not by itself prove that a process is Poisson: it is also insensitive to deviation[9]. Fig. 1C is the IH for the intervals between the impulses in the series used to construct Fig. 1D. The summed exponential line hardly deviates from the simple exponential line over the middle range of intervals, and it is only for intervals of more than 2000 msec that the difference between the two predicted distributions becomes at all obvious. Although more long intervals were observed than one would predict from

the simple exponential, there were only 14 intervals longer than 2000 msec altogether, and this is only 1.17% of the total number of intervals.

The same series of impulses illustrated by Fig. 1A, B was analysed again. The numbers of impulses occurring in corresponding 0.5-sec intervals of successive respiratory cycles were compared with the predicted numbers according to the Poisson distribution using the chi-squared test[9]. The prediction was good when each bin was considered separately ($\chi^2 = 2.7$, $df = 6$, $0.9 > P > 0.8$). If, however, the bins were all lumped together there was a significant departure from Poisson ($\chi^2 = 38.1$, $df = 6$, $P < 0.001$). This further supports the hypothesis that the generation of chemoreceptor impulses in the breathing animal can be described as a Poisson process oscillating at the frequency of respiration.

The question remains: why did Biscoe and Taylor[4] not see any evidence of oscillations or of departure of the observed from the predicted values in their IHs? Oscillations in discharge are not always present and their amplitude depends on many factors[3,7]. The fibres studied by Biscoe and Taylor[4] may not have been showing oscillatory discharge. Even if they had this would probably not have been apparent in the interval distribution histograms for two reasons. Firstly, they used fewer impulses to construct their histograms than are used here. Although one of their published histograms was constructed using 1142 intervals the mean frequency was very high, 25 imp./sec, and at these high frequencies not only would oscillations be unlikely but the discharge of the chemoreceptors shows a departure from randomness at the short interval end of the histogram anyway[4,13]. When the mean frequency was lower, they used far fewer intervals. Secondly, and more important, the oscillations in discharge must be very large before there is any significant departure from the simple exponential prediction.

In conclusion: although the discharge of chemoreceptor fibres of the carotid body is not random with respect to the respiratory cycle, the impulses are nevertheless random with respect to each other. The probability of an impulse occurring varies with a respiratory periodicity but not with the time since the previous impulse occurred. There is no paradox in describing their behaviour as both random[4,13] and oscillatory[2,3,7]. There is thus no need for any modification of the models constructed by Biscoe and Taylor[4] or Stein[13] to explain the pattern of discharge of single chemoreceptor fibres.

It could be argued that, if the low probability period of an oscillation in a Poisson process was shorter than a significant number of the intervals predicted in that period, the method of reconstructing exponentials used above would not predict the distribution of intervals correctly as too few long intervals would be seen. This criticism does not apply to the second method of prediction used. A corollary to this is that, under these circumstances of oscillating probability, a Poisson process need not necessarily obey a summed exponential distribution of the intervals between the events.

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1 BAND, D. M., CAMERON, I. R., AND SEMPLE, S. J. G., Oscillations in arterial pH with breathing in the cat, *J. appl. Physiol.*, 26 (1969) 261–267.
2 BAND, D. M., SAUNDERS, K. B., AND WOLFF, C. B., The relation between chemoreceptor discharge and respiratory fluctuation of arterial pH in the anaesthetized cat, *J. Physiol. (Lond.)*, 218 (1971) 73–74P.
3 BISCOE, T. J., AND PURVES, M. J., Observations on the rhythmic variation in the cat carotid body chemoreceptor activity which has the same period as respiration, *J. Physiol. (Lond.)*, 190 (1967) 389–412.
4 BISCOE, T. J., AND TAYLOR, A., The discharge pattern recorded in chemoreceptor afferent fibres from the cat carotid body with normal circulation and during perfusion, *J. Physiol. (Lond.)*, 168 (1963) 332–344.
5 FATT, P., AND KATZ, B., Spontaneous subthreshold activity at motor nerve endings, *J. Physiol. (Lond.)*, 117 (1952) 109–128.
6 GOODMAN, N. W., Efferent control of arterial chemoreceptors mediated by glossopharyngeal fibres and artefacts introduced by stimulation techniques, *J. Physiol. (Lond.)*, 230 (1973) 295–311.
7 GOODMAN, N. W., NAIL, B. S., AND TORRANCE, R. W., Oscillatory behaviour of single chemoreceptor fibres from the cat carotid body, in preparation.
8 HALDANE, J. S., AND PRIESTLEY, J. G., *Respiration*, 2nd ed., Oxford Univ. Press, London, 1935, pp. 16–42.
9 HUBBARD, J. I., LLINÁS, R., AND QUASTEL, D. M. J., *Electrophysiological Analysis of Synaptic Transmission*, Arnold, London, 1969, pp. 124–126.
10 NAIL, B. S., On-line biomedical data analysis, *J. Physiol. (Lond.)*, 225 (1972) 5–7P.
11 PARRATT, L. G., *Probability and Experimental Errors in Science*, John Wiley, New York, 1961, pp. 227–229.
12 PURVES, M. J., Fluctuations of arterial oxygen tension which have the same period as respiration, *Resp. Physiol.*, 1 (1966) 281–296.
13 STEIN, R. B., Some implications of the variability in chemoreceptor discharge. In R. W. TORRANCE (Ed.), *Arterial Chemoreceptors*, Blackwell, Oxford, 1968, pp. 205–210.

6.5 Oscillations and ventilation

There is a growing literature on the possible significance of oscillations in the discharge of chemoreceptors in the control of respiration (see e.g. Black & Torrance, 1971; Cunningham et al., 1973). In Paper II (i.e. Section 6.3 of this thesis) our approach was at the level of the receptor. Here I shall briefly mention where our observations are relevant to the discussion of the significance of the oscillations to ventilation.

i. The oscillation is not symmetrical about the mean if the respiratory period is longer than about 3 seconds. The pattern of discharge is not a sine wave but rather a saw-tooth with the fall more rapid than the subsequent rise.

ii. When the $\dot{V}_{O_2}$ is raised in muscular exercise the oscillations would probably be more marked, and present at higher respiratory frequencies, than normal.

iii. Cunningham and co-workers (Cunningham et al., 1973; Marsh et al., 1973) suppose that, in man, steady hypoxia amplifies the response of the respiratory centre to small changes in the wave form of P_{a,CO_2} resulting from imposed changes in the waveform

of P_{A,CO_2}. We can say that, in the cat, this does not happen at the peripheral chemoreceptors in relative terms, as the relative amplitude of the oscillation is far larger in hyperoxia than it is in hypoxia. However the absolute amplitude of the oscillation is larger in hypoxia and it is probably the absolute size of the signal that is important to the respiratory centre.

iv. We cannot say whether an oscillating P_{a,CO_2} is normally a greater stimulus to chemoreceptors than a steady one of the same mean level. In Paper II the amplitude of the oscillations is explained in terms of transient response curves that cut the steady-state response curves at the mean P_{a,CO_2}, but these transient curves take no account of any adaptation that might have occurred over the respiratory period. The relation of the frequency of discharge to the P_{a,CO_2} will not be a straight line but a hysteresis loop, and the loop could also be asymmetrically placed on the steady-state curve. We can say that in hyperoxia an oscillating P_{a,CO_2} is probably a greater stimulus than the mean P_{a,CO_2}. The excursion of the transient response curve cannot be as great below the steady-state curve as above it because it cuts the P_{a,CO_2} axis.

Chapter 7

Observations on the Homogeneity of Response of Single Chemoreceptor Fibres

7.1 Introduction

A population of receptors that respond to certain modalities of stimulus can be compared one with another in terms of their thresholds, sensitivities and peak responses to each of these stimuli. Receptors from a totally homogeneous population would all give the same response in any circumstances to a given stimulus or combination of stimuli. In a totally heterogeneous population the frequency of discharge of one receptor could not be predicted from the frequency of discharge of any other member of the population of receptors. The work reported here makes some observations on the homogeneity of the chemoreceptor population. In doing so it reveals some limitations of recording from preparations of single chemoreceptor fibres from the carotid sinus nerve.

The frequency of discharge recorded from a chemoreceptor fibre is a compound function of its response to its individual stimuli. The discharge can be modified by other factors such as the sympathetic and glossopharyngeal efferent fibres as discussed in earlier chapters of this thesis. This complex stimulus makes it difficult to compare responses to a given experimental procedure when they are recorded serially.

During the study of the oscillations in chemoreceptor discharge that occur with respiration (Chapter 6) pairs of single fibres were occasionally recorded from simultaneously. Each fibre of a pair was laid over a separate electrode and recorded on a separate recording channel. This allowed comparisons of the oscillations in discharge to the same oscillation of stimulus. The recording method allowed further comparisons between fibres, when the arterial stimulus must have been the same for each receptor, by going back over the experimental records and comparing the frequencies of discharge of the pairs of fibres at any time. In some experiments specific manoeuvres were performed to obtain further information.

