J. Physiol. (1971), **218**, *pp.* 671–689
With 10 *text-figures*
Printed in Great Britain

THE ACTION OF CARBON DIOXIDE ON SYNAPTIC TRANSMISSION IN THE CUNEATE NUCLEUS

By MARY E. MORRIS

From the Department of Research in Anaesthesia, McGill University, Montreal, Canada

(*Received 7 May 1971*)

SUMMARY

1. The excitability of synaptic structures in the cuneate nucleus was studied in eighteen decerebrate, unanaesthetized cats during acute changes in inspired P_{CO_2}.

2. Micro-electrode stimulation in the caudal half of the cuneate nucleus evoked antidromic and orthodromic responses which were recorded simultaneously from a forelimb nerve and from the medial lemniscus at the transected mid-brain surface.

3. Increases in the concentration of inspired CO_2 (2–20 %) progressively decreased the direct excitability of both the afferent fibre terminals (reflected in the antidromic potentials) and the cuneate relay neurones (reflected in the α wave of the orthodromic lemniscal response). Synaptically mediated responses, recorded as the β component of the lemniscal potentials, were also depressed.

4. The relation between input and output at the cuneate was determined by plotting antidromic against trans-synaptic (β lemniscal) responses for different intensities of stimulation. The mean slope for logarithmic values of control potential amplitudes was 1·17 ($\pm$ S.E. 0·13). It therefore appears that the transfer function for the cuneate is linear over a wide range.

5. In the majority of experiments the input–output relation was unchanged or increased by raising P_{CO_2}. It was concluded that the efficiency of synaptic transmission and release of transmitter appeared to be well maintained or possibly increased at individual active synapses during hypercarbia.

6. The depressant action of CO_2 on afferent transmission can therefore be attributed largely to a block of impulse conduction in the primary afferent fibres.

INTRODUCTION

An attempt has been made in the present study to determine the site of a transmission failure produced by CO_2 in the dorsal column–lemniscal system, which was described in the previous paper (Morris, 1971*a*). The block might occur along the path of the afferent fibres or at their synaptic connexions, and could be due to depression of excitability or a decrease in synaptic efficiency, for example by a diminished release of transmitter or lower sensitivity of receptors. The first relay of the dorsal column–lemniscal system in the cuneate nucleus, where the synapses are densely collected close to the surface, provides ready access to study changes in synaptic events. With the technique described by Wall (1958), it has been possible to examine the effects of increasing amounts of CO_2 on pre- and post-synaptic excitability and the synaptic mechanism and thus to estimate the input–output relation of the cuneate.

METHODS

The experiments were carried out on eighteen cats, decerebrated under initial halothane anaesthesia. The surgical preparation, administration of CO_2 and times of observation were as described in the previous paper (Morris, 1971*a*); eight of the animals contributed data to both groups of experiments. Four animals breathed spontaneously; all others were paralysed with 0·1% succinylcholine chloride (Anectine, Burroughs Wellcome & Co., Ltd) and ventilation was controlled.

Stimulation and recording. Coarse glass micro-electrodes, filled with 1–4 M-NaCl, with tips of 20–40 μ and resistances of 100–300 kΩ, were used for stimulation in the cuneate nucleus. Their exact placement is described in the Results section. Pulses of 10–80 V and 0·01–0·2 msec duration at 1 Hz were delivered through a series resistance of 100 kΩ.

In five experiments a forelimb nerve was stimulated with bipolar platinum electrodes and 0·1 msec pulses of < 3 mA at 1 Hz.

Monophasic antidromic potentials were recorded from a forelimb nerve with a pair of platinum electrodes. Potentials were recorded from the medial lemniscus with two platinum electrodes (diameter 0·3 mm), which were insulated to the tip and held in separate micro-manipulators. A recording electrode was first placed approximately on the transected surface of the mid-brain, according to stereotactic coordinates of Jasper & Ajmone-Marsan (1954) and the largest response to cuneate stimulation was then sought. An indifferent electrode was placed about 10 mm away to diminish the stimulus artifact by differential recording. In some experiments field potentials, in response to stimulation of a nerve, were also recorded with the cuneate micro-electrode.

Fig. 1 shows a diagram of the stimulating and recording arrangement and examples of the potentials evoked simultaneously by increasing intensities of stimulation in the cuneate. At the right are antidromic potentials recorded from a forelimb nerve. The main peak is the direct response, while the later responses which fail at low stimulation frequencies (10–20 Hz) are due to reflex activity, such as the dorsal root reflex (Andersen, Eccles, Schmidt & Yokota, 1964*b*). At the left are orthodromic potentials recorded from the medial lemniscus: the first arrow marks an early α

component, evoked by direct excitation of the cuneate neurones; the second arrow marks a trans-synaptic β component, evoked by the stimulation of fibre terminals. The stimulus–response curves below the traces give the amplitude of the first potentials of each group of responses, and represent the direct excitation of the afferent terminals and the cuneate relay neurones.

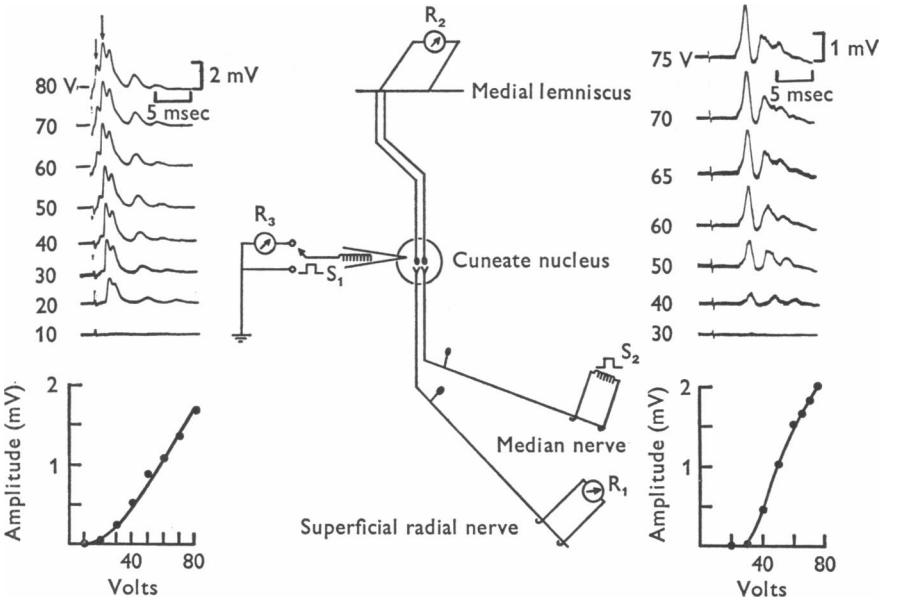

Fig. 1. Diagram showing dorsal column afferent pathway and stimulating and recording electrodes. S_1, micro-electrode stimulation. S_2, peripheral nerve stimulation. R_1, bipolar recording from superficial radial nerve. R_2, differential recording from medial lemniscus. R_3, recording with micro-electrode of field potential response to afferent volleys. At right are antidromic potentials recorded from nerve and evoked by cuneate stimulation (30–75 V). Below is stimulus–response curve for amplitude of the main peak. At left are medial lemniscal responses to cuneate stimulation (10–80 V): the direct (α) wave at first arrow, followed by two peaks of higher amplitude – the trans-synaptic (β) wave at second arrow. Graph below shows stimulus–response relation for the α wave amplitude.

