An Investigation Concerning the Results of Ischemia upon the Surface Positive Burst Response of Neuronally Isolated Cerebral Cortex of the Cat.

A Thesis presented to the

University of Manitoba

in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

by

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September, 1971

To my Mother and Father

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ABSTRACT

The effects of ischemia upon the surface positive burst response (SPBR) of the isolated cortical slab in the cat were studied. It was found that the resultant brief lowering and the more prolonged elevation of the SPBR threshold could be reproduced by the selective induction of either hypoxia or hypercarbia. The effects of these latter conditions were distinguished by their short-lived nature; the excitability of the cortex recovered to its pretreatment level within minutes despite continuation of the treatment. A similar early recovery was observed with the depressant effect produced by the carbonic anhydrase inhibitor acetazolamide and with the facilitatory effect of the β-adrenergic antagonist D-(-)-INPEA and its adrenergically inactive isomer L-(+)-INPEA.

Involvement of the putative inhibitory neurotransmitter γ-aminobutyric acid in the depressant effect of carbon dioxide was suggested by its enhancement by amino-oxyacetic acid. The lack of involvement of adrenergic transmitter substances was indicated by the lack of effect of various α- and β-blocking agents, and by the similarity of effect of the two isomeric forms of INPEA.

The site of the depressant action of carbon dioxide in the cerebral cortex appeared to be largely restricted to the superficial layers where the SPBR is initiated, rather than in the depths where it is sustained. This followed from the lack of effect of hypercarbia upon the amplitude and duration of the SPBR, and from the diversity of the several observed effects upon neuronal unit activity evoked by microelectrophoretic application of glutamic acid and by initiation of the SPBR. Such

a site of action was indicated also by the increased latency-to-onset of the SPBR-associated unit activity without a decrease of the intensity of this activity.

Comparison of the effects of carbon dioxide upon glutamate-evoked unit activity with its effects on SPBR-associated unit activity suggested that the population of cortical neurons which sustains the SPBR differs from that population which is sensitive to glutamic acid.

ACKNOWLEDGEMENTS

The interaction with many individuals on a casual as well as scientific basis was valuable and welcome in the conduct of the present work. I wish to express my deepest gratitude to these many people.

To Dr. Ian Innes thanks are due for welcoming me into his department.

I have been greatly privileged to experience the humanity of Dr. Carl Pinsky who has been my mentor in the pursuit of this project. No small measure of the imagination required in such an enquiry was fostered by his encouragement.

Warmest thanks are owing to Dr. John Phillis for his advice and for the generous use of his laboratory facilities, which made possible a significant portion of the present study.

My introduction to the surgical procedures involved in making recordings from the brain of the cat was provided by Dr. Khem Jhamandas to whom thanks are offered.

To Dr. John Armstrong and the Canadian Heart Foundation are owing thanks for their moral as well as financial support.

Much of the technical assistance was supplied by Mr. Dave Brown, whom I thank. Miss Maureen Cumming is also owed my thanks.

Mr. Stan Vivian was responsible for the writing of the computer programs which made possible most of the data analysis. My thanks are also owing to him for long conversations directed at seeking the most effective means of handling the data.

The animals used in this study were supplied by Mr. John Kiekush and his able staff, to whom warmest thanks are due.

The attractive appearance of the illustrations in this thesis is due to the assistance of Mr. Roy Simpson, and the readableness of the manuscript was enhanced by the typing skill of Mrs. Chris Elbrink. Thank you.

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INTRODUCTION

I. Prologue:

For many years it has been known that central nervous tissue is profoundly sensitive to ischemic injury, far more so than any other tissue in the body. Data is available from histological studies (reviewed by Meyer, 1958) which permit the ranking of the central nervous regions and cellular components according to the order of appearance of permanent structural changes subsequent to a period of ischemia. These indicate that the cerebral cortex is among the most susceptible structures, and that the small pyramidal cell of the third layer of the cortex is one of the most susceptible neurons. It is further known that the rapidity of onset of post-ischemic damage in cerebral cortical neurons is due largely to their peculiarities of energy metabolism (e.g. Reichelt, 1968).

On the other hand, studies which provide an explanation for the immediate functional effects of acute ischemia are incomplete. Recent studies (discussed below) have described the effects of altered respiratory gas transport upon various electrophysiological properties of the nervous system. The phenomena studied in mammalian central nervous tissues, however, are often poorly understood, and may in fact be remote from any real connection with central nervous function in the intact and normal animal.

For these reasons it was thought desirable to examine the effects of impaired circulation and of manipulation of the inhaled gas mixture upon the function of neuronally isolated cerebral cortex. The electrophysiological property to be studied was the surface positive burst response (SPBR), not because its structural substrates have been fully delineated (in fact they have not), but because 1) the SPBR is an all-or-

none response for which a threshold, duration, and amplitude can be determined with a good degree of precision; 2) it is known to be very rapidly abolished by anoxia (Burns, 1951), which may suggest that the level of integration required for the generation of this response resembles the level of integration necessary for conscious experience; and 3) it is unaffected by anticonvulsant drugs in doses which clearly antagonize evoked convulsant activity in the same preparation (Sanders & Gravlin, 1968), just as these drugs produce their anticonvulsant and antiepileptic effects in man without affecting normal cerebration.

The effects of altered gas tensions upon the firing activity of single neurons were studied also in the same preparation by extracellular microelectrode recording. All-or-none spike discharges were evoked from single neurons in the isolated slab by microelectrophoresis of glutamic acid, and by stimulating the slab to evoke the SPBR. It was therefore possible, in these single-unit studies, to examine the extent to which the population of neurons participating in the SPBR resembles the population of neurons responsive to glutamic acid (presumed to be representative of the entire neuron population in the slab), in its response to the experimental manipulation of tissue gas tension. It was hoped that such studies would help elucidate the types of cortical neurons that are involved in the generation of the SPBR, and thus strengthen the basis for studying this response.

II. <u>The role of the cerebral circulation as a determinant of cerebral activity</u>:

In 1938, Bronk suggested that the rhythmic activity of the cerebral cortex may be related to local circulatory factors. He based

this conclusion on his observations of the depressant effect of asphyxia upon peripherally recorded evoked spike discharges of deafferented respiratory center neurons and upon synaptic transmission in sympathetic ganglia. Since then, however, the converse concept has been generally accepted and it is widely held that in normal circumstances the neuronal and metabolic activity of the cortex determines the cortical circulation (e.g. Harper, 1969; Sokoloff & Kety, 1960).

Nevertheless, various pieces of evidence have appeared, particularly in the past decade, which indicate that at least under some circumstances the hierarchical order is reversed. Foremost among these are studies which demonstrate the involvement of extrinsic neurogenic control in the regulation of cerebral cortical blood flow (CBF). Even if this neurogenic factor is not the primary determinant of the caliber of cerebral resistance vessels, it must, nevertheless modify to some extent their response to local metabolic factors.

Darrow, Green, Davis & Garol (1944) observed that sectioning the facial nerves at their entrance to the internal auditory meatus led to the appearance of high-amplitude low-frequency activity in the electroencephalogram (EEG) of β-erythroiden-paralyzed hyperventilated cats. They concluded that the spasmogenic response of cerebral arterial vessels during hyperventilation is opposed by the cholinergic vasodilator nerve fibers running in the greater superficial petrosal nerve (Chorobski & Penfield, 1932). Stimulation of the distal cut end of the facial nerves was found to reduce or abolish the EEG changes brought out by sectioning the nerves, as did intravenous eserine, while atropine enhanced them (Darrow, Green, Davis & Garol, 1944; Darrow & Graf, 1945).

Interestingly, Morrice (1956) observed that patients who demonstrated an unstable EEG response to hyperventilation showed also an instability to autonomic stimulation. The latter instability was absent in patients whose EEG regularly slowed in response to hyperventilation.

An important recent observation which amplifies the foregoing is that of Mchedlishvili & Nikolaishvili (1970). The caliber of pial arteries in the rabbit was studied photographically. It was found that a moderate intravenous dose of atropine (0.2 mg/kg) could block the dilatation of these vessels which occurred during exsanguination. Other muscarinic antagonists produced the same effect.

Carlyle and Grayson (1956) found that atropine considerably reduced the increase in CBF produced by intracarotid adrenaline infusion despite a consistent rise in blood pressure. They concluded that the increase normally produced by adrenaline was due at least in part to reflex activation of the vasodilator nerves.

It would appear from this that neurogenic factors in the control of CBF may in fact modulate the activity of the cortex by affecting its metabolic state. An apparently irreconcilable objection arises from the observations that the EEG effects of hyperventilation in the gallamine-paralyzed cat can be prevented by neuronal isolation of the cortex (Sherwin, 1965)(which would leave the vasomotor nerve supply intact) and by lesions in the anterior pole of the thalamus (Sherwin, 1967). These results are reminiscent of the suggestion of Bonvallet and Dell (1956) that the EEG changes seen during hyperventilation are due to suppression of the ascending reticular activating system (ARAS). However, the consensus is that EEG changes produced by hyperventilation are due to local hypoxia

secondary to hypocarbic vasoconstriction. The evidence for the latter is contained in the observations that breathing 7% oxygen predisposes to the EEG slowing (Davis & Wallace, 1942), and that breathing pure oxygen reduces its occurrence (ibid.), as do pretreatment with vasodilator agents (Whittier, 1964; Korein, Geller, Rosenblum & Levidow, 1966) and enhancement of cerebral blood flow and of cortical oxygen tension by intravenous adrenaline (Meyer & Gotoh, 1960). Cerebral hypoxia during hyperventilation would also be expected to occur through the impairment of dissociation of oxygen from hemoglobin at low carbon dioxide gas tension (Bohr Effect) (Malette, 1959).

Sherwin's observations (1965; 1967) are countered by those of Ingvar (1955) who found that stimulation of the ARAS produced EEG suppression in the neuronally isolated cerebral cortex of the unanesthetized cat immobilized with curare. This coincided with cortical blanching, which indicated that impairment of local circulation was the proximate cause of the EEG change. That the CBF impairment was not due to blood pressure changes was indicated by the findings that midbrain section prevented the effects of ARAS stimulation, while prevention of peripheral circulatory changes by means of ganglionic blocking agents and by high spinal cord section did not. Unfortunately, the significance of Ingvar's observations on the effect of midbrain transection is largely uninterpretable because it is not made clear whether curare was used in the experiments where this was done. Comparisons cannot properly be made between CNS studies done with curare and those done in its absence, because curare and the preferred synthetic agent gallamine are now known to produce both excitant (Rech & Domino, 1960a; Morlock & Ward, 1961; Krnjevic & Phillis, 1963;

Halpern & Black, 1967, 1968) and depressant (Purpura & Grundfest, 1956; Morlock & Ward, 1961) CNS effects, despite their classification as non-depolarizing neuromuscular blocking agents having minimal CNS activity.

Ingvar concluded that at least part of the control exhibited by the ARAS over cortical activity can be explained on the basis of extrinsic neural control of the cortical circulation. Scheinberg (1968) noted that stimulation of the mesencephalic tegmentum in the cat consistently resulted in increased CBF without consistent alteration of the blood pressure or the EEG. Langfitt and Kassell (1968a; 1968b) made essentially identical observations in the rhesus monkey, but Meyer, Nomura, Sakamoto, and Kondo (1969) found that brain stem stimulation never led to increased CBF except in the presence of EEG desynchronization, suggesting to them that the observed CBF change was secondary to increased neuronal metabolism.

Shalit and co-workers observed that bathing the dog's brain stem with a cerebrospinal fluid (CSF) having a high carbon dioxide gas tension and low pH resulted in increased CBF (Shalit, Shimojyo & Rheinmuth, 1967); that perfusion of a branch of the middle cerebral artery with blood of elevated P_{CO_2} paradoxically increased the resistance of its vascular bed, while elevation of the systemic arterial P_{CO_2} lowered it (Shalit, Rheinmuth & Scheinberg, 1967; Shalit, Shimojyo, Rheinmuth, Lockhart & Scheinberg, 1968); and that certain brain stem lesions abolished the vasodilatation which occurred in response to elevation of the systemic arterial P_{CO_2} (Shalit, Rheinmuth, Shimojyo and Scheinberg, 1967). Bathing the brain stem with normocarbic CSF at low pH did not increase CBF (Shalit, Shimojyo & Rheinmuth, 1967). This indicated that the "receptors" are selectively sensitive to carbon dioxide rather than to hydrogen ions.

The conclusion appeared inescapable that brain stem receptors are responsible for CBF changes produced by CO_2.

Contrarily, Skinhoj and Paulson (1968; 1969) were unable to demonstrate any role of the brain stem in bringing about cortical vasodilatation when they compared the effects upon cortical CBF of the injection of hypercapnic blood into the internal carotid artery and into the vertebral artery in man. Kogure, Scheinberg, Rheinmuth, Fujishima & Busto (1970) also were unable to produce cerebral vasodilatation in the dog by infusions of hypercarbic blood into the vertebral artery, except at high infusion rates which probably allowed spillage to occur into the circle of Willis. However, inhalation of 8% CO_2 was found to produce a greater vasodilatation in the bed of a cannulated middle cerebral artery (autoperfused from the animal's femoral artery) than did similarly perfused blood having a P_{CO_2} greater than that obtained with the CO_2 inhalation.

Molnar (1968) observed that decerebration in the rabbit abolished the cerebrovascular dilatatory effect of CO_2, and indeed often replaced it with a constrictor response. Fujishima, Scheinberg & Rheinmuth (1970) occluded the basilar artery of the dog by means of magnetically localized ion particles, and found that the dilator effect of CO_2 was subsequently impaired.

Millar and co-workers (James, Millar & Purves, 1968; 1969; Millar, 1969), in a study on the baboon, demonstrated a neurogenic component in the responses of cerebral vessels to CO_2 and to blood pressure changes. Stimulation of cervical sympathetic nerves prevented the dilator action of CO_2 and of hypoxia, while cutting these nerves enhanced this

action. Cutting these nerves also substantially impaired the constriction which normally occurred in response to elevation of the blood pressure. Stimulation of the cephalad end of the cut nerves restored this "autoregulation".

A mild reduction of CBF produced by stimulating the cerebral sympathetic nerves has been reported by others, but blocking these nerves was not found to result in increased flow. This finding has led to the belief that in normal circumstances, as well as in hypertension and other pathologic states, the sympathetic nerves play a negligible role in determining cerebrovascular resistance (Forbes & Cobb, 1938; Harmel, Hakfenschiel, Austin, Crumpton & Kety, 1949; Scheinberg, 1959; Meyer, Yoshida & Sakamoto, 1967).

In further discussing the role of the noradrenergic innervation of intracranial vessels it is necessary to examine the body of work done on arterial spasm. Noradrenergic innervation does not appear to mediate the spasm induced by topical application of fresh arterial blood or 5-hydroxytryptamine (5-HT) (Fraser, Stein, Barrett & Pool, 1970) or by hyperventilation (Fraser, Stein & Pool, 1971). These workers found that 1) tyramine, which releases noradrenaline from nerve endings, and was observed by these workers to abolish the catecholamine fluorescence of these vessels, produced only a transient constriction, without impairment of the spasm produced by subsequent application of blood and by hyperventilation; 2) phenoxybenzamine (POB) and 2-brom-LSD, both antagonists of 5-HT, fully prevented induction of spasm by blood; 3) denervation by bilateral sympathectomy performed two weeks previously did not impair the spasm induced by blood and 5-HT. From the observation that POB reverses the spasm by

hyperventilation in tyramine-pretreated arteries, the authors concluded that blockade of the α-adrenergic receptor somehow interferes with the sensitivity of the hydrogen ion receptor. However, the poor pharmacologic specificity of POB (Nickerson, 1970) leaves open the possibility that other mediators may be responsible for this spasm. They also concluded that blood-induced spasm is mediated by a blood-borne constrictor substance acting at the α-adrenergic receptor site. This possibility is borne out by the observation of Lende (1960) that phentolamine, a blocker of α-adrenergic receptors which, however, also lacks specificity (Nickerson, 1970), was the best agent for prevention as well as for relief of spasm induced by both electrical and mechanical means. On the other hand, β-adrenergic blockers appear to be the most effective means for preventing barium-induced spasm (Rosenblum, 1969). It is of interest also that the β-blocker INPEA appears to oppose the vasoconstriction produced by hyperventilation (Falck, Nielsen & Owman, 1968), although these observations are confounded by a recent report that β-receptors in the cerebrovascular bed mediate dilatation (Lowe & Gilboe, 1971).

Cholinergic mediation of dilatation in response to CO_2 was indicated by the abolition of such responses after bilateral section of the vagi (James, Millar, & Purves, 1969; Millar, 1969) (i.e. the afferent limb of the vasodilator reflex (Wolff, 1936)). Contrarily, ipsilateral facial nerve section reduced only slightly the dilator action of CO_2. Stimulation of the facial nerve after vagal nerve section produced a powerful dilator effect, regardless of the arterial P_{CO_2}.

Studies which support the idea that the cerebral circulation is an important determinant of normal cerebral function have been described

by Davis & Bronk (1957), and by Silver (1965). They found that the partial pressure of oxygen at points in the cerebral cortex distant from capillaries is often so low as to fail to satisfy the critical P_{O_2} (Jobsis, 1964; 1967) for neuronal energy metabolism. Furthermore, cortical P_{O_2} is known to fluctuate rhythmically at a frequency of 6-12/minute. This rhythmicity might conceivably contribute to rhythmic variations in neuronal activity, particularly in regions of low P_{O_2}. In any event, it would seem self-evident, in view of the finding of very low intercapillary oxygen tensions, that any alteration of the regional circulation would modify the balance between supply and demand and thereby alter neuronal activity.

There has been a recent report (Reivich, Isaacs, Evarts & Kety, 1968) of increased blood flow during slow-wave sleep (non-Rapid Eye Movement, n-REM) in ten of the 25 regions studied in the cat's brain with an autoradiographic technique (Reivich, Jehle, Sokoloff & Kety, 1969). This confirms an earlier finding, made in the human whole brain with the nitrous oxide technique (Mangold, Sokoloff, Kleinerman, Therman & Kety, 1955). On the basis of an unaltered oxygen consumption during n-REM sleep, observed in the latter study, the flow should be the same as during wakefulness. Hence the possibility must be seriously entertained that neurogenic factors are important in the occurrence of the increased flow during n-REM sleep, since the blood O_2 and CO_2 tensions are unaltered from those of the wakeful state (Mangold et al., 1955; Reivich et al., 1968).

The same conclusion follows from observations that drug-induced depression of cerebral function and of oxygen uptake is often accompanied by dilatation of cerebral resistance vessels (Schmidt, 1960), which cannot be attributed to vasomotor paralysis (Pierce, Lambertsen,

Deutsch, Chase, Linde, Dripps & Price, 1962), but may rather be due to an altered balance of vasomotor nerve activity.

III. Hypoxia as a factor in the effects of impaired cerebral blood flow:

The effects of ischemia upon the activity and excitability of the nervous system have been extensively studied. However, no clear distinction has yet been made between the component of the ischemic response which is due to hypoxia and that which is due to retention of carbon dioxide. There is some agreement that depression by ischemia is preceded by a variable period of enhancement. This has been observed in cortical responses evoked by stimulating the optic radiations (Nicholson, MacNamara, & Borland, 1968), and in EEG recordings (Sugar & Gerard, 1938; Hossmann & Sato, 1970). The negative-wave Direct Cortical Response (nDCR) is also initially enhanced (Ochs, 1959) but the interpretation of alterations of nDCR amplitude is complicated by the fact that enhancement can result from decreased ongoing activity (Ochs, 1966), presumably through decreased refractoriness in the responsive elements. This would allow recruitment of more elements into the response. The same explanation has been advanced by Purpura & Grundfest (1956) to explain their observation that nDCR amplitude is greater in cats anesthetized with pentobarbital than in cats paralyzed with curare.

Brief enhancement of many forms of central nervous activity is known to occur with the induction of hypoxia. Spindle-burst activity develops for a short while in the electrocorticogram (ECoG) of the monkey given 8% oxygen to breathe (Massopust, Wolin, Kadoya & White, 1969). Inhalation of pure nitrogen produces a transient enhancement of the nDCR in cat cortex (Chang, 1951). Initial enhancement also occurs with the mono-

synaptic response of cat spinal cord subjected to hypoxia (Eccles, Loyning & Oshima, 1966). This is regarded as being due to an increase in the number of transmitter quanta released by the afferent volley since there is enhancement of the excitatory postsynaptic potential (EPSP) despite a decrease in the number of presynaptic fibers activated. Altered postsynaptic sensitivity is excluded by the absence of any change in the motoneuron membrane resistance and in the time course of the EPSP.

Lloyd (1970b), on the other hand, reported that the EPSP disappears after an initial period of enhancement by asphyxia while the motoneuron spike discharge persists. Lloyd concluded that the EPSP, which according to this scheme reflects activation of the L-(labile) fraction of the postsynaptic membrane, is not essential for monosynaptic transmission, since activation of the Q-(quick) fraction of the membrane can occur in the absence of activation of the L-fraction (Lloyd, 1970a). He further concluded that "the postsynaptic potential is not the agent for engendering monosynaptic reflex motoneuron impulse discharge" (Lloyd, 1970b).

Fox & Kenmore (1967) observed that rapidly-conducting fibers in the proximal portion of the canine tibial-sciatic nerve were more susceptible to ischemic deterioration than were more slowly-conducting fibers in the distal portion. This contrasted with their finding that along the total nerve segment the slowly-conducting fibers were more sensitive to ischemia than the more rapidly-conducting fibers. They concluded that the density of packing of nerve fibers is an important parameter of their sensitivity to ischemia, possibly through modification of the rate of accumulation of a metabolite (CO_2?) or of an ion (K^+?).

Lehmann (1937b) found that replacement of oxygen by nitrogen caused a lowering of the threshold for excitation of mammalian A-fibers bathed in bicarbonate-buffered Krebs solution. This phase of facilitation, which lasted six to eight minutes, was followed by an abrupt decrease in excitability, with total loss of responsiveness by about 30 minutes. Afterpotentials disappeared by about ten minutes.

Hypoxia in Aplysia giant neurons will induce the firing of spike potentials in response to a previously subliminal stimulus which had produced only graded EPSPs. This has been attributed, however, to the observed coincident depolarization of the postsynaptic resting potential (Chalazonitis, 1963). The possibility of a presynaptic mechanism does not appear to have been examined.

Gradual diminution of the membrane potential is an attractive mechanism to explain the increased excitability seen so often in the early phase of hypoxia. This occurs almost ubiquitously, as reported for Aplysia giant neurons (Chalazonitis, 1963; 1968), cat spinal cord motoneurons and interneurons (Kolmodin & Skoglund, 1959; Collewijn & Harreveld, 1966a; 1966b; Eccles, Loyning & Oshima, 1966; Niechaj & Harreveld, 1968), and in guinea pig cerebral cortical neurons (Li & McIlwain, 1957). It is difficult, however, to reconcile the idea of a reduction of the presynaptic resting potential with the increase in quantal release postulated by Eccles, Loyning & Oshima (1966) as the cause for augmentation by hypoxia of the EPSP.

IV. Hypercarbia as a factor in the effects of impaired cerebral blood flow:

In the intact cerebral cortex, inhalation of carbon dioxide in

low concentrations tends to produce solely depression; in moderate concentrations, excitation; while anesthesia is produced with high concentrations. The phase of excitation is demonstrable with electroshock thresholds in the rat and mouse (Woodbury & Karler, 1960), with strychnine spike frequency in the cat (Gellhorn & French, 1953), and with EEG analysis in the cat (Ivanov, 1963; Lavy & Carmon, 1969), dog (Ivanov, 1963), and human (Swanson, Stavney & Plum, 1958).

It is fairly clear that excitation is not due to a direct action of the gas upon the cortex, but, rather, arises at a subcortical site. This follows from the fact that it is prevented by partial as well as complete undercutting of the cortex (Gellhorn & French, 1953; Soderberg, 1964), and by interruption of reticulocortical pathways (Lavy & Carmon, 1969). In isolated cortex, carbon dioxide appears to exert a purely depressant action at all concentrations (Gellhorn & French, 1953; Ingvar, 1955; Maiti & Domino, 1964; Soderberg, 1964). Krnjevic, Randic & Siesjo (1965), however, reported that depression of neuronal unit activity evoked by micro-electrophoretically applied glutamic acid in the partially and completely undercut cortex is regularly preceded by a brief facilitation. These workers reported also that the phase of depression is accompanied by hyperpolarization of the transmembrane potential in six units from which successful intracellular recording was possible.

In Aplysia depilans visceral ganglion giant neurons, carbon dioxide is known to produce depolarization (Chalazonitis, 1963; Chalazonitis & Nahas, 1965), but a recent report of work done in a different species indicates that hyperpolarization occurs also. Brown & Berman (1970) and Walker and Brown (1970) found that some of the giant cells of

A. californica visceral ganglion were depolarized while others were hyperpolarized. These effects were attributed solely to changes of the extracellular pH since exposure to inorganic acids reproduced the effects, while the effect of carbon dioxide could be blocked by holding the extracellular pH constant by titration. These workers (Brown, Walker & Sutton, 1970; Walker & Brown, 1970) further concluded that the effects of extracellular pH upon transmembrane potentials are due to a selective increase of the membrane chloride conductance. Cells which were hyperpolarized were found to have an intracellular chloride activity which was less than the extracellular, while depolarized cells had a high chloride activity. Ion-selective microelectrodes were used to make these measurements. Thus, the changes of the membrane resting potential seen during exposure to carbon dioxide was elegantly explained on the unitary basis of the membrane potential approaching its equilibrium potential for chloride.

There is still considerable controversy as to whether the effects of carbon dioxide upon mammalian nervous tissue can be explained on the basis of altered extracellular pH. Swanson, Stavney & Plum (1958) examined the relationships between arterial pH, arterial carbon dioxide content, alveolar pCO_2, and EEG activity in the d-tubocurarine-succinyl choline paralyzed cat. They found that sustained metabolic acidosis (arterial pH less than 6.5) had no effect upon the EEG except during a transient elevation of the alveolar pCO_2.

Meyer, Gotoh & Tazaki (1961b)measured extracellular cortical pH, in addition to the arterial and cortical oxygen and carbon dioxide gas tensions in d-tubocurarine paralyzed cats and monkeys. In contrast to Swanson, Stavney and Plum (1958), they found that EEG "slowing" occurred

during metabolic acidosis when the pH was reduced below 6.6 - 6.9, despite a minimal concurrent increase of the pCO_2. Significantly, EEG slowing occurred during CO_2 inhalation only when the brain pH fell below 6.6 - 6.9.

The observation of Krnjevic, Randic & Siesjo (1965) that cerebral cortical neurons are hyperpolarized during inhalation of carbon dioxide cannot be explained, without question, on the basis of alteration of the transmembrane pH gradient. Carter, Rector, Campion & Seldin (1967), by using refined techniques with pH microelectrodes, have demonstrated a very rapidly responding Donnan equilibrium for hydrogen ions across the cell membrane of rat skeletal muscle. It is not unreasonable to suppose that a property as fundamental as this also exists in the neuronal cell membrane. Unfortunately, the findings of these workers are not in agreement with those of earlier workers (Kostyuk & Sorokina, 1960; Caldwell, 1968). Some of the many as yet unresolved problems regarding a Donnan equilibrium for the hydrogen ion have been recently reviewed (Waddell & Bates, 1969).

Wyke (1963), on the basis of an extensive review of earlier work, concluded that the effects of carbon dioxide upon mammalian and amphibian nervous tissue cannot be mimicked by changing the extracellular pH. These effects, therefore, have been attributed to the peculiar ability of carbon dioxide to cross freely into the cell interior where it can be hydrated and subsequently give rise to hydrogen ions, thus lowering the intracellular pH. It has been postulated that the intracellular accumulation of hydrogen ions results in a decreased sodium permeability (Krnjevic, Randic & Siesjo, 1965), possibly through the mobilization of intracellular calcium. The increased intracellular calcium activity is

thought to stabilize the membrane by closing the sodium channels (Woodbury & Karler, 1960).

It must be pointed out, however, that the Donnan equilibrium hypothesis for hydrogen ions contradicts the foregoing hypothesis. If in fact a Donnan equilibrium exists for hydrogen ions across the neuronal membrane, then selective lowering of the intracellular pH would be expected to produce depolarization of the transmembrane potential (Carter, Rector, Campion & Seldin, 1967).

It is of interest that in the spinal cord carbon dioxide depresses the monosynaptic reflex to a greater extent than it does the polysynaptic reflex (Esplin & Rosenstein, 1963). Inhalation anesthetics show a similar selectivity (De Jong, Robles, Corbin & Nace, 1968), but other depressants (pentobarbital and mephenesin, Esplin & Rosenstein, 1963; local anesthetics, Taverner, 1960; meprobamate and morphine, Domino, 1962) show selectivity for the polysynaptic reflex. Lidocaine differs from other local anesthetics in enhancing the monosynaptic reflex while depressing the polysynaptic reflex (De Jong, Robles & Corbin, 1969). None of these effects has been adequately studied to permit conclusions concerning the exact locus or mechanism of action. It is, however, noteworthy that carbon dioxide most resembles the inhalation general anesthetics with regard to the effect produced in the spinal cord reflexes.

It has been further established that the depression of the monosynaptic reflex by carbon dioxide does not involve either presynaptic or postsynaptic inhibition (Miyahara, Esplin & Zablocka, 1966; Weakly, Esplin & Zablocka, 1968).

Lehmann (1937a) found that peripheral nerve is stabilized by

carbon dioxide. The threshold for evoking a propagated spike potential is elevated, and the durations of the positive and negative afterpotentials are increased. The afterpotential changes probably indicate increased ion fluxes during the spike potential, since the positive afterpotential in desheathed rabbit cervical sympathetic trunk has been found to be due to active sodium extrusion (Ritchie & Straub, 1957). The positive afterpotential of squid giant axons has been found to reside in a selective increase of the membrane permeability to potassium (Frankenhaeuser & Hodgkin, 1956).

Carpenter (1963) found that the stabilizing actions of carbon dioxide upon rat sciatic nerve and respiratory center neurons could not be due to a pH effect upon ionization of extracellular calcium, since the effect was observed in nerve fibers labilized by complete removal of extracellular calcium by the addition of citrate.

An alternative to the calcium hypothesis for the stabilizing actions of carbon dioxide is provided by Sears and Eisenberg (1961), who found that CO_2 increases the surface tension of lipid. They concluded that carbon dioxide has a physiological role in determining the molecular arrangement of cell membranes, through the removal of hydrated cations.

Contrary to the idea that CO_2 exerts a direct action upon the cell membrane is the finding of Ellis (1969) that the changes in action potential amplitude and in conduction time produced in phrenic nerve by carbon dioxide correlated better with the pH of the bathing medium than with the P_{CO_2}.

V. <u>The surface-positive burst response (SPBR)</u>.

The SPBR was first described by Burns (1951). It was evoked in

neuronally isolated slabs of cerebral cortex which had been prepared in unanesthetized decerebrate (cerveau isolé, Bremer, 1935) cats. Burns found that the response could be initiated by the application of a single stimulus pulse with a duration in the order of 0.5 msec. Threshold voltage was about four volts, applied at the pial surface through bipolar electrodes which had 0.5 mm interpole spacing.

The response was all-or-none in that its amplitude did not increase when the stimulus intensity was increased above threshold. Spread of the response occurred throughout the isolated slab, without attenuation, at a velocity of 10-20 cm/sec. Duration of the response was about 2-4 seconds. The "bursting" consisted of oscillatory potentials superimposed upon the slow positive wave; these oscillations were said to have a frequency of 60-75/sec. The SPBR was rapidly abolished by anoxia, and by the induction of full general anesthesia by various agents. This was unlike the surface-negative response (SNR) which Burns observed invariably to precede the SPBR. The SNR was described as being very resistant to anoxia and general anesthesia (Burns, 1951). It is now generally agreed that the SNR which Burns described is identical with the negative-wave direct cortical response (nDCR, discussed below in the context of the origin of the SPBR).

The involvement of synaptic links in the transmission of the SPBR was inferred from its non-decremental spread, and, later, from the observation of neuronal spike discharges occurring throughout the isolated slab (Burns & Grafstein, 1952; Burns, Grafstein & Olszewski, 1957).