7.2 Methods

General methods were as described in Chapter 2.

Recordings were made simultaneously from two single units of the carotid sinus nerve each in a separate fine slip of nerve and each lifted on to a separate stainless steel electrode. Both signal electrodes were referred to a single indifferent electrode to minimise the problem of earth loops.

The frequencies of discharge from each of a pair of fibres were compared in the following ways:

(a) "Stable conditions". The ratio between the frequencies of discharge of the two fibres of a pair was calculated over periods of 30 or 60 seconds during long periods (5-16 minutes) when the cat's respiration was in an apparently steady state as judged by the mean frequencies of discharge. For each pair the fibre with the higher overall frequency of discharge was used as the numerator of the fraction.

(b) "Long-term transients". The level of arterial stimulus was allowed to alter progressively for about 30-60 seconds usually followed by a return to control. All occasions in the experimental

records when the frequencies of discharge changed markedly were analysed, whether intentional or not. Intentional manoeuvres included disconnecting the ventilatory pump from the cat for a number of cycles during artificial ventilation, or switching the pump from one gas mixture to another. Gas mixtures were made up in Douglas bags. Mixtures used: 100% O_2, 35% O_2 in N_2, 8-12% O_2 in N_2, 100% N_2, 7-10% CO_2 + 21% O_2 in N_2.

The records were divided into 5-second intervals and the number of impulses in each interval counted for each fibre of the pair. The frequency of discharge of one fibre was plotted against that of the other for 10-second intervals, each 5-second count being included in two consecutive 10-second points on the graphs. This was done because the inherent randomness of chemoreceptor discharge caused too much variability between 5-second counts, but if simple 10-second counts were plotted there were not enough points on the graph.

(c) "Rapid transients". 0.2 ml slugs of saline equilibrated with 100% CO_2 were injected retrogradely into the lingual artery with the external carotid artery clamped. The superior thyroid artery and a few other easily accessible branches of the common

carotid artery were tied off. The background discharge was reduced to virtually nil by hyperventilation with 100% O_2 to make identification of the first impulse of each fibre's response unambiguous.

(d) "Oscillatory behaviour". Impulses recorded during stable conditions were summed over many respiratory cycles as described in Chapter 6.

7.3 Results

Twenty-four fibres from 8 cats were used in 14 pairs. It was not possible to apply all four methods of comparison to every pair of fibres. It can be difficult holding one single fibre for long enough to perform the experimental tests one wishes to, holding two single fibres is more than twice as difficult.

Ideally one would plot the instantaneous frequency of discharge of one fibre of a pair against the instantaneous frequency of the other, but this is not possible because of the inherent randomness of chemoreceptor discharge (Biscoe & Taylor, 1963). The average frequencies over convenient periods were calculated and plotted instead, and this "f_1 : f_2" plot is the most convenient way of presenting and discussing the "R_1 : R_2" relation, the relation of

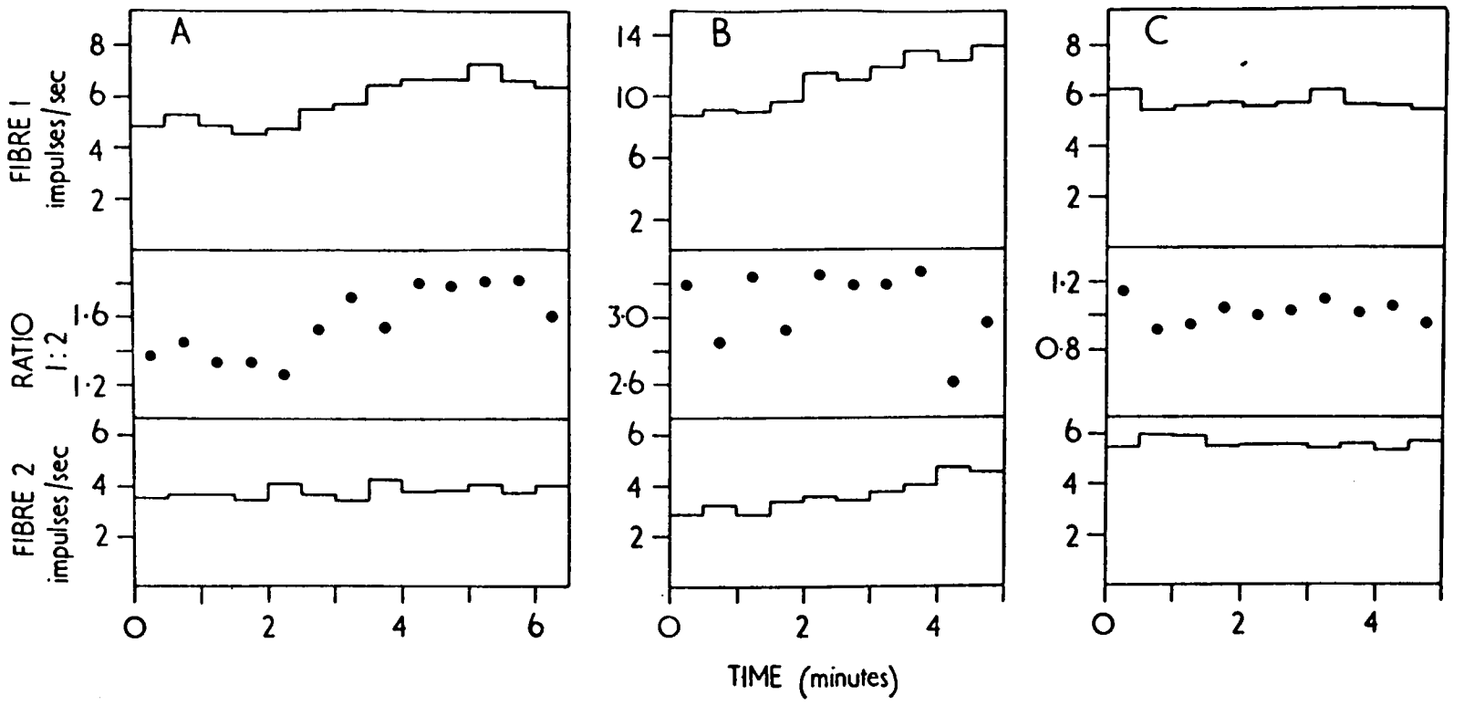

Fig. 13: Cat: nembutal. Simultaneous recording on separate electrodes from two single chemoreceptor fibres of the sinus nerve. A and B from one pair of fibres, C from another pair from a different cat. The upper section of each block is a histogram of the mean frequency of discharge (counted over 30 sec) of one fibre of each pair, the lower section is the histogram for the other fibre of each pair. The middle section shows the "pair-ratio" for each 30-sec period. A: spontaneous ventilation, air. The pair-ratio increases by about 50% during the course of the recording. B: the same pair of fibres, the cat now paralysed with gallamine and artificially ventilated with air. The pair-ratio is even higher than in A, but, whilst the mean rates of discharge of both the fibres increase steadily, the pair-ratio shows no trend. C: from a recording in a different cat showing steady-state rates of discharge and a steady pair-ratio.

the discharge of one receptor to the discharge of another. The term "pair-ratio" is a useful descriptive term that refers simply to the ratio of frequencies of a pair of fibres at any time. Although a constant pair-ratio implies a constant $R_1 : R_2$ relation the reverse is not necessarily true, as will be discussed later.

Stable conditions (9 pairs from 5 cats)

Five out of nine pairs of fibres showed constant pair-ratios, allowing for the randomness of the discharge. These ratios were maintained for the whole of the recordings (5-16 minutes), and the same ratios seen during any later recordings, i.e. if the mean rates of discharge had altered because of a different ventilatory regime they had done so in the same direction and to the same degree.