RESULTS

Criteria for the placement of the stimulating electrode

The stimulating electrode was inserted with a micro-manipulator vertically into the dorsal surface of the cuneate nucleus to depths of 0·3–1·0 mm. The area studied was 1–4 mm caudal to the obex, and 1–2 mm lateral from the mid line. The tip of the electrode was considered to be in the densest synaptic regions by at least one of the following criteria:

(i) *reversal of field potentials.* The slow surface-positive potential, evoked in the cuneate by an afferent volley and which has been ascribed to a sustained depolarization of presynaptic terminals, reverses at depths < 1 mm (Therman, 1941; Andersen *et al.* 1964*a*). Fig. 2*A* shows an example of potentials at the surface and near the depth of reversal.

(ii) *maximal presynaptic facilitation by a conditioning volley.* The change in excitability of afferent fibres has been shown to be greatest near their terminals (Wall,

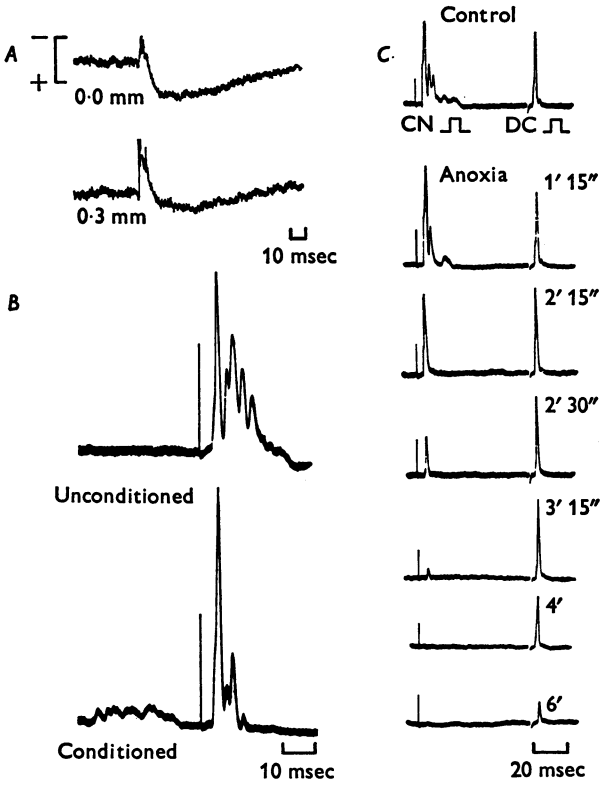

Fig. 2. *A. Changes in field potentials* recorded with micro-electrode at 0·0 and 0·3 mm depth in cuneate in response to forelimb nerve stimulation. Early surface negative potential increases and later positive potential decreases and reverses as electrode passes into synaptic region.

B. Facilitation of antidromic potentials of superficial radial nerve, when cuneate micro-electrode stimulation is preceded by a conditioning afferent volley from the median nerve (interval 50 msec). Effect increases as electrode penetrates nucleus, and here was maximal at depth 1 mm.

C. Differential sensitivity to anoxia of terminal and more distal parts of afferent fibres. Antidromic responses of superficial radial nerve to micro-electrode stimulation in the cuneate nucleus (CN) and 15 mm caudal in the dorsal column (DC) show that excitability of terminals fails earlier than that of dorsal column fibres. Duration of inhalation of 100% nitrogen is given in min and sec at right-hand side of figure.

1958). When a stimulating micro-electrode is inserted into the cuneate, the facilitation of an antidromic nerve potential by a conditioning afferent volley becomes progressively greater and reaches a maximum at depths of $\leqslant$ 1·mm (Andersen *et al.* 1964*b*). Fig. 2*B* shows the largest increase in the direct response in one experiment where the electrode depth was 1·0 mm.

(iii) *Differential changes in amplitude of potentials evoked at different electrode depths.* As shown in Fig. 3, when a stimulating electrode penetrates the cuneate, the responses to excitation of the afferent terminals – the antidromic potentials at the nerve and the β lemniscal potentials (with latency long enough to include a synaptic

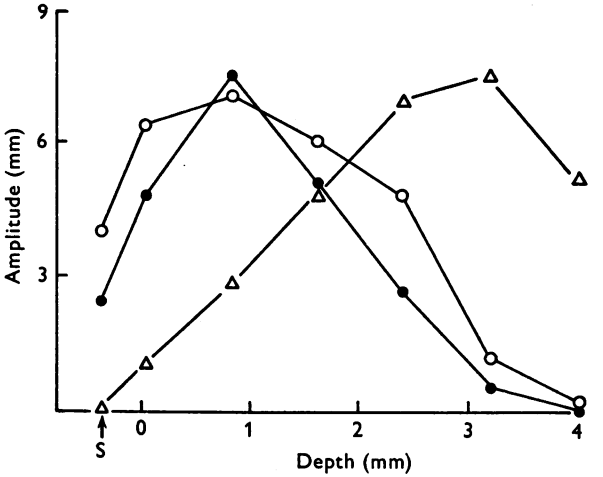

Fig. 3. Amplitude changes of potentials evoked by 70 V stimulation through a micro-electrode at increasing depths in the cuneate (1 mm caudal to obex). Changes in antidromic potentials in superficial radial nerve (○) correspond closely to those in first trans-synaptic (β) potential of medial lemniscal response (●) while the direct (α) lemniscal potential (△) increases independently. S, electrode near surface of cuneate in irrigating fluid. (Amplitude scale is in mm from photographic record of oscilloscope traces.)

delay) – change concomitantly. At the same time the direct α lemniscal potential progressively increases. The depth at which there is a maximal response to terminal excitation corresponds to the point of maximal presynaptic interaction and reversal of the field potential. Therefore this can be considered as the region of greatest density of afferent terminals.