A related response had been studied earlier in the chloralose-anesthetized cat (Burns, 1949; 1950). This surface-positive response was

considerably abbreviated in duration (less than one second) compared to the response of unanesthetized cortex, and its oscillatory character was much less apparent. The velocity of transmission was similar to that in unanesthetized cortex, and the spread remained non-decremental. The threshold stimulus intensity was greater with light chloralose anesthesia than without a general anesthetic. The response did not occur until the precedent SNR was 60-70% of its maximal amplitude, whereas in the unanesthetized cortex, the SPBR was evoked with a stimulus which produced a SNR which was only 30-35% of maximal SNR amplitude.

The surface-positive response of unoperated intact cerebral cortex (Chang, 1951) has a duration of about 50 msec. Increasing the stimulus intensity leads to the appearance of a late negative wave close upon the positive wave. A further increase causes the late negative wave to increase in size and encroach upon the positive wave, until the latter is considerably reduced in duration (to ten msec at the highest stimulus intensity used by Chang). The positive component apparently never showed any oscillations in Chang's experiments. The second negative wave, which lasted more than 800 msec with strong stimulation, was interpreted as being due to reinvasion of the superficial cortical layers by the activity in the deep layers which produced the surface-positive wave. Other cortical layers were assumed to participate in the late negativity since this component of the response did not show reversal when recorded from a depth of 1.0 mm in the cortex.

From Burns's early study in the anesthetized cat (Burns, 1949; 1950), he concluded that the SPBR was related to the "deep" response studied fifteen years earlier by Adrian (1936) in the intact cortex of

the Dial-anesthetized cat. This response appeared only after a period of repetitive stimulation at a frequency in the order of 10/sec. The "deep" response, which followed soon after the "superficial" (surface-negative) response, consisted of a single positive wave having a duration of about 50 msec. It is likely that the true duration was longer, however, since Adrian was recording with bipolar electrodes. Transmission of this positive wave proceeded with a velocity of about 25 cm/sec (range 10-40 cm/sec). Spread was said to be non-decremental, but the extent of spread was found to increase with increasing the duration of stimulation, as did the response amplitude. (Such facilitation has not been reported to occur with the SPBR). The stimulus intensity required was 2-3 times the threshold intensity for the "superficial" cortical response.

The SPBR of isolated cortex was further characterized (Burns & Grafstein, 1952) as showing reversal of the steady potential component at a depth in the slab of about 0.6 mm, with peak negativity at 1-1.5 mm. The oscillatory component also was maximal at this depth. These findings were interpreted to indicate a region where neuronal structures were more extensively depolarized than at any other depth in the cortex. Cuts made from the surface of the cortex to a depth of 1.0 mm, and from the white matter up to 0.5-0.75 mm below the surface of the cortex, failed to block transmission of the response. It was therefore concluded that there is an essential pathway for transmission of the response which lies in the middle depths of the cortex, and involves synapses made on basilar dendrites and/or somata. These cells were named "type B" cells, to distinguish them from the "type A" cells which were proposed to mediate the SNR (Burns & Grafstein, 1952). Spike potentials which coincided with the

SPBR could be recorded from a small number of deep-lying structures during an examination of potential distributions through the cortex (Burns & Grafstein, 1952). They were regarded as a further basis for the "type B" cell hypothesis.

A more detailed study of unit activity coincident with the SPBR (Burns, Grafstein & Olszewski, 1957) revealed that participating units could be found at almost all depths in the cortex (between 0.2-1.3 mm). A hypothetical group of cells, postulated to be essential for lateral spread of the response, was given the name of "primary type B" cells while other cells which fired during the response were categorized as "secondary type B" cells. This classification arose mainly from the observation that the response could be obtained in chronically isolated cortex, despite the finding (ib.) of total degeneration of large pyramidal cells. Such degeneration would imply that these cells lack intracortical axonal ramifications. However, Weisman (1969) in a more extensive study of the histology of chronically isolated cortex, has demonstrated that many large pyramidal cells persist, even after as much as twelve months duration of isolation.

Burns, Grafstein & Olszewski (1957) further suggested that the largest neurons of the cortex are preferentially activated during the SPBR. This proposal was based on their observation that the spike discharges recorded during the SPBR were always larger in amplitude than injury discharges recorded by the same micro-electrode at the same location in the cortex, despite the likelihood that the electrode tip was much closer to the unit generating the injury discharge than to the unit which fired during the SPBR. These workers failed to acknowledge, however, that

an injury discharge by its very nature must be of lesser amplitude than the discharge of which the unit is normally capable, since the injury is most likely accompanied by a substantial reduction of the transmembrane steady potential. Moreover, it has never been established that a direct or even clear relationship exists between the amplitude of extracellularly recorded spike discharges and the size of the cell body of the neuron from which the spikes originate. It is likely only that the units whose activity they observed had large axons, since an overwhelming sampling bias for large-axoned neurons has been demonstrated in extracellular microelectrode recording from the cerebral cortex (Towe & Harding, 1970).

It thus appears that the available studies shed very little light on the nature of the neurons which are activated during the SPBR.

Burns & Grafstein (1952) drew the conclusion that the SPBR "must be a component of normal physiological activity in the unanesthetized animal since its magnitude and duration are clearly dependent upon local cortical conditions and not upon the nature of the stimulus used to start it." However, the present writer has been unable to find documented the evidence to which these workers refer, other than the fact that the SPBR is rapidly abolished by nitrogen-breathing and is sensitive to anesthetics.

Sanders and Gravlin (1968) have provided very convincing evidence in favor of the contention that the substrates of the SPBR are somehow related to the substrates of the normal functioning of the cerebral cortex. They found that both anti-grand mal and anti-petit mal agents in therapeutic doses were without effect upon the SPBR whereas the same doses suppressed the epileptiform afterdischarge (EAD) and the epileptiform sustaining response (ESR).

Burns (1950) proposed that some neuronal structure was involved in the genesis of both the SNR and the SPBR. This was suggested by his observation that the SPBR was always preceded by the SNR, and that the SPBR always followed the SNR whenever the latter exceeded a certain proportion of its maximum amplitude. The fact that two carefully spaced subthreshold stimuli could summate temporally to evoke an SPBR indicated to him the presence of synaptic links between the two responses. Adrian (1935), however, had earlier rejected a causal relation between the superficial response and the deep response of intact cortex. He argued that the greater stimulus intensity necessary to evoke the deep response indicated a direct activation of the deeper layers of the cortex.

Burns & Grafstein (1952), observing a deep positivity in association with the SNR, concluded that the response was mediated by superficial tangential branches of neuronal processes which pass radially into the depths of the cortex (which they tentatively termed "axons"). The excitation of these processes was not discussed, but it may plausibly be inferred that the investigators felt that such excitation is involved in the generation of the SPBR.

Eccles (1951), drawing a parallel with the synaptic potential of spinal motoneurons, postulated that the SNR is the post-synaptic potential of radially oriented neurons, activated by tangential presynaptic fibers of other neurons. Burns & Grafstein (1952) rejected his view, on the ground that the ability to evoke the SNR by a stimulus applied in the depth of the cortex indicated volume conduction along radial portions of the superficial processes. This observation, however, does not exclude the possibility of synaptic excitation of superficial

neuronal processes, particularly since the dendritic tree of the pyramidal cell is known to be a major receptive field of impulses delivered both from within and from without the cortex (Scheibel & Scheibel, 1970).

Burns (1958) suggested also that the persistence of the SNR during anoxia is evidence against its being a synaptic potential, on the unstated assumption that synaptic transmission is exquisitely vulnerable to abolition by anoxia. However, Eccles, Loyning & Oshima (1966) found that a ten-minute period of inhalation of 5% oxygen actually enhanced the monosynaptic EPSP in the cat spinal cord, as did a period of inhalation of pure nitrogen in excess of two minutes. Burns (1951) did not state the duration of nitrogen-breathing in his experiments, but it could not have been much longer than this, since the animal recovered when given air to breathe.

Support for Eccles's view (1951) is provided by the nDCR studies of Ochs. Block of transmission of the response by tetrodotoxin application between the stimulating and recording electrodes indicated that conduction of the activation is not passive (electrotonic) but active (Ochs & Clark, 1968a). Frank & Pinsky (1966) made the same finding for the transmission of the SNR (which is identical to the nDCR save that it was first described in isolated cortex). The involvement of axons in the transmission of the nDCR was indicated by the ineffectiveness of γ-aminobutyric acid (GABA) applied between the stimulating and recording electrodes to block conduction of the response (Ochs & Suzuki, 1965). GABA is known not to affect transmission along axonal fibers (Curtis, Phillis & Watkins, 1959), so that the ability of GABA to block the response when applied in the vicinity of the recording electrode indicates that generation of the

response must almost certainly occur in dendrites (Ochs & Suzuki, 1965). The failure to detect a spike potential from the axons postulated to conduct the excitation is explained on the basis of 1) their being very fine, with a short space constant; 2) the very short duration of the action potential; and 3) the minute current created by the very fine fibers. In contrast, EPSPs would be easily detected because of their long time course and their synchronous occurrence in numerous processes. The conclusion was made by Ochs and Suzuki (1965) that axons are excited initially and that the excitation is transferred synaptically to dendrites, in which the EPSP is detected by surface recording electrodes as the nDCR. The subsequent failure (Ochs & Clark, 1968b) to demonstrate spatial summation (by closely spaced stimuli at two sites) introduced a doubt as to the validity of this model, but a more recent study (Phillis & Ochs, 1971) suggests that the occlusive interaction of such paired stimuli may be due to long-lasting refractoriness or to some form of inhibition, rather than to non-synaptic transmission. Interestingly, spatial summation has been clearly demonstrated for the SNR in neuronally isolated cortex (Frank & Pinsky, 1964).

That the dendritic post-synaptic potential can be transmitted into the cortex was shown by the unmasking of positivity after the application of tetrodotoxin to the surface of the cortex in the vicinity of the recording electrodes (Ochs & Clark, 1968a), and after the induction of long-lasting depression in the superficial responding fibers by a prior strong stimulus (Ochs & Clark, 1968b). The discovery of negative waves in the depth of the cortex during this reversal of the response also indicated invasion of the deeper layers. Furthermore, unit dis-

charge in the depth of the cortex which coincides with the nDCR has been demonstrated by several investigators (Li & Chou, 1962; Stohr, Goldring & O'Leary, 1963; Phillis & Ochs, 1971).

From the foregoing, it appears likely that activation of the deep neuronal structures which generate the SPBR, is causally linked to the generation of the SNR. The necessary involvement of synapses in the activation of the deep-lying structures is by no means a certainty, however, since direct activation of cortical neurons by surface stimulation, as indicated by the very brief delay from stimulus to spike discharge, has been well documented (Phillips, 1956b; Li & Chou, 1962; Rosenthal, Waller, & Amassian, 1967). Also, Phillis & Ochs (1971) observed spike discharges in the depth of the cortex which preceded the nDCR.

Less is known about the mechanisms responsible for sustaining the activity of the SPBR for several seconds, or for its termination, which is usually so abrupt as to confer the appearance of a rectangular wave to the recording of the response. As has already been mentioned, general anesthesia greatly abbreviates the response duration (Burns, 1950). Both duration and amplitude are reduced by all of the central depressants studied to date (Frank & Jhamandas, 1970), while lowering of the temperature appears to permit an increased duration (Harwood & Sanders, 1969). Burns (1951) visualized a process of reexcitation brought about by the interaction between a primarily excited ring of neurons interconnected by dendrites, and the neurons located on the periphery of the ring. Thus, the excitation spreads both circumferentially and radially from the stimulus site. He thought that the termination of the response was due to progressive fatigue in the participating elements.

In the light of more recent evidence, it is now clear that inhibition is an integral part of neuronal activity in the cerebral cortex (Phillips, 1956a; Li & Chou, 1962; Stefanis & Jasper, 1964; Krnjevic, Randic & Straughan, 1966a; Rosenthal, Waller & Amassian, 1967). It follows from this that termination of the SPBR is, in all probability, due to a change in the balance between excitatory and inhibitory synaptic activity, although the finding by Phillis & Ochs (1971) of long-term refractoriness in certain cortical neurons leaves open the question of "fatigue".

Production of recurrent surface positive bursts ("afterbursts", Burns, 1954) by a period of repetitive stimulation is more difficult to understand. Burns proposed that the recurrent activity is due to different rates of recovery at the superficial and deep ends of the neurons, but the only evidence he could muster in support of this idea was in the form of a model (Burns, 1955).

The nature of the SPBR is further discussed in a later section of the present thesis (Discussion).

EXPERIMENTAL METHODS

I. Surgical preparation

Cats of either sex weighing 1.9-3.5 kg were fasted overnight. Anesthesia was induced with ether, as follows. The animal was placed inside the lower compartment of an air-tight wooden box. The door of this compartment was fitted with a glass window which permitted observation. Diethyl ether (Ether, Squibb) was introduced by pouring approximately 30 ml onto a cotton-wool pad in the upper compartment of the box, to evaporate into the lower compartment through a wire screen. When the cat appeared to be unconscious, a cone consisting of cloth stitched over a wire frame, containing an ether-soaked pad of cotton-wool, was placed over the animal's face. Failure of the animal to react to inhalation of the increased concentration of ether from the cone was taken as evidence of surgical anesthesia (Cohen & Dripps, 1970).

When this had been achieved, the cat was removed from the box with the cone still over its face. It was then secured, supine, to an operating table by pieces of twine tied to its legs. Areas of skin to be incised were shaved with a small animal clipper (Oster, model A2, size 40 blades). The trachea was exposed by a midline incision in the neck from the glottis to the manubrium sterni, and a conventional brass T-cannula was inserted one cm below the glottis. A 30 cm length of rubber tubing provided connection to a variable-bypass ether bottle. In order to reduce the effects of the respiratory dead space of the rubber tubing a small hole was kept open in the cannula, providing access to room air. The neck wound was closed with a suture. The depth of anesthesia was carefully adjusted and monitored so as to maintain a satisfactory pattern of respiration, since shallow respirations would result in alveolar hypoxia,

while hyperventilation would produce cerebral hypoxia.

In those experiments where it was desired to investigate the effects of brief carotid artery occlusion upon the surface positive burst response threshold, the common carotid arteries were bilaterally mobilized and threads were passed behind them to provide a means of traction. Traction was not applied at any time during surgery.

In experiments where stimulation of the right vagus nerve was to be carried out in order to arrest the heart, this nerve was mobilized in the neck and carefully separated from the adherent sympathetic nerve trunk. An electrode was secured to the nerve as described below in Methods: Stimulation.

The left femoral artery and vein were next exposed. A serrefine was placed on the artery at its most proximal portion, and cannulae made of Teflon tubing (0.038" I.D. x 0.070" O.D.), fitted with luer-lok adaptors and stop-cocks, and filled with 0.9% sodium chloride solution, were inserted into these vessels and secured by threads tied tightly around them. The arterial cannula contained heparin sodium (Connaught) 10 I.U./ml and was used for recording the systemic arterial blood pressure. The venous cannula was used for infusion and injection of solutions. The serrefine was left in place until the arterial cannula was connected to the pressure gauge. A temporary suture was used to close the wound.

The cat was then turned over and the head was secured, by means of a Czermak holder mounted on a stand attached to the operating table. The scalp was shaved and opened by a midline sagittal incision, and the edges retracted by means of retractors consisting of a spring-loaded clip, a 60 cm length of twine, and a lead weight. The muscles taking

origin from the dorsal median sagitall crest and the lambdoid ridges, and inserting into the external ear and the skin (levator auris longus, intermedius scutulorum) were cut. Care was taken to avoid the large vessels located about the origin of these muscles behind the ear. The left temporalis muscle was mobilized from its origin on the calvarium by means of a bone scraper. Prior to transecting the belly of the muscle to remove most of it, a stout thread was tied around it near the zygomatic arch after being secured to it by means of a curved needle inserted tangentially through the sheath on its superficial aspect, and similarly through the deep surface of the belly of the muscle. This ensured that no bleeding occur from the cut surface at any time after cutting it.

Following removal of this muscle the left parietal plate was cut away, by means of a compact hand-held high-speed drill (Dremel Moto-Tool, model 260 series 55, 30,000 rpm), fitted with a dental burr (Jota, type 9, size 8). To ensure against electrocution by inadvertent wetting of the interior of the drill (which never happened), the power line of the drill was isolated from ground by means of a 100 watt line-isolation transformer (Hammond, type 200881).

A drip of 0.9% saline solution at room temperature was directed at the area where the cut was being made. Although it was found easier to visualize the dura mater during craniectomy when the bone was kept dry, it was necessary to wet the bone for several reasons. First, air embolism has been demonstrated to occur through surgically interrupted diploic sinuses. Second, in a few cats prepared without wetting of the bone, a line of coagulated brain tissue was seen along parts of the path

of the dental burr. It was feared that spreading cortical depression (Leao, 1944; Ochs, 1962) might originate from such regions of injury and thereby compromise the usefulness of an isolated slab prepared in adjacent cortex. Hence the saline drip was always maintained for cooling.

The craniectomy was made along a path about 3 mm lateral of the sagittal suture, 3 mm anterior of the coronal suture, thence down to a point about 3 mm from the zygomatic arch, and back up toward the sagittal suture along a line about 1 cm from the lambdoid ridge.

Bone wax (3 parts hard paraffin, with 1 part yellow soft paraffin and 0.02 parts phenol) was used to control bleeding from the diploë, and to prevent air embolism. After removal of the parietal plate, bleeding from torn veins in the dura mater was controlled by applying a small piece of absorbent paper, about which a blood clot usually rapidly formed. A hole was made in the dura near the edge of the craniectomy by means of a small curved needle, and the dura was cut away with blunt-tip scissors. Care was taken to observe the presence of Rolandic veins so that they could be sealed by means of a radio frequency electrocautery (Birtcher Hyfrecator, model X-712) and then cut to prevent tearing of the intact pial portion of the veins during removal of the dura.

After removal of the dura the surface of the cerebral hemisphere was intermittently moistened with warm saline solution.

Decerebration:

Prior to insertion of the decerebrating knife, the lateral border of the temporal lobe was gently lifted by means of a broad stainless steel spatula and the underlying space was examined for the presence of Rolandic veins. These were sealed and cut whenever possible. If

bleeding prevented their being sealed, the subdural space beneath the temporal lobe was packed with absorbent paper after completion of decerebration to reduce the risk of a hematoma forming around the brainstem.

Decerebration was performed by inserting a blunt knife (Pinsky, 1957) beneath the temporal lobe, along the surface of the dura covering the tentorium cerebelli, and through the midbrain at the plane of the intracranial border of the foramen magnum, above the level of the corpora quadrigemina. (Insertion behind the tentorium produces a cut through the superior colliculi (Brown & Pinsky, 1970). See Fig. 1.) The knife was gently pressed against the nearby borders of the occiput and tentorium, to ensure transection of any portions of the disrupted midbrain pushed aside during the initial pass through it. The knife was then removed and the undersurface of the brain was liberally irrigated with warm saline in order that a minimum of blood remain to clot and press against the brainstem and cerebral hemispheres.

Bleeding from the decerebration would usually stop spontaneously, but if necessary the region was packed with absorbent paper. The ether bottle was disconnected from the tracheal cannula at this time. The adequacy of the decerebration was assessed some time after removal of the ether on the basis of: 1) absence of voluntary movements, either spontaneous or after manipulation of limbs; and 2) failure to respond to pin-pricks applied to the skin. If deafferentation proved to be inadequate, the decerebration procedure was repeated. Widely dilated pupils and postural rigidity, the hallmarks of midbrain transection, were not always present, presumably as a consequence of the high level of the cut and the resulting intact condition of the Edinger-Westphal nucleus and

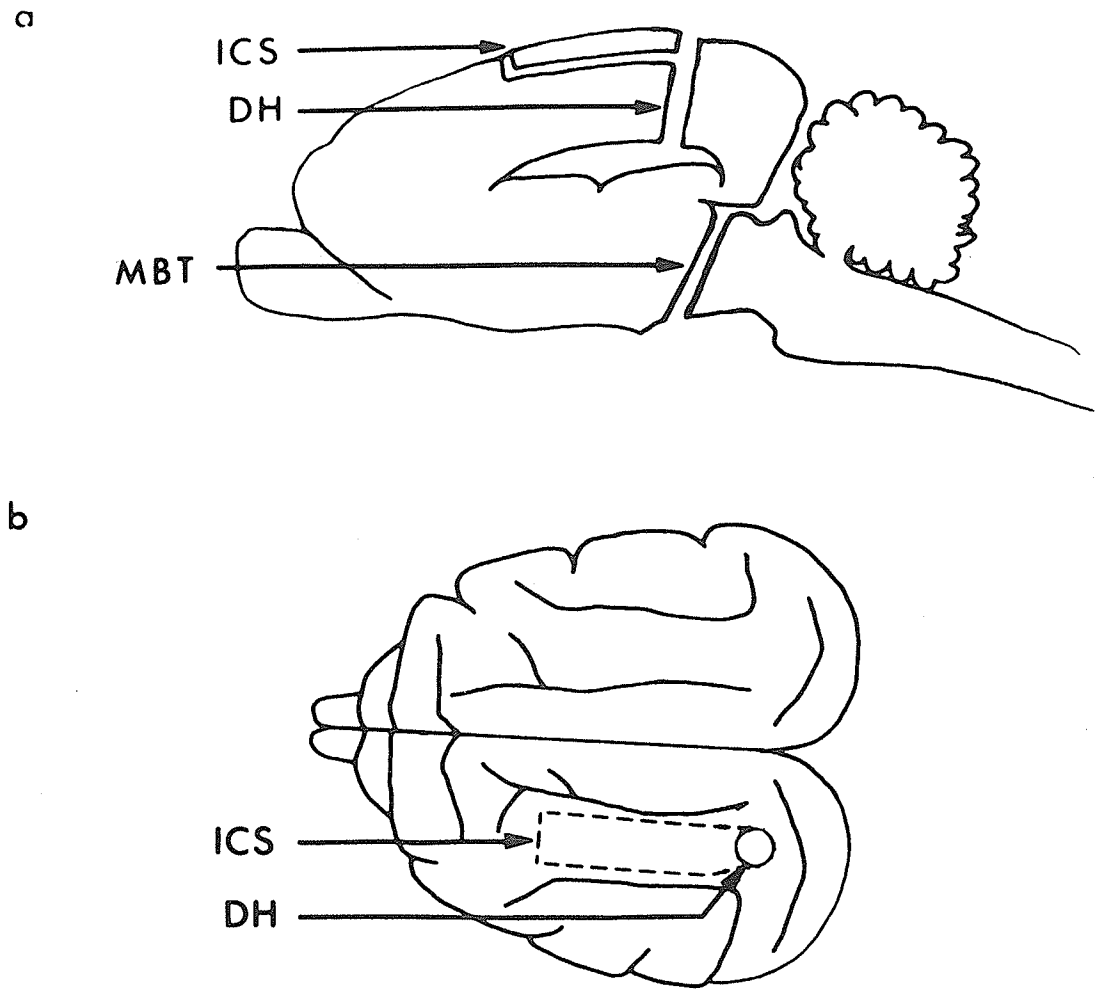


Fig. 1. Brain of cat.

a) sagittal section; b) dorsal surface. DH, drainage hole.

ICS, isolated cortical slab in the suprasylvian gyrus.

MBT, midbrain transection.

its efferents. The forelimbs were rigid much more often than the hindlimbs. Rate and depth of respiration, as measured by the air temperature in the tracheal cannula (See Methods: Recording) varied slightly from cat to cat, but were quite constant and regular in the great majority of cats.

Since the cat had been deprived of hypothalamic thermoregulation by transection of the midbrain, two 40 watt light bulbs mounted beneath the stainless steel top of the operating table, were usually turned on just after decerebration.

Neuronal Isolation of Cerebral Cortex:

Attention was next directed to the exposed left suprasylvian gyrus (Fig. 1). A portion of this gyrus in the region bordering the posterior composite gyrus was rendered avascular by means of the electrocautery, then removed by suction to create a channel to the lateral cerebral ventricle. The continuity of this channel was verified by directing a jet of warm saline at its opening. This resulted in the surface of the entire hemisphere rising if the saline was able to enter the ventricle. This channel permitted removal by suction of blood and blood clots whenever these had resulted from bleeding into the ventricular system at the time of decerebration; provided a route for escape of cerebrospinal fluid secreted into the ventricle, which could no longer be drained through the aqueduct of Sylvius; and provided a route for inserting the slab-cutting instruments beneath the suprasylvian gyrus.

The slab was prepared by first making an undercut of the cortex with a blade fashioned either from a razor blade (0.011 inch thick) or an automotive feeler gauge (0.009 inch thick), measuring 4 x 25 mm, and brazed to the end of a brass handle. This was inserted tangentially 3-4 mm below

the pial surface and pushed toward the rostral end of the gyrus. The side-cuts of the slab were made with a blunt-tipped stainless steel wire bent at the tip to give a 4 mm cutting edge, and mounted in a handle fashioned from an acrylic plastic rod. Clear visualization of the tip of the wire through the pia mater ensured that all layers of the cortex, including the uppermost, were cut. The pial vasculature along the side cuts was essentially undisturbed, as was evidenced by the identical appearance of the vessels overlying the isolated slab, to that of the vessels of adjacent intact cortex, viewed through a dissecting microscope.

Slabs prepared in this manner measured 3-4 mm in width and 15-25 mm in length.

After preparation of the slab the surface of the hemisphere was kept moist by a thin covering of surgical gauze soaked with saline.

The scalp retractors were then shifted to expose the right side of the calvarium; the right hemisphere was exposed, and a slab of isolated cortex was prepared in the right suprasylvian gyrus in a manner essentially identical to that outlined above for the left side.

Finally, threads were inserted by means of a curved needle along the edges of the incised skin, to be tied later to an iron ring mounted above the cat's head. This permitted covering the surface of the brain with paraffin oil, as detailed below in Methods: Recording. Until transfer of the animal to the recording table, the edges of the scalp were held together by a clamp or alligator clip, in order that no dehydration of the brain occur.

As a rule, a minimum of two hours elapsed after termination of the ether anesthesia until commencing recording.

II. Recording

After surgery had been completed the animal was transferred to the recording table. The head was secured by the Czermak holder in the experiments where unit activity was not recorded, and by a stereotaxic frame (Narashige Co.) in experiments where it was. In all experiments the scalp flaps were secured by threads to an ion ring mounted just above the animal's head, so that the flaps formed a pool. This was filled with paraffin oil (Mineral Oil U.S.P., Fisher), to prevent drying of the brain and the silk wick electrode, to serve as an electrical insulator, and to buffer the brain against temperature changes.

A vinyl-sheathed thermistor probe (Yellow Spring Instruments, model 402) inserted into the oil pool was connected to a temperature controller (YSI, model 63) which regulated a heating pad placed beneath the animal. This pad was enclosed in an electrically grounded stainless steel case, to minimize interference and to eliminate the danger of electrocuting the animal through wetting of the pad. The controller was adjusted to maintain the oil pool at a temperature of about 33°C, as indicated by a mercury thermometer placed in the pool. This temperature was found to correspond to a rectal temperature of 37°C.

In the experiments where neuronal unit recording was done, a rectal thermistor probe, coupled to a solid state thermal controller (Krnjevic & Mitchell, 1961), was used to maintain the body temperature constant at 37°C, as indicated by a second rectal probe (YSI, model 401) coupled to an electrical thermometer (YSI Telethermometer, model 43).

To minimize pick-up of stray radio frequency signals, recording was done in a shielded enclosure. In most experiments this consisted of

a cage built onto the recording table. The table top, covered by stainless steel sheet, formed the bottom of the cage, while removable panels made of bronze screen stretched over a wooden frame formed the walls and top. Two layers of screen separated by a 3/4 inch space were used in order to prevent focusing of high-frequency radiation by the screen. All components of the cage were connected to the electrical ground of the recording instruments. Access to the preparation was provided by opening one side of the cage.

In the experiments where unit activity was recorded, shielding was provided by a cubicle measuring approximately 8 x 6 x 7 feet. Galvanized sheet steel provided shielding beneath the floor covering and over the surface of the door, while bronze screen covered the walls and ceiling. Three 19-inch instrument racks were fitted into one wall of the cubicle.

The details pertaining to the various variables recorded are presented in the following sections.

(i) Blood pressure:

Systemic arterial blood pressure was recorded from a femoral artery through a 20 cm-long cannula connected by a luer-lock adaptor and stop-cock to a pressure gauge (Statham, model P23). Teflon was used for the cannula, for its ability to support tightly tied threads without collapsing, and for the facility with which it could be removed at the end of the experiment. The cannula and the acrylic plastic dome of the gauge were filled with 0.9% saline solution containing 10 I.U./ml heparin sodium (Connaught). If clotting occurred in the cannula, the cannula was flushed from a syringe containing the same solution, via the stop-cock.

The output of the gauge was amplified by a preamplifier (Grass, model 5P1) and recorded on paper by a penwriter (Grass, model 5).

(ii) Respiration:

The respiratory movements of the animal were monitored by a temperature-sensitive device mounted in the trachael cannula. This consisted of a glass-coated thermistor bead (Victory Engineering, part no. 41A7) mounted in a brass tube which fitted snugly in the side-arm of the T-cannula. Electrical connection was made to a Wheatstone bridge circuit contained in a compact metal box mounted on one side of a preamplifier of the penwriter. The thermistor formed one arm of the bridge circuit, so that changes in the electrical resistance of the thermistor (brought about by changes in its temperature) created an imbalance of potential across the two sides of the bridge. This potential difference was amplified and recorded by the penwriter, so that comparisons of the animal's respiratory rate and approximate depth could be made from moment to moment.

(iii) Electrocorticogram (ECoG)

Input from the surface of the neuronally isolated slab of cerebral cortex for the DC-recording of potentials was provided by a silk-wick electrode. This consisted of a glass tube (3/64 - 1/16" I.D. x 3/32 - 18" O.D.) bent one inch from the end and tapered to provide an opening at the tip of about one mm, through which a silk thread was passed so as to protrude about three mm from the end. The glass tube was filled with a solution of 0.9% sodium chloride and about 1% agar-agar (Fisher), the latter to prevent the salt solution and silk wick from escaping out of the end. A chlorided silver wire formed a non-polarizable lead that could be inserted into the other end of the electrode. The silk-wick

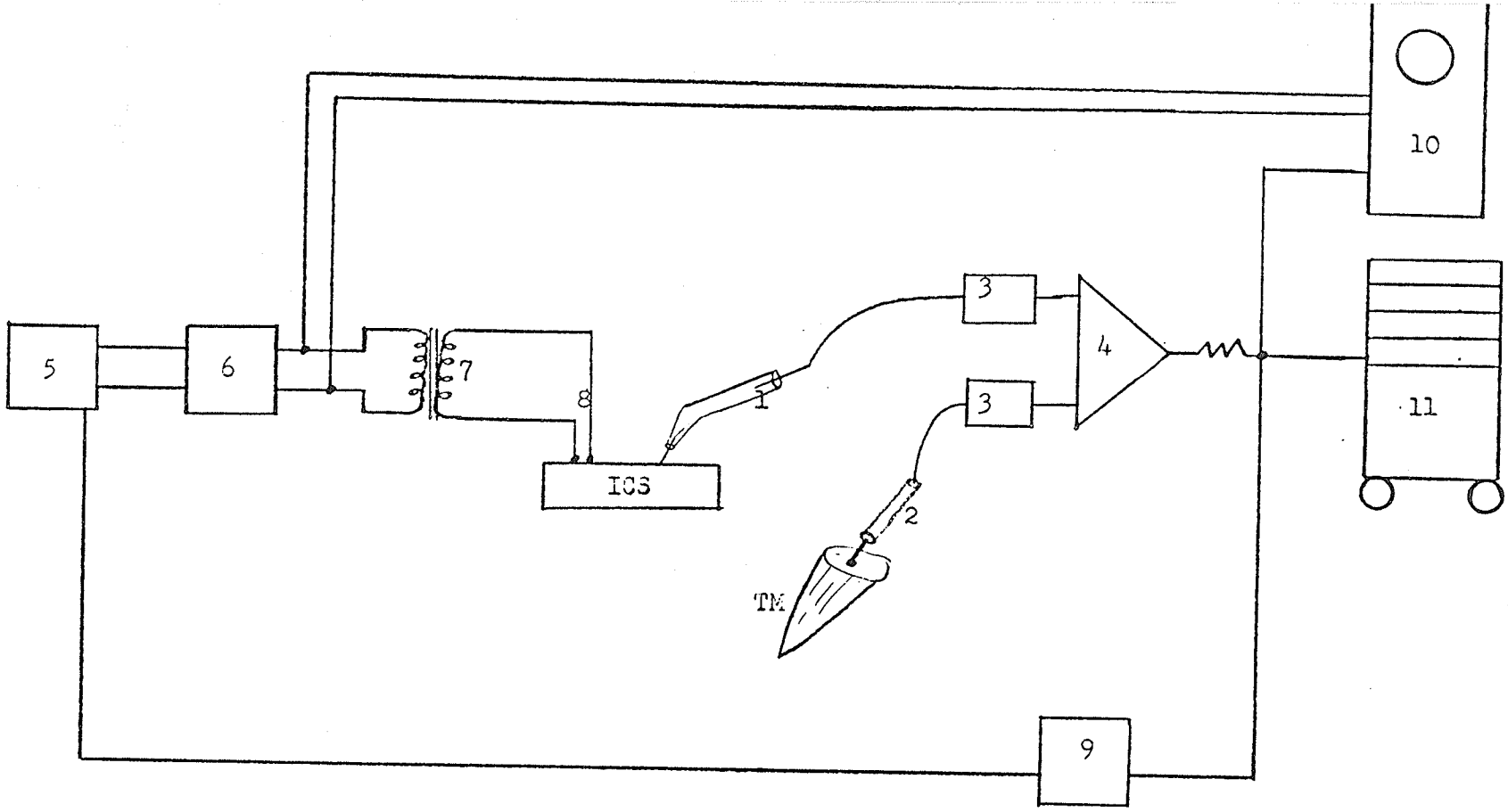
electrode may be thought of as a salt bridge between the cortical surface and the chlorided silver lead.