The other 4/9 pairs did not show constant pair-ratios. They showed either different pair-ratios in at least one subsequent stable recording, or the pair-ratio altered progressively during a recording. Two pairs showed both phenomena. With either of these types of change of pair-ratio, one of the fibres commonly had an

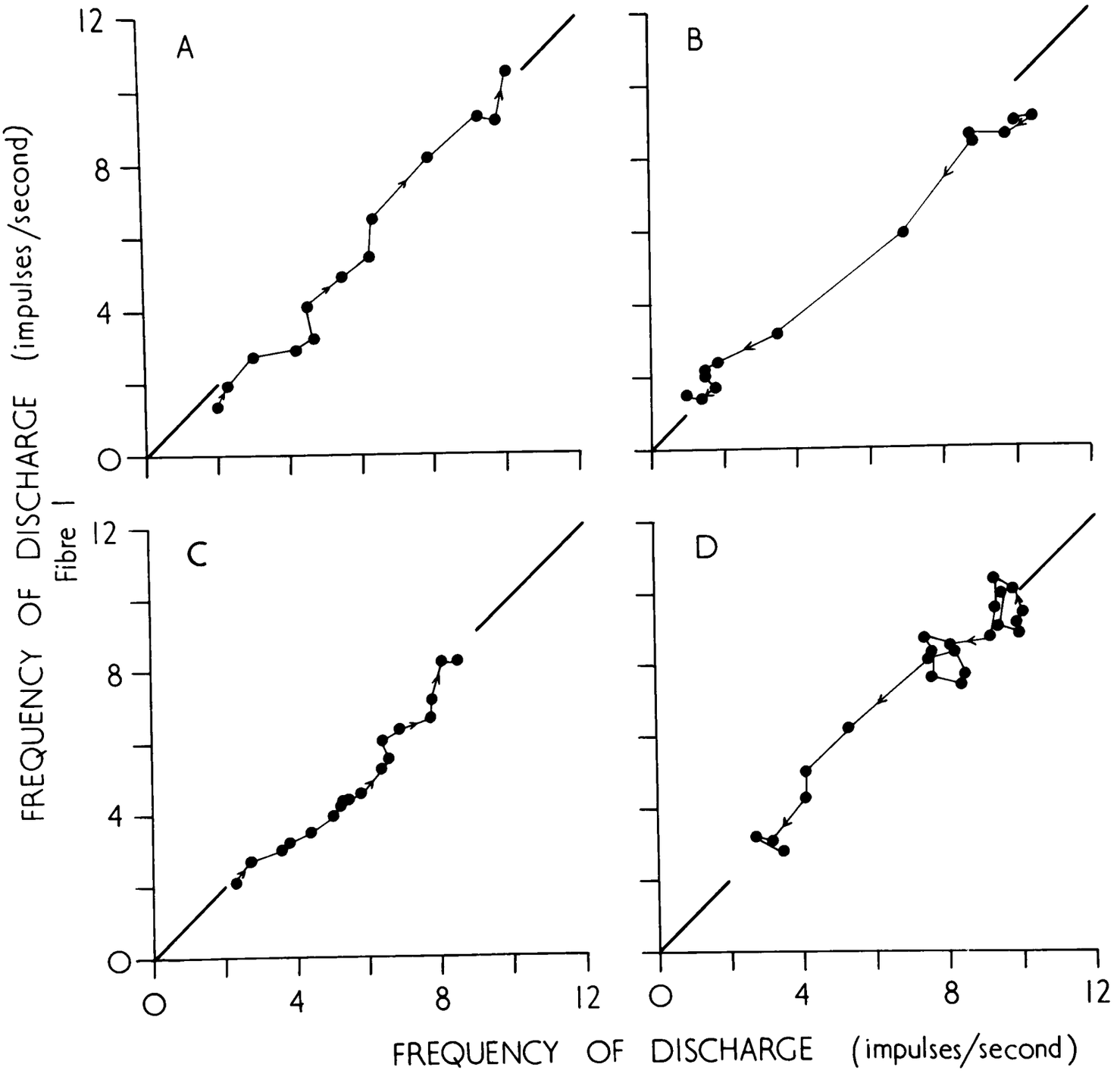
A
B
C
D
12
8
4
0
0
4
8
12
FREQUENCY OF DISCHARGE (impulses/second)
Fibre 1
FREQUENCY OF DISCHARGE (impulses/second)
Fibre 2

Fig. 14: Cat: nembutal, spontaneous ventilation. Simultaneous recording on separate electrodes from two single chemoreceptor fibres of the sinus nerve. Graphs of frequency versus frequency constructed during periods when the frequencies were changing. The records were divided into 5-sec intervals and each point on the graphs represents 10 sec of discharge, each 5-sec count being included in two consecutive points. The points are joined in sequence in each graph. All 4 graphs are from the same pair of fibres.
A: from 100% O_2 to 10% O_2 in N_2. B: from 10% O_2 in N_2 to 100% O_2.
C: from 100% O_2 to 7% CO_2 + 21% O_2 in N_2. D: from 7% CO_2 + 21% O_2 in N_2 to air and then to 100% O_2. The heavy lines are reference lines at a pair ratio of 1 : 1.

unchanged rate of discharge (Fig. 13).

There was no correlation between the behaviour of a particular pair of fibres and any other measured parameter, e.g. systemic blood pressure, or any experimental factor, e.g. whether the sympathetic supply to the carotid body was intact or had been cut.

Long term transients (13 pairs from 7 cats)

The pair-ratio for any one pair of fibres remained constant over any particular transient, i.e. the $f_1 : f_2$ plot could be approximated by a straight line that could be extrapolated to the region of the origin whether the pair-ration was 1 or 15.

Of the 5 pairs which showed a constant pair-ratio under stable conditions, 4 showed this same ratio during the long-term transients (Fig. 14). Those pairs which had not shown a constant pair-ratio under stable conditions had pair-ratios during long-term transients which might be the same as one or other of the stable values, or might vary from one test to another.

This left 4 pairs which were subjected to long-term transients but could not be studied under stable conditions. Two pairs (one tested 7 times, the other 10 times) showed the same pair-ratios in

all the long-term transients. Another pair was only tested once. The pair-ratio of the remaining pair showed a gradual change from 2 to 1.5 over 6 long-term transients, alternately asphyxic and hypoxic, due to a steady fall in the rate of discharge of one of the fibres. In the control periods before each long-term transient the other fibre's rate of discharge remained constant. The seventh and last test of this particular pair of fibres was an intravenous injection of DNP (2:4-Dinitro-phenol, British Drug Houses), and the pair-ratio was still 1.5, although the drug caused an increase in the frequencies of discharge to $2\frac{1}{2}$ times the maximum frequencies reached in the earlier tests. This provides a good example of the general principle that, with pairs of fibres having a different pair-ratio in different long-term transients, there was no correlation between the change of pair-ratio and the stimulus used, whether asphyxic, hypoxic or hypercapnic.

While it is true that the $f_1 : f_2$ plots could be approximated by straight lines, this statement requires some modification. Two pairs of fibres showed a change in pair-ratio at low frequencies of discharge (about 10 impulses per minute). Pairs of fibres were rarely taken to zero discharge because of the inordinately long

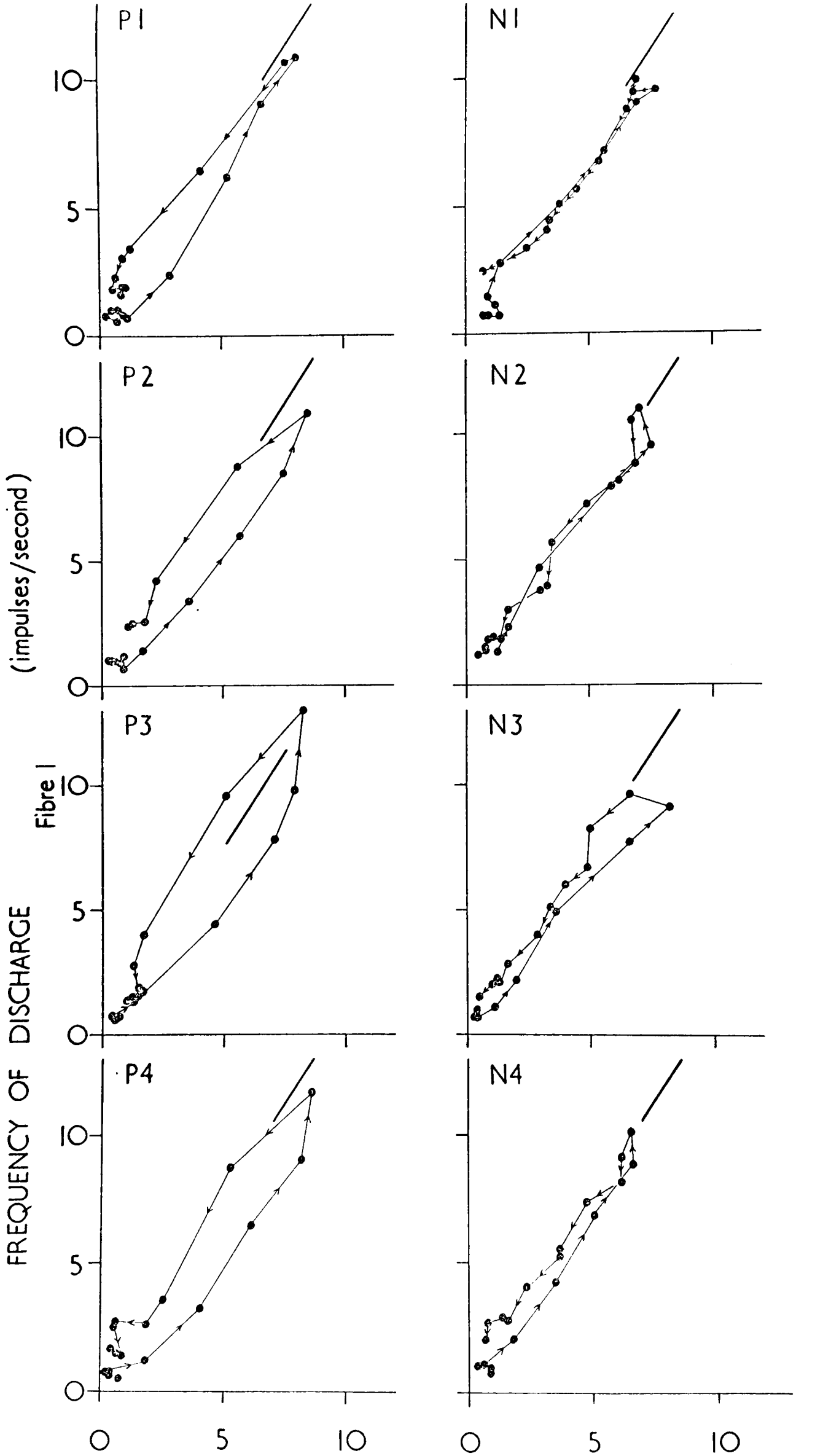
P1
N1
P2
N2
P3
N3
P4
N4
0
5
10
(impulses/second)
Fibre 1
FREQUENCY OF DISCHARGE
FREQUENCY OF DISCHARGE (impulses/second)
Fibre 2