(iv) *sensitivity to anoxia.* During anoxia the excitability of the afferent terminals in the spinal cord and in the cuneate nucleus is depressed more easily than that of the parent fibres (Edisen, 1957; Galindo, 1969). This was confirmed by the inhalation of pure nitrogen at the end of most experiments, when antidromic responses from the cuneate disappeared. The stimulating electrode was either withdrawn into the zone of the ascending afferent fibres, close to the surface and away from the terminal regions, or re-introduced at a point about 20 mm more caudal in the dorsal column; or two separate electrodes were used to stimulate at the same time the cuneate and the dorsal column (Fig. 2*C*). The antidromic response from the cuneate consistently failed earlier than that obtained by stimulation of the dorsal column fibres. This

suggested that it was indeed the excitability of the terminal part of the afferent fibre which was being tested during the experiments.

Effects of CO_2 on afferent terminal excitability

The inhalation of 2–20 % CO_2 caused a gradual diminution of the potentials antidromically conducted to a forelimb nerve in response to stimulation of the afferent terminals. Fig. 4 illustrates, in the upper row of traces, the decreases with 20 % CO_2 in one experiment.

Changes in the amplitude of the directly evoked potentials reflect the number of excited fibres, which in turn are related to the strength of stimulus (Wall, 1958).

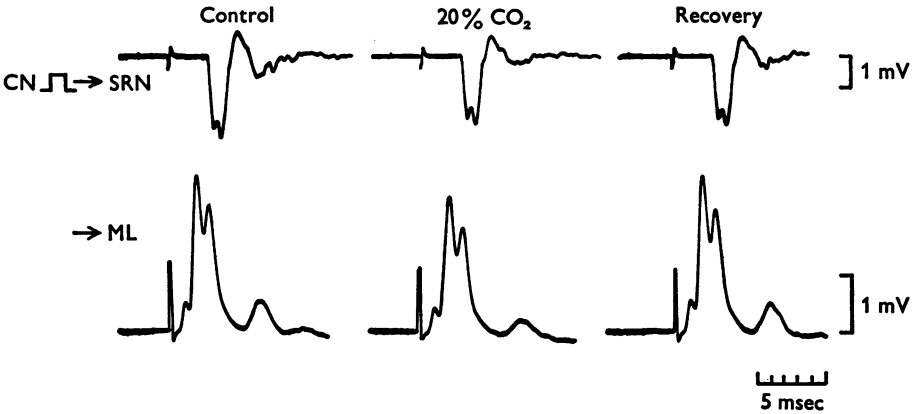

Fig. 4. Effect of breathing 20 % CO_2 for 7 min on potentials evoked by stimulation of synaptic regions in the cuneate. Above, antidromic potentials recorded from superficial radial nerve (SRN) in response to micro-electrode stimulation in cuneate (70 V, 0·2 msec pulse, 1 Hz). Below, medial lemniscal (ML) responses evoked at the same time. Recovery traces 10 min after CO_2 administration stopped.

Method of calculating excitability. Changes in excitability can be calculated as a ratio of stimulus intensities:

(i) The amplitude of the changed response to a given stimulus (S_2) is referred to a calibration curve to determine the strength of stimulation (S_1) which would have produced the same response (Graham & Lorente de Nó, 1938). For example, an excitability change $(S_1/S_2) \times 100 = (40/80) \times 100 = 50\,\%$ would be obtained if, in the right stimulus–response curve of Fig. 1 the antidromic response to 80 V decreased from 2·15 to 0·45 mV.

(ii) An alternate method is to compare stimulus–response curves (Eccles, Magni & Willis, 1962). The curves were derived from four to eight responses to stimulus intensities in the range 3–10 times threshold, and compared by calculating a mean of three stimulus intensity ratios. Fig. 5 illustrates the method in one experiment where 10 % CO_2 was given. A mean excitability change was calculated from the stimulus ratios at the middle and extremes of a linear range of the stimulus–response relation, before (S_1) and during (S_2) the inhalation of CO_2. Ratios of S_1/S_2 = 73/80,

61/68 and 51/56 gave a mean change to 90% of the control value. The mean coefficient of variation, determined from the means of three ratios for each of 30 administrations of CO_2 in fourteen experiments, was 2·74% (±S.D. 3·86).

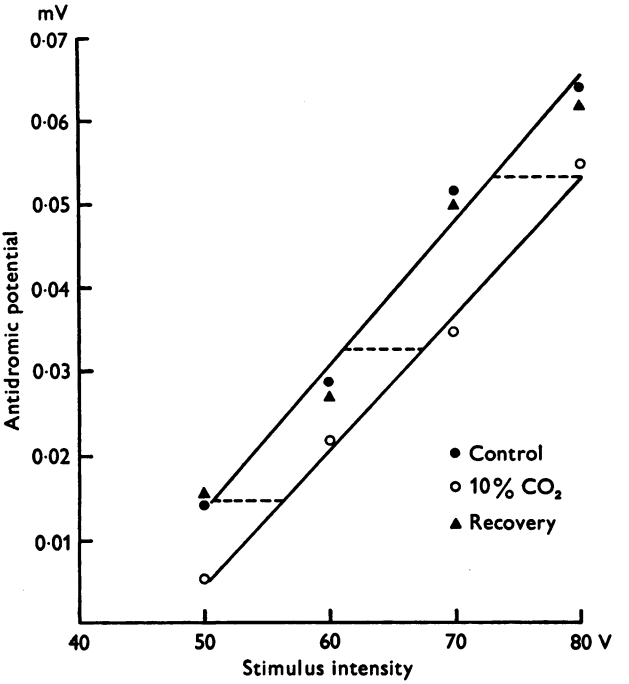

Fig. 5. Example of method of calculating excitability from stimulus–response curves. Effect of inhalation of 10% CO_2 on amplitudes of antidromic responses to different intensities of cuneate stimulation. Control ●; CO_2 (6 min) ○; recovery (10 min) ▲. Continuous lines drawn by eye. At dashed horizontal lines, three ratios of the stimuli required to produce the same response during high CO_2 (S_2) and during the control period (S_1) are calculated ($S_1/S_2 \times 100$). The excitability was changed by CO_2 to 90·4% of control level (mean calculated from ratios of 73/80, 61/68 and 51/56).

Fig. 6 shows how the excitability of the afferent terminals was reduced in one experiment when 2, 4, 10 and 20% CO_2 were administered.

The excitability changes from 30 observations in fourteen experiments, calculated from stimulus–response curves, are summarized in the graph of Fig. 7. Terminal excitability was inversely related to the concentration of inspired CO_2 (correlation coefficient $r = -0{\cdot}81$, $P < 0{\cdot}001$). On the average, excitability was depressed to 84% with 10% CO_2, and to 73% with 20% CO_2.