This lead was connected to one input terminal of a differential cathode-follower-input DC preamplifier (Grass, model P6-12) (see Fig. 2). The other terminal was connected to a chlorided silver electrode placed on the cut dead end of a temporalis muscle. The preamplifier output was displayed on one beam of a dual beam cathode-ray oscilloscope (Tektronix, model 502), and recorded simultaneously on paper by the penwriter.

In the experiments where neuronal unit activity was recorded, the output was also displayed on the lower beam of a second dual-beam oscilloscope (Tektronix, model 565), which permitted photographic recording of the surface positive burst response (SPBR) and spike potentials of neuronal units, as they occurred in relation to one another (see Fig. 4).

(iv) Cerebral cortical blood flow (CBF)

At the outset it was intended that a technique be developed for continuous measurement of CBF in small tissue volumes (in the order of 1 mm^3) which would indicate: a) the true flow at any given point in time; b) the magnitude and time course of experimentally imposed changes; and c) the degree of recovery following termination of the maneuver by which the CBF was altered. Upon reviewing the available methods, it became apparent that a continuous recording could be obtained only by monitoring of thermal conductivity. Calibration was to have been made by means of either a wash-out technique (Cameron, 1970), or a stop-flow technique (Stow & Schieve, 1959). However during development



ICS - Isolated Cortical Slab
TM - Temporalis Muscle (cut)

1. silk-wick electrode
2. chlorided silver electrode
3. cathode follower probe
4. DC-preamplifier
5. stimulator
6. stimulus amplitude control
7. isolation transformer
8. beaded platinum electrodes
9. stimulator (synchronized with (5))
10. cathode-ray oscilloscope
11. penwriter

Fig. 2. Arrangement of instruments for stimulating and recording at the surface of the slab.

of the method, it became apparent that the many problems to be solved could not be given priority equal to that of other aspects of the study, and therefore the instrumentation designed to satisfy the three objectives outlined above has been used only for semi-quantitative monitoring of regional CBF in a small portion of the cortical slab, in terms of thermal clearance (Grahn, Paul, & Wessel, 1968). The details of the method are provided below.

Two glass-coated thermistor beads (Victory Engineering, part no. 32A129) were mounted 4 mm apart in a small acetate platform secured to the pial surface of the cortical slab (See p. 47 for details of this platform). This arrangement effectively ensured that the electrode assembly follow movements of the cortex brought about by circulatory pulsations and by respiration.

The electrical current flowing through each thermistor was maintained constant in order that the potential drop across each thermistor be a measure of its resistance. This was achieved by using two separate independent collector-follower transistor circuits (See Fig. 3) adjusted so that one thermistor (called the "heated" thermistor) was heated to one Centigrade degree above the ambient temperature, with the other thermistor (called the "unheated" thermistor), heated only to 0.05 Centigrade degrees above ambient.

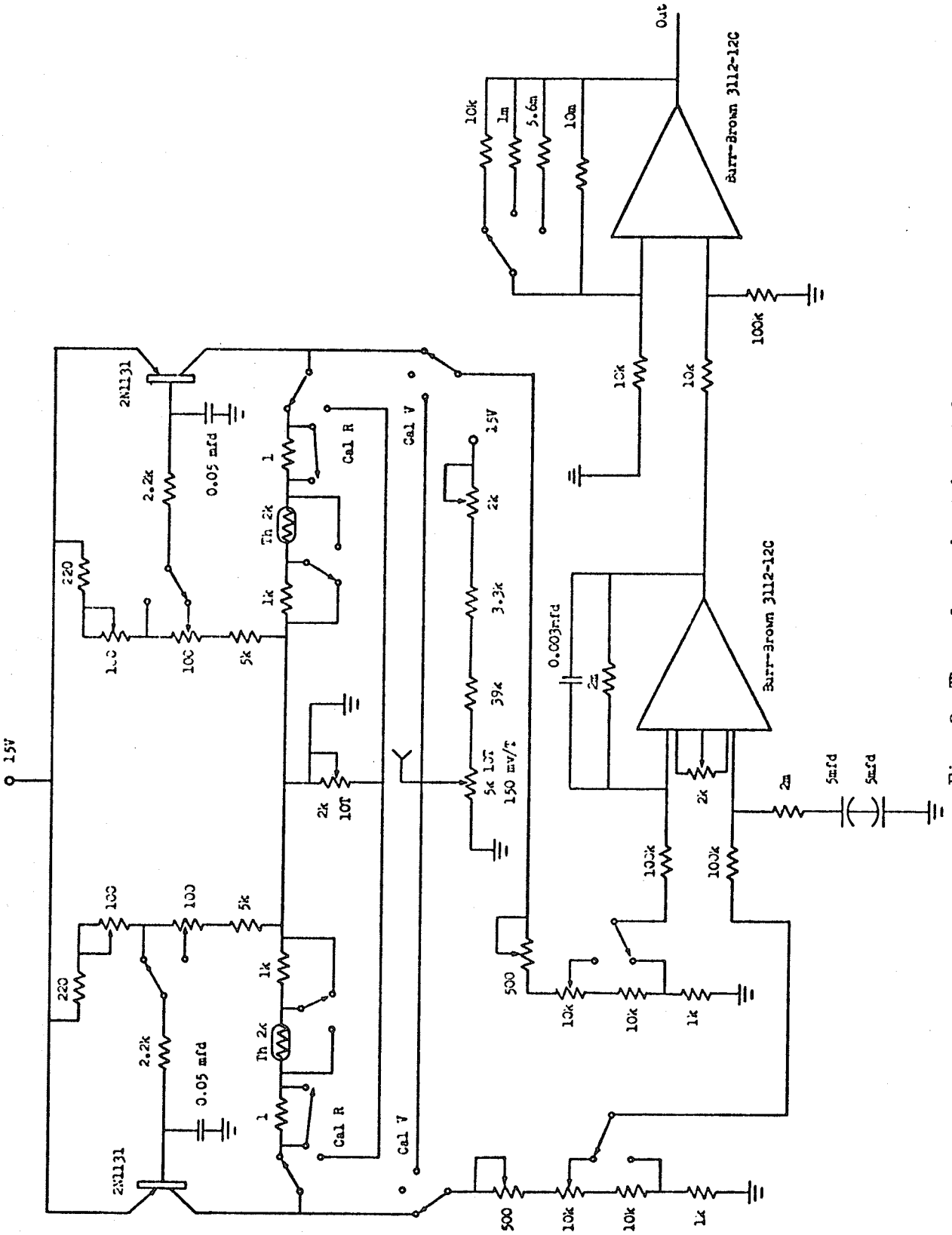


Fig. 3. Thermal cerebral blood flow recorder.

The appropriate currents were determined by solving the formula $T-T_A = EI/\delta$ (James, Paul & Wessel, 1965).

Since the electrical resistance of a thermistor is determined by its temperature, the resistance of the two thermistors would differ because of the unequal degree of heating. When placed in a flowing medium, or in contact with a tissue through which blood is flowing, the temperature (and therefore the resistance) of the heated thermistor would tend to approach that of the unheated thermistor, so that the difference in potential drop across the two thermistors would be determined by the blood flow.

The use of two thermistors ensured that changes of the temperature of the tissue not appear as flow changes, since for any change of the ambient temperature the resistance of the two thermistors would change equally, and the potential difference between them would not alter.

The potential across each thermistor was applied to an input of a differential amplifier (see Fig. 3) which subtracted the potentials and amplified the difference. The output was recorded by the penwriter.

(v) Neuronal unit activity:

In experiments where extracellular microelectrode recordings were made from the isolated slab, the animal's head was mounted in a stereotaxic frame (Narashige Co.). An acrylic plastic pressor of the type first described by Phillips (1956), mounted in a micromanipulator, was gently applied to the surface of the slab at the site where the micropipette was to be inserted. The pressor effectively eliminated movement of the underlying cortex without compressing it. Since it was transparent, the pial vessels could be visualized through a dissecting

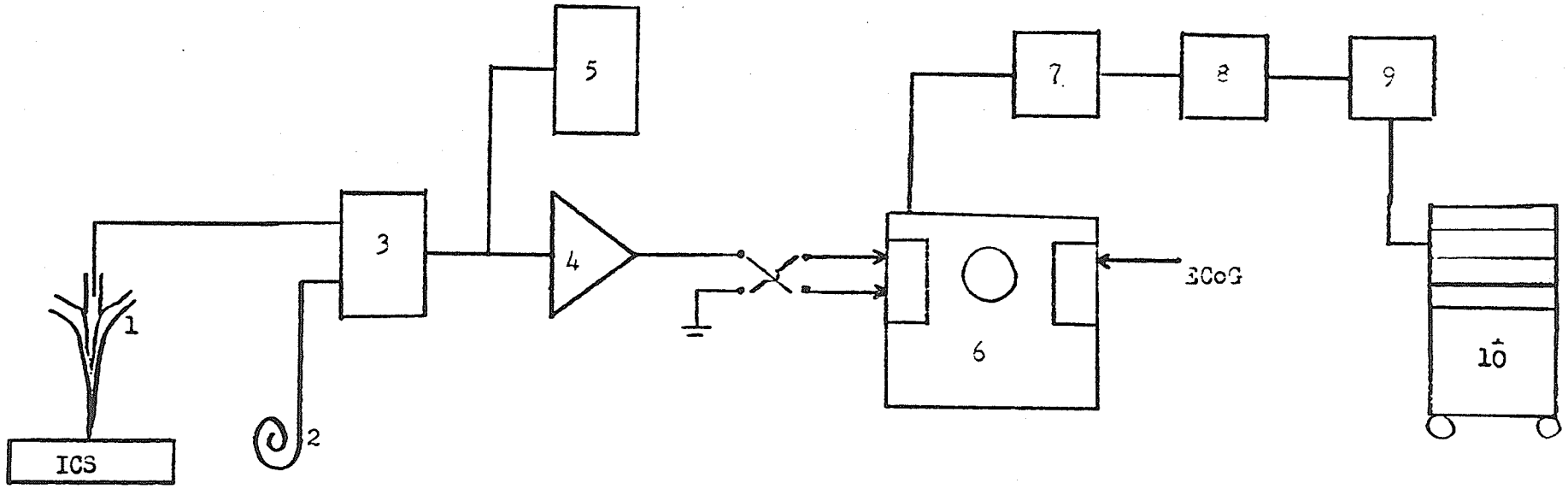
microscope during and after placement of the pressor to ensure that they were not compressed. A one mm hole drilled through the pressor provided access to the cortex for the micropipette. Two beaded platinum stimulating electrodes 0.5 mm in diameter were mounted one mm apart in the pressor, so as to protrude a small fraction of a mm below its undersurface.

As detailed below, spike potentials detected by the micropipette were amplified, monitored auditorily and visually, and counted electronically after being examined by a gating device; the output of the counter was recorded by the penwriter. See Fig. 4.

Five-barrel micropipettes were used. A detailed description of the technique of preparing these electrodes is available (Curtis, 1964). In brief, five hard glass tubes fused together were heated and drawn to a fine tip by a heavy-duty electrode puller of the type described by Winsbury (1956) and manufactured by Narashige Co. The barrels were filled by injecting solutions into them through a fine pliable tube, then spinning the electrode in a centrifuge to force the solutions to the tips of the barrels. The electrodes were stored at 4°C. with the tips immersed in Ringer solution.

The central barrel was filled with 2.7-3 M sodium chloride solution for extracellular recording. Overall tip diameter was 6-12 μm. The resistance of the central barrel was in the range of 2-10 megohms.

The micropipette was mounted over the cortex in the electrode holder of a stepping hydraulic micro-drive (David Kopf Instruments, model 1207B), which permitted movement of the micropipette in one μm calibrated steps. Because of dimpling of the pia mater at the time of



ICS - Isolated Cortical Slab

1. 5-barrel micropipette electrode
2. silver wire indifferent electrode
3. cathode follower electrometer
4. CR-coupled preamplifier
5. audio monitor
6. cathode-ray oscilloscope
7. gated trigger
8. electronic counter
9. digital-to-analog converter
10. penwriter

Fig. 4. Arrangement of instruments for recording of single neuronal unit activity.

insertion, the depths indicated by the micro-drive probably underestimated the true depth by about 100 μm.

A bare stainless steel wire lead inserted into the central barrel provided electrical connection to a cathode follower probe (WPI, model M4A Electrometer), whose output was amplified by a CR-coupled preamplifier (Tektronix, model 122). The indifferent lead of the cathode follower was a spiral of silver wire inserted under the skin at the incision overlying the femoral vessels.

The output of the cathode follower was applied also to the input of an audio monitor (Grass, model AM6).

The output of the preamplifier was applied through a switch which permitted polarity reversal, to one channel of a dual-beam oscilloscope (Tektronix, model 565; input module 3A9). In experiments where the latency to onset of SPBR-evoked unit activity was examined, the sweep of this oscilloscope was triggered by the stimulator synch output. A camera (Grass, model C4N) was used to photograph the image on the face of the oscilloscope tube. The output of the oscilloscope vertical amplifier (type 3A9) was brought out to a gated trigger circuit so that the voltage level of the input to the gate could be controlled by the position control of the vertical amplifier. The output of the gate circuit was led to an electronic digital counter (Hewlett Packard, model 5214L), preset to count for 0.5 second intervals, which automatically reset to zero at the beginning of each interval. The output of the instrument was converted to analog potentials of 0.5 second duration whose amplitude was proportional to the count made during the previous half-second. These potentials were recorded by the penwriter.

III. Stimulation

(i) Evoking the SPBR:

To evoke the SPBR, two beaded platinum electrodes, 0.5 mm in diameter and spaced one mm apart, were placed on the pial surface of the isolated cortical slab, toward its rostral end. In the experiments where thermistors were also applied to the cortical surface, the platinum electrodes were mounted together with the thermistors in an acetate platform measuring 8 mm square. In order that this assembly follow the movements of the surface of the cortex produced by respiration, it was secured by four stainless steel pins inserted into the cortex bordering the slab. See Fig. 5. Silicon rubber was cemented around the base of these pins to provide spacing so as to prevent the thermistors and platinum electrodes from compressing the cortex.

In the iontophoresis experiments, the platinum electrodes were mounted in the acrylic plastic pressor that was applied to the surface of the slab.

Single bipolar pulses, isolated from ground by means of an audio transformer (Hammond, type 835), were delivered to the cortex through the platinum electrodes from a stimulator (Grass, model SD5) modified to provide a frequency as low as 0.05 Hz. See Fig. 2. In early experiments, the pulse amplitude was determined by means of the output control of the stimulator. In later experiments an emitter-follower transistor circuit (Fig. 6) was used which provided 20 mv precision in the resolution of pulse amplitudes. In this circuit, the base potential of the transistor was controlled by a ten-turn potentiometer over the range of 0-20 volts, the upper voltage being fixed by a zener diode.

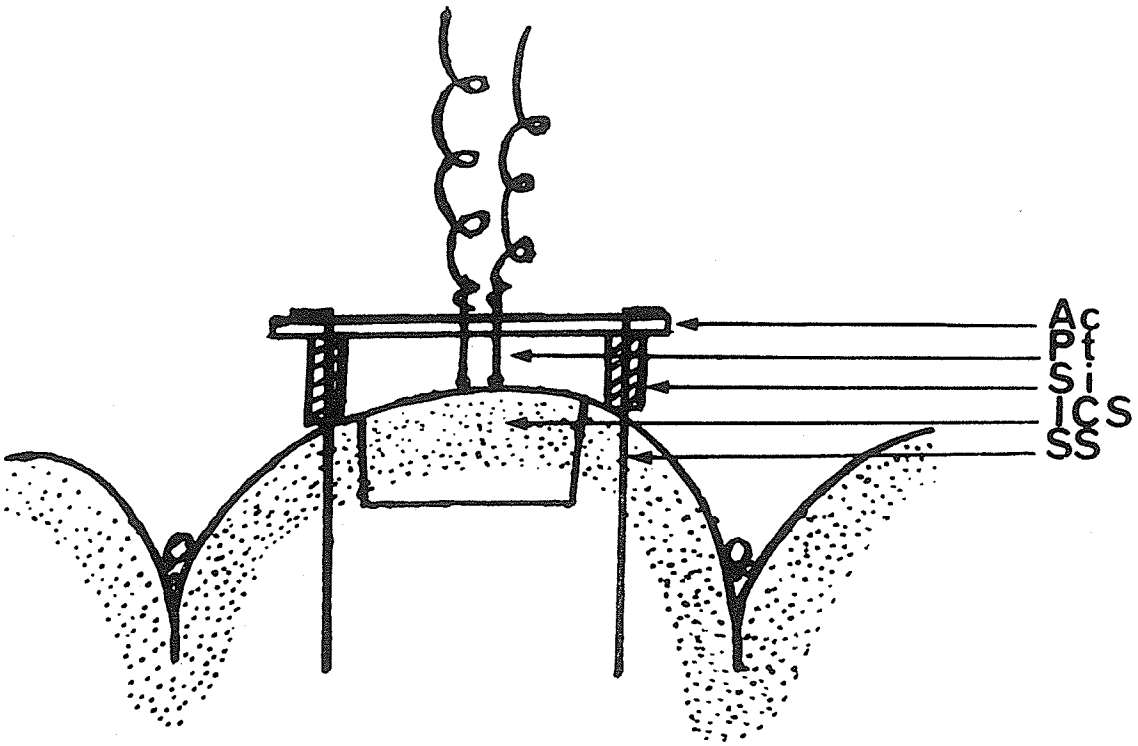
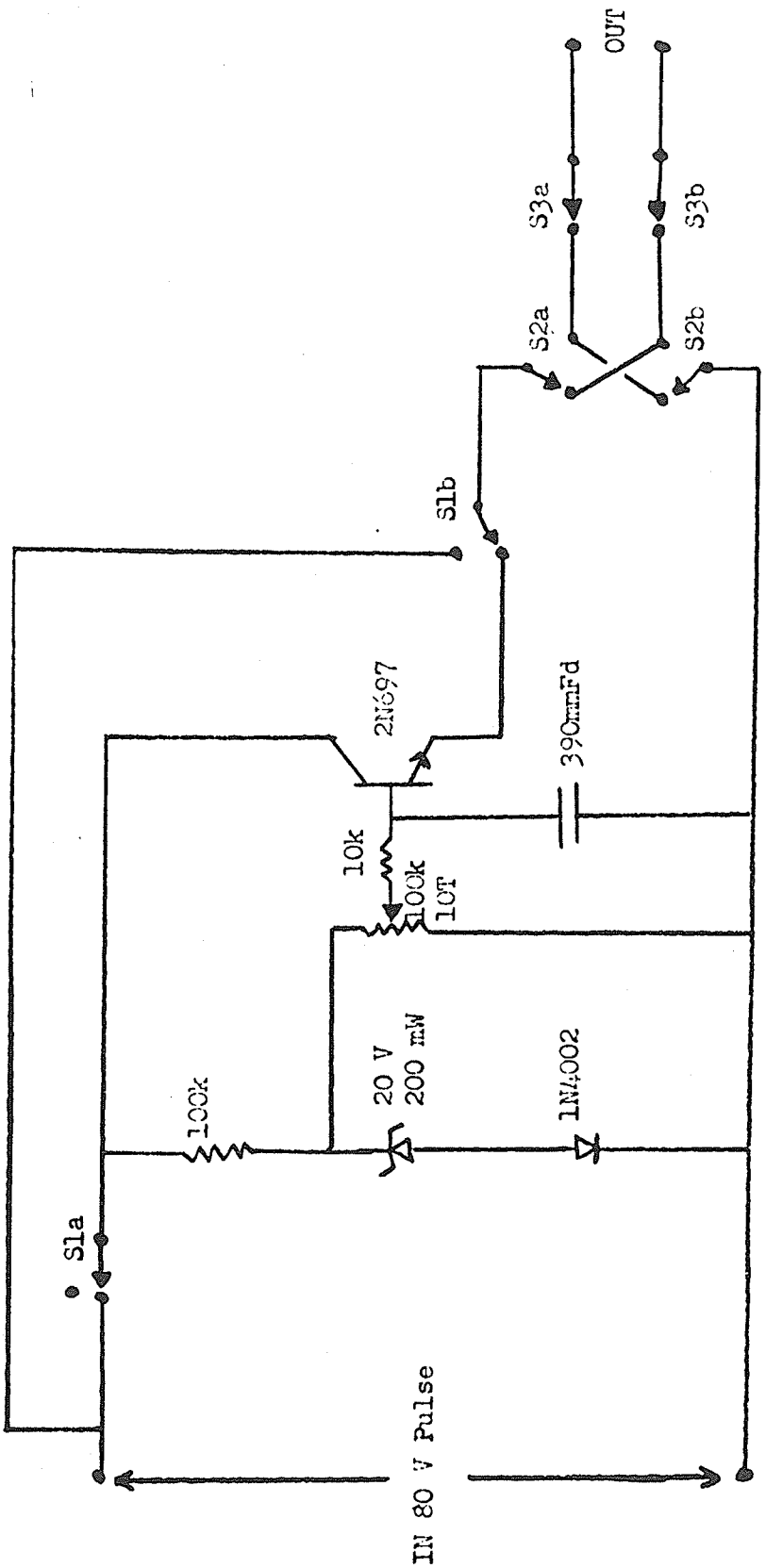


Fig. 5. Electrode platform. Coronal section through suprasylvian gyrus.

Ac, acetate sheet.
Pt, platinum electrode for stimulation.
Si, silicon rubber for spacing.
ICS, isolated cortical slab.
SS, stainless steel wire.

Not shown are the 2 thermistors mounted 2 mm apart along the length of the platform.



S1 bypass
S2 polarity
S3 safety

Fig. 6. Stimulus amplitude controller.

Since the counter dial of the potentiometer had 100 small divisions, the resolution of the instrument was 20 mv. Input to this circuit was provided by the stimulator, which determined pulse duration and frequency. The output voltage of the controller was found to be virtually independent of any load impedance above 5 kilohm. Since the cerebral cortex underlying the platinum electrodes usually had an impedance in the order of 10 kilohm or greater, this instrument effectively prevented the occurrence of artifactual changes of stimulus thresholds brought about by changes in cortical impedance.

A synchronous electric motor was coupled by bevelled gears to the shaft of the potentiometer to drive it at the rate of 1/6 rpm, the interval between 20 mv increments of the output being 3.6 seconds. The stimulator frequency was adjusted so as to deliver pulses in synchrony with the increments. This arrangement provided automatic augmentation of the stimulus applied to the cortex. The potentiometer was manually reset by momentarily disengaging the gears.

In all experiments the pulse duration was 0.5 msec.

a) <u>100%-response method for determining SPBR threshold:</u>

In the early experiments where stimulus amplitude was determined by means of the stimulator, pulses were delivered at 10 second intervals, and the minimum pulse height, in volts, was determined which would yield a response with every pulse. This method is referred to henceforth as the 100% response method. Its shortcomings reside in the poor resolution provided by the stimulator, the inability to provide confident measurements of threshold changes at specific moments in time, and the inevitable delay in detecting a fall in threshold. In its favor

is the rapid detection of a threshold rise.

b) Incremental method for determining SPBR threshold:

In the experiments where the transistor controller circuit was used for determining the stimulus amplitude, the minimum pulse height for evoking the SPBR was determined by an incremental method. See Fig. 7.

Subthreshold stimuli were delivered at intervals of 3.6 seconds (See Results (i)), each pulse being increased by 20 mv over the one preceding, until a response occurred. The amplitude of the pulse which evoked the response was read off the counting dial of the instrument and written down beside the ECoG recording on the penwriter paper. To allow recovery of the slab from the activity occurring during the response, no pulses were delivered during the ensuing 20 seconds (See Results (ii)), during which time the controller was reset to a subthreshold level. If a response occurred to the very first stimulus after the 20 second recovery period, it was ignored, and stimulation was resumed at a still lower level after a further 20 second wait. This protocol provided discrete measurements of SPBR threshold, having a resolution of 20 mv. It is henceforth referred to as the incremental method.

In order to confirm that the stimulus pulse was reaching the slab, the stimulus was monitored on one channel of a dual-beam oscilloscope (Tektronix, model 502). If delivery was not made, the pulse appeared as an undistorted rectangular wave; if delivery was made, the pulse had a rapid rise and slow return to base line (presumably because of retention of the polarization of the stimulating electrodes by the capacitance in the cortex). Differentiation of the two wave forms was most easily made by turning off the horizontal sweep of the oscilloscope.

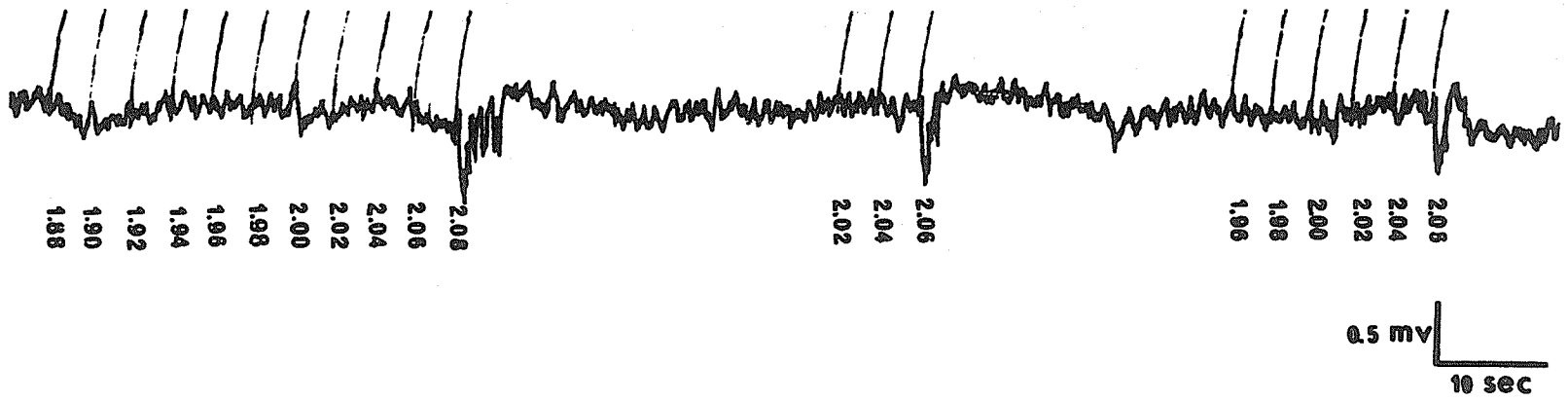


Fig. 7. Stimulation protocol for SPBR threshold determination by the incremental method. Stimuli are separated by a 3.6 second interval, with 20 seconds after each response.

If delivery was not made, the spot on the screen appeared stationary, whereas if delivery was made, the spot abruptly rose and slowly fell. For this reason the horizontal sweep was switched off as the standard procedure, so that routine checks of stimulus delivery could easily be made whenever the stimulus failed to evoke a response.

Since it was desirable to have marked on the recording paper the exact moment at which stimuli were delivered, a second stimulator (Grass, model SM6) was synchronized with the output of the first stimulator to deliver a 25 msec marker pulse to the driver amplifier of the penwriter. This was necessitated by the sometimes inadequate amplitude of the stimulus artifact picked up in the ECoG signal, and by the relatively slow response of the pen recorder galvanometers.

(ii) Inducing cardiac arrest:

In order to bring about circulatory arrest for brief periods to study its effect upon the SPBR, the right vagus nerve was exposed in the neck, separated from the sympathetic nerve trunk, cut, and the distal end mounted in a specially constructed stimulating electrode. This electrode consisted of a cylindric acrylic plastic rod, 18 mm long, by ¼ inch in diameter. A channel one mm deep by one mm wide was cut along its length and two platinum wires were mounted five mm apart, one in each wall of the channel. A second channel was located around the circumference of the rod three mm from one end, for the purpose of securing the nerve to the rod by means of a thread tied around it. The nerve was laid in the longitudinal channel with the cut end overlying the circumferential channel and secured to it. A drop of paraffin oil was placed upon the nerve as it lay in the longitudinal channel, in order to minimize

shunting of stimulus current and to prevent drying of the nerve. Finally, a tubular acrylic plastic collar was slipped onto the electrode so as to close over the longitudinal channel and hold the nerve inside. Stimulation was provided by pulses of 15-80 volts, 1-2 msec in duration, delivered at a frequency of 20-50 Hz from a stimulator (Grass, model SM6). The stimulator output was isolated from ground by an audio transformer (Hammond, type 835).

(iii) Micro-iontophoresis:

Two of the four outer barrels of a five-barrel micro-pipette (See Methods: Recording, (v))were filled with a saturated solution of sodium L-glutamate and a 3M solution of acetylcholine hydrochloride. For locating cortical units the micro-pipette was inserted into the isolated slab and advanced in one μm calibrated steps while glutamate ions were ejected from the tip of one of the barrels by a current of 40-50 nanoamp (nA), provided by a calibrated constant-current polarizer (designed by Mr. Hugh Spencer). When the audio and oscilloscope monitors indicated the presence of spike discharges having a satisfactory signal-to noise ratio the micro-drive was stopped and the depth read off and written down. The polarizer was switched from its continuous mode to a pulse mode, the pulses being six seconds in duration and their frequency 3/min. Between pulses, a backing current of about 5 nA was applied to prevent diffusion of glutamate ions from the electrode tip. If necessary, the current was adjusted to optimize the frequency of firing, after which recording was begun.

The currents used during iontophoretic pulses were in the range 20-60 nA, with a constant current for a given unit.

In some experiments, iontophoretically-applied glutamate was not used to locate units. Instead, the microelectrode was slowly inserted into the cortex in twenty μm steps, while SPBR's were evoked at ten second intervals by application of suprathreshold stimuli to the surface of the slab. Units located in this manner almost always fired only during a simultaneously-occurring SPBR. In the few instances where spike potentials were not coincident with the SPBR, they usually disappeared after a short time, and were regarded as being due to injury of a nerve cell by the micro-pipette.

In some instances iontophoresis was used to test the sensitivity to acetylcholine and glutamate of units located in the manner just detailed.

IV. Analysis of results

In most instances, statistical analysis was done with the aid of a programmed electronic calculating machine (Olivetti Programma 101). In some instances this was infeasible due to the quantity of data, and the necessity arose for grouping the data from such experiments and averaging them with respect to some chosen initial state. Such averaging was done with a small digital computer (Digital Equipment Corp., model PDP-8/1).