Fig. 15: Cat: nembutal, artificial ventilation. Simultaneous recording on separate electrodes from two single chemoreceptor fibres of the sinus nerve. Graphs of frequency versus frequency constructed as in Fig. 14. All 8 graphs are from the same pair of fibres.

Control conditions: pump on air. P1-4: the pump was disconnected from the cat for 10 pump cycles. N1-4: the pump was switched to 100% N_2 for 10 pump cycles. The heavy lines are reference lines at a pair-ratio of 1.5 : 1.

recording times needed at very low frequencies but no evidence was seen to suggest that the $f_1 : f_2$ plots did not go through the origin. Hayes & Torrance (private communication) have occasionally seen apparent thresholds when testing strands containing two or three fibres to silence.

The peak frequencies seen during the long-term transients were usually 12-20 impulses per second (lower in the very low firing fibres). Only one pair of fibres showed any change of pair ratio at these frequencies. This was only from 1 to 1.2 but it was consistent. However, at the very high frequencies (50-70 impulses per second) seen during the rapid transients (see below) the pair-ratios did change.

Finally, two pairs of fibres showed clear hysteresis during asphyxic long-term transients but not during hypoxic ones (Fig. 15). The mean pair-ratios remained the same, hysteresis occurring about a line of that gradient. The pair illustrated also showed a tendency to an increased pair-ratio (to 2.5 - 3.5) over the first 30 seconds or so at the return to control frequencies, irrespective of whether the stimulus had been asphyxic or hypoxic.

Some of the other 11 pairs showed hysteresis occasionally but not in a consistent way.

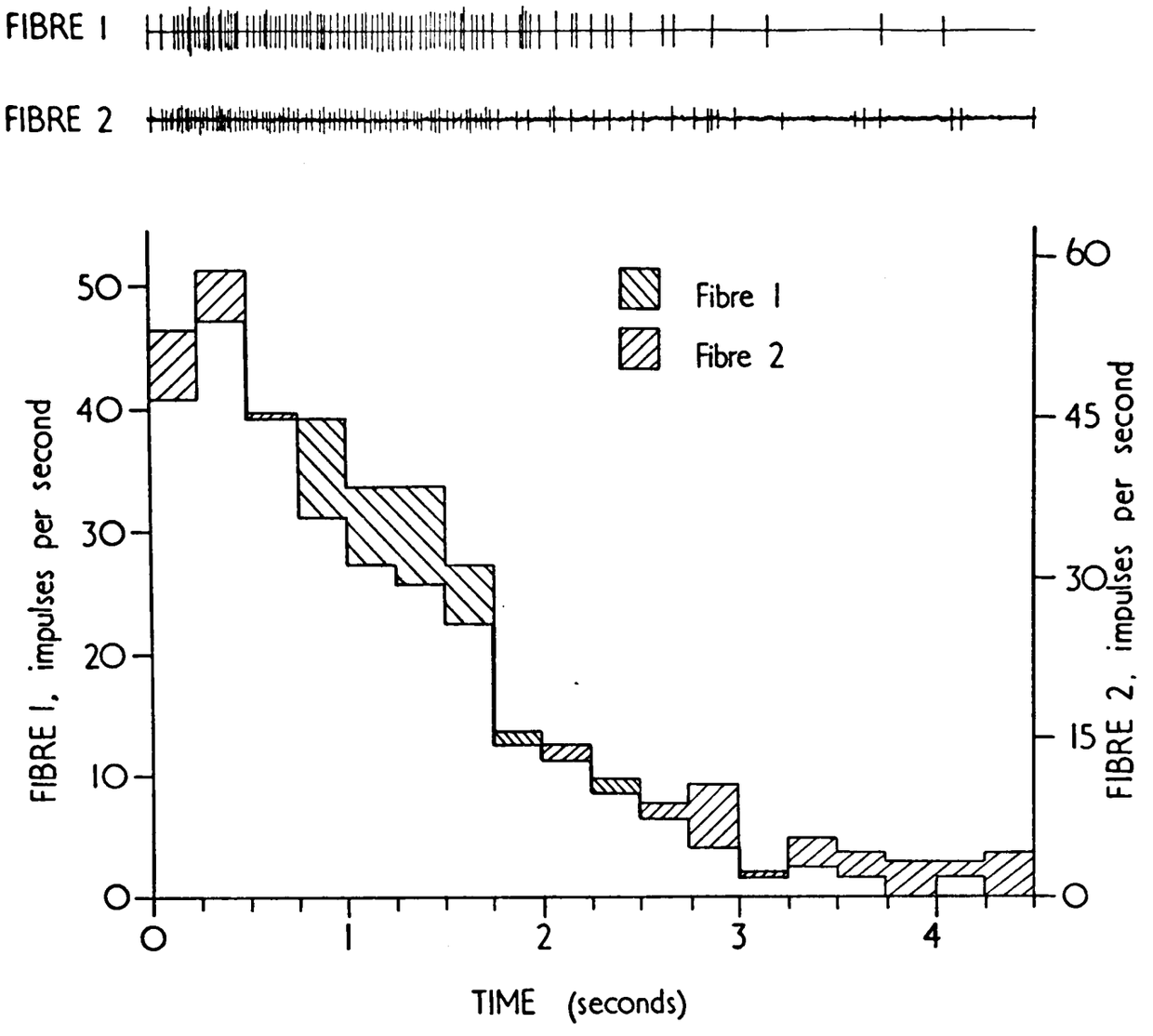

Fig. 16: Cat: nembutal, hyperventilation with 100% O_2. Simultaneous recording on separate electrodes from two single chemoreceptor fibres of the sinus nerve. The length of experimental record shows the response of a pair of fibres to a slug of 100% CO_2-saline injected retrogradely into the lingual artery. The histograms are of 5 such responses summed for each fibre of the pair. The summed responses have been scaled to the total number of impulses recorded from each fibre during the response (fibre 1 = 391 impulses, fibre 2 = 451 impulses) and are presented as equivalent frequency of discharge on the ordinate.

Rapid transients (4 pairs from 1 cat)

In one cat, one fibre was held for the whole of the experiment and compared serially with 3 other fibres. With 3 of the pairs 5 injections of CO_2-saline were made, only one injection was possible with the other pair.

Fibres of a pair responded within 30 milliseconds of one another (to the nearest 10 msec), but the largest constant delay between 2 fibres was 20 msec. The same fibre of a pair did not necessarily always respond first. The peak occurred over the same time for each fibre of a pair, in the first or second 250 msec. The total length of each summed transient was about the same for each fibre of a pair. When the number of impulses for each fibre of a pair in a summed transient were totalled, and the summed transients then scaled, the shape of the transient responses was very similar (Fig. 16). As mentioned above, the pair-ratios revealed by this procedure were somewhat different from those seen in stable or long-term transient conditions.

Oscillations in the discharge with the respiratory cycle

The discharge of chemoreceptors of the carotid body oscillates in phase with the respiratory cycle, probably responding to the oscillating level of P_{CO_2} in the carotid blood (Chapter 6). The fibres of 10 of the 11 pairs analysed in this way showed oscillations in discharge.

The corresponding oscillations were remarkably similar, despite the wide range of mean frequencies (Fig. II/4). The ratio of the relative amplitudes of the oscillations seen in the fibres of seven out of ten pairs was 1-1.3. The others ranged between 1.1 - 1.5, 1.2 - 1.5 and 1.2 - 1.6. The same fibre in each pair always had the larger relative amplitude. Any procedure that reduced the amplitude of oscillation of the discharge from one fibre always reduced the amplitude of oscillation of the discharge of the other. There was no correlation between relative amplitude and mean frequency of discharge within a pair, the fibre firing at either the higher or lower mean frequency could show the larger relative amplitude of oscillation.