Effects of CO_2 on the excitability of cuneate relay neurones

Stimulation in the region of the afferent terminals in the cuneate nucleus, in addition to evoking antidromic potentials in peripheral nerves, also produced in the lemniscal relay fibres the orthodromic potentials

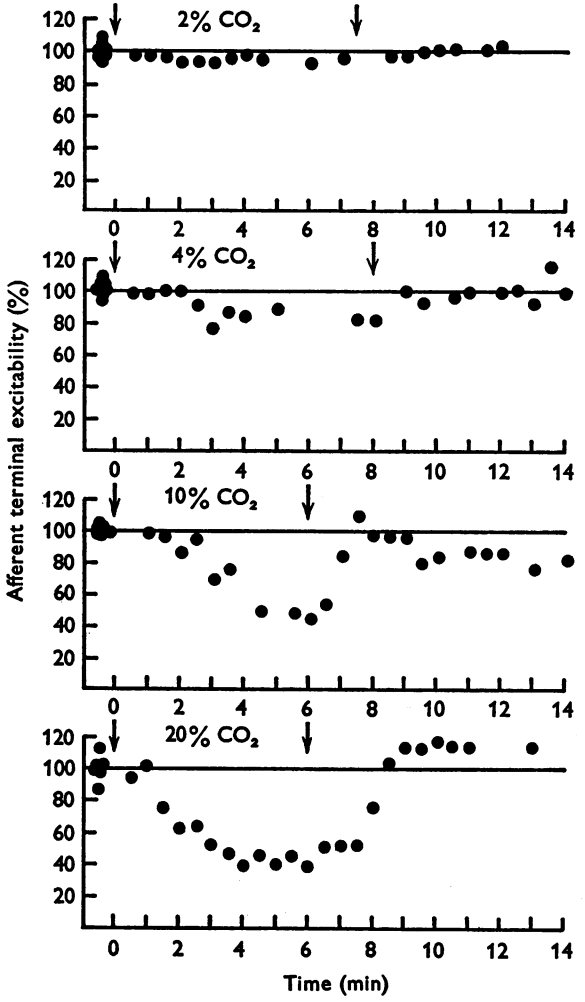

Fig. 6. Effects of inhalation of 2, 4, 10 and 20 % CO_2 on excitability of afferent cuneate terminals, as reflected in antidromic potentials of the superficial radial nerve. Arrows mark times of administration of CO_2. Control values to left of first arrow are consecutive responses during one minute before start of CO_2. 80 V stimulation in cuneate with micro-electrode. Excitability calculated as a stimulus-equivalent ratio from a control calibration curve and expressed as a % of the control mean.

which were recorded at the cut surface of the mid-brain (see Fig. 1). The first component, the α wave, because of its brief latency and ability to follow > 200 Hz frequencies of stimulation, probably represents the *direct* electrical response of the cuneo-thalamic relay cells and/or their fibres (Andersen, Eccles, Oshima & Schmidt, 1964). The second component, the biphasic β wave, is probably *trans-synaptic*, arising from excitation of the fibre terminals; this is suggested by its longer latency and ability to follow

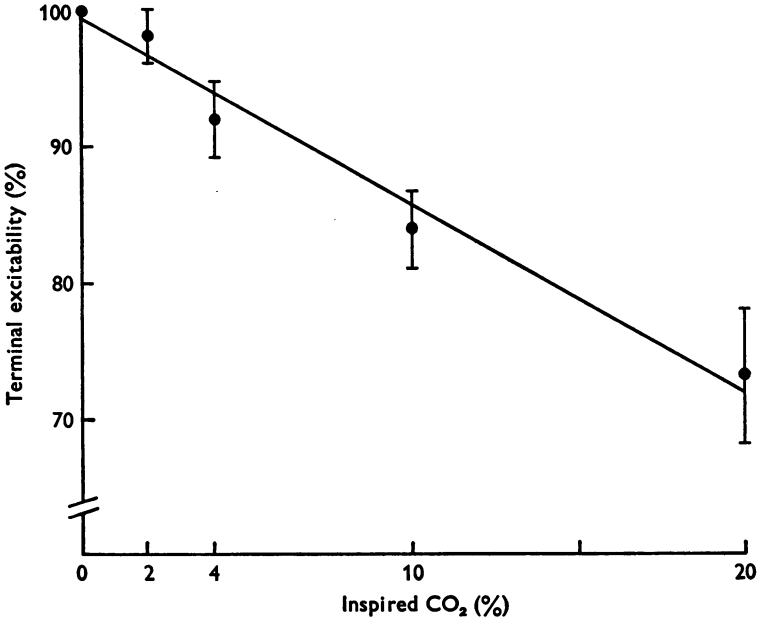

Fig. 7. Relation of afferent terminal excitability to concentration of inspired CO_2. Data from thirty observations in fourteen cats. Mean values of excitability, calculated from changes in stimulus–response curves of antidromic potentials are from four, seven, eleven and eight cats during administrations of 2, 4, 10 and 20 % CO_2 respectively. Vertical bar ± 1 S.E. Regression equation, calculated from all values, is $Y = 99\text{–}1{\cdot}39X$; S.E. of regression coefficient $\pm 0{\cdot}13$.

a high frequency of stimulation but with more rapid attenuation than the α wave, and by the observation that its amplitude varies in parallel with the amplitude of the antidromic potentials in the peripheral nerve, when the depth of the stimulating micro-electrode is changed.

Direct responses. The direct excitability of the cuneate relay neurones, as reflected in the amplitude of the α wave of the medial lemniscal potentials, was also depressed by the inhalation of CO_2. A small but distinct depression of this component during the inhalation of 20 % CO_2 can be seen in the second row of responses of Fig. 4. The progressive effects of

increasing amounts of CO_2 are shown in the example of Fig. 8 and by the grouped data from twelve cats (twenty-nine administrations of CO_2) of Fig. 9. These changes were not as great as those in the afferent terminals (cf. Fig. 7). For example, the inhalation of 20% CO_2 depressed the excitability of the terminals to 73% (± S.E. 5·2), and that of the post-synaptic neurones to 83% (± S.E. 3·1). The regressions of excitability on