This measure was necessary in the studies on the effect of 10% CO_2 inhalation upon the SPBR threshold and upon single unit activity. The data for such response averaging was punched manually onto paper tapes for convenient loading into the computer. Since the control level of the SPBR threshold varied from slab to slab, and since the control level of unit activity varied from unit to unit, the crude data was converted by the program to percentage values of the last datum obtained before commencement of the treatment. The times of commencement and of termination of the treatment were made reference points in time, in order properly to align the data points.

In the SPBR threshold experiments, where measurements were not spaced at constant intervals, the time elapsed between the data points was marked off on the punched tape in intervals of 30 seconds. The markers were used to instruct the program to skip locations where no data was available, thus ensuring that each datum would be grouped according to the actual elapsed time at which it had been obtained.

Since the durations of the pretreatment, treatment, and post-treatment phases varied from one trial to another, a specific number of

locations was allocated to each phase for the cumulation of data. The allocation of a specific number of locations to the treatment phase resulted in the omission of data points and of spaces from trials whose treatment phase contained more than this number. It resulted, also, in the addition of an artifactual blank period at the end of the treatment phase in those trials which contained fewer than the specified number of locations. Hence, the computation introduced a discontinuity at the end of the treatment phase, in order that the averaging of the post-treatment phase data be synchronized with respect to the beginning of this phase. No such discontinuity was introduced at the end of the pretreatment phase since in this phase the programmed omission of data or addition of a blank period was done at the beginning of the phase.

Because of the variability of the effect of carbon dioxide upon unit firing rates, simple averaging of the entire body of data was worthless because opposite effects would be obscured. To permit unbiased selection of data on the basis of uniform effect, a second computer program was written to provide statistical comparisons within single experiments of the mean firing rate prevailing during the pretreatment phase, with the mean firing rate of the treatment phase. Those experiments showing a statistical difference at the $p < 0.05$ level were then grouped according to whether the effect was to increase or to decrease the firing rate, and then averaged according to the procedure previously described.

In addition, the occasional observation of a dual effect (stimulation followed by depression) prompted a comparison within the treatment phase of the mean of the first three data points with the mean of the next three. This procedure served to screen out the experiments

where this dual effect occurred, which could then be averaged in order to illustrate this effect.

RESULTS

Part I. Studies of the surface positive burst response

1. Effect of the duration of the interstimulus interval upon the adequacy of a stimulus which follows a response

While using the '100% response' method for determining SPBR threshold, it was found that stimuli spaced ten seconds apart sometimes would evoke a response only to each alternate stimulus. This suggested that some degree of refractoriness to stimulation persisted for some time after an evoked SPBR, and that ten seconds was not a sufficient period of time to remove this refractoriness.

A search of the literature failed to disclose any study of refractoriness following the evocation of the SPBR. Most workers have accepted ten to fifteen seconds as an appropriate interval between suprathreshold stimuli (Sanders & Gravlin, 1968; Harwood & Sanders, 1969; Frank & Jhamandas, 1970; Brown & Pinsky, 1971). Brown (1968) justified an interval of ten seconds on the ground that shorter intervals tend to increase spontaneous activity, probably referring to the production of afterbursts (Burns, 1954; 1955).

Refractoriness following the SPBR was therefore investigated in five cats as detailed immediately below, in order to learn what recovery time must be allowed in order to be certain of avoiding artifactual elevation of the threshold.

SPBR threshold was determined by the incremental method. Incrementally augmented subthreshold stimuli were delivered at intervals of ten seconds, and at least twenty seconds were allowed to pass after termination of each response before stimulation was recommenced. When the threshold was found to be stable to within a few percent, for about

twenty minutes, the stimulus voltage was increased by five percent above threshold, and the effect of varying the interval between these supra-threshold stimuli upon the percent response to a given number of stimuli was determined. Intervals were measured from the termination of the preceding response, and ten stimuli were tested at each interval. The percent response was plotted against the interval as shown in Fig. 8.

On the basis of five such experiments, it was found that eighteen seconds was the minimum recovery time which was certain to yield 100% response to stimulation, i.e. to allow for complete disappearance of refractoriness to stimulation. Therefore, in all experiments where the incremental method for SPBR threshold determination was used, an interval of twenty seconds or longer was allowed after each response.

2. Effect of the frequency of application of subthreshold stimuli upon the SPBR threshold:

Inhibition of single unit firing has been reported to occur following the application of single brief current pulses to the surface of the cerebral cortex (Krnjevic, Randic & Straughan, 1964; 1966). It was inferred from this that the application of subthreshold stimuli to the surface of the isolated cortical slab might affect the SPBR threshold. This possibility was supported by the results of some early experiments where the incremental method was used. In these, subthreshold stimuli were applied incrementally at a rate of one every two seconds. When threshold was reached and the responseevoked, an interval of twenty seconds was allowed to elapse before another stimulus, of lesser magnitude than the threshold just determined, was delivered. Occasionally such a stimulus would evoke a response. This result was interpreted as indicating

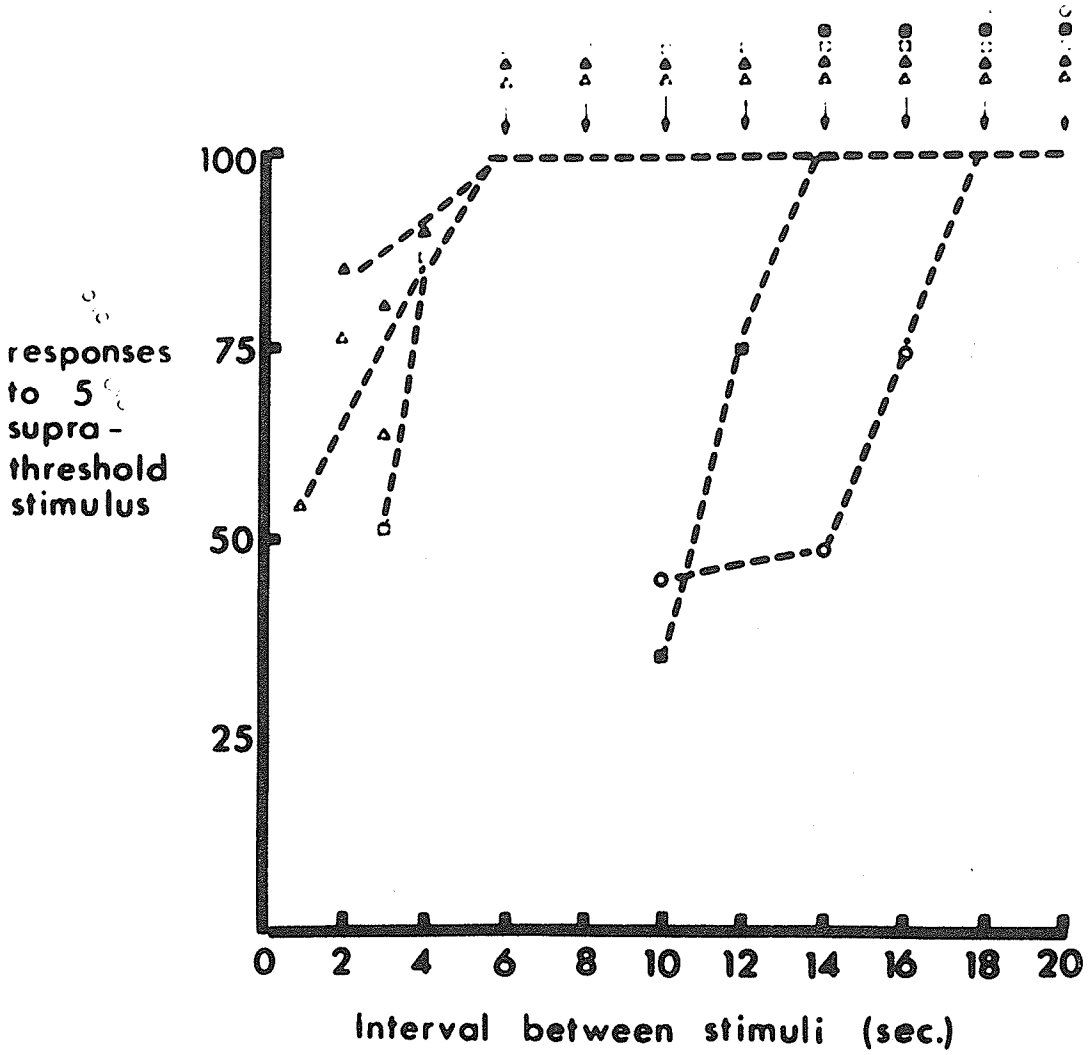


Fig. 8. Relation between responsiveness of the isolated cortical slab, and the interval duration between suprathreshold stimuli. Each symbol indicates the percent response to 10 stimuli in one animal.

that subthreshold stimuli too often applied (in this case, one every two seconds) can raise the SPBR threshold by some form of induction of refractoriness, and that the long interval (20 seconds) allowed to elapse after a response, permits this refractoriness to subside. In order to avoid this artifactual complication, the relation between threshold and the interval between incrementally augmented subthreshold stimuli was examined, to make possible the choice of a suitable interval for routine use in the study of SPBR threshold by the incremental method.

At least ten thresholds were determined using each test interval. The arithmetic means of the thresholds were plotted against the intervals, and comparisons were made by means of Student's t-test. This was done in four cats, of which Fig. 9 is representative. In this experiment there was no significant elevation of SPBR threshold by subthreshold stimuli applied at intervals exceeding 0.5 second in duration. In only one of these four cats did intervals as long as two seconds produce threshold elevation.

On this basis the speed of the motor-driven stimulus controller was chosen to augment the stimulus amplitude by one increment (20 mv) at intervals of 3.6 seconds. The stimulator frequency was set to deliver pulses which coincided with the addition of each increment.

3. Effects of impaired cerebral blood flow (CBF) upon the SPBR threshold:

A. Cardiac arrest.

In three cats, a total of 72 cardiac arrests each having duration of about five seconds induced by right vagus nerve stimulation. An interval of at least two minutes separated successive trials, because it was found that the blood pressure always restabilized by this time,

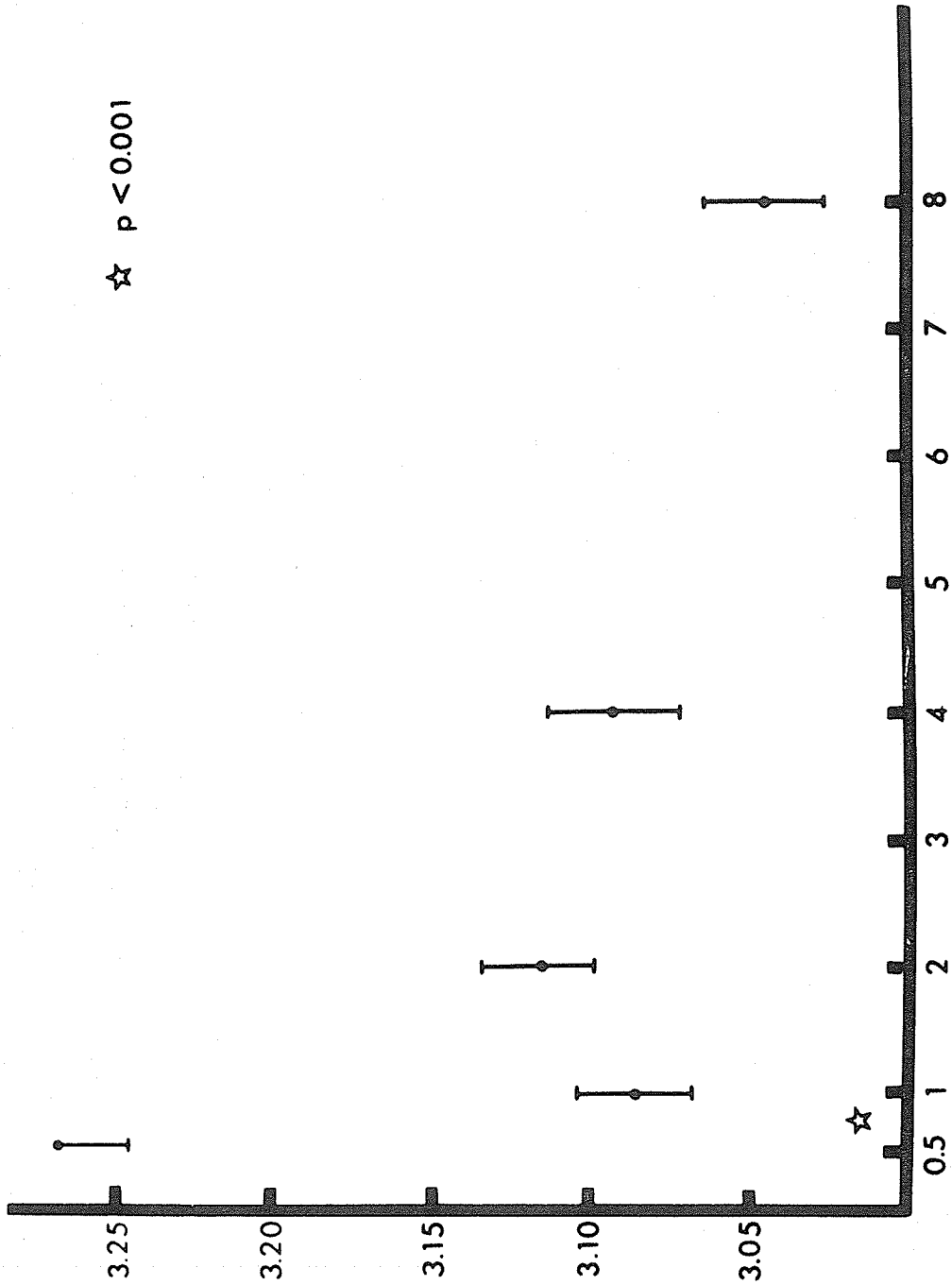


Fig. 9. Relation between the determined SPBR threshold and the frequency of application of incrementally augmented subthreshold stimuli.

as did the SPBR threshold.

As a rule, femoral arterial blood pressure rapidly fell to about 20 mm Hg, where it remained for the duration of the arrest. The 100% response method of threshold determination was used in these experiments.

It was found that the threshold rose in 28 of the 72 trials. An example of this is shown in Fig. 10. Such threshold elevation rarely lasted for more than 30 seconds after the beginning of the arrest.

B. Carotid occlusion.

The effect of ipsilateral occlusion of the common carotid artery was studied in two cats. Occlusion was brought about by applying tension to a thread loosely tied around the artery. Duration of occlusion was 20-25 seconds. SPBR threshold was determined by the 100% response method. In each of four trials there was an immediate elevation of threshold which persisted for up to thirty seconds after termination of the occlusion. One of these trials is represented in Fig. 11.

Two trials of bilateral carotid occlusion in two cats also produced an immediate elevation of threshold, but in these instances it led to an apparently permanent abolition of the response, as indicated by the inability to evoke any response using any stimulus amplitude up to 20 volts. In experiments such as these where the response became apparently unobtainable, attempts to evoke a response were made periodically for several hours, after which, if no success occurred, the experiment was terminated.

C. Acute hypotension produced by a short-acting ganglionic blocking agent.

Acute hypotension was produced by intravenous injection of tri-

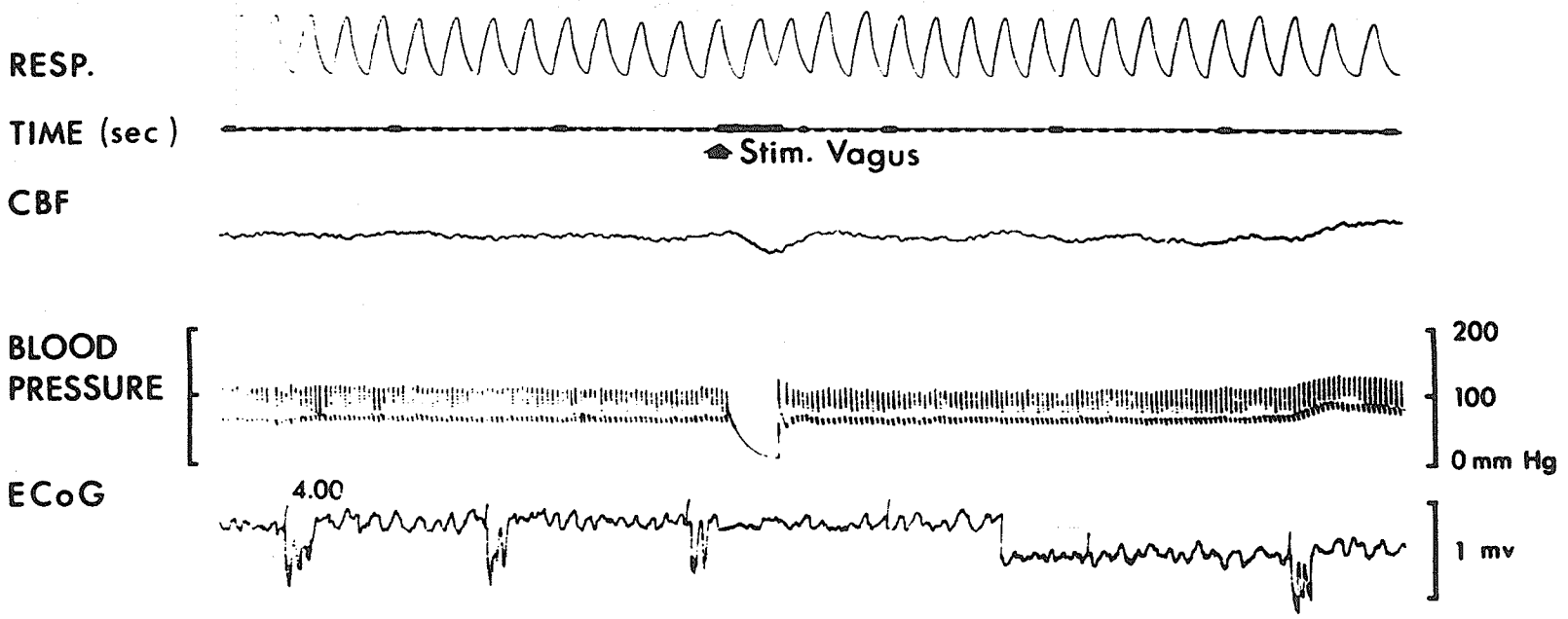


Fig. 10. The effect of brief cardiac arrest upon the SPBR threshold (100% method). Note the absence of response to the two stimuli which follow the arrest. The number which appears in the electrocorticogram (ECoG) trace in this and subsequent figures is the stimulus voltage.

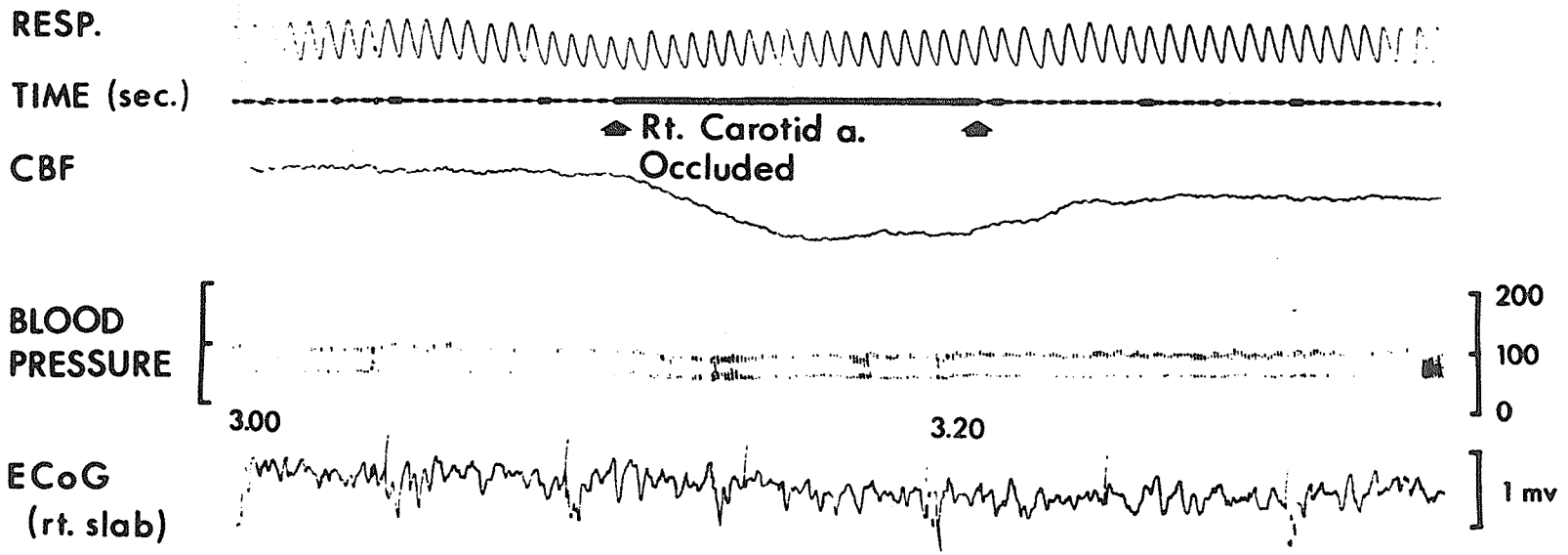


Fig. 11. The effect of occlusion of the ipsilateral common carotid artery upon the SPBR threshold. Note that the response fails during the occlusion, and that a subsequent elevation of the stimulus voltage fails to yield 100% response.

methaphan camphorsulfonate (Arfonad- Roche) 0.05-0.10 mg/kg and the effect upon SPBR threshold was studied in three cats.

In two cats studied by the 100% response method, the threshold was elevated in 8 trials, lowered in one, and unaltered in 5. An instance where the threshold was elevated is shown in Fig. 12.

In one cat studied by the incremental method, twelve trials were made and the effects determined in two slabs (not simultaneously). In five instances there was a transient fall in threshold followed by a rise. One of these is shown in Fig. 13, as is one of the two instances in which the threshold fell without a rise. In two instances it rose, and in three there was no change.

These results are summarized in Table 1.

D. Slow hemorrhage.

Slow hemorrhage was induced by connecting the femoral arterial cannula by the side-arm of its stopcock to a heparinized syringe, the plunger of which was withdrawn by an infusion-withdrawal pump (Harvard Apparatus Co., model 600-950). Blood was withdrawn at the rate of about one ml/kg/min, in order to minimize reflex activation of the adrenal medulla (Manger, Bollman, Maher & Berkson, 1957).

This was done in two cats, one studied by the 100% response method, the other by the incremental method. In both instances there was no change of SPBR threshold until the blood pressure had fallen to 50/25, systolic/diastolic (Fig. 14) at which time the response abruptly became unobtainable. This coincided with the cortex turning white. Prompt replacement of the blood led to recovery of the response in both cats.

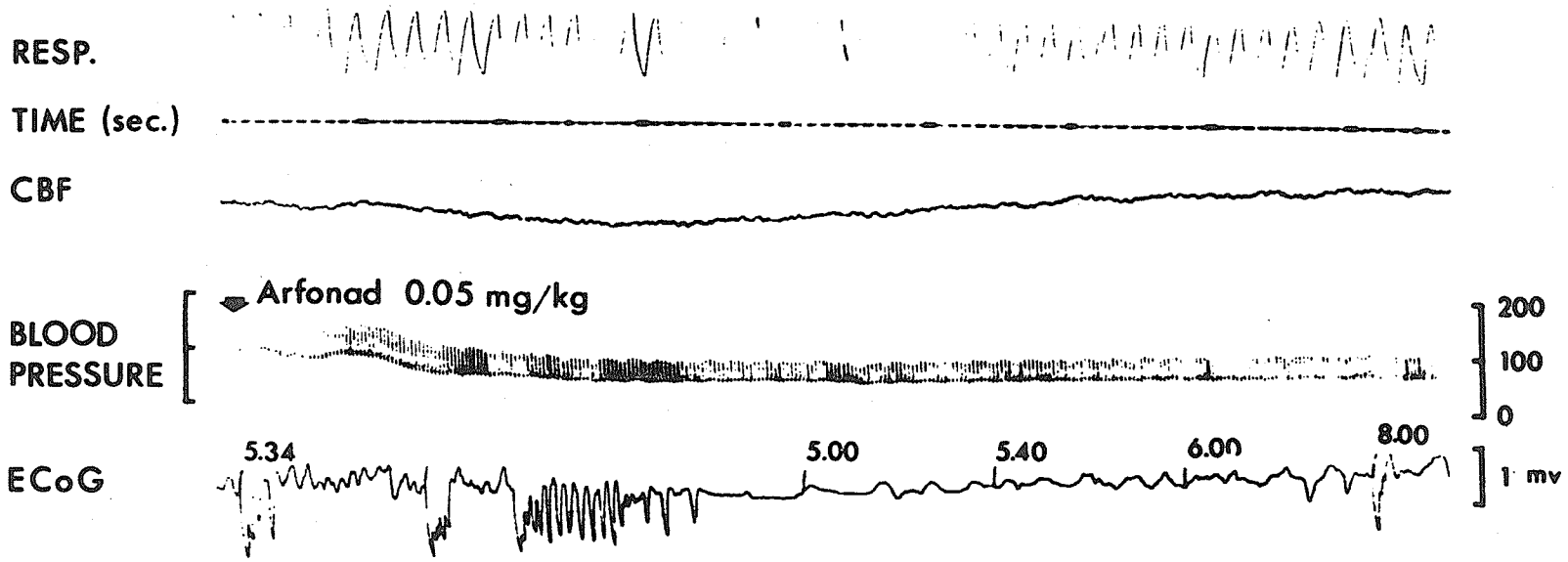


Fig. 12. The effect of hypotension induced by an intravenous ganglionic blocking agent upon the SPBR threshold. Arfonad, trimethaphan camphorsulfonate.

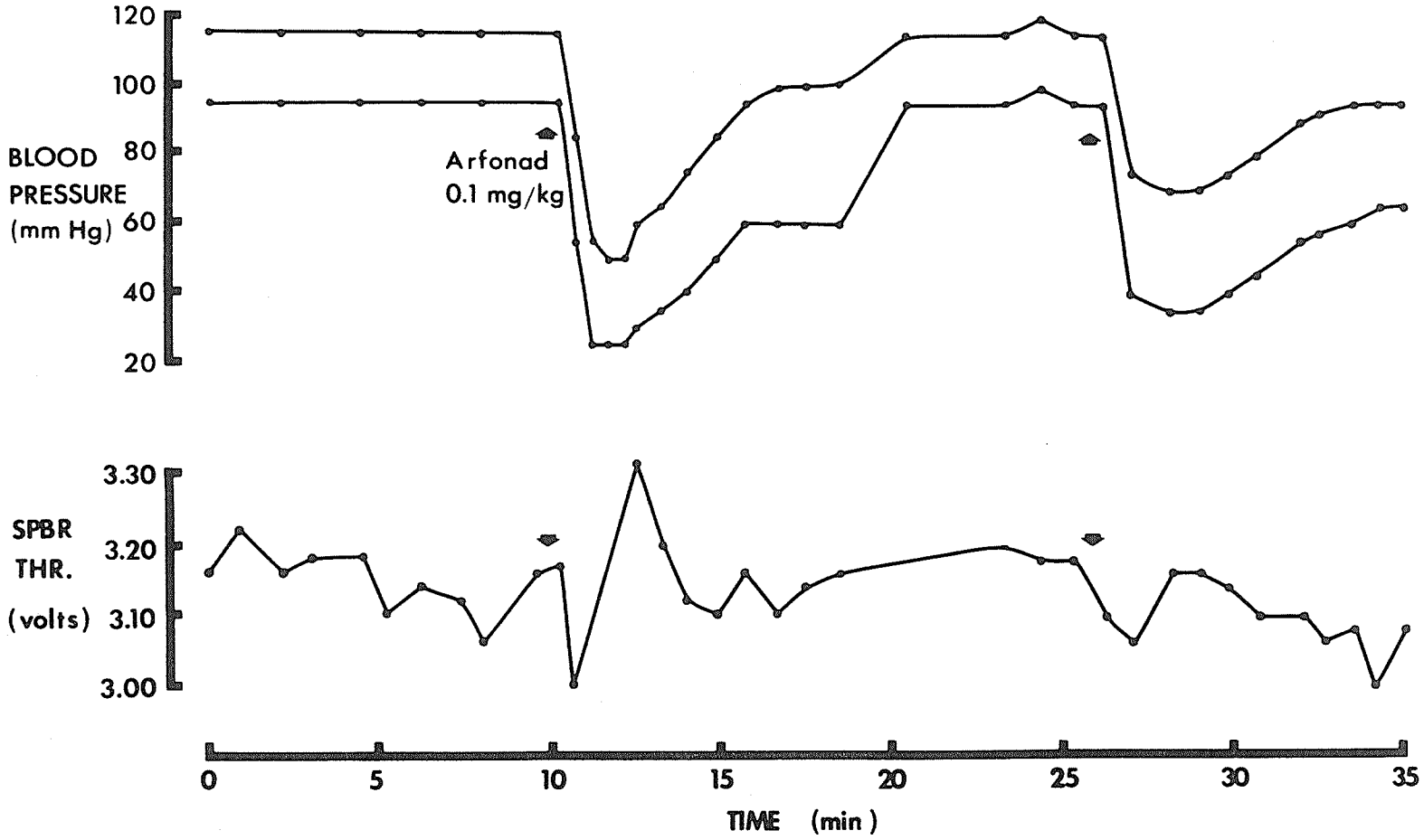


Fig. 13. The effect of hypotension induced by an intravenous ganglionic blocking agent upon the SPBR threshold (measured in this and in succeeding figures by the incremental method). Time of injection of the drug is indicated by the arrows.

TABLE 1

Effect of arfonad-induced hypotension upon the SPBR threshold

Method	No. of Trials				
	Elevated	Lowered	Lowered, then Elevated	No Change	Total
100% Response					
Expt. 1	4	1	0	0	5
Expt. 2	4	0	0	5	9
Incremental					
rt. slab	0	2	2	1	5
left slab	2	0	3	2	7
Total	10	3	5	8	26

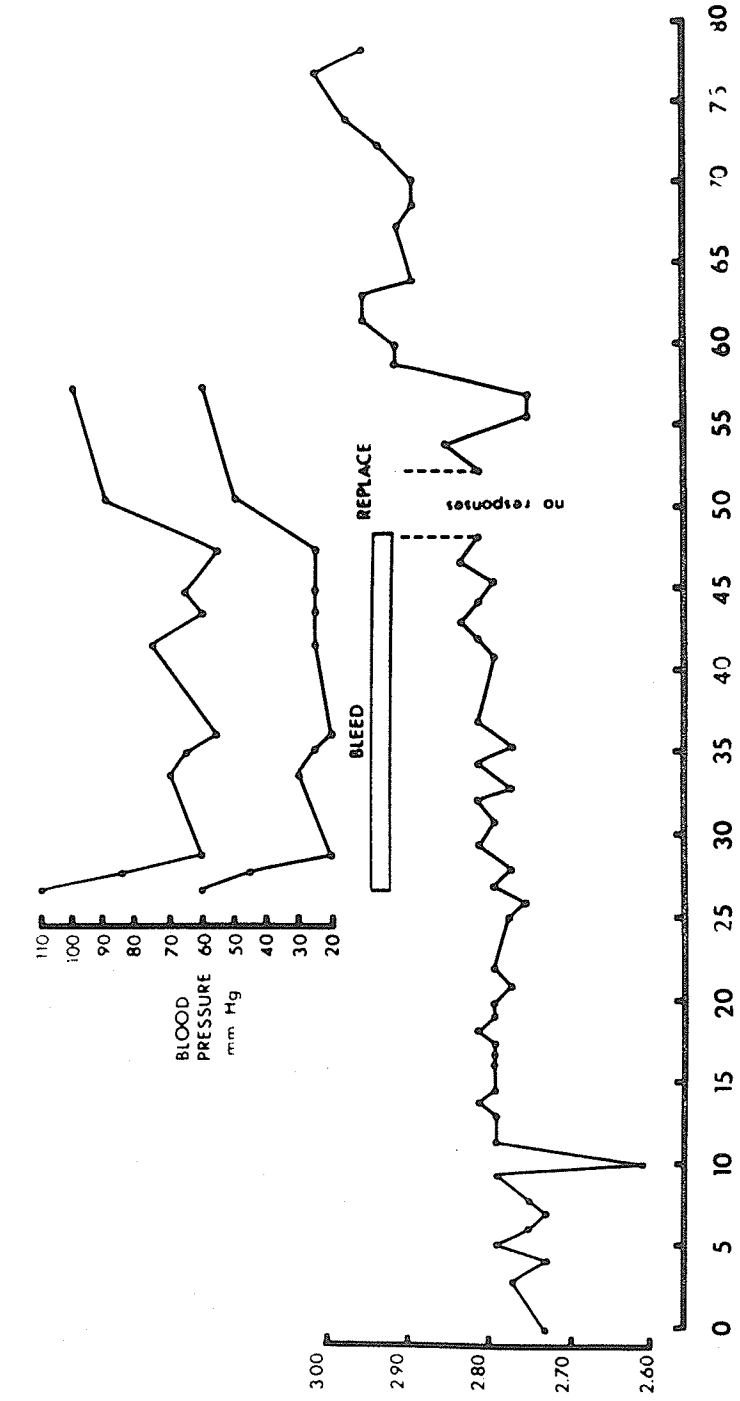


Fig. 14. The effect of hypotension induced by slow hemorrhage upon the SPBR threshold.