There were no phase differences between the oscillations recorded from any pair of fibres not attributable to the irregularity

of chemoreceptor discharge. The bin width used in the PST's was 200 msec, so this was the smallest detectable phase shift, but one needs to collect a very large number of impulses (about 6000-8000) using this bin width before the PST assumes a profile smooth enough to enable one to detect small phase differences with confidence. This was not always possible. The largest difference was a pair that appeared to have a 2 bin (400 msec) phase difference in a 3600 msec respiratory cycle (i.e. a 20° phase difference) but only one recording was possible from this particular pair.

7.4 Discussion

Consider two receptors R_1 and R_2 that each respond to either or both of two stimuli S_1 and S_2, ignoring for the moment any dynamic response the receptors might show. If R_1 and R_2 come from a totally homogeneous population, then their rates of discharge will always be the same no matter to what combination of S_1 and S_2 they be exposed. The $R_1 : R_2$ relation can be described by the $f_1 : f_2$ plot and will be a straight line passing through the origin. With this starting point we can describe how certain inhomogeneities within the population could alter the $f_1 : f_2$ plot.

Differing characteristic frequencies of discharge for a given intensity of stimuli would give a line of fixed gradient, or pair-ratio, varying from pair to pair. Differing thresholds would result in a straight line cutting one of the axes. If the sensitivity of the receptor varied with the intensity of the stimuli then the $f_1 : f_2$ plot need not be a straight line. With either of these last two conditions the pair-ratio would not be constant, although there would still be a unique relation between the frequencies of discharge of the two receptors: the $f_1 : f_2$ plot still describes the $R_1 : R_2$ relation. If the receptors are differentially sensitive to the two stimuli then the $f_1 : f_2$ plot can no longer be used to describe the $R_1 : R_2$ relation because there will no longer be a unique relation between their frequencies. Nevertheless the receptors could still be homogeneous in their sensitivities to each of the individual stimuli. The maximum degree of heterogeneity would be a population of receptors having differing thresholds and sensitivities to both S_1 and S_2, with no correlation between the threshold of a receptor, its characteristic frequency, and its sensitivity.

The chemoreceptors are not a totally homogeneous population. It is already well recognized that they have a range of characteristic frequencies of discharge (Fidone & Sato, 1969). The present results suggest that this is the major inhomogeneity of the population. The $f_1 : f_2$ plots were straight lines (but see below) that could be extrapolated to the region of the origin. Any change of pair-ratio could not be correlated with the type of manoeuvre (hypercapnic, hypoxic or asphyxic) that had caused the change in the level of the arterial stimulus. I conclude that the mechanism of impulse generation is a process common to the responses to hypoxia and hypercapnia. This is at variance with Fitzgerald & Parks (1971) who studied the effect of hypoxia on the integrated response of the sinus nerve to hypercapnia. However, I studied relatively few pairs of fibres and the range of differential sensitivity may be small. Neil reported a preparation of two chemoreceptor units, one of which responded principally to hypoxia and the other to hypercapnia (see pp.187-188 in Heymans & Neil, 1958): perhaps more would be revealed by a larger study using stimuli which were more purely hypoxic or hypercapnic.

There are differences between some receptors in the development of a response, as shown by hysteresis in long-term transients and the small differences in the relative amplitudes of oscillations, and possibly also at the extremes of frequency.

Hysteresis will occur if one receptor of a pair responds more quickly or adapts more slowly to a change of stimulus, whether it is an inherent difference between the receptors or a difference in the transmission of the change to the receptor. It will also occur if there is a differential sensitivity to hypercapnia and hypoxia and these stimuli develop at different rates during an asphyxic manoeuvre, but there was no other evidence of differential sensitivity.

Hysteresis was marked only during asphyxic manouevres and not in hypoxic or hypercapnic ones, but the pairs that showed hysteresis were not subjected to hypercapnic manoeuvres, and those pairs that were did not show hysteresis. The maximum constant delay between the response of two fibres to a sudden large CO_2 stimulus is short, and the lack of phase differences between the oscillations recorded from pairs of fibres suggests that this is also true for small CO_2 stimuli. This makes it unlikely that the speeds of response to changes of P_{a,CO_2} are different, and hysteresis did not occur in

hypoxic manoeuvres so the same is probably true for the changes in P_{a,O_2}. One must suppose, therefore, that hysteresis is due to differences in the degrees of adaptation of receptors to CO_2, at least in the asphyxic situation, and this would fit with the small observed differences in the relative amplitudes of oscillations. Also, during an asphyxic manoeuvre, the responses developed more quickly than during simple hypoxic or hypercapnic ones. Further, systematic, investigation is required on this point. The systemic blood pressure rose to its highest levels during asphyxia, and this could have played a role in the hysteresis.

On the question of thresholds, Biscoe, Purves & Sampson (1970) studied some strands containing two fibres which had different thresholds of hypoxia-sensitivity. In the present work two pairs showed changes in pair-ratio at low frequencies, although the frequencies at which this became apparent were far lower than those indicated by Biscoe et al. (1970).

During the rapid transients, when the frequencies reached 50-70 impulses per second, the pair-ratios differed from the stable or long-term transient values. However, the stimulus was very

large, the picture may have been complicated by threshold effects as the stimulus was delivered against a background of virtually zero discharge, and the decay of the response probably indicated the wash-out of the CO_2-saline from the receptor site rather than adaptation to the stimulus.

The practical difficulties of recording from single fibres are realised by all who have attempted it, but little criticism has been made of the method. Each receptor can be thought of as metering the arterial stimulus but it seems that the sensitivity of the meter can change with time. This was most striking when, in apparently steady-state conditions, the discharge of one fibre steadily changed whilst that of the other of the pair remained constant.

Thus, in any experiment in which a single chemoreceptor fibre is held for any length of time, for instance to construct a stimulus-response curve, the response of the receptor could change whilst the fibre was under study. This could explain the hysteresis that Biscoe et al. (1970) saw in their pH_a response curves. It might also have played a part in the shift of the P_{a,O_2}-response

curve claimed to have been due to the disconnection of the glosso-pharyngeal efferent innervation (Sampson & Biscoe, 1970; Neil & O'Regan, 1971). One can only speculate on why the discharge varies with time. It is possible that the sensitivity of the receptor itself alters, or that the metabolism of the glomus cells changes, or that there are changes in the local blood flow.

In conclusion: for the 11 pairs of fibres for which it is possible to judge, 4 always showed the same pair-ratio over the range likely to be encountered in life, or in most experiments, and, taken at any one moment of time, the chemoreceptors are a surprisingly homogeneous population. The discharge of one fibre could give as much information as can the whole population, but the variability of the discharge precludes this and a large number of receptors is needed to reduce the error in the signal (Stein, 1967).

References

Acker, H., Lübbers, D. W. & Purves, M. J. (1971). Local oxygen tension field in the glomus caroticum of the cat and its change at changing arterial P_{O_2}. Pflügers Arch. ges. Physiol. 329, 136-155.

Al-Lami, F. & Murray, R. G. (1968). Fine structure of the carotid body of normal and anoxic cats. Anat. Rec. 160, 697-718.

Ballard, K. J. & Jones, J. V. (1970). The fine structural localization of cholinesterases in the carotid body of the cat. J. Physiol. 219, 747-753.

Band, D. M., Cameron, I. R. & Semple, S. J. G. (1969). Oscillations in arterial pH with breathing in the cat. J. appl. Physiol. 26, 261-267.

Band, D. M., Saunders, K. B. & Wolff, C. B. (1971). The relation between chemoreceptor discharge and respiratory fluctuations of arterial pH in the anaesthetized cat. J. Physiol. 218, 73-74P.

Band, D. M. & Wolff, C. B. (1973). The effect of hypoxia on the respiratory fluctuations of chemoreceptor discharge in the cat. J. Physiol. 234, 71-72P.

Biscoe, T. J. (1963). Some effects of drugs on the isolated, superfused carotid body. Nature 208, 294-295.

Biscoe, T. J. (1971). Carotid body: structure and function. Physiol. Rev. 51. 437-495.

Biscoe, T. J., Bradley, G. W. & Purves, M. J. (1969). The relation between carotid body chemoreceptor activity and carotid sinus pressure in the cat. J. Physiol. 203, 40P.

Biscoe, T. J., Bradley, G. W. & Purves, M. J. (1970). The relation between carotid body chemoreceptor discharge, carotid sinus pressure and carotid body venous flow. J. Physiol. 208, 99-120.

Biscoe, T. J., Lall, A. & Sampson, S. R. (1970). Electron microscopic and electrophysiological studies on the carotid body following intracranial section of the glossopharyngeal nerve. J. Physiol. 208, 133-152.

Biscoe, T. J. & Pallot, D. (1972). Serial reconstruction with the electron microscope of carotid body tissue. The Type I cell nerve supply. Experientia 28, 33-34.