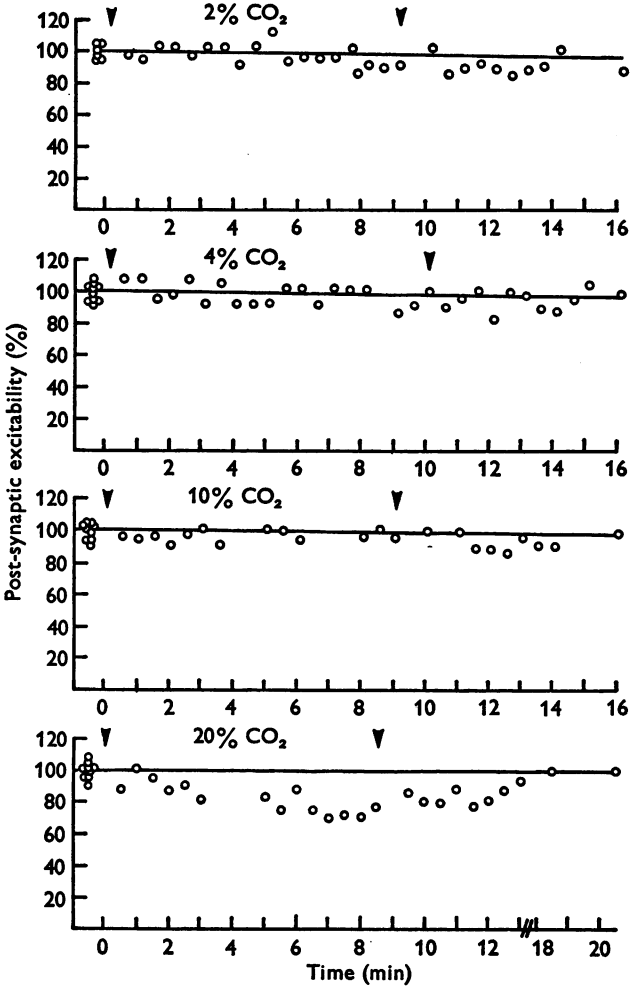

Fig. 8. Effects of inhalation of 2, 4, 10 and 20% CO_2 on post-synaptic excitability of cuneate relay neurones, as reflected in amplitudes of α medial lemniscal response to cuneate micro-electrode stimulation (80 V). Arrows mark periods of CO_2 inhalation. Control values to left of first arrow are consecutive responses during 1 min before start of CO_2. Excitability (expressed as a % of a control mean) is calculated as a stimulus–equivalent ratio from a control calibration curve.

the concentration of inspired CO_2 were significantly different ($P < 0{\cdot}001$; regression coefficients 1·39 (± S.E. 0·13) for the terminal and 0·87 (± S.E. 0·09) for the post-synaptic effect).

Trans-synaptic responses. The trans-synaptic (β) lemniscal responses to stimulation of the terminals in the cuneate were also significantly depressed by CO_2, as would be expected from the changes in terminal excitability. This may be seen in the lower traces of Fig. 4 (second and third peaks). The changes in the β wave were not as profound as might have been predicted from the depression of pre- and post-synaptic excitability, and this led to the following analysis of synaptic transmission.

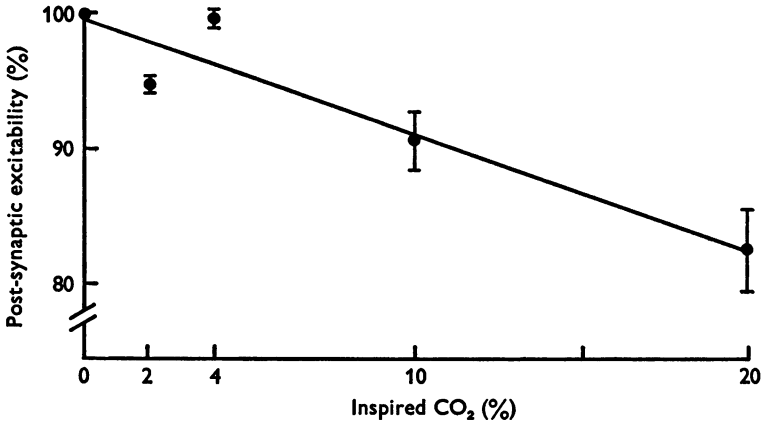

Fig. 9. Relation of post-synaptic excitability to concentration of inspired CO_2. Data from twenty-nine observations in twelve cats. Mean values of excitability, calculated from changes in stimulus–response curve of α lemniscal potentials are from four, five, eleven and nine cats during inhalation of 2, 4, 10 and 20 % CO_2. Vertical bar ± 1 S.E. Regression equation, calculated from all values, is $Y = 99{\cdot}9 - 0{\cdot}87X$; S.E. of regression coefficient ± 0·10.

Effects of CO_2 on the input–output relation of cuneate transmission

In order to assess the effects of CO_2 on the synaptic transmission in the cuneate, the efficiency of output for a given input was estimated by comparing the orthodromic and antidromic responses evoked simultaneously by direct excitation of the afferent terminals. The antidromic nerve potential gives an estimate of input, on the assumption that its amplitude is proportional to the number of active terminals. The trans-synaptic (β) lemniscal potential estimates output, on the assumption that its amplitude represents the number of responding neurones.

The input–output relation. Input–output plots were constructed from the data of sixteen experiments, in each of which a range of between five

and twelve different intensities of cuneate stimulation was used. Some of the curves did not pass through the origin (see Fig. 10), while in those which did, output appeared to increase rapidly for an increase in input.

The highest significance for the regression of output on input was obtained when straight lines were fitted to values plotted on log-log graphs (correlation coefficient, r: $P < 0{\cdot}001$ in 37 control plots and