4. Effect of altered blood gases upon the SPBR:

Manipulations of cerebral blood flow can be expected to alter the delivery of oxygen to, and removal of carbon dioxide from the brain. (The involvement of other substances such as glucose does not fall within the scope of this study, although hypoglycemia is known to reduce markedly the tolerance to hypoxia (Pieroni, Broderick, & Levine, 1971), while hyperglycemia markedly increases tolerance (Davis & Wallace, 1942; Hershgold & Riley, 1959)).

The possibility that such changes might have been the proximate cause of the effects seen in the experiments with altered CBF was therefore considered. To examine this, cats were administered controlled gas mixtures while the SPBR threshold was monitored by the incremental method.

The gases were metered through the calibrated flow gauges of an anesthetic machine (Heidbrink Kinetometer) fitted with cylinders containing pure oxygen, nitrogen, and carbon dioxide, respectively (supplied by Canadian Liquid Air, Medical Gases Division). The relative proportion of each gas in the administered mixture was determined by its flow. In hypercarbia experiments the carbon dioxide was mixed with twenty percent oxygen, with nitrogen as the diluent.

A one-way valve (Warren & Collins Inc., Braintree, Mass.) fitted to the tracheal cannula served the two purposes of preventing access to room air, and of limiting the respiratory dead space to the volume of the valve. A breathing bag was incorporated along the tubing joining the anesthetic machine to the breathing valve.

A. Hypoxia.

Seven cats were given gas mixtures containing oxygen in a concentration of ten percent and less. In three instances ten percent oxygen

caused a short-lived lowering of the SPBR threshold (e.g. Fig. 15), and in the remaining four had no effect. This depression of SPBR threshold was not accompanied by changes either of the SPBR peak amplitude or of the duration.

In four of the cats the oxygen concentration was further lowered in steps, until abolition of the response occurred. In one instance the threshold rose immediately prior to abolition (Fig. 16), in two it fell, and in one the loss of response was not preceded by any change of threshold.

B. Hypercarbia: 10% CO_2.

Ten percent carbon dioxide was given to nineteen cats, and a total of 30 exposures were examined in 21 slabs for their effect upon the SPBR threshold. (Additional exposures which followed the administration of various drugs are discussed in later sections of this thesis.)

The most frequently observed effect was a short-lived threshold elevation above the preinhalation level, together with a short-lived fall of threshold below the preinhalation level upon withdrawal of the gas mixture and return to room air. (The latter effect will henceforth be referred to as "withdrawal hyperexcitability".) See Fig. 17, 18. This pattern was seen in 24 of the 30 exposures. Of the other six, one had no discernible effect; three had no effect during the inhalation but were followed by withdrawal hyperexcitability (e.g. Fig. 19); and two produced a lowering of the threshold (e.g. Fig. 20). The last-mentioned effect was seen in only one cat, in which an earlier inhalation of the gas mixture had produced the pattern of effects first described above (i.e. short-lived threshold elevation, with hyperexcitability upon withdrawal).

Averaging of the SPBR threshold measurements obtained in all

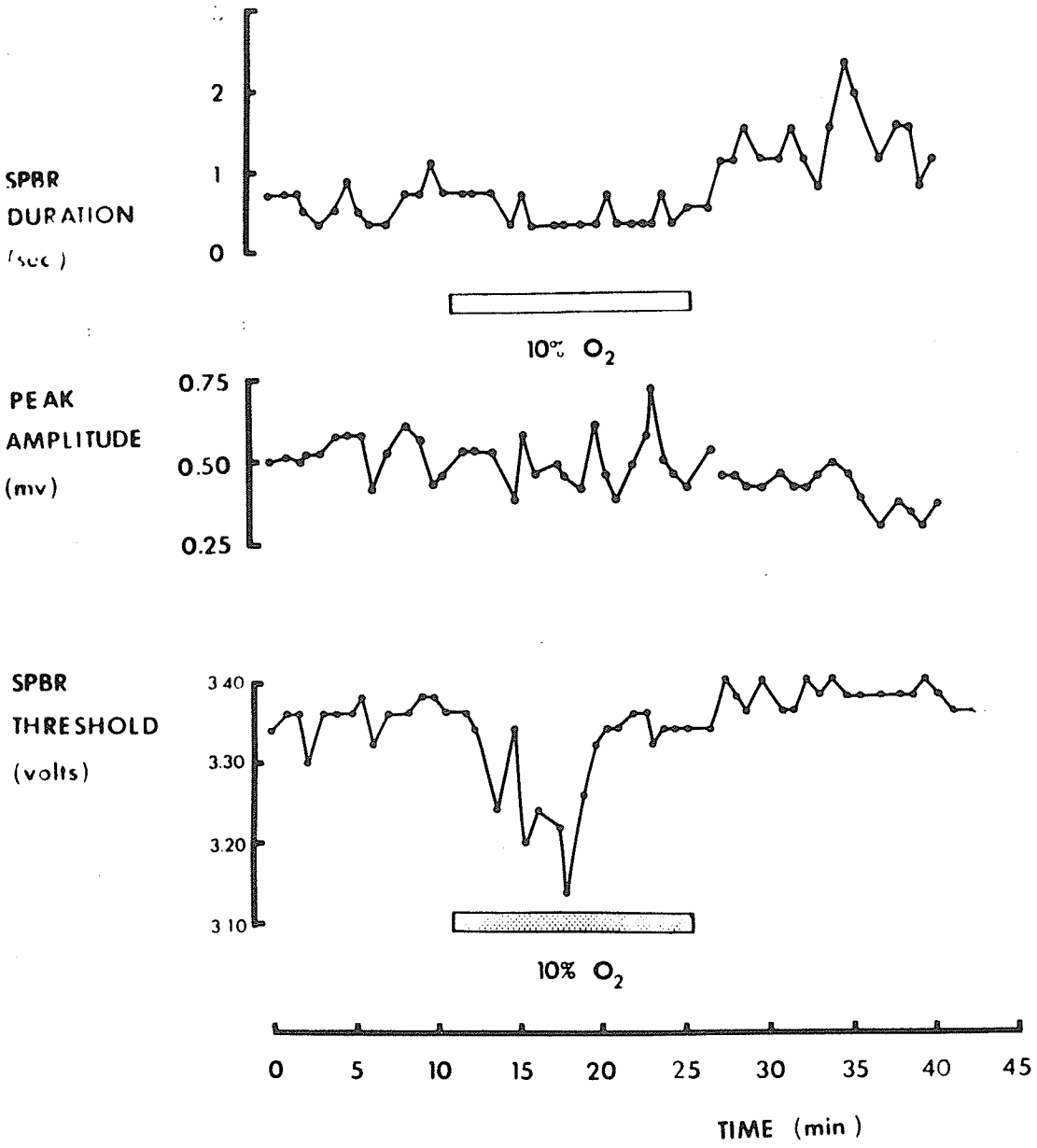


Fig. 15. The effect of hypoxia upon the SPBR threshold. Note that the threshold recovers to the pre-hypoxic level before the period of hypoxia is terminated. Also note the absence of any effect upon the duration and amplitude of the response.

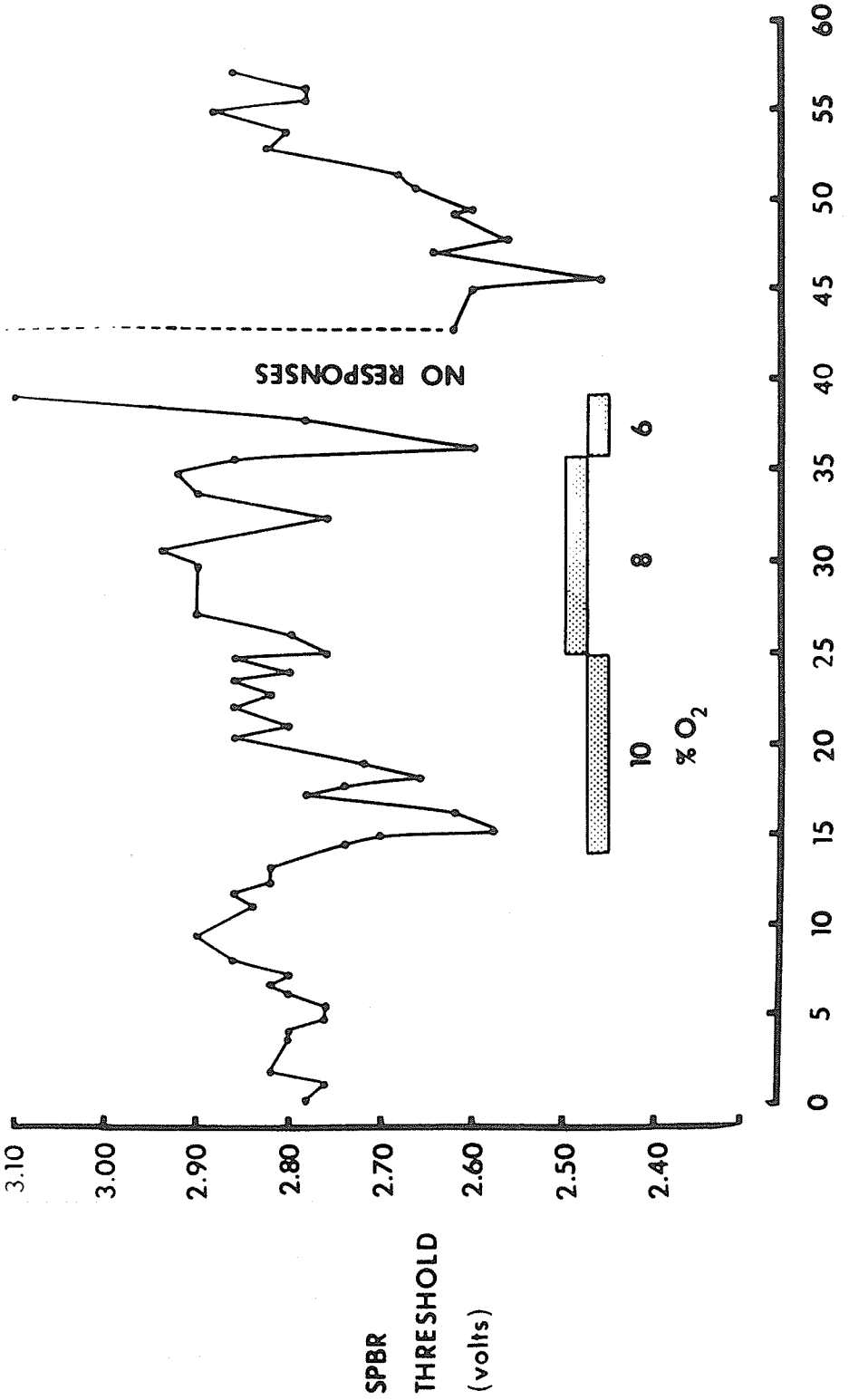


Fig. 16. The effects of progressive hypoxia upon the SPBR threshold. Note the transient nature of the initial fall of the threshold.

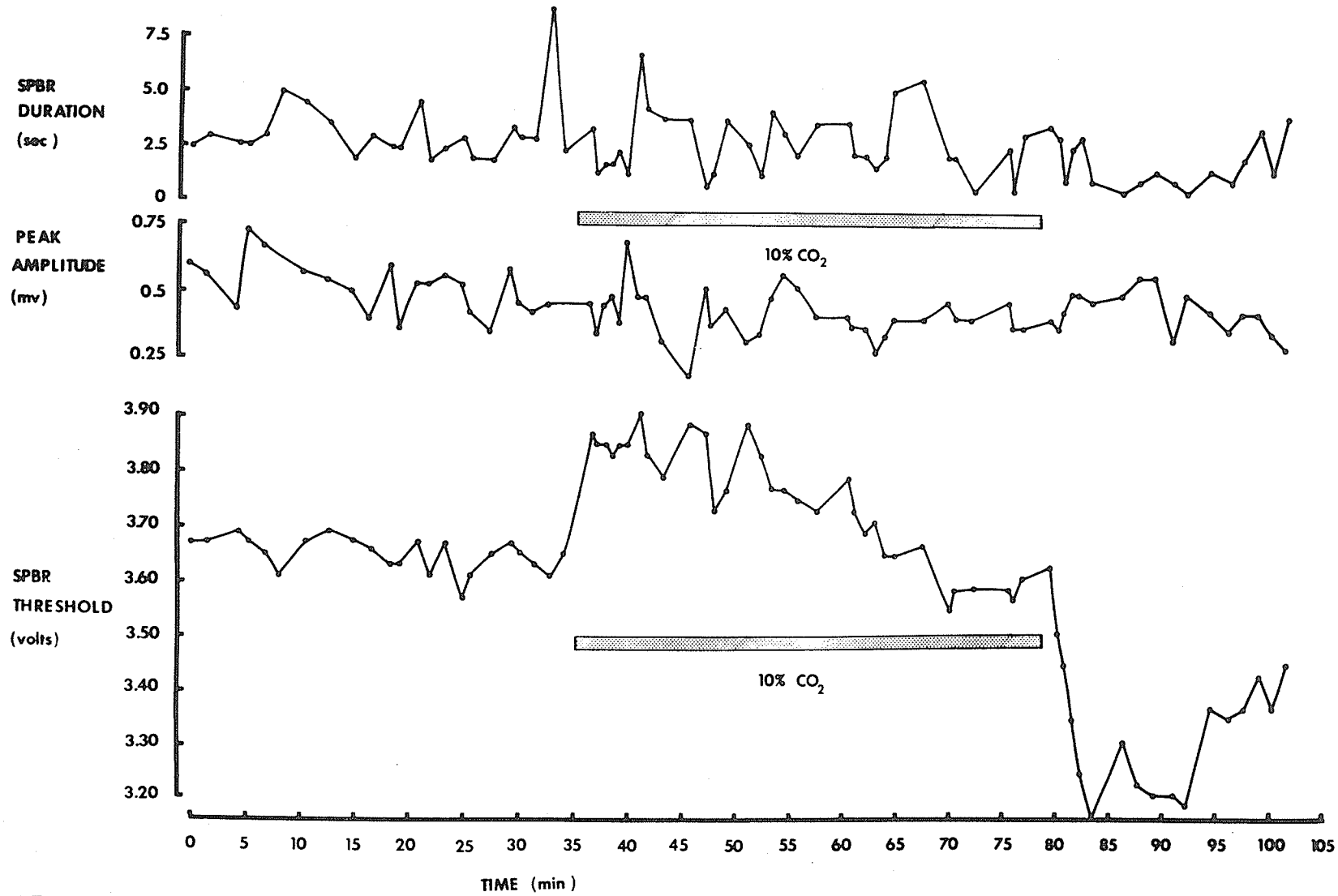


Fig. 17. The SPBR threshold-elevating effect of inhalation of 10% CO_2. Note: 1) the tendency for recovery of the threshold to the control level to occur before termination of the gas inhalation; 2) the absence of any effect upon the duration and amplitude of the response. (The absence of some points in the SPBR amplitude record is due to a small instability of the DC-level which resulted in the occasional overloading of the penwriter's galvanometer during the response.)

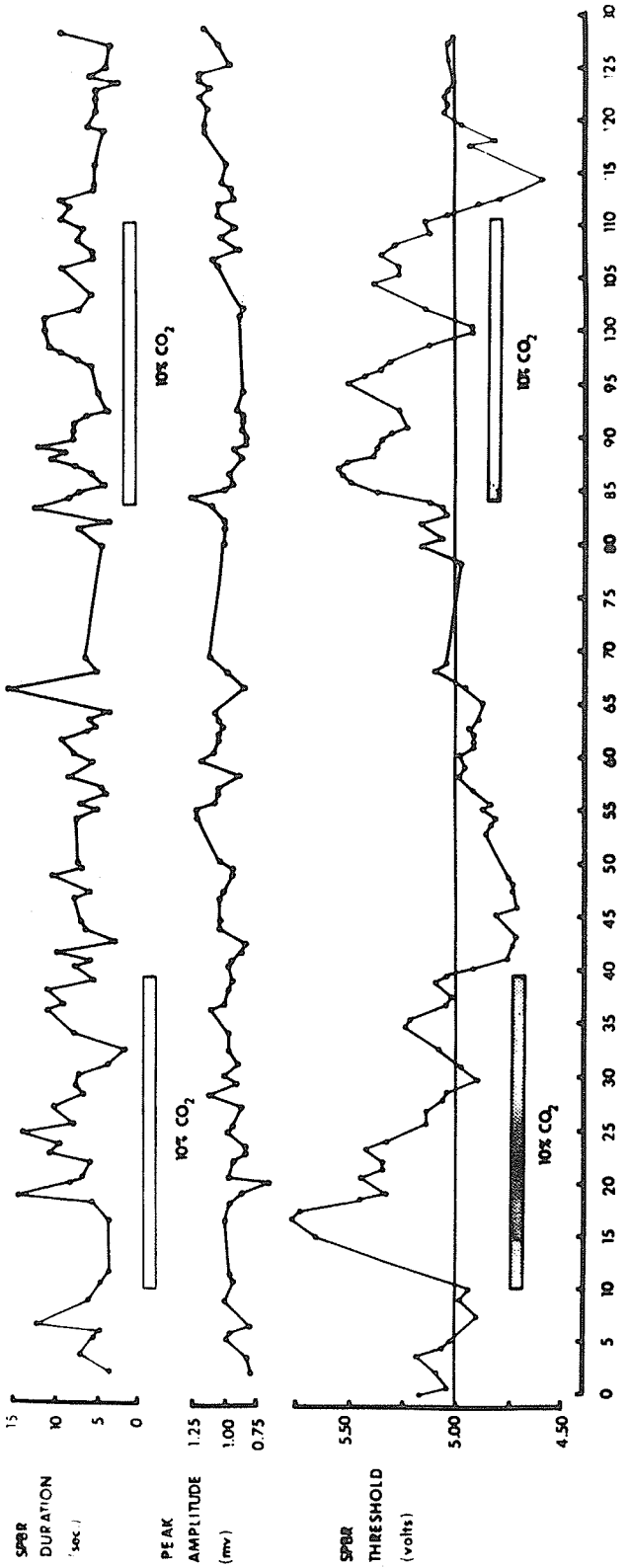


Fig. 18. The SPBR threshold-elevating effect of inhalation of 10% CO_2.

Two trials in the same slab.

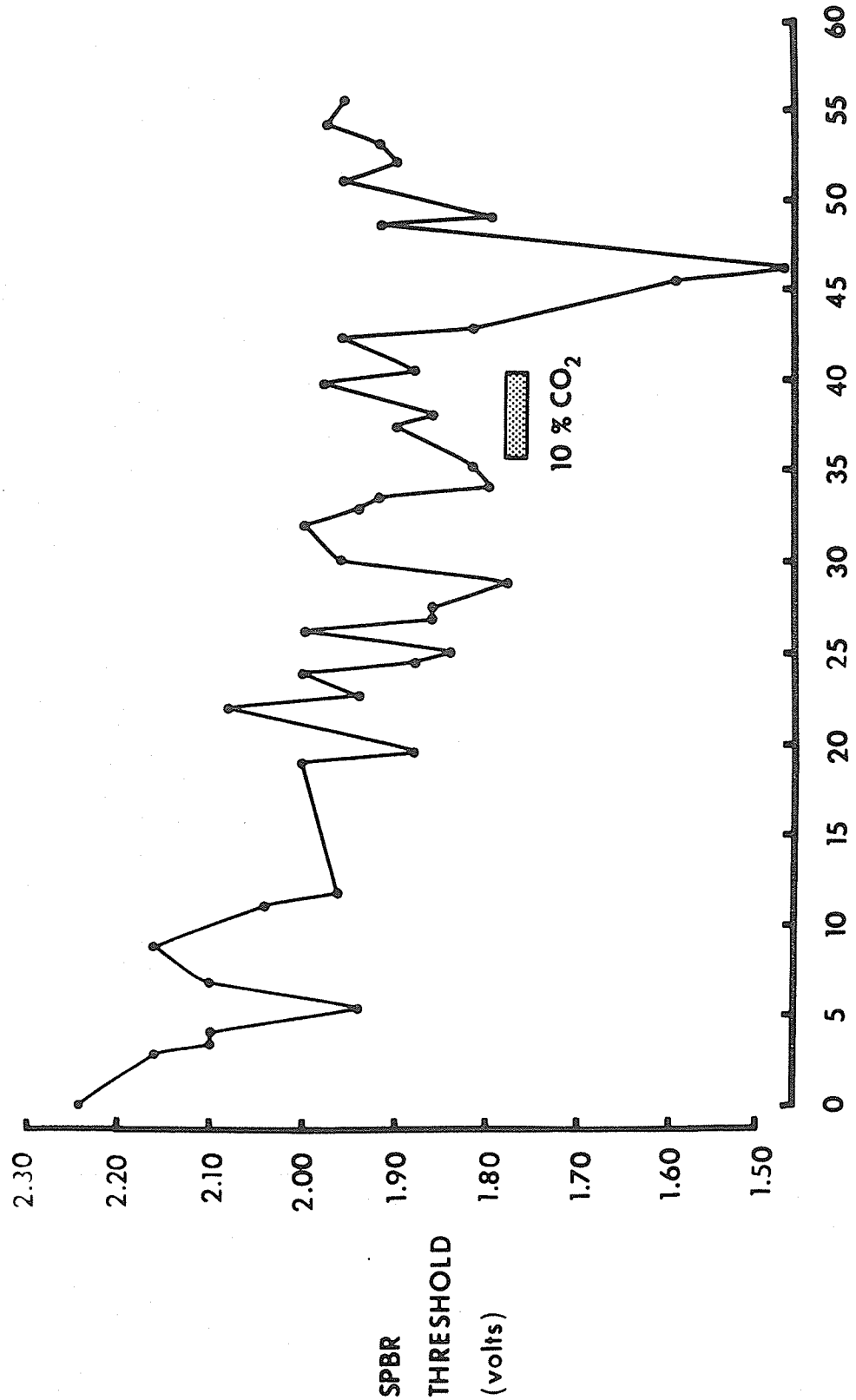


Fig. 19. Hyperexcitability upon withdrawal of 10% CO_2, in the absence of any alteration of the SPBR threshold during the gas inhalation.

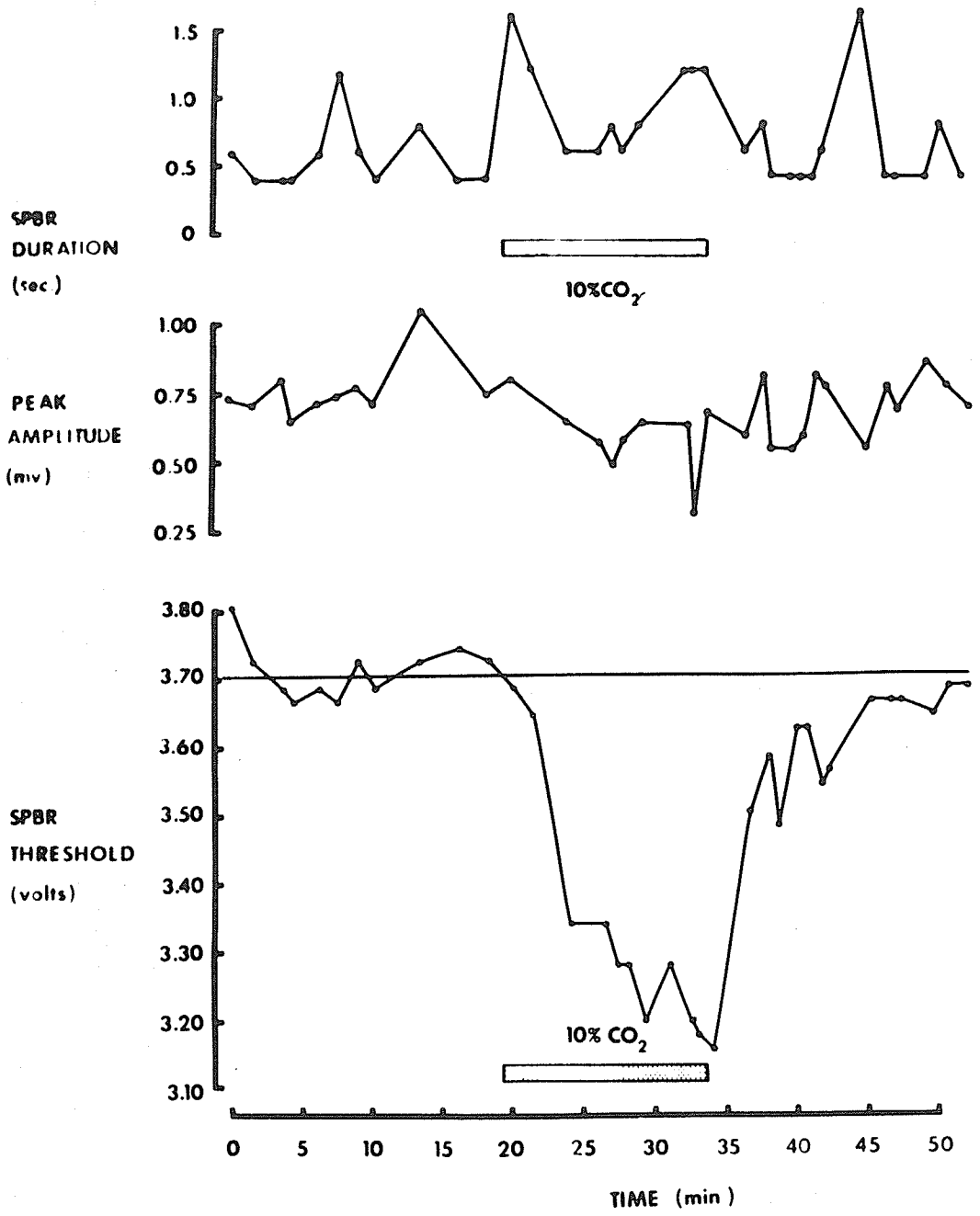


Fig. 20. The SPBR threshold-lowering effect of 10% CO_2. This effect was noted in 2 trials in one cat, out of a total of 30 trials conducted in 19 cats.

thirty trials was carried out, and the result is shown in Fig. 21. The result for the 28 experiments exclusive of the two in which threshold lowering occurred is shown in Fig. 22. For the thirty experiments, the mean and standard error of the peak threshold displacements from the control level, and of the threshold at the time of termination of the treatment are shown in Fig. 23.

Of the 24 trials where the threshold was elevated, it was possible in twelve instances to demonstrate complete return of the threshold during CO_2 inhalation to the preinhalation level. Failure of accommodation to be complete in the other eleven instances appears to have been largely due to an inadequate duration of exposure to the gas mixture, as indicated by the regression of the SPBR threshold at the time of withdrawal of the gas mixture, upon the duration of inhalation of the mixture (Fig. 24).

In the twelve trials where accommodation was complete, peak elevation of threshold occurred 3.94 ± 0.48 (mean $\pm$ standard error) minutes after beginning the inhalation, and the return to the preinhalation level was complete at 12.89 ± 1.81 minutes. See Fig. 25. The corresponding curve for the twelve experiments where accommodation was incomplete is shown in Fig. 26.

The regression of the extent of rebound hyperexcitability upon the duration of CO_2 inhalation is statistically significant at the $p < 0.05$ level (Fig. 27), and there is excellent correlation ($r = 0.70$, $p < 0.001$) between peak rebound and peak threshold elevation (Fig. 28). These relationships suggested the existence of a causal relation between the elevation of threshold during CO_2 inhalation, and the rebound hyper-

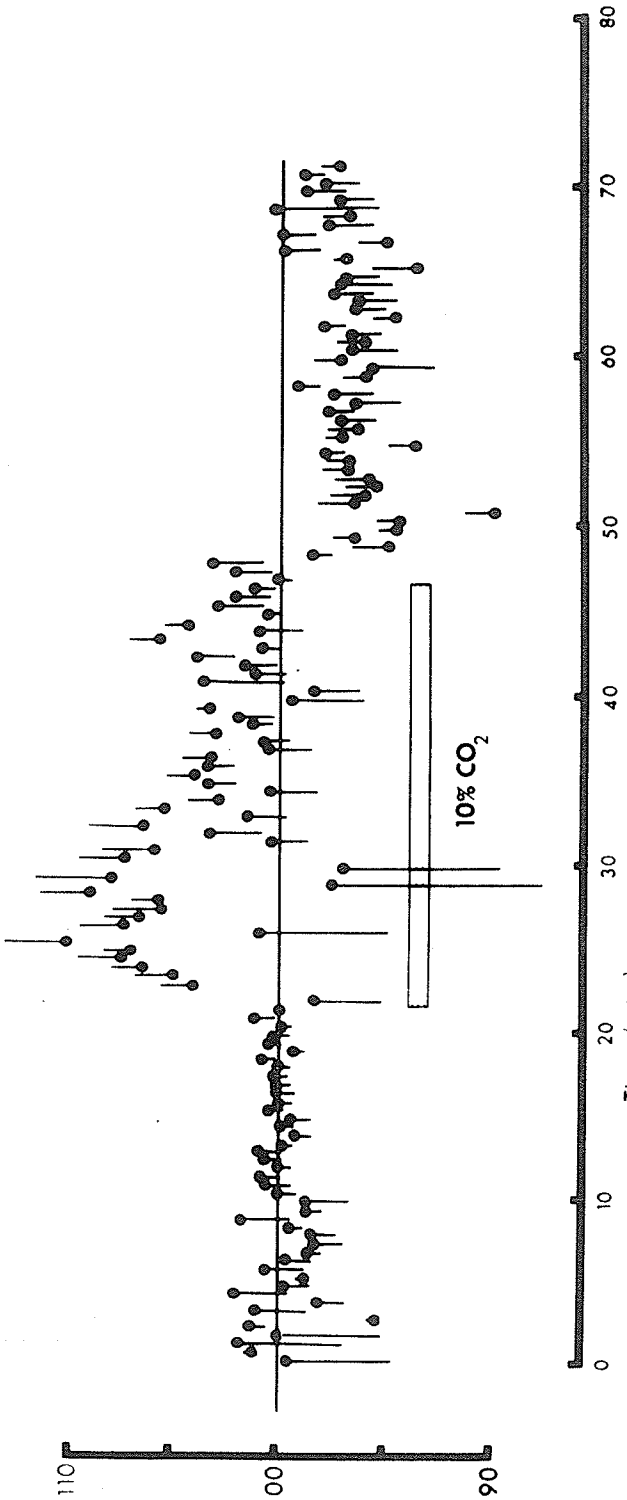


Fig. 21. The SPBR threshold-elevating effect of inhalation of 10% CO_2. Averaged data from 30 trials in 19 animals.