Biscoe, T. J. & Purves, M. J. (1967). Observations on the rhythmic variation of the cat carotid body chemoreceptor activity which has the same period as respiration. J. Physiol. 190, 389-412.

Biscoe, T. J., Purves, M. J. & Sampson, S. R. (1970). The frequency of nerve impulses in single carotid body chemoreceptor afferent fibres recorded in vivo with intact circulation. J. Physiol. 208, 121-131.

Biscoe, T. J. & Sampson, S. R. (1968). Rhythmical and non-rhythmical spontaneous activity recorded from the central cut end of the sinus nerve. J. Physiol. 196, 327-338.

Biscoe, T. J. & Silver, A. (1966). The distribution of cholinesterases in the cat carotid body. J. Physiol. 183, 501-512.

Biscoe, T. J. & Stehbens, W. E. (1965). Electron microscopic observations on the carotid body. Nature, 208, 708-709.

Biscoe, T. J. & Stehbens, W. E. (1966). Ultrastructure of the carotid body. J. cell. Biol. 30, 563-568.

Biscoe, T. J. & Stehbens, W. E. (1967). Ultrastructure of the denervated carotid body. Q. Jl exper. Physiol. 52, 31-36.

Biscoe, T. J. & Taylor, A. (1963). The discharge pattern recorded in chemoreceptor afferent fibres from the cat carotid body with normal circulation and during perfusion. J. Physiol. 168, 332-344.

Black, A. M. S., Goodman, N. W., Nail, B. S., Rao, P. S. & Torrance, R. W. (1973). The significance of the timing of chemoreceptor impulses for their effect upon respiration. Acta Neurobiol. exper. 33, 139-147.

Black, A. M. S., McCloskey, D. I. & Torrance, R. W. (1971). The responses of carotid body chemoreceptors in the cat to sudden changes of hypercapnic and hypoxic stimuli. Respir. Physiol. 13, 36-49.

Black, A. M. S. & Torrance, R. W. (1971). Respiratory oscillations in chemoreceptor discharge in the control of breathing. Respir. Physiol. 13, 221-237.

Blümcke, S., Rode, J. & Niedorf, H. R. (1967). The carotid body after oxygen deficiency. Z. Zellforsch. 80, 52-77.

Bogue, J. Y. & Stella, G. (1935). Afferent impulses in the carotid sinus nerve (nerve of Hering) during asphyxia and anoxaemia. J. Physiol. 83, 459-465.

Burgess, P. R. & Perl, E. R. (1973). In Handbook of Sensory Physiology, Vol. II "Somatosensory System", ed. Iggo, A., pp.29-78. Berlin: Springer-Verlag.

Calvin, W. H. (1972). Synaptic potential summation and repetitive firing mechanisms: input-output theory for the recruitment of neurons into epileptic bursting firing patterns. Brain Res. 39, 71-94. (Appendix A).

Chalazonitis, N. & Arvanitaki, A. (1970). Neuromembrane electrogenesis during changes in P_{O_2}, P_{CO_2}, and pH. In Advances in Biochemical Psychopharmacology, Vol. 2 "Biochemistry of simple neuronal models", eds. Costa, E. & Giacobini, E., pp.245-284. New York: Raven Press.

Chen, I-Li & Yates, R. D. (1968). An electron microscopic cytochemical study of the effects of hypoxia and reserpine on the glomus cells of the carotid body. Anat. Rec. 160, 330.

Christie, R. V. (1933). The function of the carotid gland (glomus caroticum). I. The action of extracts of a carotid gland tumour in man. Endocrinology 17, 421-432.

Comroe, J. H. & Schmidt, C. F. (1938). The part played by reflexes from the carotid body in the chemical regulation of respiration in the dog. Am. Jl Physiol. 121, 75-97.

Cunningham, D. J. C., Howson, M. G. & Pearson, S. B. (1973). The respiratory effects in man of altering the time profile of alveolar carbon dioxide within each respiratory cycle. J. Physiol. 234, 1-28.

Daly, M. de B., Lambertsen, C. J. & Schweitzer, A. (1954). Observations on the volume of blood flow and oxygen utilization of the carotid body of the cat. J. Physiol. 125, 67-89.

Dearnaley, D. P., Fillenz, M. & Woods, R. I. (1968). The identification of dopamine in the carotid body of the rabbit. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.189-192. Oxford: Blackwell.

de Castro, F. (1926). Sur la structure et l'innervation de la glande intercarotidienne (Glomus caroticum) de l'homme et des mammifères, et sur un nouveau système d'innervation autonome du nerf glossopharyngien. Etudes anatomiques et éxperimentales. Trab. Lab. Invest. biol. Univ. Madr. 24, 365-432.

de Castro, F. (1928). Sur la structure et l'innervation du sinus carotidien de l'homme et des mammifères. Nouveaux faits sur l'innervation et la fonction du glomus caroticum. Etudes anatomiques et physiologiques. Trab. Lab. Invest. biol. Univ. Madr. 25, 331-380.

de Castro, F. (1951). Sur la structure de la synapse dans les chémorécepteurs: leur mécanisme d'excitation et rôle dans la circulation sanguine locale. Acta physiol. scand. 22, 14-43.

de Castro, F. & Rubio, M. (1968). The anatomy and innervation of the blood vessels of the carotid body and the role of chemo-receptive reactions in the autoregulation of the blood flow. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.267-270. Oxford: Blackwell.

de Kock, L. L. (1954). Intraglomerular tissues of the carotid body. Acta Anat. 21, 101-116.

de Kock, L. L. & Dunn, A. E. G. (1964). Ultra-structure of carotid body tissue as seen in serial sections. Nature 202, 821-822.

de Kock, L. L. & Dunn, A. E. G. (1966). An electron microscope study of the carotid body. Acta Anat. 64, 163-173.

de Kock, L. & Dunn, A. E. G. (1968). Electron-microscope investigation of the nerve endings in carotid body. In Arterial Chemo-receptors, ed. Torrance, R. W., pp.179-187. Oxford: Blackwell.

Detweiler, P. B. & Alkon, D. L. (1973). Hair cell interaction in the statocyst of Hermissenda. J. gen. Physiol. 62, 618-642.

Douglas, W. W. (1952). The effects of a ganglion blocking drug hexamethonium on the response of the cat's carotid body to various stimuli. J. Physiol. 118, 373-383.

Douglas, W. W. & Ritchie, J. M. (1957). A technique for recording functional activity in specific groups of medullated and non-medullated fibres in whole nerve trunks. J. Physiol. 138, 19-30.

Duke, H. N., Green, J. H. & Neil, E. (1952). Carotid chemoreceptor impulse activity during inhalation of carbon monoxide mixtures. J. Physiol. 118, 520-527.

Edwards, M. W. Jr & Mills, E. (1969). Arterial chemoreceptor oxygen utilization and oxygen tension. J. appl. Physiol. 27, 291-294.

Eränko, O. & Harkonen, M. (1965). Monoamine-containing small cells in the superior cervical ganglion of the rat and an organ composed of them. Acta physiol. scand. 63, 511-512.

Eyzaguirre, C. & Koyano, H. (1965a). Effects of hypoxia, hypercapnia and pH on the chemoreceptor activity of the carotid body in vitro. J. Physiol. 178, 385-409.

Eyzaguirre, C. & Koyano, H. (1965b). Effects of some pharmacological agents on chemoreceptor discharges. J. Physiol. 178, 410-431.

Eyzaguirre, C. & Koyano, H. (1965c). Effects of electrical stimulation on the frequency of chemoreceptor discharges. J. Physiol. 178, 438-462.

Eyzaguirre, C., Koyano, H. & Taylor, J. R. (1965). Presence of acetylcholine and transmitter release from carotid body chemoreceptors. J. Physiol. 178, 463-476.

Eyzaguirre, C., Leitner, L-M., Nishi, K. & Fidone, S. (1970). Depolarisation of chemosensory nerve endings in carotid body of the cat. J. Neurophysiol. 33, 685-696.

Eyzaguirre, C. & Lewin, J. (1961a). Chemoreceptor activity of the carotid body of the cat. J. Physiol. 159, 222-237.

Eyzaguirre, C. & Lewin, J. (1961b). Effect of different oxygen tensions on the carotid body in vitro. J. Physiol. 159, 238-250.

Eyzaguirre, C. & Lewin, J. (1961c). The effect of sympathetic stimulation on carotid nerve activity. J. Physiol. 159, 251-267.

Eyzaguirre, C., Nishi, K. & Fidone, S. (1972). Chemoreceptor synapses in the carotid body. Fedn Proc. 31, 1385-1393.

Eyzaguirre, C. & Zapata, P. (1968a). Pharmacology of pH effects on carotid body chemoreceptors in vitro. J. Physiol. 195, 557-588.