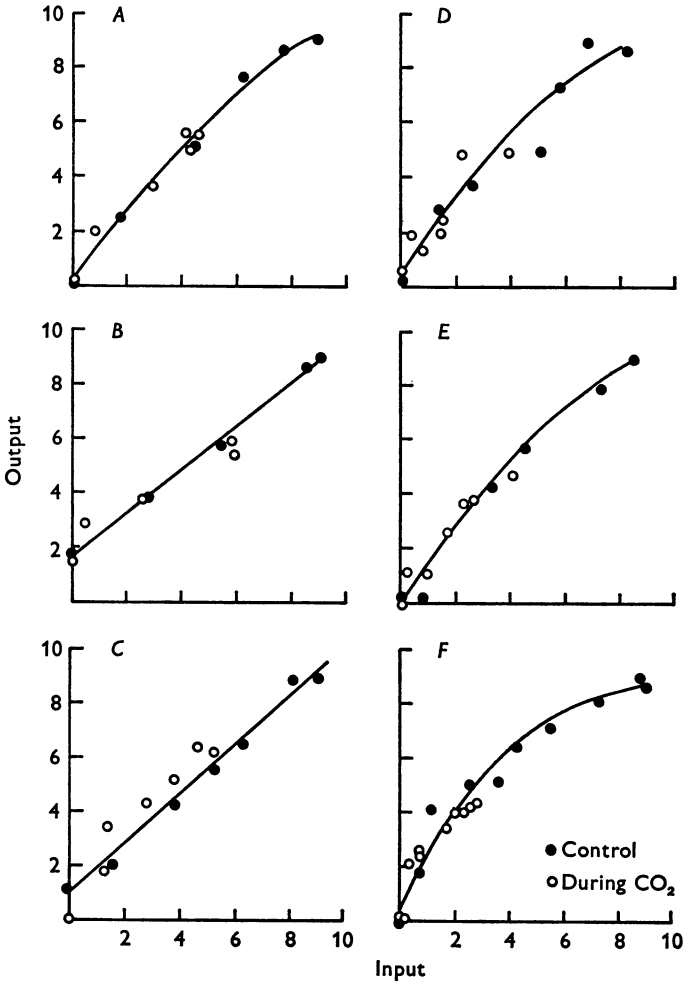

Fig. 10. Effects of inhalation of CO_2 (10 %, *A*, *B*, *C*; 20 %, *D*, *E*, *F*) on the input–output relation of synaptic transmission, evoked by different intensities of stimulation in the cuneate. Input represented by amplitude of antidromic responses in superficial radial nerve. Output represented by amplitude of trans-synaptic (β) responses in medial lemniscus. ●, control values; ○, while CO_2 was given (7–10 min). (Data from five cats; *A* and *F* in same cat. Amplitudes in arbitrary units; curves drawn by eye).

$P < 0{\cdot}05$ in three others). The mean slope was 1·17 (± S.E. 0·13), while the median was 1·01.

Effects of CO_2. In the majority of experiments the relation of output to input did not alter during administrations of 2–20 % CO_2. Fig. 10 shows in six plots (from five different animals) the effects of 10 % CO_2 (*A*, *B*, *C*) and 20 % CO_2 (*D*, *E*, *F*). Decreases in output corresponded to decreases in input, and so the shape of the curves was little changed.

TABLE 1. Effects of CO_2 on the input–output relation of the cuneate nucleus. A regression analysis of the slopes of the logarithmic amplitude plots of trans-synaptic (β) lemniscal and antidromic potentials, evoked by different intensities of cuneate stimulation (cf. Fig. 10). Data from sixteen cats (forty observations). Figures represent numbers of slope comparisons, categorized at the 5 % significance level (*F*-test)

Slope of input–output relation, compared to control	4 % CO_2	10 % CO_2	20 % CO_2
With full recovery*			
Unchanged	5	8	7
Increased	1	1	0
Decreased	0	2	2
Total	6	11	9
Without full recovery			
Unchanged	3	5	2
Increased	1	2	0
Decreased	0	0	1
Total	4	7	3

* Recovery defined as full when slopes before and after CO_2 were not significantly different ($P > 0{\cdot}05$).

Table 1 summarizes a statistical comparison of the logarithmic input–output regressions from sixteen cats before and during the effects of CO_2 (*F*-test, Snedecor (1956)). The slopes were either unchanged or increased by 4 % CO_2. They did not change significantly ($P > 0{\cdot}05$) during the inhalation of 10 and 20 % CO_2 in sixteen of twenty cats where there was full recovery. When control and recovery slopes were dissimilar, there was in most cases also no decrease in slope when CO_2 was given.

DISCUSSION

Excitability of cuneate neurones

In these experiments micro-electrodes were inserted to depths < 1 mm into the cuneate, and were judged by several criteria to be near the presynaptic terminals. Since the space constant of vertebrate nerve fibres is 1–2 mm (Barron & Matthews, 1935; Lussier & Rushton, 1951), it seems

reasonable to conclude that the direct (α) lemniscal potential evoked by stimulation in the cuneate substantially reflects the excitability of the post-synaptic neurones (Andersen *et al.* 1964).

Excitability of afferent terminals

An analysis of possible failure of synaptic transmission must include an estimate of the relation of output to input. The β lemniscal response and the antidromic response to direct stimulation of the afferent cuneate terminals provided some relevant data.

Trans-synaptic response. The β peak of the lemniscal potential follows the α peak at an interval of $\geqslant$ 1 msec, which is greater than the mean inter-spike interval of spontaneously firing cuneate units (Amassian, Macy, Waller, Leader & Swift, 1964; Schwartz, 1965). These neurones can follow peripheral stimulation rates of 300 Hz and have negligible positive after-potentials (Amassian & De Vito, 1957; Andersen, Eccles, Oshima & Schmidt, 1964). Since the response of relay neurones to antidromic invasion from juxtaliminal stimulation of the lemniscus is a single discharge (Amassian & De Vito, 1957; Gordon & Seed, 1961; Andersen *et al.* 1964*c*), there is probably little or no repetitive firing from their direct stimulation. Therefore the β trans-synaptic response will not fall in the refractory period following the α wave. It may reasonably be considered an index of synaptic output.

Antidromic response. Antidromic responses to cuneate stimulation can be recorded from more than one nerve at the same time. In addition, the dorsal roots receive fibres from different nerves and have been shown to have a localized distribution in the cuneate (Keller & Hand, 1970). Therefore, the potentials recorded from one nerve represent only part of the total number of afferent fibres excited.

The tests used to determine the final position of the stimulating electrode in the cuneate suggested that impulses were initiated in the afferent fibres near their endings. Even so, there is probably greater security for antidromic conduction in the parent fibres than for orthodromic transmission in the diverging terminal branches. Synaptic input will, in this respect, be over-estimated by the antidromic potential.

The response at the nerve may therefore be considered to provide an approximate index of the number of active synapses in the cuneate.

Changes in excitability

Variations in the amplitude of the monophasic antidromic potentials evoked by a constant stimulus can be ascribed to corresponding changes in the excitability of the afferent terminals and therefore in the number of fibres excited (Wall, 1958). The only necessary assumptions are that the

amplitude and synchronization of individual fibre spikes are not significantly altered.

During the inhalation of CO_2 any change in the spike height of both the afferent fibres and the relay neurones is probably small, and is likely to be an increase rather than a decrease. Observed variations in membrane potential caused by CO_2 in vertebrate peripheral nerve fibres have been in the order of only a few millivolts and mainly hyperpolarizing in direction (Straub, 1956; Lorente de Nó, 1947). During intracellular recording in the toad motoneurone, CO_2 caused a hyperpolarization, an increase in spike amplitude and eventually abrupt loss of the response (Washizu, 1960). Krnjević, Randić & Siesjö (1965) recorded hyperpolarization in the mammalian motoneurone although Papajewski, Klee & Wagner (1969) found a loss of excitability with both depolarizing and hyperpolarizing changes.