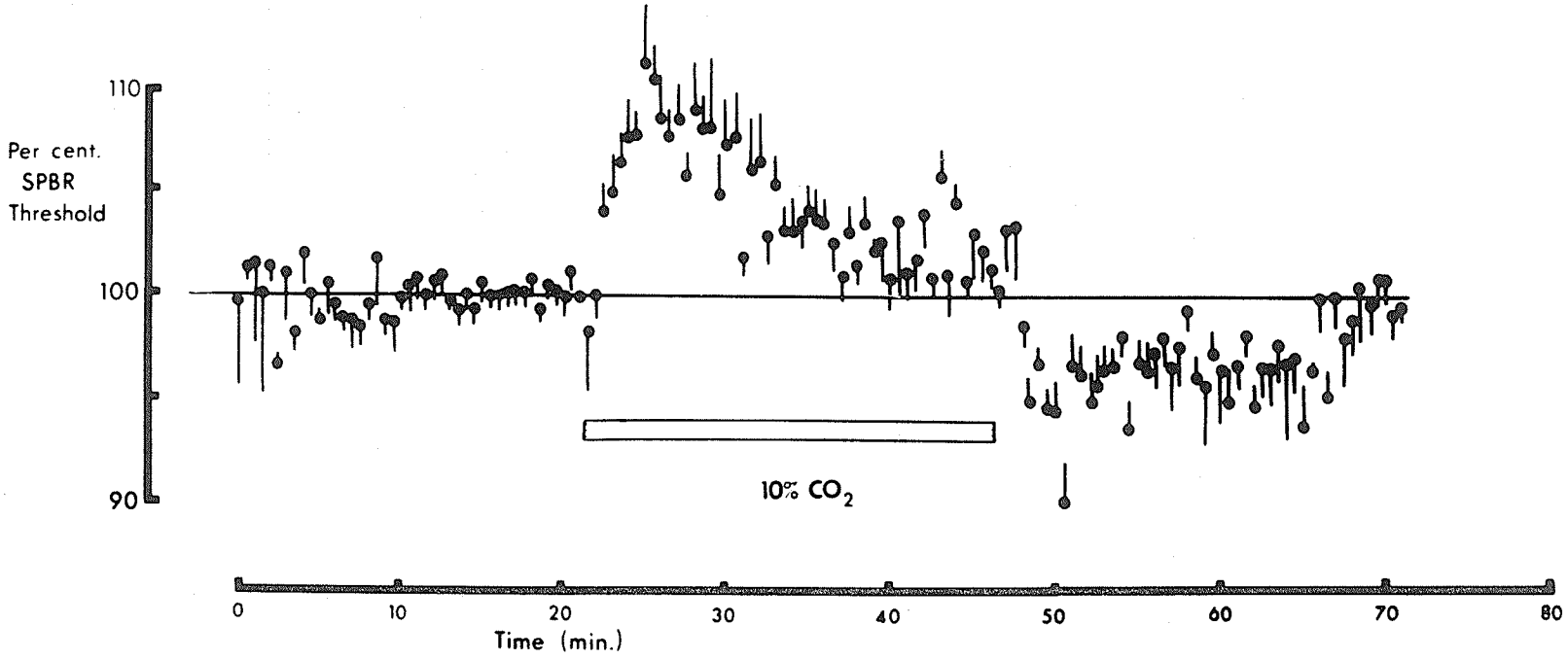


Fig. 22. The SPBR threshold-elevating effect of inhalation of 10% CO_2. Averaged data from 28 trials in 19 animals, exclusive of the 2 trials in which the threshold was lowered.

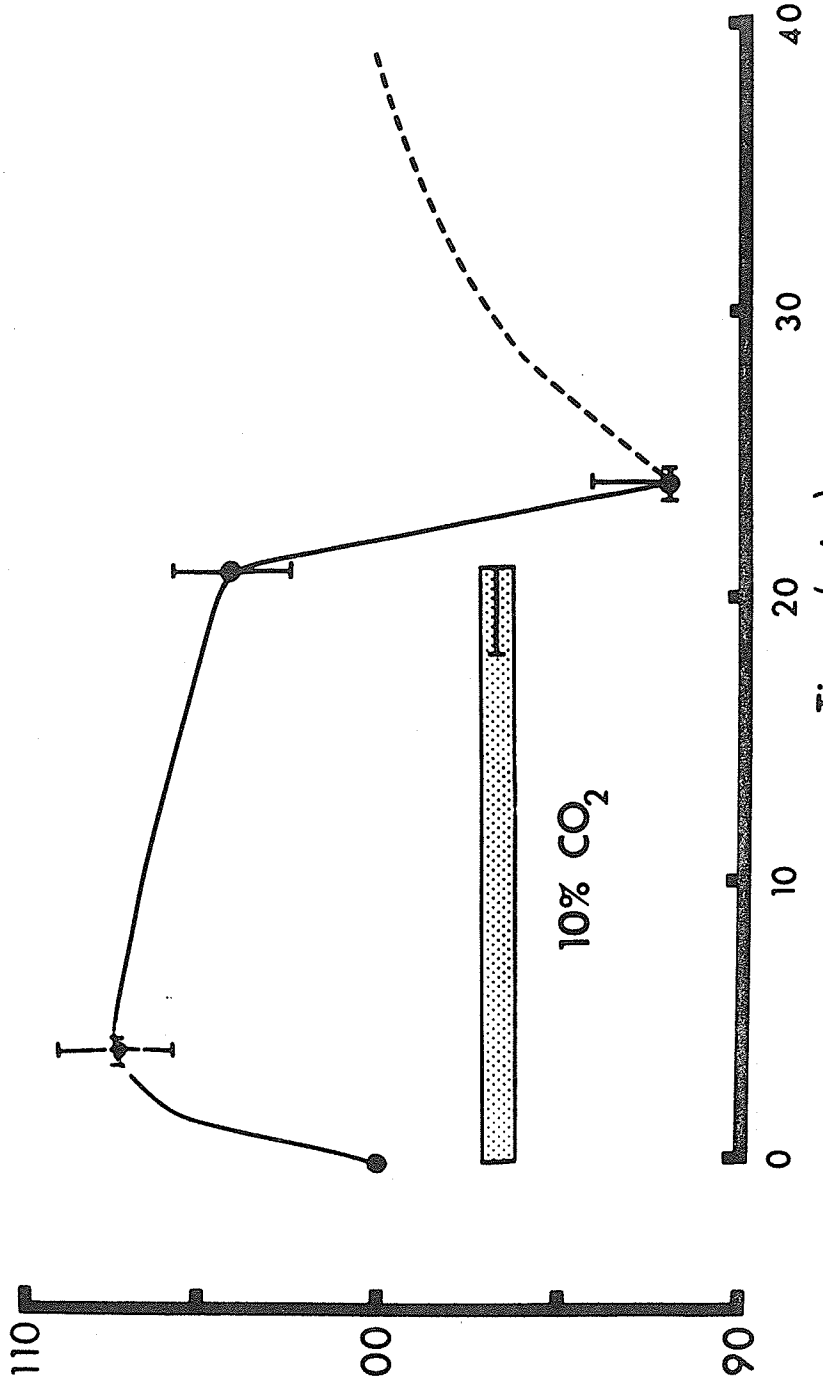


Fig. 23. Means and standard errors of the SPBR threshold and time, of the peak threshold elevation, the end of the gas inhalation, and of the withdrawal rebound for the entire group of 30 trials in 19 animals.

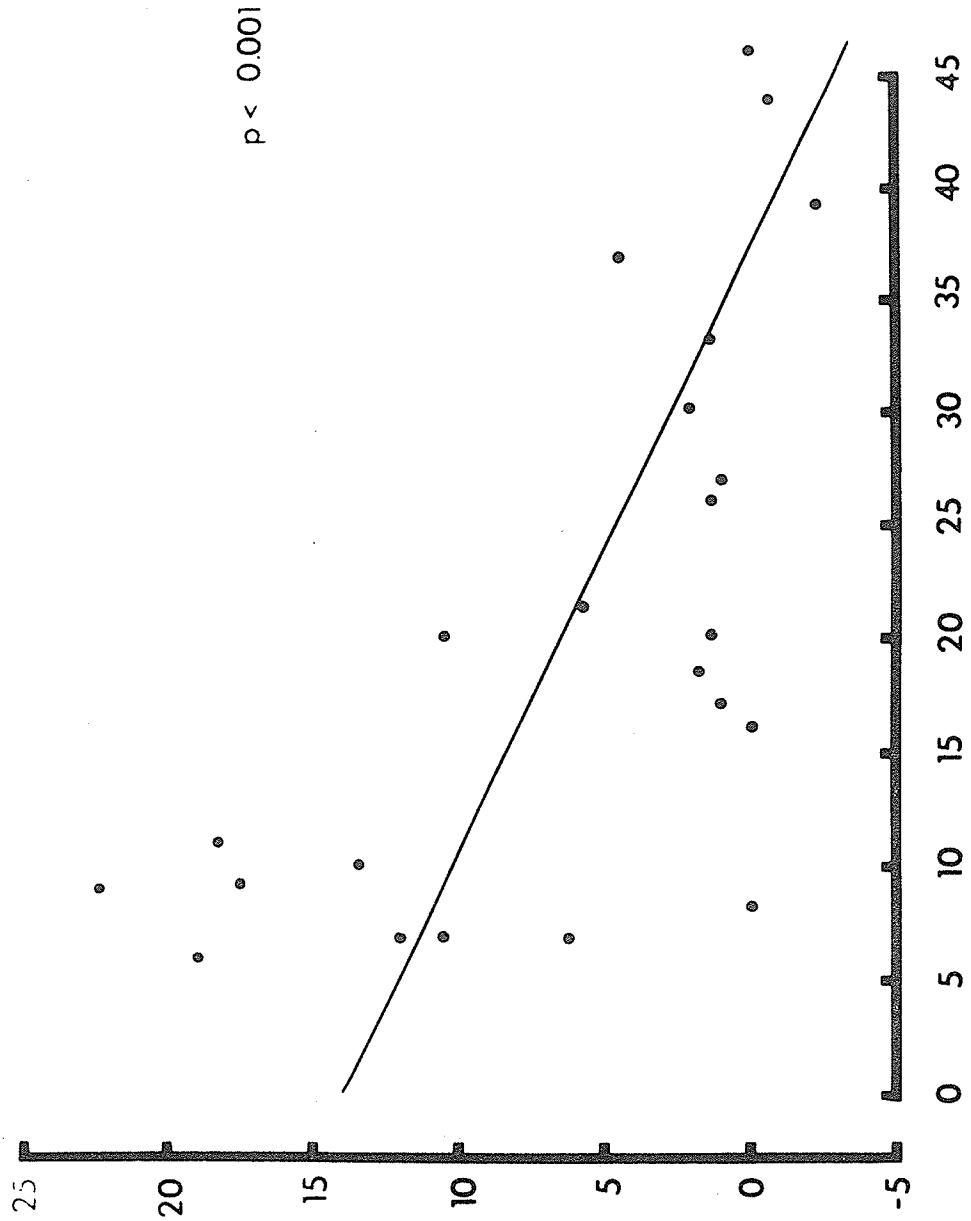


Fig. 24. The regression of the SPBR threshold measured just prior to termination of the 10% CO_2 inhalation, upon the duration of the inhalation.

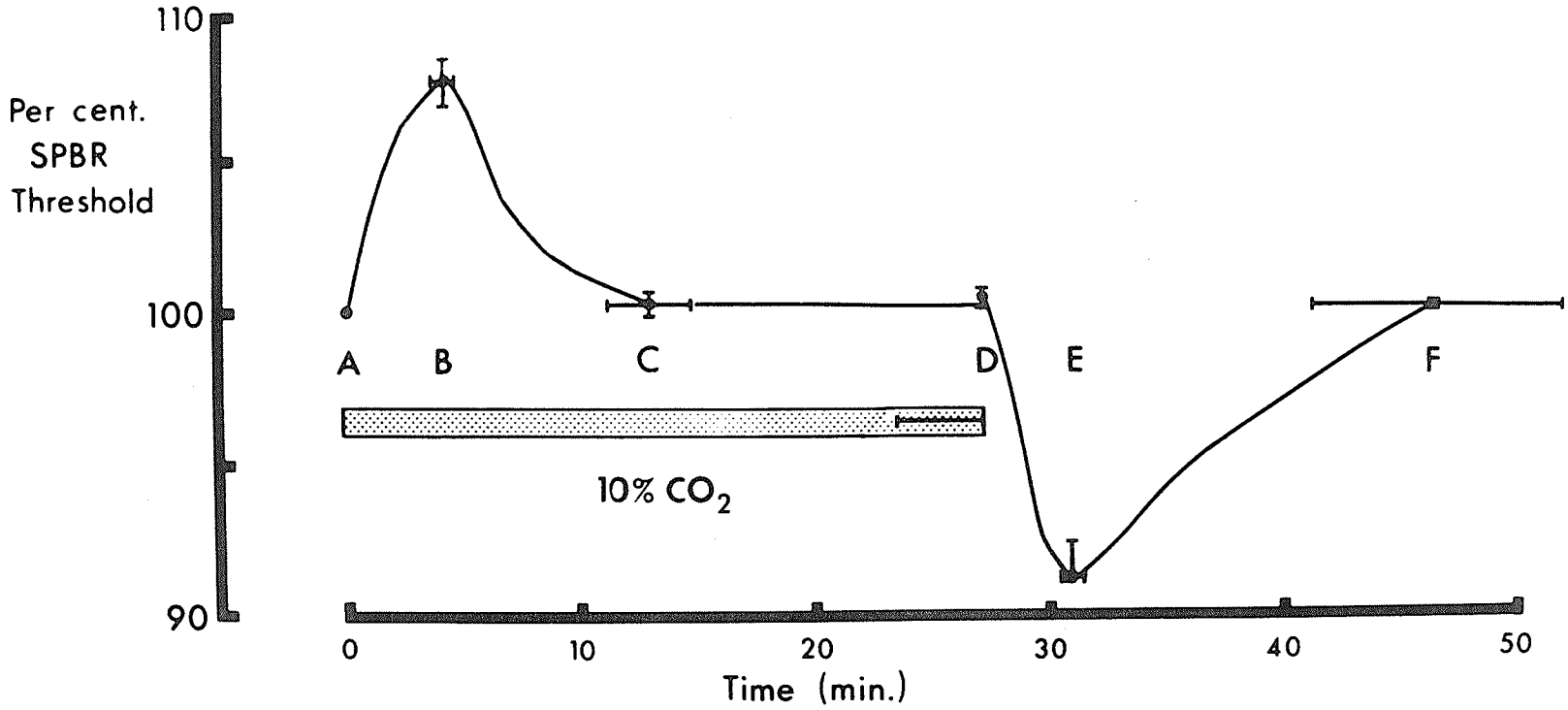


Fig. 25. Means and standard errors of the SPBR threshold and time of the peak threshold elevation (B), earliest return to the pre-inhalation level (C), end of gas inhalation (D), withdrawal rebound (E), and final return to pre-inhalation level (F). The times of (E) and (F) are measured from (D). The data are derived from the 12 trials in 11 animals where the SPBR threshold returned during the gas inhalation to within 1% of the pre-inhalation level.

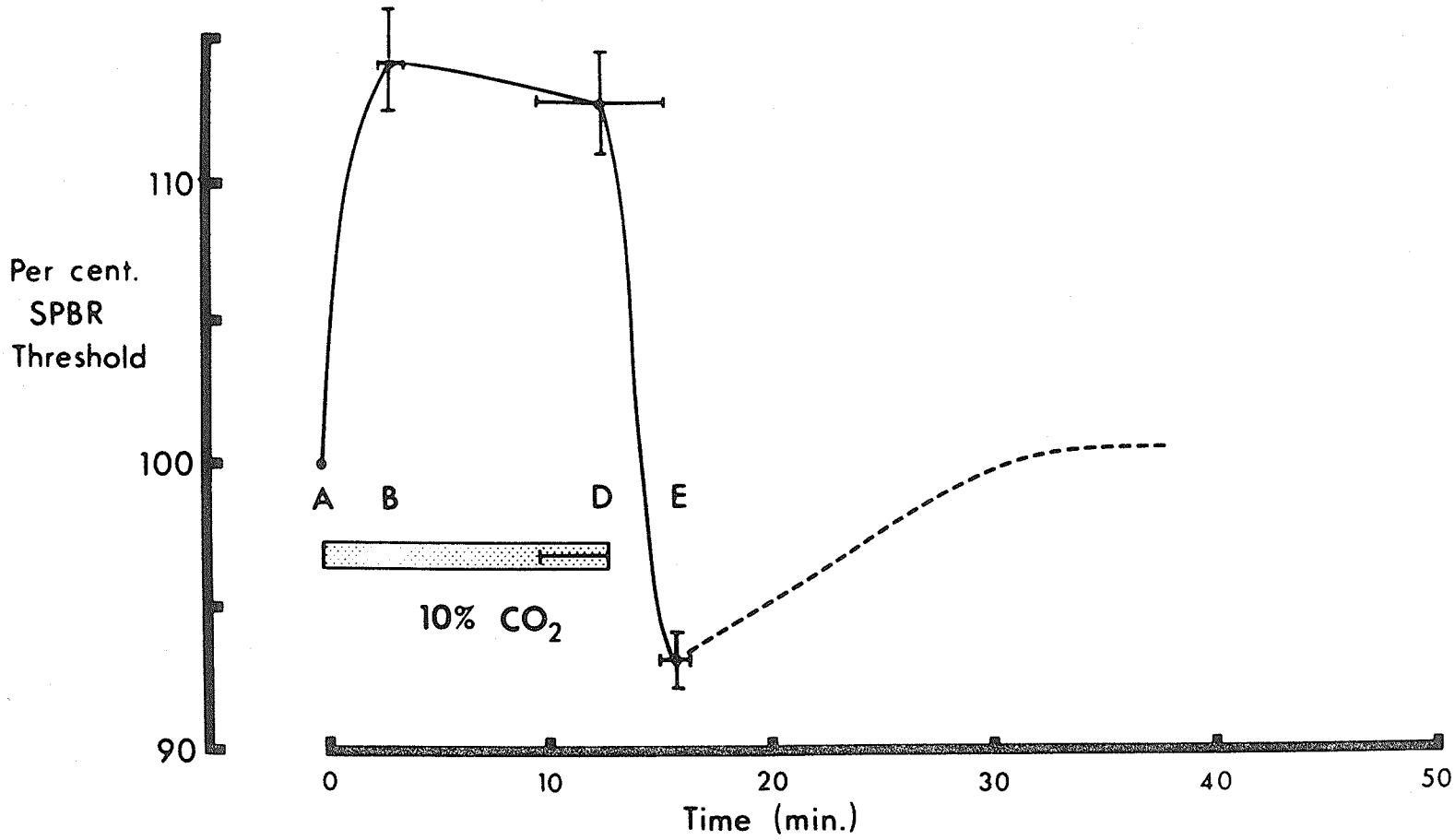


Fig. 26. Means and standard errors of the SPBR threshold and time for the 12 trials in 10 animals where the SPBR threshold was elevated during inhalation of 10% CO_2 but failed to return during the gas inhalation to within 1% of the pre-inhalation level. The identifying letters are explained in the caption to Fig. 25.

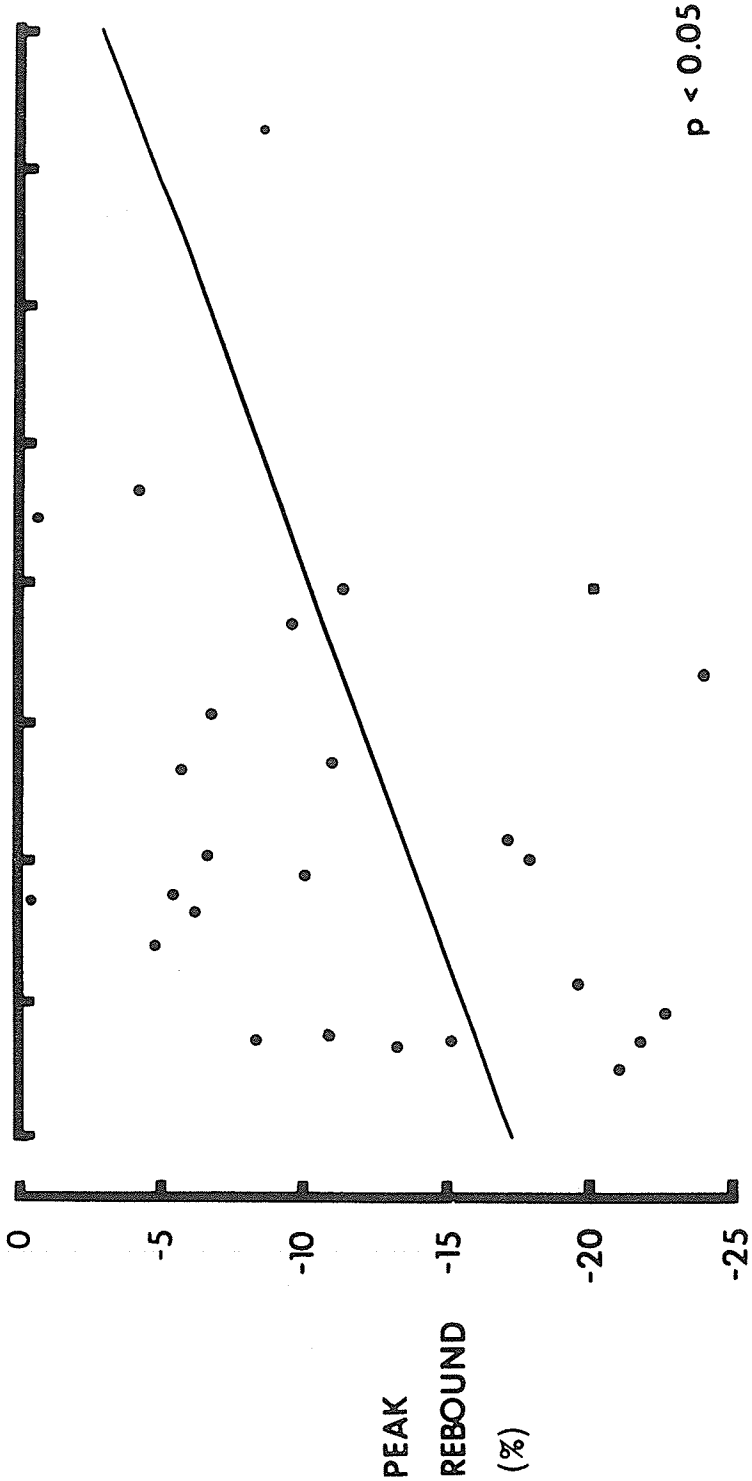


Fig. 27. Regression of the peak fall of the SPBR threshold after withdrawal of 10% CO_2 upon the duration of inhalation.

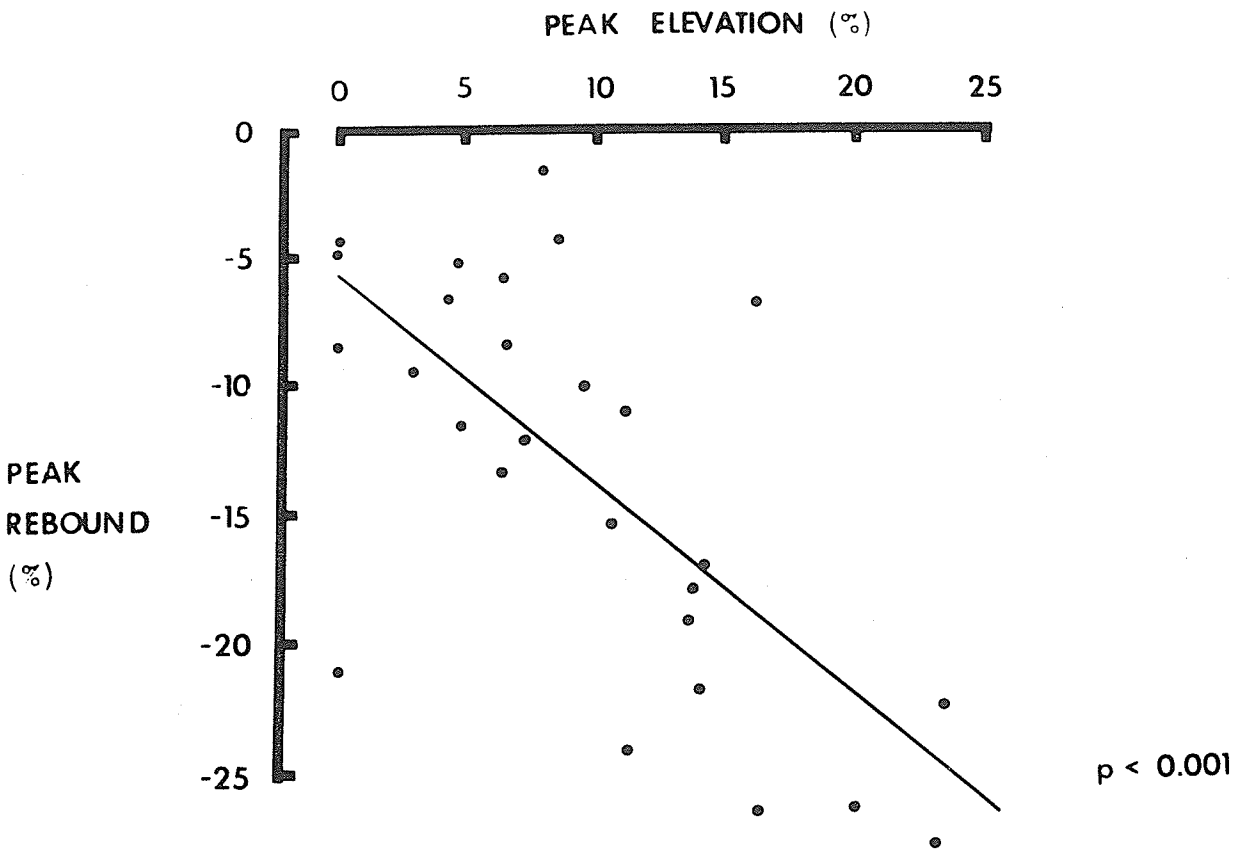


Fig. 28. Correlation between peak elevation of the SPBR threshold during 10% CO_2 inhalation, and the peak lowering of threshold after withdrawal of the gas (measured from the threshold level at the time of termination of the gas inhalation).

excitability which follows withdrawal of the gas. However, withdrawal hyperexcitability occurred in three trials, in the absence of an earlier elevation of threshold (e.g. Fig. 19).

No orderly relation was apparent between the peak elevation of threshold and the time to peak (Fig. 29), or between the peak of rebound hyperexcitability and the time to peak (Fig. 30).

The relation between duration of CO_2 inhalation and accommodation and rebound was investigated further in one cat. Inhalation of 10% CO_2 was carried out for periods of time too brief to allow accommodation to occur, and repeated several times (Fig. 31). It was found that the peak elevation of threshold diminished with each succeeding inhalation. Also, there was no rebound hyperexcitability after the first withdrawal from CO_2, but there was a distinct rebound after withdrawal from the fourth inhalation.

Administration of 10% CO_2 had no discernible effect upon the amplitude and duration of the evoked SPBR. (e.g. Fig. 17, 18, 20).

C. Hypercarbia: 3% CO_2.

Administration of 3% CO_2 was carried out seven times in four cats. On three occasions (two cats) it had no effect; on two occasions (one cat) the threshold was lowered for a short period with subsequent recovery to the preinhalation level (Fig. 32); and on two occasions (two cats) the threshold was elevated to a small extent.

On two of the three occasions when there was no effect, withdrawal of the gas mixture was followed by hyperexcitability (Fig. 33). Withdrawal hyperexcitability was also seen on one of the two occasions when the threshold was lowered (Fig. 34).

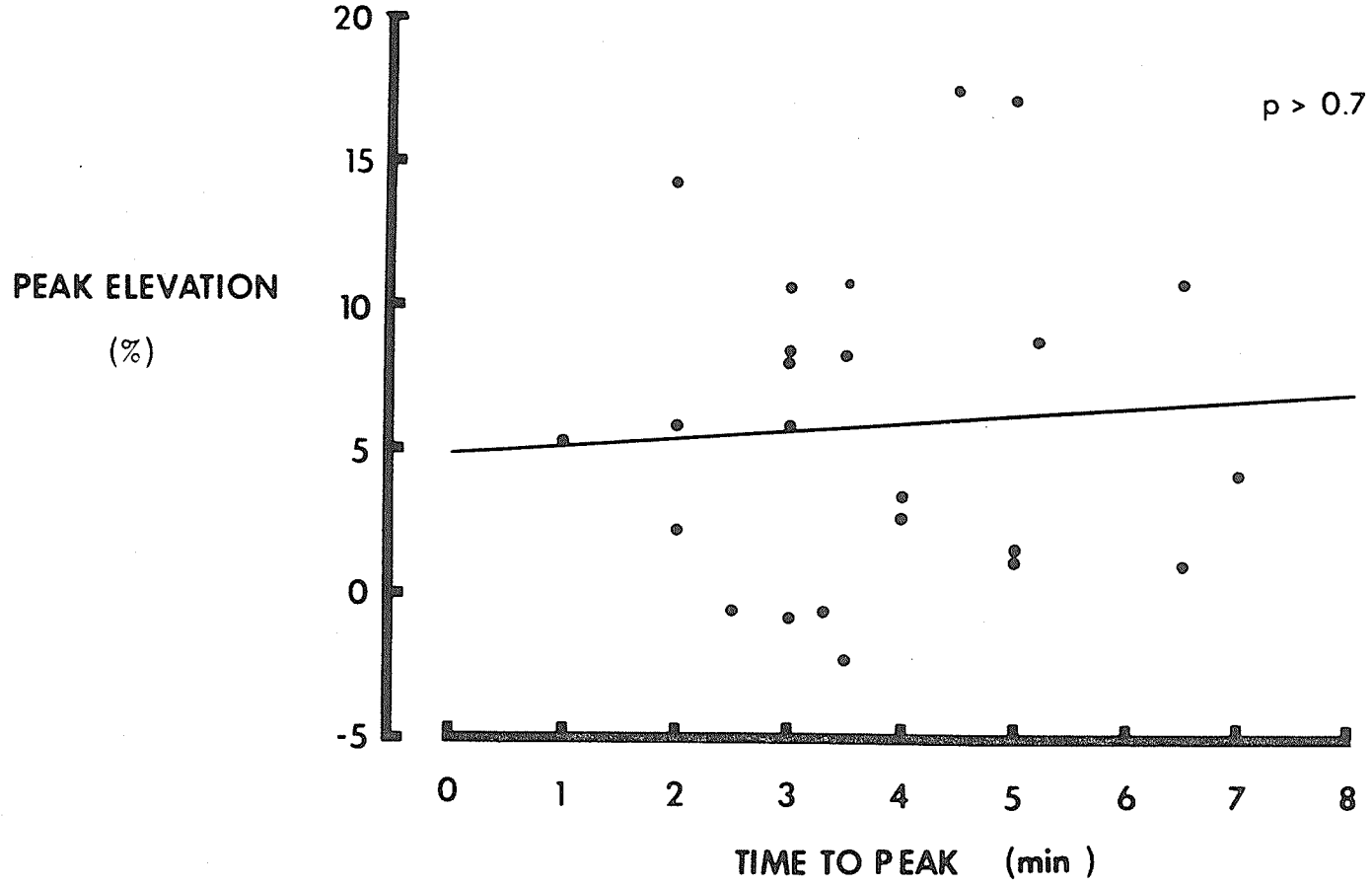


Fig. 29. Lack of correlation between peak elevation of the SPBR threshold during inhalation of 10% CO_2, and the time to peak.