Eyzaguirre, C. & Zapata, P. (1968b). The release of acetylcholine from carotid body tissues. Further study on the effects of acetylcholine and cholinergic blocking agents on the chemosensory discharge. J. Physiol. 195, 589-607.

Eyzaguirre, C. & Zapata, P. (1968c). A discussion of possible transmitter or generator substances in carotid body chemoreceptors. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.213-251. Oxford: Blackwell.

Fatt, P. & Katz, B. (1952). Spontaneous subthreshold activity at motor nerve endings. J. Physiol. 117, 109-128.

Fay, F. S. (1970). Oxygen consumption of the carotid body. Am. Jl Physiol. 218, 518-523.

Fidone, S., O'Leary, T. & Eyzaguirre, C. (1971). A simple method for the preparation and recording from thin slices of living tissue. Brain Res. 30, 401-406.

Fidone, S. J. & Sato, A. (1969). A study of chemoreceptor and baroreceptor A and C-fibres in the cat carotid nerve. J. Physiol. 205, 527-548.

Fidone, S. J. & Sato, A. (1970). Efferent inhibition and antidromic depression of chemoreceptor A-fibres from the cat carotid body. Brain Res. 22, 181-193.

Fillenz, M. (1973). The dopamine cell. Title only of a paper presented at an International Workshop on Chemoreceptors held in Bristol, July 1973. Proceedings to be published by Cambridge University Press.

Fitzgerald, R. S., Leitner, L-M. & Liaubet, M-J. (1969). Carotid chemoreceptor response to intermittent or sustained stimulation in the cat. Respir. Physiol. 6, 395-402.

Fitzgerald, R. S. & Parks, D. C. (1971). Effect of hypoxia on carotid chemoreceptor response to carbon dioxide in cats. Respir. Physiol. 12, 218-229.

Flock, A. & Russell, I. (1973). Efferent nerve fibres: postsynaptic action on hair cells. Nature (New Biology) 243, 89-91.

Floyd, W. F. & Neil, E. (1952). The influence of the sympathetic innervation of the carotid bifurcation on chemoreceptor and baroreceptor activity in the cat. Archs int. Pharmacodyn. Ther. 91, 230-239.

Forster, R. E. (1968). The diffusion of gases in the carotid body. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.115-128. Oxford: Blackwell.

Gerhich, J. L. & Moore, G. P. (1970). Cyclic variations in carotid body chemoreceptor activity. Physiologist 13, 203.

Gillis, D. B. & Mitchell, R. A. (1972). The role of the cat's carotid bodies in the regulation of erythrocyte production. Physiologist, 15, 145.

Goodman, N. W. (1972). Efferent control of arterial chemoreceptors. J. Physiol. 225, 31-32P.

Goodman, N. W. (1973). Efferent control of arterial chemoreceptors mediated by glossopharyngeal fibres and artefacts introduced by stimulation techniques. J. Physiol. 230, 295-311.

Goodman, N. W. & McCloskey, D. I. (1972). Intracellular potentials in the carotid body. Brain Res. 39, 501-504.

Goodman, N. W. & Nail, B. S. (1973). Oscillatory behaviour and randomness of firing of chemoreceptor fibres in the cat. Brain Res. 59, 379-383.

Goodman, N. W., Nail, B. S. & Torrance, R. W. Oscillations in the discharge of single carotid chemoreceptor fibres of the cat. Respir. Physiol. (in the Press).

Granit, R., Leksell, L. & Skoglund, C. R. (1944). Fibre interaction in injured or compressed region of nerve. Brain 67, 125-140.

Gray, B. A. (1968). Response of the perfused carotid body to changes in pH and pCO_2. Respir. Physiol. 4, 229-245.

Haldane, J. S. & Priestley, J. G. (1935). Respiration, 2nd edn, pp.16-42. London: O.U.P.

Hansen, A. J., Fogh, J., Møllgård, K. & Sørensen, S. C. (1973). Evidence against erythropoietin production by the carotid body. Respir. Physiol. 18, 101-106.

Hebb, C. O. (1968). In Arterial Chemoreceptors, ed. Torrance, R. W. pp.138-139. Oxford: Blackwell.

Helpap, B. (1970). Untersuchungen zum Aminosaeurestoffwechsel des Carotikoerperchens der Ratte. Virchows Arch. Abt. B Zell. Pathol. 6, 294-301.

Hess, A. (1968). Electron microscopic observations of normal and experimental cat carotid bodies. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.51-56. Oxford: Blackwell.

Hess, A. & Zapata, P. (1972). Innervation of the cat carotid body: normal and experimental studies. Fedn Proc. 31, 1365-1383.

Heymans, C. & Neil, E. (1958). Eds. Reflexogenic Areas of the Cardiovascular System. London: Churchill.

Heymans, C. & Rijlant, P. (1933). Le courant d'action du nerf du sinus carotidien intact. C. R. Soc. Biol. (Paris) 113, 69-73.

Hornbein, T. F. (1968). The relation between stimulus to chemoreceptors and their response. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.65-78. Oxford: Blackwell.

Hornbein, T. F., Griffo, Z. J. & Roos, A. (1961). Quantitation of chemoreceptor activity: interrelation of hypoxia and hypercapnia. J. Neurophysiol. 24, 561-568.

Howe, A. & Neil, E. (1972). Arterial chemoreceptors. In Handbook of Sensory Physiology, Vol. III/1 "Enteroceptors", ed. Neil, E., pp.47-80. Berlin: Springer-Verlag.

Hubbard, J. I., Llinás, R. & Quastel, D. M. J. (1969). Electrophysiological Analysis of Synaptic Transmission, pp.124-126. London: Arnold.

Huxley, A. F. (1961). A micromanipulator. J. Physiol. 157, 5-7P.

Joels, N. & Neil, E. (1964). The action of some metabolic inhibitors on the chemoreceptor activity of the carotid body. J. Physiol. 171, 43P.

Joels, N. & Neil, E. (1968). The idea of a sensory transmitter. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.153-176. Oxford: Blackwell.

Keller, H. P. & Lübbers, D. W. (1972). Flow measurement in the carotid body of the cat by the hydrogen clearance method. Pflügers Arch. ges. Physiol. 336, 217-224.

Kobayashi, S. (1971a). Comparative cytological studies of the carotid body. 1. Demonstration of mono-amine storing cells by correlated chromaffin reaction and fluorescence histochemistry. Arch. histol. jap. 33, 319-339.

Kobayashi, S. (1971b). Comparative cytological studies of the carotid body. 2. Ultrastructure of the synapses on the chief cell. Arch. histol. jap. 33, 397-420.

Kobayashi, S. & Uehara, M. (1970). Occurrence of afferent synaptic complexes in the carotid body of the mouse. Arch. histol. jap. 32, 193-201.

Lahiri, S. & DeLaney, R. G. (1973). A possible molecular mechanism of carotid chemoreceptor excitation in the cat. Physiologist, 16, 371.

Laporte, Y. & Bessou, P. (1959). Modifications d'excitabilité de motoneurones homonymes provoquées par l'activation physiologique de fibres afférentes d'origine musculaire du groupe II. J. Physiol. (Paris) 51, 897-908.

Lee, K. D. & Mattenheimer, H. (1964). The biochemistry of the carotid body. Enzymol. Biol. Clin. 4, 199-216.

Lee, K. D., Mayou, R. A. & Torrance, R. W. (1964). The effect of blood pressure upon chemoreceptor discharge to hypoxia, and the modification of this effect by the sympathetic-adrenal system. Q. Jl exper. Physiol. 49, 171-183.

Leitner, L.-M. & Dejours, P. (1968). The speed of response of chemoreceptors. In Arterial Chemoreceptors, ed. Torrance, R. W., pp.79-90. Oxford: Blackwell.

Lever, J. D., Lewis, P. R. & Boyd, J. D. (1959). Observations on the fine structure and histochemistry of the carotid body in the cat and rabbit. J. Anat. 93, 478-490.

Liang, C.-S. & Hood, W. B. Jr (1973). Comparison of cardiac output responses to 2,4-dinitrophenol-induced hypermetabolism and muscular work. J. clin. Invest. 52, 2283-2292.

Libet, B. (1970). Generation of slow inhibitory and excitatory post-synaptic potentials. Fedn Proc. 29, 1945-1956.

Lindblom, U. F. (1958). Excitability and functional organization within a peripheral tactile unit. Acta physiol. scand. 44; Suppl.153, 1-84.

Lishajko,F. (1970). Release and uptake of dopamine in isolated granules from a human carotid body tumour. Acta physiol. scand. 79, 533-536.

McCloskey, D. I. & Torrance, R. W. (1971). Autoregulation of blood flow in the carotid body. Respir. Physiol. 13, 23-35.

Marazzi, A. J. & Lorente de No, R. (1944). Interaction of neighbouring fibres in myelinated nerve. J. Neurophysiol. 7, 83-101.