A decrease in conduction velocity probably makes only a minimal contribution to the changes in potentials: the increases in transmission time observed for both antidromic and lemniscal potentials in these experiments were $< 4\%$. From the narrow range of thresholds and conduction velocities of the dorsal column and lemniscal fibres, it may be assumed that the potentials are produced by relatively homogeneous groups of fibres (Rudin & Eisenman, 1953) and that all fibres in a group would be affected to the same extent by CO_2. That this is so is suggested by the observation that during the inhalation of CO_2 the stimulus–response relation remained linear; moreover changes in stimulus–equivalent ratios were reasonably similar over the range of voltages used. The more homogeneous the fibre population, the more reasonable is the approximation of the measurement of the potential amplitude to that of the area, and the less significant are any changes in conduction velocity.

Hence, one can conclude that the depression of antidromic and lemniscal potentials represents mainly a decrease in the number of responding fibres and therefore a reduction in the excitability of the afferent terminals and the cuneate relay neurones.

The excitability changes of the afferent terminals may be even greater than reflected in the antidromic potentials, since thresholds will be highest for small final branches, and the impulse is likely to arise from adjacent regions of lower threshold. Antidromic impulses from several branches of a single parent axon are less likely to fail than those ascending orthodromically as fibres branch and decrease in size, the myelin cover is lost, and threshold rises. Impulse transmission in the primary afferent fibres has been shown to have a much higher safety factor for antidromic conduction from the dorsal columns than for orthodromic conduction from nerve to the spinal cord (Barron & Matthews, 1935; Wall, Lettvin, McCulloch & Pitts, 1955; Raymond, 1969; Morris, 1970). It seems unlikely that there will be losses in the antidromic potential, but rather that changes in synaptic input may be somewhat underestimated.

Synaptic transmission

It is of some interest to consider the nature of the transfer function at the cuneate nucleus (Eccles, 1966; Mountcastle, 1966). The input–output plots derived from amplitudes of the antidromic and trans-synaptic potentials showed variation, possibly owing to some disparity in the representations of input and output caused by differences in the position of the stimulating electrode in the cuneate. It was therefore not surprising that the exponents of the input–output functions had a wide range

(S.D. $\pm$ 0·8). However, the median and mean slopes had values close to 1·0. This strongly suggests that transmission through the cuneate is predominantly linear, as has been found in some other regions of the central nervous system (Lloyd, 1957; McIntyre & Mark, 1960).

From the changes in terminal and post-synaptic excitability, it might have been predicted that the trans-synaptic responses would be depressed by CO_2. However, the input–output analysis, relating the trans-synaptic and antidromic potentials, showed that for a given input the output at the cuneate was usually well maintained or even increased, in spite of a significant depression of the direct excitability of the relay neurones. Thus, one can conclude that for impulses which reach the synapse, the efficiency of synaptic transmission is not only not obviously diminished but is even likely to be enhanced, possibly by an increase in the release of transmitter or in post-synaptic chemical sensitivity.

Hyperpolarization of the afferent fibre membrane could potentiate the release of transmitter at individual terminals. The amount of transmitter released has been found to depend on the amplitude of the presynaptic spike in the squid ganglion (Tasaki & Hagiwara, 1957; Katz & Miledi, 1966, 1967) and the rat diaphragm (Liley, 1956).

There is little evidence that membrane sensitivity is altered by an increase in acidity or in P_{CO_2} (Krnjević & Phillis, 1963; Krnjević *et al.* 1965); although the ionization of the proposed chemical transmitters for the cuneate (Galindo, Krnjević & Schwartz, 1967) would not be significantly altered by the likely changes in pH, the ionization of the membrane receptors might be. The efficiency of transmission could possibly be raised by an increase in membrane resistance causing a larger EPSP, as found with acetylcholine at the neuromuscular junction (del Castillo, Nelson & Sanchez, 1962).

The fact that CO_2 depresses overall dorsal column–lemniscal transmission (Morris, 1971*a*) without lowering the efficiency of synaptic transfer at the cuneate can only be explained by a significant block of the synaptic input, i.e. of conduction in the afferent fibres. Regions of low safety factor, such as points of branching (Krnjević & Miledi, 1958) would be most vulnerable: the most likely sites of block are therefore in the spinal cord near the entry of the dorsal roots (Wall *et al.* 1955; Raymond, 1969; Morris, 1971*b*) and in the preterminal regions in the cuneate. Analysis of quantal aspects of transmission in the spinal cord confirms that CO_2 causes a conduction block in afferent fibres (D. W. Esplin, B. A. Esplin, & R. Čapek, personal communication).

A single mechanism which could explain increased release of transmitter, decreased terminal and post-synaptic excitability and conduction block in afferent fibres, would be hyperpolarization and increased conductance of the membrane. Hydrogen ions and CO_2 increase chloride permeability in vertebrate muscle (Hutter & Warner, 1967) and in neurones of *Aplysia* (Brown & Berman, 1970; Brown, Walker & Sutton,

1970). At present, there is insufficient data for the mammalian central nervous system to allow any conclusions about the permeability change which CO_2 might produce.

These studies were supported by grants from the Canadian Medical Research Council and Hoffmann-La Roche Ltd. Ayerst Laboratories and Burroughs Wellcome & Co. Ltd. kindly provided supplies of Fluothane and Anectine respectively. The assistance of Norma Lake during many experiments was very much appreciated.

REFERENCES

AMASSIAN, V. E. & DE VITO, J. L. (1957). La transmission dans le noyau de Burdach. *Colloques int. Cent. natn. Rech. scient.* **67**, 353–393.

AMASSIAN, V. E., MACY, J., WALLER, H. J., LEADER, M. S. & SWIFT, M. (1964). Transformation of afferent activity at the cuneate nucleus. In *Information Processing in the Nervous System*, pp. 235–255. *Proc. int. Union Physiol. Sci.*, vol. 3. Amsterdam: Excerpta Medica Foundation.

ANDERSEN, P., ECCLES, J. C., OSHIMA, T. & SCHMIDT, R. F. (1964). Mechanisms of synaptic transmission in the cuneate nucleus. *J. Neurophysiol.* **27**, 1096–1115.

ANDERSEN, P., ECCLES, J. C., SCHMIDT, R. F. & YOKOTA, T. (1964*a*). Slow potential waves produced in the cuneate nucleus by cutaneous volleys and by cortical stimulation. *J. Neurophysiol.* **27**, 78–91.

ANDERSEN, P., ECCLES, J. C., SCHMIDT, R. F. & YOKOTA, T. (1964*b*). Depolarization of presynaptic fibres in the cuneate nucleus. *J. Neurophysiol.* **27**, 92–106.

ANDERSEN, P., ECCLES, J. C., SCHMIDT, R. F. & YOKOTA, T. (1964*c*). Identification of relay cells and interneurons in the cuneate nucleus. *J. Neurophysiol.* **27**, 1080–1095.

BARRON, D. H. & MATTHEWS, B. H. C. (1935). Intermittent conduction in the spinal cord. *J. Physiol.* **85**, 73–103.