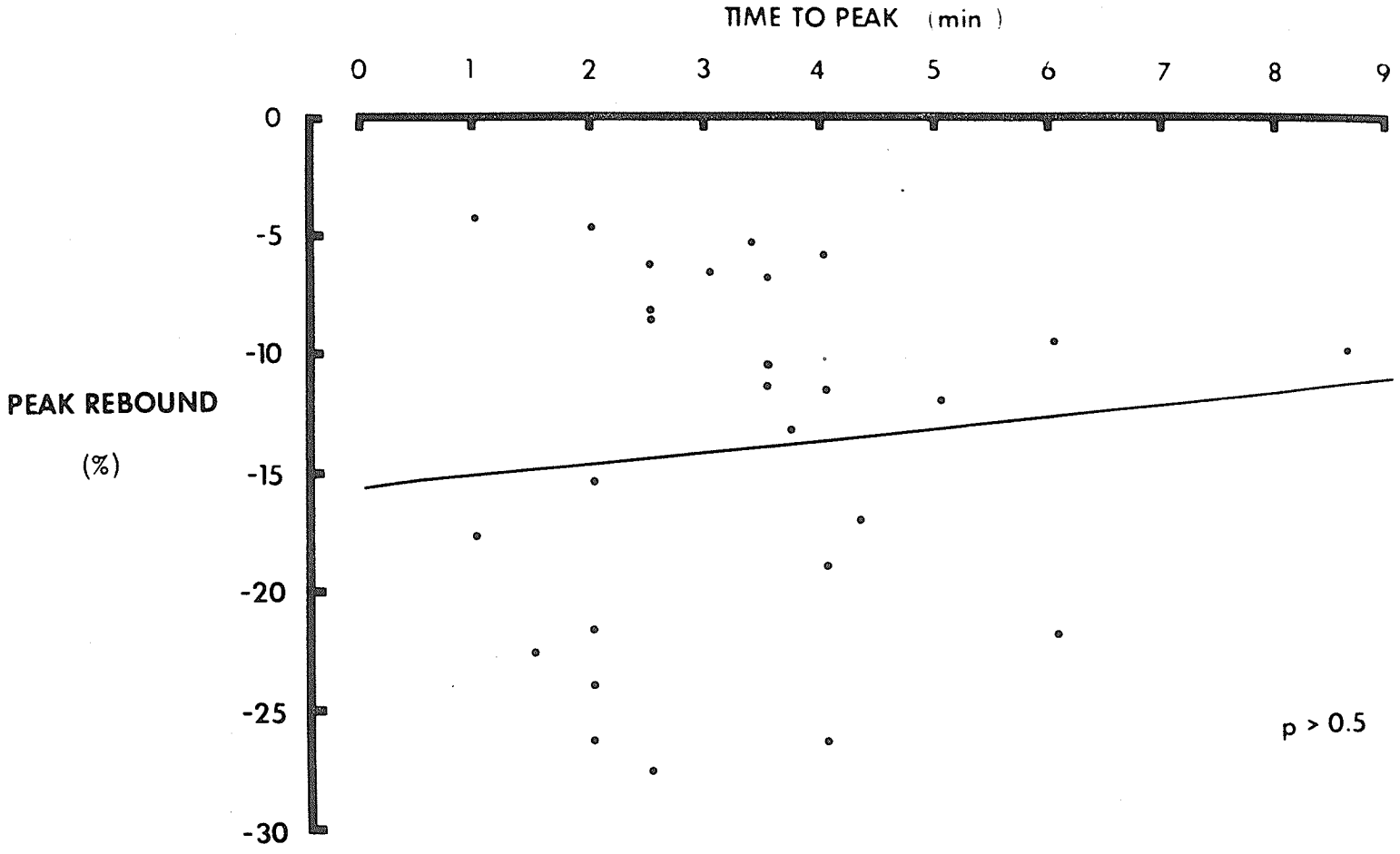


Fig. 30. Lack of correlation between peak lowering of the SPBR threshold upon withdrawal of 10% CO_2, and the time to peak (measured from the time of termination of the gas inhalation.)

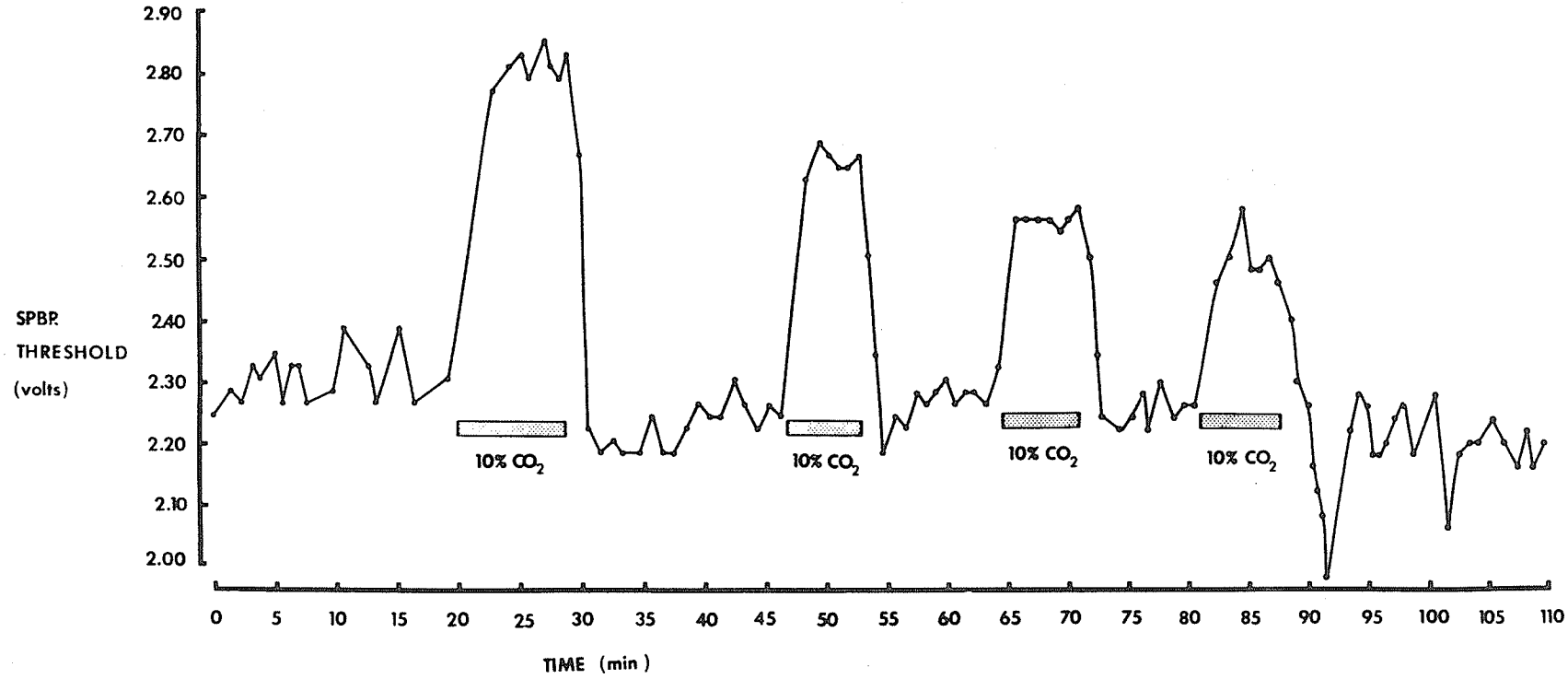


Fig. 31. The effect of repeated brief inhalations of 10% CO_2 upon the SPBR threshold.

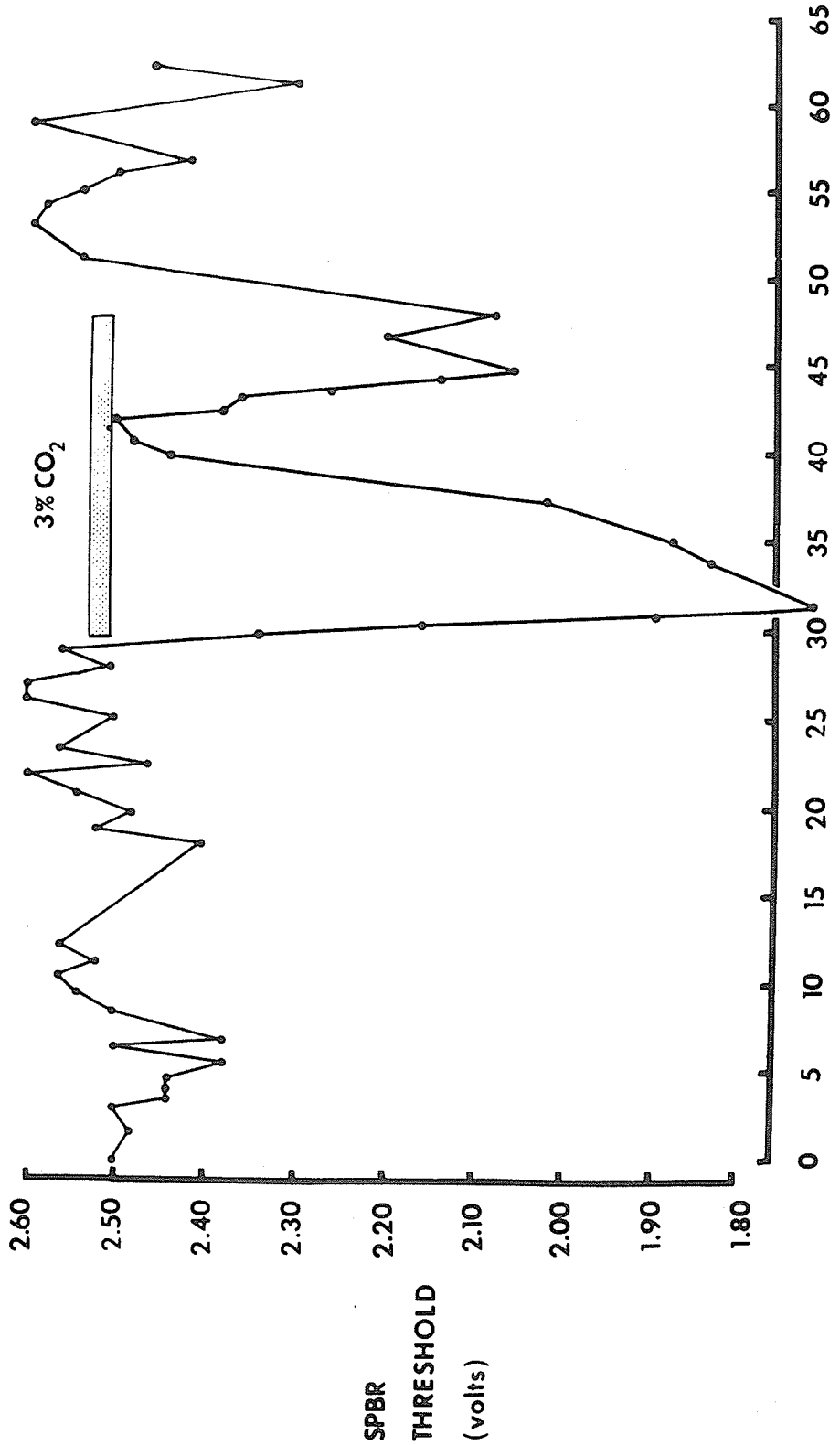


Fig. 32. The effect of inhalation of 3% CO_2 upon the SPBR threshold. Note the tendency for the effect to terminate before withdrawal of the gas mixture.

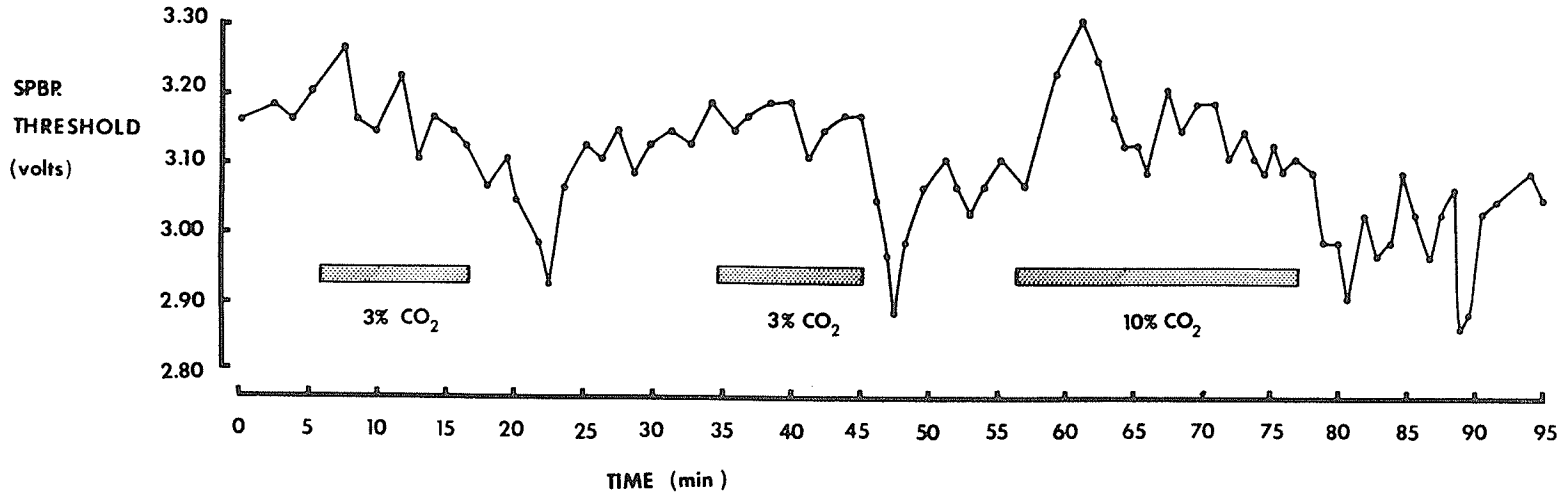


Fig. 33. Hyperexcitability upon withdrawal of 3% CO_2, in the absence of any alteration of the SPBR threshold during the gas inhalation. Note that 10% CO_2 clearly produces elevation of the threshold.

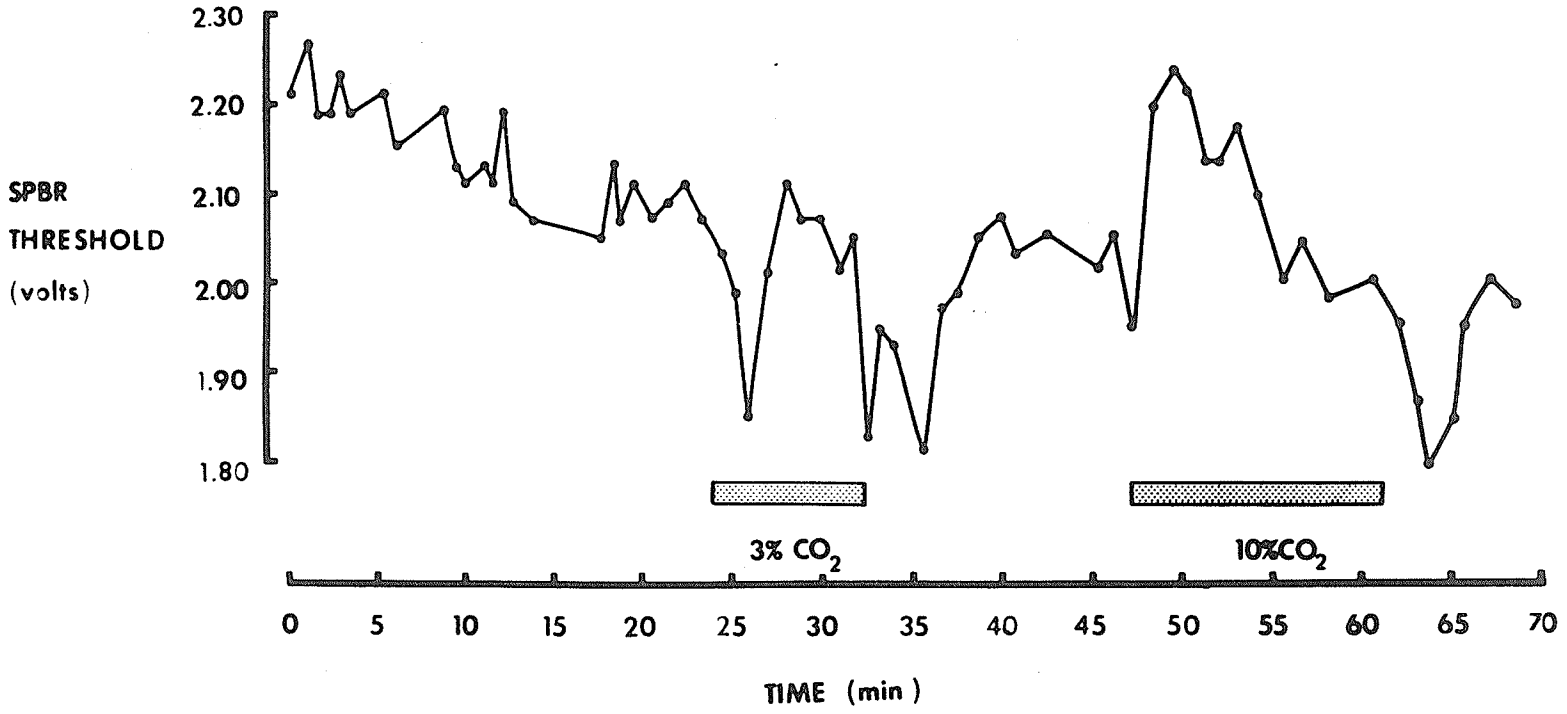


Fig. 34. The occurrence of hyperexcitability both during and after inhalation of 3% CO_2.

D. Hypercarbia: 20% & 30% CO_2.

The effects of inhalation of 20% CO_2 were examined on one occasion and those of 30% CO_2 on four occasions. In all instances the SPBR was rapidly abolished. On only one occasion did the response fail to return after withdrawal of the gas mixture.

No rebound hyperexcitability was observed upon return of the response, but in one instance withdrawal of 30% CO_2 was followed by the development of spontaneous recurrent burst activity ("afterbursts", Burns, 1954, 1955).

5. Investigations of the mechanism of CO_2-induced elevation of SPBR threshold:

The observations made here on the cortical depressant action of 10% CO_2, just described in the preceding section, are intriguing for several reasons. First, the effect is reproducibly short-lived, and is associated with a remarkably consistent return of cortical excitability to the precise level prevailing before exposure to CO_2. Second, termination of CO_2 inhalation results in a short-lived increase of excitability which also is followed by a return to the preinhalation level of excitability. This sequence of events is highly suggestive of some homeostatic mechanism which opposes the depressant action of CO_2, but which, after withdrawal of the gas mixture, persists unopposed for a short while to lower the threshold even further.

Because of the inevitable interest enjoined by the homeostasis of the cerebral cortex, an attempt was made in this study to investigate the mechanism of these effects. This initially took the form of a study of the interaction of CO_2 inhalation with various pharmacologic treatments.

Later, the activity of cortical neuronal units was studied before, during, and after CO_2 inhalation. The drug interaction observations are presented first, with a preamble concerning the use of each agent. It must be stressed that because of the unsettled state of present knowledge about the structural and pharmacologic complexity of the cerebral cortex these studies can be viewed only as preliminary.

All drugs were given by intravenous injection of approximately isotonic solutions. Topical application onto the cortex was not attempted because of the almost inevitable shunting of stimulating electrodes and consequent artifactual changes in excitability.

A. Carbonic anhydrase inhibition.

The effect of acetazolamide upon the SPBR and its interaction with 10% CO_2 were examined in five cats. Six trials were made. Each animal was submitted to a control inhalation of 10% CO_2 lasting from 10 to 25 minutes. After withdrawal of the gas mixture the animal was allowed to breathe room air for 10 to 25 minutes to ensure stabilization of the SPBR threshold. Acetazolamide sodium (Diamox - Lederle) was then injected in a dose ranging from 26 to 70 mg/kg. Such doses are known to suppress epileptiform activity evoked by intravenous strychnine and penicillin in cat cortex (Meyer, Gotoh & Tazaki, 1961).

Upon injection of the drug there was an immediate elevation of SPBR threshold, followed in a few minutes by a return to the control level. This was seen in four of the five trials made while the cat was breathing room air (e.g. Fig. 35, 36, 37). Injection of an identical volume of isotonic saline, with flushing of the venous cannula in a similar manner did not produce this or any other effect. No effect developed

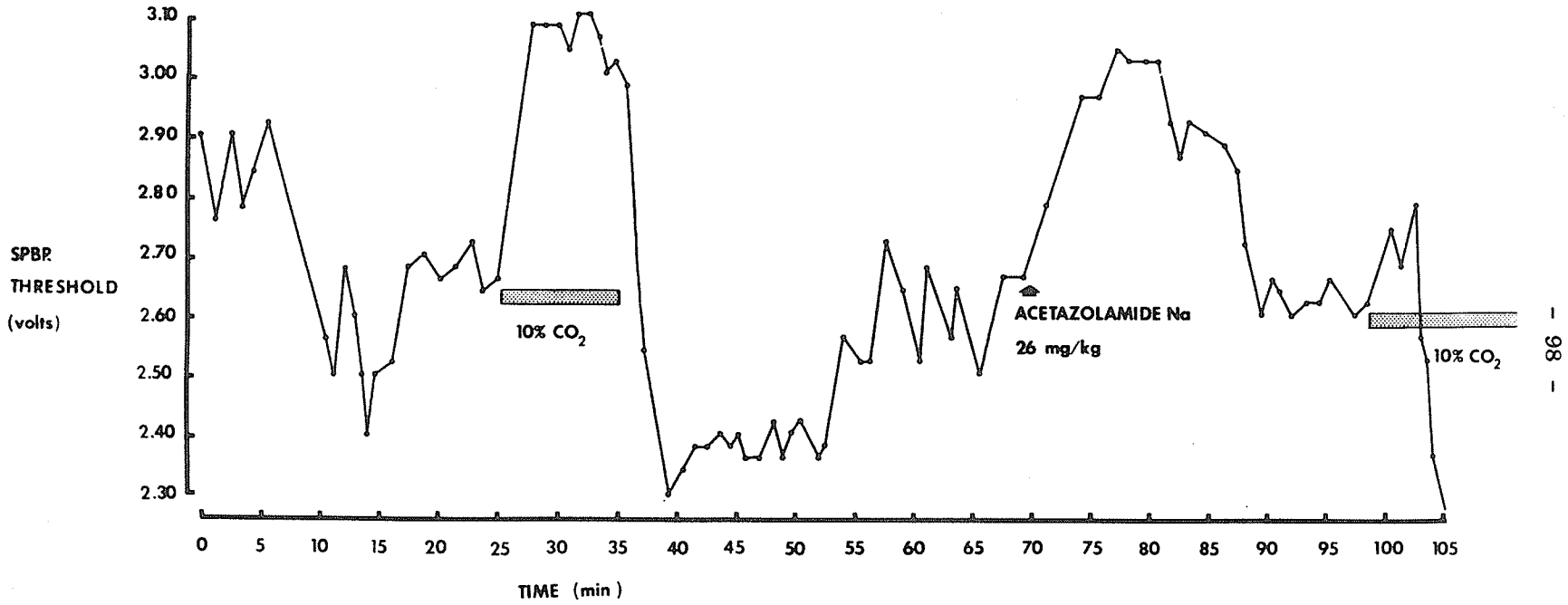


Fig. 35, part 1. The effect of acetazolamide upon the SPBR threshold, and its tendency to reverse the effect of subsequent 10% CO_2 inhalation. (This figure overlaps with the figure shown on the next page.)

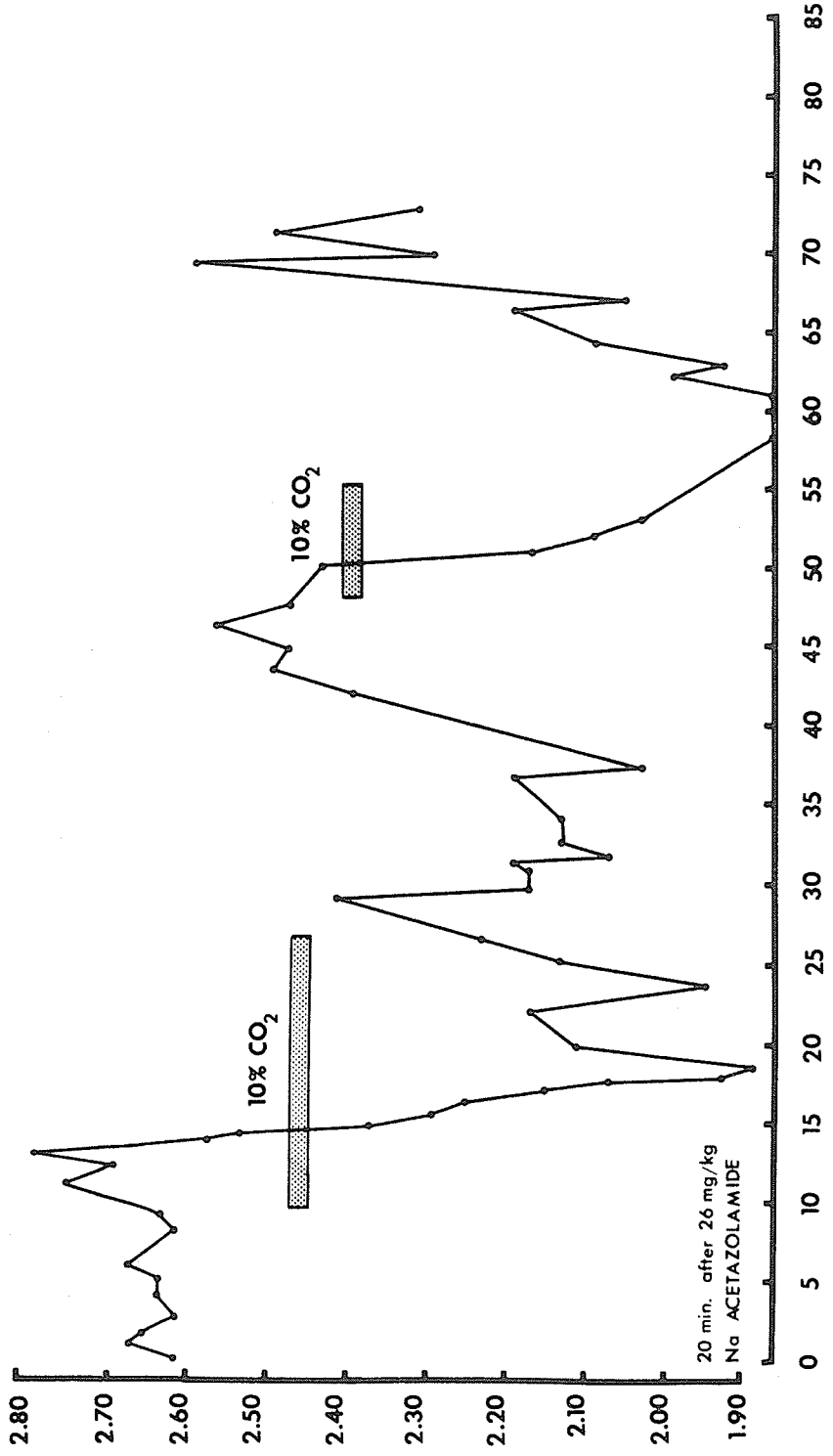


Fig. 35, part 2. The effect of acetazolamide upon the SPBR threshold, and its tendency to reverse the effect of subsequent 10% CO_2 inhalation. (This figure overlaps with the figure shown on the next page.)

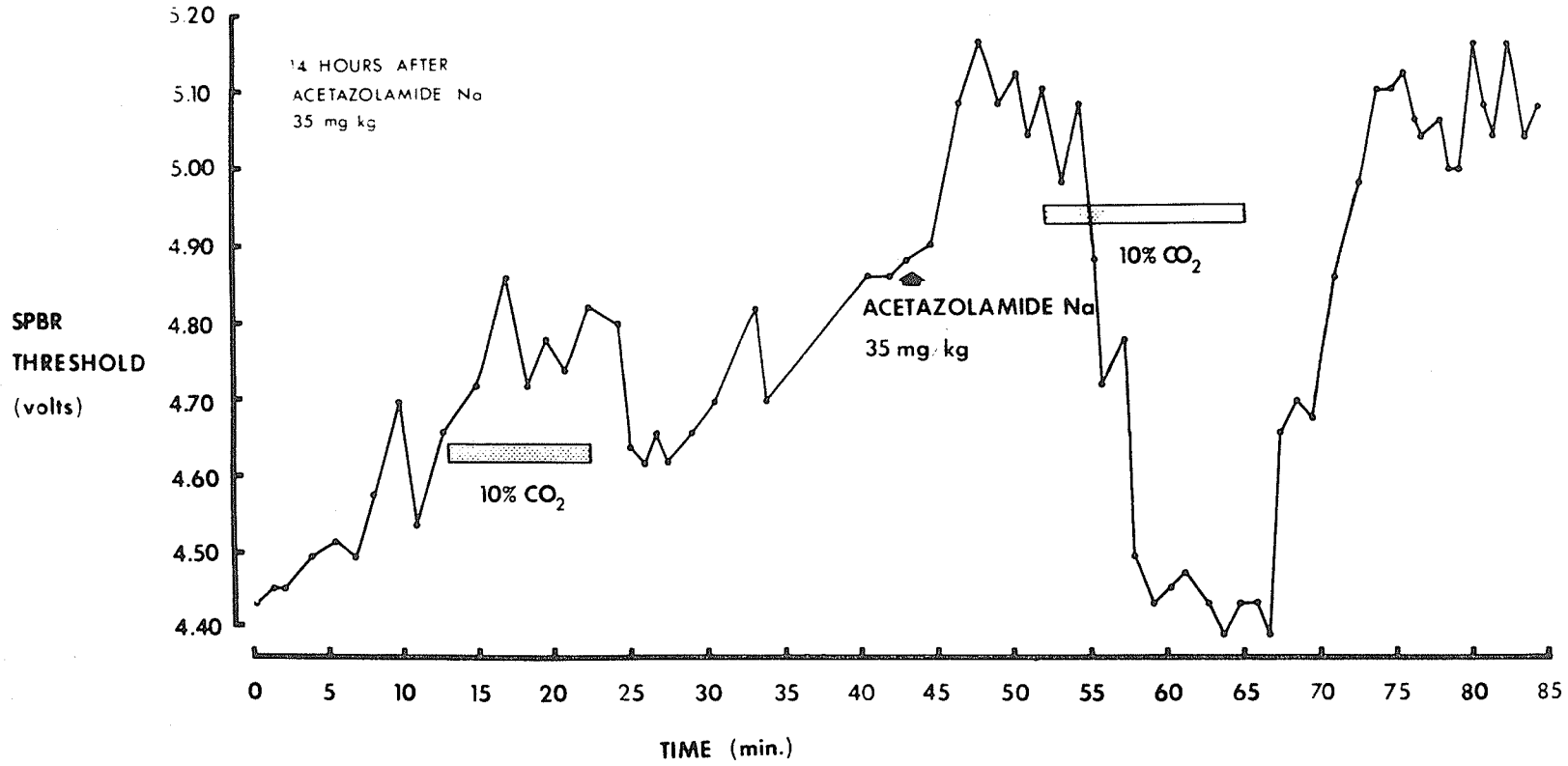


Fig. 36. Effect of acetazolamide upon the SPBR threshold and its tendency to reverse the effect of subsequent CO_2 inhalation.

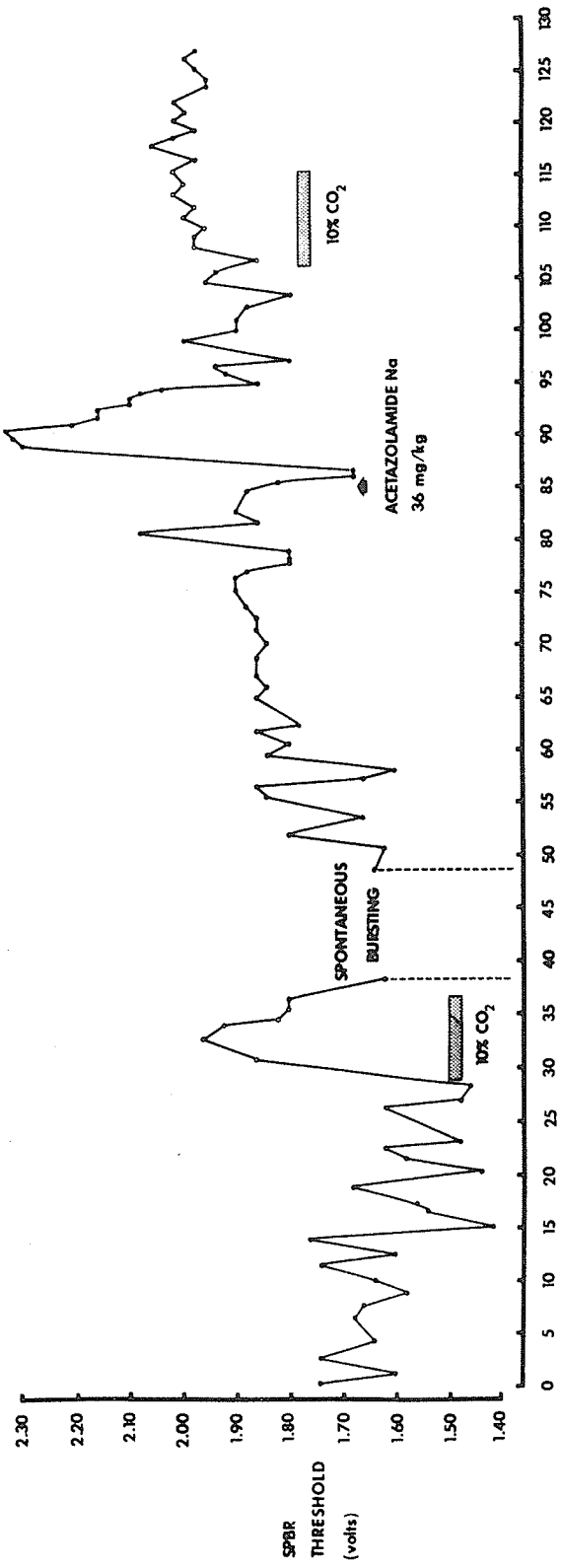


Fig. 37. Effect of acetazolamide upon the SPBR threshold and its tendency to block the effects of subsequent CO_2 inhalation.

in the fifth instance.