Maren, T. H. (1967). Carbonic anhydrase: chemistry, physiology and inhibition. Physiol. Rev. 47, 595-781.

Marsh, R. H. K., Lyen, K. R., McPherson, G. A. D., Pearson, S. B. & Cunningham, D. J. C. (1973). Breath-by-breath effects of imposed alternate-breath oscillations of alveolar CO_2. Respir. Physiol. 18, 80-91.

Matthews, B. H. C. (1933). Nerve endings in mammalian muscle. J. Physiol. 78, 1-53.

Metz, B. (1969). Release of acetylcholine from the carotid body by hypoxia and hypoxia plus hypercapnia. Respir. Physiol. 6, 386-394.

Mills, E. (1968). Activity of aortic chemoreceptors during electrical stimulation of the stellate ganglion in the cat. J. Physiol. 199, 103-114.

Mills, E. & Edwards, M. W. Jr (1968). Stimulation of aortic and carotid chemoreceptors during carbon monoxide inhalation. J. appl. Physiol. 25, 494-502.

Mills, E. & Jöbsis, F. F. (1972). Mitochondrial respiratory chain of carotid body and chemoreceptor response to changes in oxygen tension. J. Neurophysiol. 35, 405-428.

Mitchell, J. H. & McCloskey, D. I. (in preparation). Chemoreceptor responses to sympathetic stimulation and changes in blood pressure.

Mitchell, R. A. (1970). Respiration. Annu. Rev. Physiol. 32, 415-438.

Mitchell, R. A. & McDonald, D. M. (1973). Adjustment of chemoreceptor sensitivity in the cat carotid body by reciprocal synapses. Pre-circulated abstract of a paper presented at an International Workshop on Chemoreceptors held in Bristol, July 1973. Proceedings to be published by Cambridge University Press.

Mitchell, R. A., Sinha, A. K. & McDonald, D. M. (1972). Chemoreceptive properties of regenerated endings of the carotid sinus nerve. Brain Res. 43, 681-685.

Nail, B. S. (1972). On-line biomedical data analysis. J. Physiol. 225, 5-7P.

Neil, E. & Joels, N. (1963). The carotid glomus sensory mechanism. In The Regulation of Human Respiration, eds. Cunningham, D. J. C. & Lloyd, B. B., pp.163-171. Oxford: Blackwell.

Neil, E. & O'Regan, R. G. (1969a). Effects of sinus and aortic nerve efferents on arterial chemoreceptor function. J. Physiol. 200, 69-71P.

Neil, E. & O'Regan, R. G. (1969b). Efferent and afferent impulse activity in the 'intact' sinus nerve. J. Physiol. 205, 20-21P.

Neil, E. & O'Regan, R. G. (1971a). The effects of electrical stimulation of the distal end of the cut sinus and aortic nerves on peripheral chemoreceptor activity in the cat. J. Physiol. 215, 15-32.

Neil, E. & O'Regan, R. G. (1971b). Efferent and afferent activity recorded from few-fibre preparations of otherwise intact sinus and aortic nerves. J. Physiol. 215, 33-47.

Niedorf, H. R., Rode, J. & Blumcke, S. (1970). Feinstruktur und Catecholamingehalt des Glomus caroticum der Ratte nach einmaliger Reserpin-Injektion. Virchows Arch. Abt. B Zell. Pathol. 5, 113-123.

Ott, N., Kiwull, P. & Wiemer, W. (1971). Activation of carotid chemoreceptors by local decrease of arterial blood pressure in rabbits and cats. Pflugers Arch. ges. Physiol. 325, 28-39.

Paintal, A. S. (1967). Mechanism of stimulation of aortic chemoreceptors by natural stimuli and chemical substances. J. Physiol. 189, 63-84.

Paintal, A. S. (1969). Further evidence that acetylcholine is not a transmitter at chemoreceptors. J. Physiol. 204, 94-95P.

Paintal, A. S. (1971). The response of chemoreceptors at reduced temperatures. J. Physiol. 217, 1-18.

Parratt, L. G. (1961). Probability and Experimental Errors in Science, pp.227-229. New York: John Wiley.

Pearse, H. G. E. (1969). The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J. Histochem. Cytochem. 17, 303-313.

Purves, M. J. (1966). Fluctuations of arterial oxygen tension which have the same period as respiration. Respir. Physiol. 1, 281-296.

Purves, M. J. (1970a). The effects of hypoxia, hypercapnia and hypotension upon carotid body blood flow and oxygen consumption in the cat. J. Physiol. 209, 395-416.

Purves, M. J. (1970b). The role of the cervical sympathetic nerve in the regulation of oxygen consumption of the carotid body of the cat. J. Physiol. 209, 417-431.

Rosenblueth, A. (1941). The stimulation of myelinated axons by nerve impulses in adjacent myelinated axons. Am. Jl Physiol. 132, 119-128.

Ross, L. L. (1959). Electron microscopic observations of the carotid body of the cat. J. Biophys. Biochem. Cytol. 6, 253-262.

Russell, I. J. (1971a). The role of the lateral-line efferent system in Xenopus laevis. J. exper. Biol. 54, 621-641.

Russell, I. J. (1971b). The pharmacology of efferent synapses in the lateral-line system of Xenopus laevis. J. exper. Biol. 54, 643-658.

Sampson, D., Nelson, M., Mirand, E. A. & Murphy, G. P. (1972). Relationship between the carotid body and erythropoietin activity in the baboon. J. Med. exper. clin. 3, 293-299.

Sampson, S. R. (1971). Effects of mecamylamine on responses of carotid body chemoreceptors in vivo to physiological and pharmacological stimuli. J. Physiol. 212, 655-666.

Sampson, S. R. (1972). Mechanism of efferent inhibition of carotid body chemoreceptors in the cat. Brain Res. 45, 266-270.

Sampson, S. R. & Biscoe, T. J. (1970). Efferent control of the carotid body chemoreceptor. Experientia 26, 261-262.

Sampson, S. R. & Hainsworth, R. (1972). Responses of aortic body chemoreceptors of the cat to physiological stimuli. Am. Jl Physiol. 222, 953-958.

Schweitzer, A. & Wright, S. (1938). Action of prostigmin and acetylcholine on respiration. Q. Jl exper. Physiol. 28, 33-37.

Stein, R. B. (1967). The information capacity of nerve cells using a frequency code. Biophys. J. 7, 797-826.

Stein, R. B. (1968). Some implications of the variability in chemoreceptor discharge. In Arterial Chemoreceptors, ed. Torrance, R. W., pp. 205-212. Oxford: Blackwell.

Torrance, R. W. (1969). The idea of a chemoreceptor. In The Pulmonary Circulation and Interstitial Space, eds. Fishman, A. P. & Hecht, H. H., pp.223-237. Chicago: University Press.

Torrance, R. W. (1974). Arterial chemoreceptors. Chapter 9 in Int. Rev. Sci., Respiration, ed. Widdicombe, J. G. Lancaster: MTP.

Tramezzani, J. H., Morita, E. & Chiocchio, S. R. (1971). The carotid body as a neuroendocrine organ involved in the control of erythropoiesis. Proc. Nat. Acad. Sci. (USA) 68, 52-55.

Travis, D. M. (1971). Molecular CO_2 is inert on carotid chemoreceptors: demonstration by inhibition of carbonic anhydrase. J. pharm. exper. Ther. 178, 529-540.

Weigelt, H., Seidl, E., Acker, H. & Lübbers, D. W. (1973). Determination of local blood flow in the carotid body of the rabbit (Oryctolagus cuniculus) by the hydrogen clearance method. Pflügers Arch. ges. Physiol. 343, Suppl. R10.

Whalen, W. J. & Nair, P. (1973). Microelectrode measurements of tissue pO_2 in the carotid body of the cat. In Oxygen Supply. Theoretical and Practical Aspects of Oxygen Supply and Microcirculation of Tissue, eds. Kessler, M. et al., pp.236-237. Baltimore: University Park Press.

Yates, R. D., Chen, I-Li. & Duncan, D. (1970). Effects of sinus nerve stimulation on carotid body glomus cells. J. cell. Biol. 46, 544-552.

Yokota, H., Hoofd, L. J. C. & Kreuzer, F. (1973). Alveolar oxygen tension in anaesthetized, artificially ventilated dogs. Pflügers Arch. ges. Physiol. 340, 273-290.

Yokota, H. & Kreuzer, F. (1973). Alveolar to arterial transmission of oxygen fluctuations due to respiration in anaesthetized dogs. Pflügers Arch. ges. Physiol. 340, 291-306.

Zapata, P., Hess, A., Bliss, E. L. & Eyzaguirre, C. (1969). Chemical, electron microscopic and physiological observations on the role of catecholamines in the carotid body. Brain Res. 14, 473-496.

Zapata, P., Hess, A. & Eyzaguirre, C. (1969). Reinnervation of carotid body and sinus with superior laryngeal nerve fibres. J. Neurophysiol. 32, 215-228.