BROWN, A. M. & BERMAN, P. R. (1970). Mechanism of excitation of *Aplysia* neurons by CO_2. *J. gen. Physiol.* **56**, 543–558.

BROWN, A. M., WALKER, J. L. & SUTTON, R. B. (1970). Increased chloride conductance as the proximate cause of hydrogen ion concentration effects in *Aplysia* neurons. *J. gen. Physiol.* **56**, 559–582.

CASTILLO, J. DEL, NELSON, T. E. & SANCHEZ, V. (1962). Mechanism of the increased sensitivity of skeletal muscle in low pH solutions. *J. cell. comp. Physiol.* **59**, 35–44.

ECCLES, J. C. (1966). Linearity of transmission along the perceptual pathway. In *Touch, Heat and Pain*, p. 80, ed. DE REUCK, A. V. S. & KNIGHT, J. London: J. and A. Churchill Ltd.

ECCLES, J. C., MAGNI, F. & WILLIS, W. D. (1962). Depolarization of central terminals of Group I afferent fibres from muscle. *J. Physiol.* **160**, 62–93.

EDISEN, A. E. U. (1957). Effects of asphyxia and repetitive stimulation on intramedullary afferent fibres. *Am. J. Physiol.* **191**, 225–232.

GALINDO, A. (1969). Effects of procaine, pentobarbital and halothane on synaptic transmission in the central nervous system. *J. Pharmac. exp. Ther.* **169**, 185–195.

GALINDO, A., KRNJEVIĆ, K. & SCHWARTZ, S. (1967). Micro-iontophoretic studies on neurones in the cuneate nucleus. *J. Physiol.* **192**, 359–377.

GORDON, G. & SEED, W. A. (1961). An investigation of nucleus gracilis of the cat by antidromic stimulation. *J. Physiol.* **155**, 589–601.

GRAHAM, H. T. & LORENTE DE NÓ, R. (1938). Recovery of blood-perfused mammalian nerves. *Am. J. Physiol.* **123**, 326–340.

HUTTER, O. F. & WARNER, A. E. (1967). The pH sensitivity of the chloride conductance of frog skeletal muscle. *J. Physiol.* **189**, 403–425.

JASPER, H. H. & AJMONE-MARSAN, C. (1954). *A Stereotaxic Atlas of the Diencephalon of the Cat.* Ottawa: The National Research Council of Canada.

KATZ, B. & MILEDI, R. (1966). Input/output relation of a single synapse. *Nature, Lond.* **212**, 1242–1245.

KATZ, B. & MILEDI, R. (1967). The release of acetylcholine from nerve endings by graded electric pulses. *Proc. R. Soc.* B **167**, 23–38.

KELLER, J. H. & HAND, P. J. (1970). Dorsal root projections to nucleus cuneatus of the cat. *Brain Res.* **20**, 1–17.

KRNJEVIĆ, K. & MILEDI, R. (1958). Failure of neuromuscular propagation in rats. *J. Physiol.* **140**, 440–461.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. *J. Physiol.* **165**, 274–304.

KRNJEVIĆ, K., RANDIĆ, M. & SIESJÖ, B. K. (1965). Cortical CO_2 tension and neuronal excitability. *J. Physiol.* **176**, 105–122.

LILEY, A. W. (1956). The effect of presynaptic polarization on the spontaneous activity at the mammalian neuromuscular junction. *J. Physiol.* **134**, 427–443.

LLOYD, D. P. C. (1957). Input–output relation in a flexor reflex. *J. gen. Physiol.* **41**, 297–306.

LORENTE DE NÓ, R. (1947). A study of nerve physiology. *Stud. Rockefeller Inst. med. Res.* **131**, 1–496.

LUSSIER, J. J. & RUSHTON, W. A. H. (1951). The relation between the space constant and conduction velocity in nerve fibres of the A group from the frog's sciatic. *J. Physiol.* **114**, 399–409.

MCINTYRE, A. K. & MARK, R. F. (1960). Synaptic linkage between afferent fibres of the cat's hind limb and ascending fibres in the dorsolateral funiculus. *J. Physiol.* **153**, 306–330.

MORRIS, M. E. (1970). Carbon dioxide and sensory transmission. Ph.D. Thesis, McGill University, Montreal, Canada.

MORRIS, M. E. (1971*a*). The action of carbon dioxide on afferent transmission in the dorsal column–lemniscal system. *J. Physiol.* **218**, 651–669.

MORRIS, M. E. (1971*b*). Conduction block in afferent fibres during hypercarbia. *Proc. Can. Fed. Biol. Soc.* **14**, 143.

MOUNTCASTLE, V. B. (1966). Discussion – Transfer functions of the skin and muscle senses. In *Touch, Heat and Pain*, pp. 21–22, ed. DE REUCK, A. V. S. & KNIGHT, J. London: J. and A. Churchill Ltd.

PAPAJEWSKI, W., KLEE, M. R. & WAGNER, A. (1969). The action of raised CO_2 pressure on the excitability of spinal motoneurones. *Electroen. Neurophysiol.* **27**, 618.

RAYMOND, S. A. (1969). Physiological influences on axonal conduction and distribution of nerve impulses. Ph.D. Thesis, Massachusetts Institute of Technology.

RUDIN, D. O. & EISENMAN, G. (1953). After-potentials of spinal axons in vivo. *J. gen. Physiol.* **36**, 643–657.

SCHWARTZ, S. T. (1965). An analysis of resting activity in the cuneate nucleus. Ph.D. Thesis, Albert Einstein College of Medicine, New York.

SNEDECOR, G. W. (1956). *Statistical Methods.* 5th edn. Ames: The Iowa State University Press.

STRAUB, R. (1956). The action of CO_2 and pH on the resting potential of myelinated nerve fibres. *XX Int. Physiol. Congr., Brussels.* Abstracts of communications, pp. 858–860.

TASAKI, I. & HAGIWARA, S. (1957). Capacity of muscle fibre membrane. *Am. J. Physiol.* **188**, 423–429.

THERMAN, P. O. (1941). Transmission of impulses through the Burdach nucleus. *J. Neurophysiol.* **4**, 153–166.

WALL, P. D. (1958). Excitability changes in afferent fibre terminations and their relation to slow potentials. *J. Physiol.* **142**, 1–21.

WALL, P. D., LETTVIN, J. Y., MCCULLOCH, W. S. & PITTS, W. H. (1955). Factors limiting the maximum impulse transmitting ability of an afferent system of nerve fibres. *3rd London Symposium on Information Theory*, pp. 329–344. London: Butterworths.

WASHIZU, Y. (1960). Effect of CO_2 and pH on the responses of spinal motoneurones. *Brain Nerve, Tokyo* **12**, 757–766.