The sixth trial was made while the cat was breathing 10% CO_2 (Fig. 38). Here acetazolamide had no effect so long as the gas inhalation was continued, and did not prevent the development of accommodation to the depressant action of CO_2. However, upon withdrawal of the gas mixture, hyperexcitability failed to occur. In the same cat, 10% CO_2 inhalation, repeated three hours later, produced a short-lived elevation of threshold followed by a profound fall. After removal of the gas mixture, the threshold returned to the preinhalation level.

Another example of what may be termed CO_2-reversal by acetazolamide is shown in Fig. 35. An initial inhalation of 10% CO_2 elevated the threshold and rebound hyperexcitability followed its termination. After acetazolamide injection, 10% CO_2 produced a sharp fall in threshold for a period of time lasting considerably longer than the duration CO_2 inhalation.

The cat from which Fig. 38 was obtained was also studied fourteen hours after the initial dose of acetazolamide; the results are shown in Fig. 36. At this time 10% CO_2 inhalation appeared to have no effect other than to produce a minor fall in threshold. After the second dose of the drug, however, 10% CO_2 administration caused a pronounced fall of threshold.

In another cat, acetazolamide blocked all effects of 10% CO_2 inhalation. This is illustrated in Fig. 37. Three control trials of inhalation of the gas mixture were carried out in this experiment, of which one is shown in the figure. All of these produced a short-lived rise in threshold, followed by the development of spontaneous recurrent

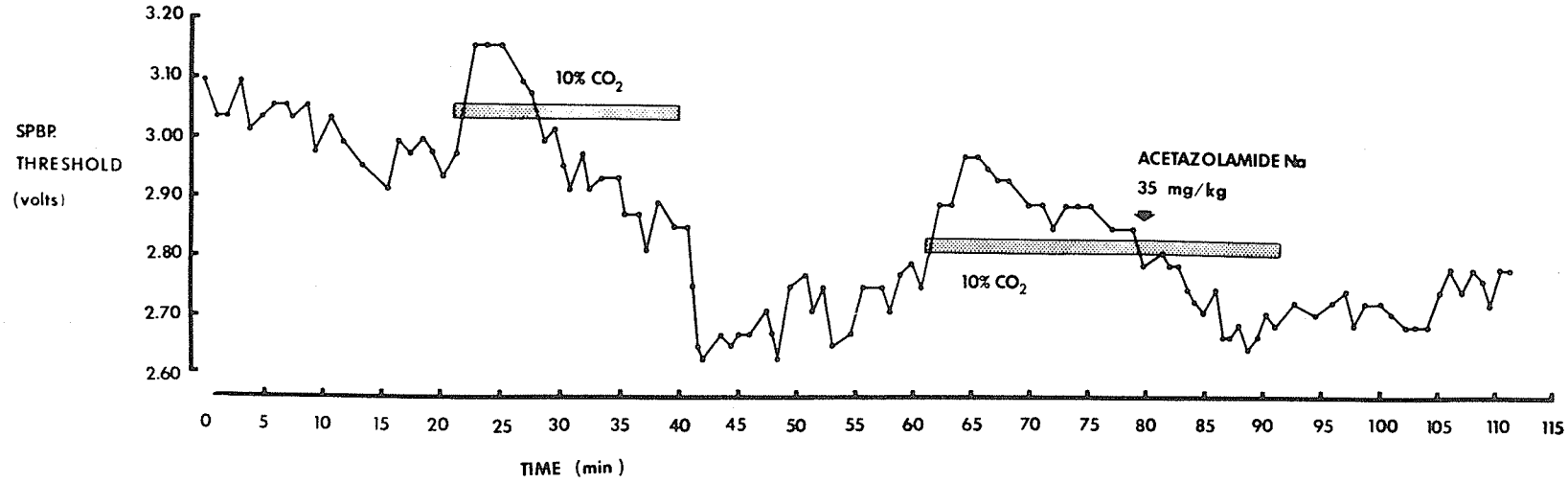


Fig. 38. Effect of acetazolamide upon the SPBR threshold when given during CO_2 inhalation. Note the absence of withdrawal hyperexcitability after the drug has been injected.

burst activity upon removal of the gas mixture. Acetazolamide in this cat produced a sharp elevation of threshold, and prevented the development of any effects either during or after a subsequent inhalation of 10% CO_2.

B. Cholinergic antagonism.

It was considered possible that 10% CO_2 might bring about an alteration of cortical excitability by altering the balance between cholinergic neurohumoral transmission (Evidence in favor of whose occurrence in the cerebral cortex is accumulating; Hebb, 1970) and other factors or transmitters influencing cortical excitability. Hence the interaction between various cholinergic antagonists and 10% CO_2 inhalation was examined.

i) mecamylamine.

The effects of the nondepolarizing ganglionic blocking agent mecamylamine were examined in one cat. Since mecamylamine is an uncharged secondary amine it can be expected not to be excluded from the brain.

A control inhalation of 10% CO_2 produced elevation of the SPBR threshold, with accommodation, and rebound hyperexcitability occurred upon removal of the gas mixture (Fig. 39). Subsequent injection of mecamylamine HCl (Inversine - Merck Sharp & Dohme) 0.5 mg/kg produced no direct effect, but the drug appeared to potentiate the depressant action of CO_2, as evidenced as a reversible abolition of the response by a succeeding inhalation of 10% CO_2.

ii) nicotine.

The effects of nicotine were studied in three cats. Nicotine sulfate (Sigma Chemical Co.) in doses of 22 and 50 μg/kg had no effect nor did it alter the effects of 10% CO_2 inhalation. In the third experiment, however, in a dose of 0.1 mg/kg it rapidly abolished the response

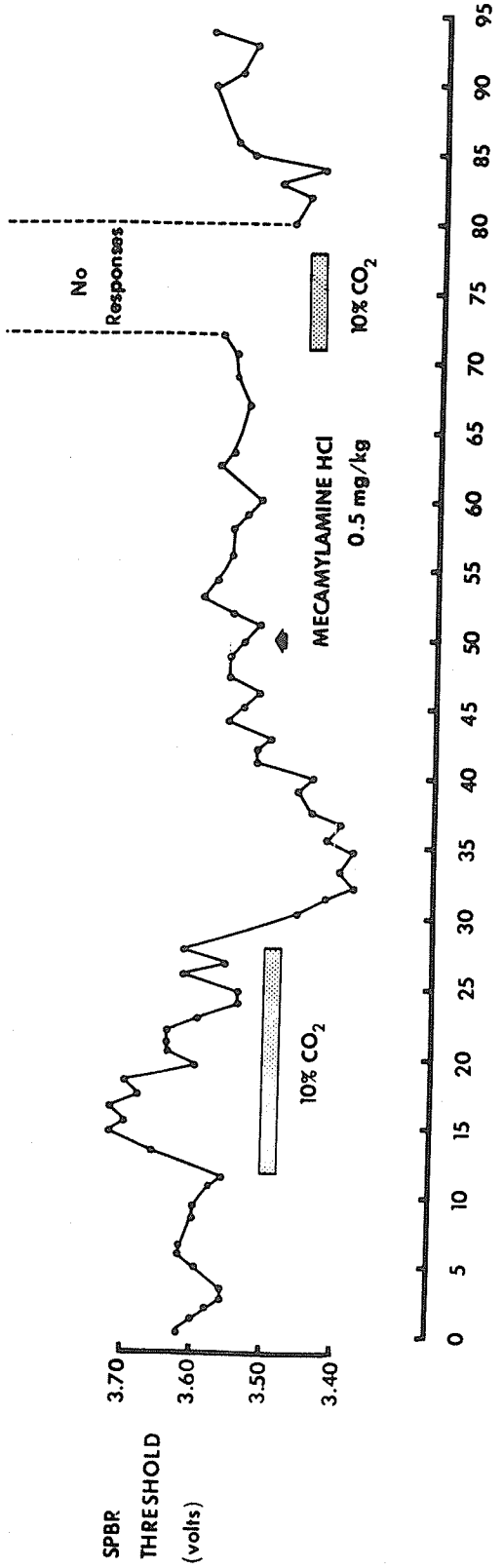


Fig. 39. Lack of effect of mecamylamine upon the SPBR threshold and its tendency to increase the depressant action of CO_2.

after an initial transient fall of threshold. Return of the response did not occur till several hours later. The effect upon respiration in this experiment was to increase rate and depth, while the blood pressure was lowered from 140/60 to 85/35 ten minutes after the injection. At the time of return of the response the blood pressure had recovered to 120/55, and the respiratory pattern had also normalized.

iii) scopolamine and atropine.

The muscarinic cholinergic antagonist scopolamine was studied in one cat (Fig. 40). Control inhalations of 10% CO_2 elevated the SPBR threshold. Removal of the gas mixture was followed by a phase of hyper-excitability characterized by development of spontaneous recurrent burst activity which precluded measurement of the SPBR threshold. Scopolamine HCl (Sigma) produced no apparent effect, nor did it alter the effects of a subsequent inhalation of 10% CO_2, or the rebound spontaneous activity which followed withdrawal of the gas mixture.

In three other experiments the muscarinic antagonist atropine (atropine sulfate - British Drug Houses) was studied in doses of 17, 48, and 70 μg/kg. These had no apparent effect nor did they modify the effects of 10% CO_2.

C. γ-Aminobutyric acid (GABA)

Cortical synaptic inhibition (but not excitation) is associated with increased release of GABA from the surface of the cerebral cortex (Mitchell & Srinivasan, 1969; Iversen, Mitchell & Srinivasan, 1971) and iontophoretically applied GABA depresses cortical neuronal discharge in the cerebral cortex (Krnjevic & Phillis, 1963). The mechanism of this effect has been shown to reside probably in hyperpolarization of the

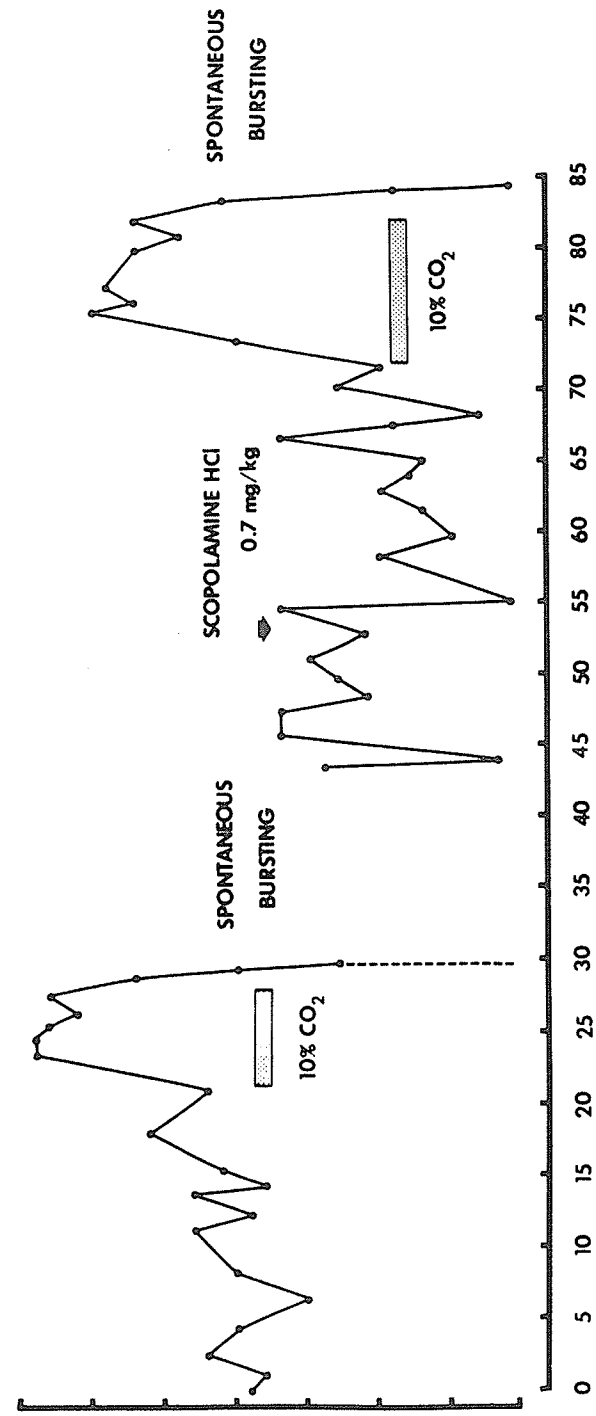


Fig. 40. Lack of effect of scopolamine upon the SPBR threshold and lack of interaction with CO_2.

membrane potential (Krnjevic & Schwartz, 1966; 1967b). Since this effect is reversed by iontophoretic injection of chloride into the interior of the neuron (Krnjevic & Schwartz, 1967b), its ionic basis is probably the same as that for the inhibitory postsynaptic potential (IPSP) studied in spinal cord neurons (Coombs, Eccles & Fatt, 1955), if reversal potentials of iontophoretically evoked phenomena can be compared to reversal potentials of synaptic potentials (Calvin, 1969). In any event, reversal potentials for GABA-induced polarization of cortical neurons have been found to correlate closely with reversal potentials for IPSP's (Dreifuss, Kelly & Krnjevic, 1969). It would thus appear that GABA may be a physiologic inhibitory transmitter in the cerebral cortex. Since CO_2 is known to augment cerebral GABA (Woodbury & Vernadakis, 1958; Woodbury & Karler, 1960), it was thought possible that it might be involved in the effects of CO_2 upon the SPBR. To examine this, the effects of amino-oxyacetic acid, D,L-GABA, and bicuculline were studied.

i) Amino-oxyacetic acid (AOAA).

AOAA is a pyridoxal phosphate antagonist which inhibits the enzymes glutamic decarboxylase and GABA transaminase *in vitro* (Roberts, Wein & Simonsen, 1964). Since the latter is selectively inhibited *in vivo* (Roberts & Kuriyama, 1968), AOAA will impair degradation of GABA and increase its steady-state intracellular level in those cells where these enzymes occur. AOAA has this effect in the cerebral hemispheres, where it also raises the threshold for electroshock-induced seizures (Kuriyama, Roberts & Rubinstein, 1966).

AOAA hemihydrochloride (Sigma) was studied in four cats. A period of at least one hour was allowed to elapse after its intravenous

administration before testing its interaction with 10% CO_2. This procedure took account of the long latency that has been reported for the onset of the action of AOAA (Kuriyama, Roberts & Rubinstein, 1966; van Gelder, 1966).

In one cat, injection of AOAA 15 mg/kg was followed by a prolonged period of spontaneous recurrent burst activity which precluded study of the SPBR threshold.

In a second cat AOAA 20 mg/kg resulted in a prolonged period of epileptiform activity which also prevented further study.

In a third cat AOAA in an initial dose of 9 mg/kg had no effect upon the SPBR, but a second dose of 30 mg/kg abolished the response and led eventually to respiratory arrest.

The fourth cat was given a dose of 20 mg/kg and was amenable to subsequent study of the SPBR and the effects of 10% CO_2 (Fig. 41). An earlier control inhalation of the gas mixture elevated the SPBR threshold. After withdrawal of the gas and the passage of a sufficient period of time for stabilization of the threshold, the drug was given. There was an immediate brief fall in threshold, followed thirty minutes later by an abrupt rise to a new level where it remained for the next thirty minutes. At the end of this period 10% CO_2 was administered; the response disappeared, apparently permanently.

ii) <u>D,L-GABA.</u>

D,L-GABA (Koch-Light Laboratories, Colnbrook, England) was administered to four cats in ten doses ranging between 17 and 174 mg/kg, but it never had any effect upon the SPBR threshold. High doses were used because these apparently penetrate the blood-brain barrier in amounts

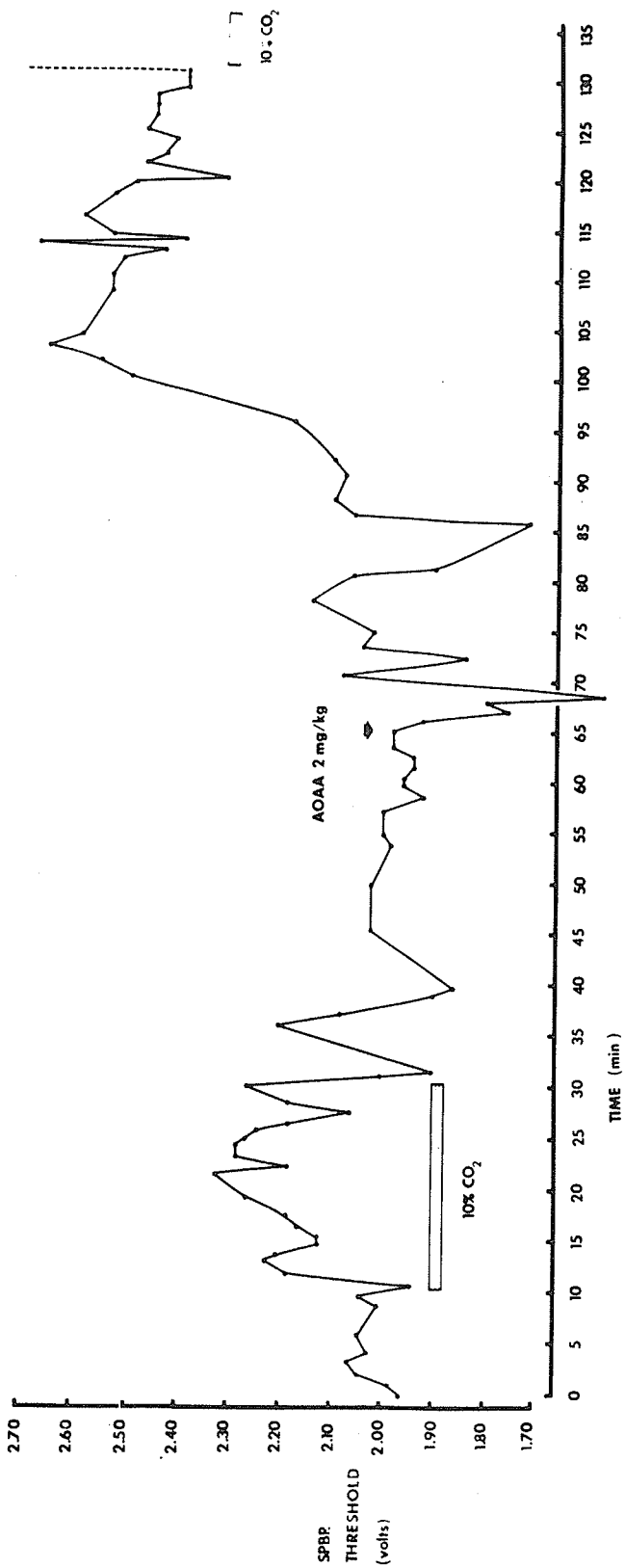


Fig. 41. Effect of amino-oxyacetic acid (AOAA) upon the SPBR threshold and its tendency to increase the depressant action of CO_2.

adequate to affect cortical function, as evidenced by block of strychnine spikes in isolated dog cortex (Rech & Domino, 1960b). Nevertheless, in none of the present experiments was there any effect.

iii) Bicuculline.

The substance bicuculline is known to be a convulsant when administered parenterally (Welch & Henderson, 1934), and there have been reports of specific antagonism of GABA by both parenteral and iontophoretic administration of this agent in subconvulsant doses (Curtis, Duggan, Felix & Johnston, 1970a; 1970b; McLennan, 1970).

Therefore, bicuculline alkaloid (Koch-Light) in a subconvulsant dose, 0.15 mg/kg, was studied in two cats. In neither cat did a single dose have any effect upon SPBR threshold, but two additional doses given to one cat led to loss of the response.

D. Adrenergic blocking agents.

Although the catecholamine-containing nerve terminals in the cerebral cortex belong to neurons whose somata lie elsewhere in the central nervous system (Fuxe, Hokfelt & Ungerstedt, 1970), some capability of catecholamine neurosecretion in the neuronally isolated cortical slab might be expected to persist for many hours (Malmfors & Sachs, 1965; Moore & Heller, 1967; Hokfelt & Ungerstedt, 1969; Fuxe, Hokfelt & Ungerstedt, 1970). In any event, CO_2 inhalation is known to activate the sympathoadrenal axis (Sechzer, Egbert, Linde, Cooper, Dripps & Price, 1960; Tenney, 1960; Morris & Millar, 1962) with the result of elevated levels of circulating catecholamines. Catecholamines do not penetrate the blood-brain barrier (Weil-Malherbe, Axelrod & Tomchick, 1959; Bertler, Falck, Owman & Rosengrenn, 1966), but since the blood-brain

barrier may not be intact in the surgically isolated cortical slab, the possibility of the involvement of catecholamines in the effects of CO_2 could not be neglected.

To examine this, the effect of α- and β-adrenergic blocking agents upon the SPBR threshold and, when possible, their interaction with 10% CO_2 inhalation, were investigated. The excitation of cortical neurons by iontophoretically applied noradrenaline (Johnson, Roberts, Sobieszek & Straughan, 1968; 1969; Johnson, Roberts & Straughan, 1969a; 1969b) is known to be blocked reversibly by both α and β blockers (Johnson, Roberts & Straughan, 1969a), while the excitation produced by other agonists is not affected, although noradrenaline-excitation of cortical neurons has been called into question because of its long latency and slow disappearance (Phillis, Tebecis & York, unpublished observations, in Phillis, 1970).

i) α-adrenergic blockade.

Phentolamine HCl (Aldrich Chemical) was tested in two cats, one at a dose of 0.075 mg/kg and the other at a dose of 0.180 mg/kg. In neither experiment did the drug alter the SPBR threshold.

ii) β-adrenergic blockade.

D-(-)-INPEA (Selvi & Co., Milan), d,l-propranolol (Ayerst, McKenna & Harrison), and pronethalol (Ayerst, McKenna & Harrison) were each tested in the same cat at respective doses of 10.5, 4.5, and 1.5 mg/kg. Three-hour intervals separated the administration of each drug. Neither propranolol nor pronethalol had any effect upon SPBR threshold. D-(-)-INPEA, which lacks the local anesthetic activity possessed by the other two agents (Somani & Lum, 1965) and is known to differ in its activity upon the CNS

(Murmann, Almirante & Saccani-Guelfi, 1966a; 1966b), after a latent period of about fifteen minutes produced a marked fall in threshold which lasted about 30 minutes. In two other cats, in respective doses of 4.0 and 4.75 mg/kg, the effect had a similar latency and lasted less than ten minutes.

Since the other two β-blockers did not produce such an effect, it was questionable whether β-adrenergic blockade was involved. This was investigated by examining the effect of the adrenergically inactive optical isomer of INPEA.

L-(+)-INPEA (Selvi) was given to one cat some time after the effect of D-(-)-INPEA had been determined (Fig. 42). It was found that the SPBR threshold was affected in a fashion very similar to that observed with the active isomer; the latency was similar, as were the duration and magnitude of the effect.

MJ-1999 (Sotalol - Mead Johnson), another blocker which lacks local anesthetic activity (Frankl & Soloff, 1968) and also lacks intrinsic sympathomimetic activity (Fitzgerald, 1969), was examined in one experiment in a dose of 22 mg/kg and was found not to alter the SPBR threshold. Administration of 10% CO_2 45 minutes after MJ-1999 produced virtually the same pattern of effects as had 10% CO_2 given before MJ-1999.

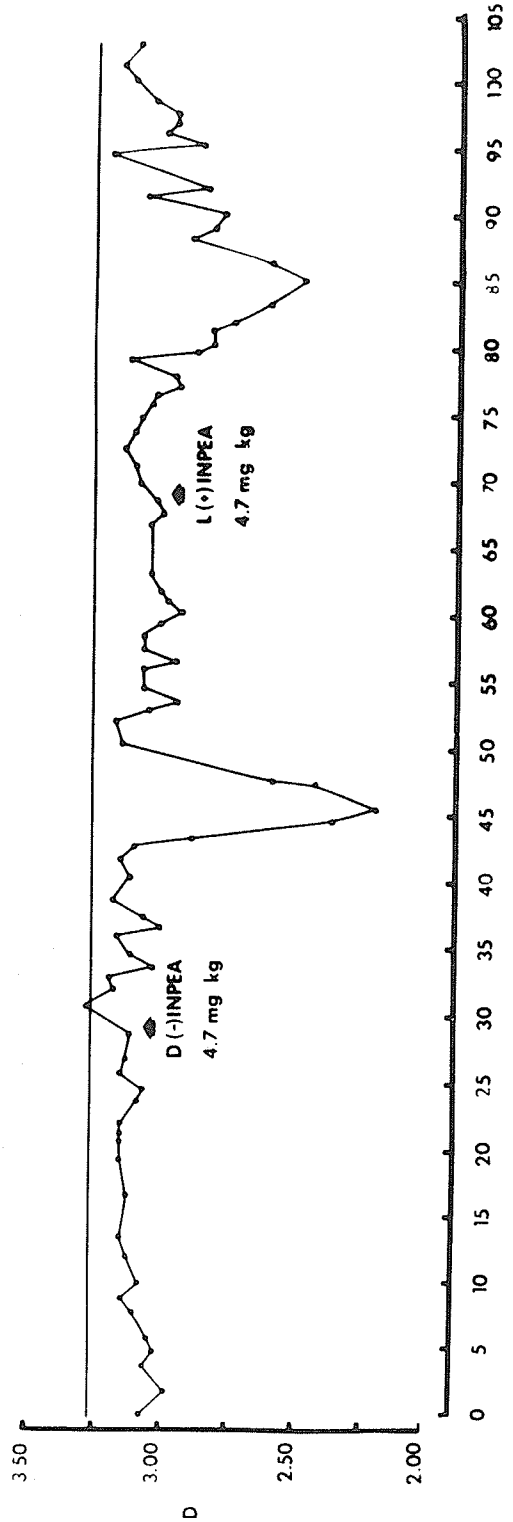


Fig. 42. Effect of INPEA upon the SPBR threshold. D-(-)-INPEA is the adrenergically active isomer. Note that both isomers produce a similar effect.

Part II. Studies of evoked neuronal unit activity

1. Relation between neuronal unit activity evoked by microelectrophoretically applied glutamic acid and activity coincident with the SPBR:

On many occasions it was observed that the electrode location at which unit activity could be induced by glutamate would not yield activity when the SPBR was evoked. However, when a continuous subthreshold glutamate current was applied, unit activity became quite evident during subsequent SPBRs. An example of a recording from such a unit is shown in Fig. 43.

The converse result was also observed on occasion. In such instances unit activity could be recorded coincident with the SPBR but not during the application of even relatively high glutamate currents. Examples of three such units recorded in two cats are shown in Fig. 44 and 45.

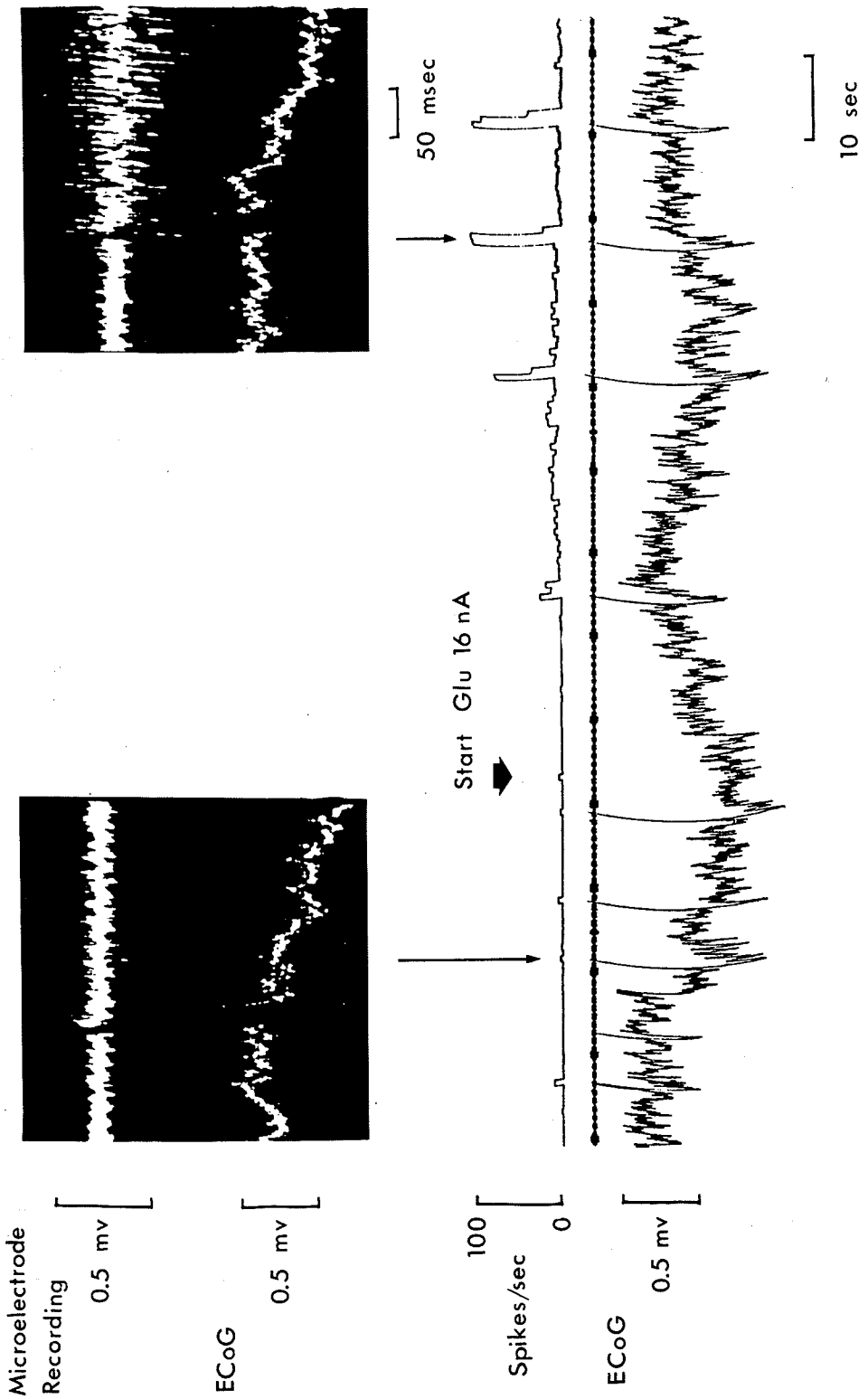
2. Effects of 10% CO_2 inhalation upon unit acivity evoked by microelectrophoretically-applied glutamic acid:

In five cats, neuronal unit activity evoked by pulses of glutamate ions was studied. After a suitable control period of recording from a unit (about five minutes), 10% CO_2 was administered to the animal for not less than five, and usually more than ten minutes. If the first five minutes of recording made during the gas inhalation revealed no change in the firing rate of the unit, the gas was usually withdrawn. A further five to ten minutes of recording was done after withdrawal of the gas before commencing the search for another unit.

The possibility existed, because of the time involved in obtaining

(facing p. 116)

Fig. 43. Facilitation of SPBR-associated neuronal unit activity by a subliminal current of glutamate ions. Oscilloscope trace recordings in this and in subsequent figures are slightly retouched.


Microelectrode Recording
0.5 mv
ECoG
0.5 mv
Start Glu 16 nA
50 msec
100
Spikes/sec
0
ECoG
0.5 mv
10 sec

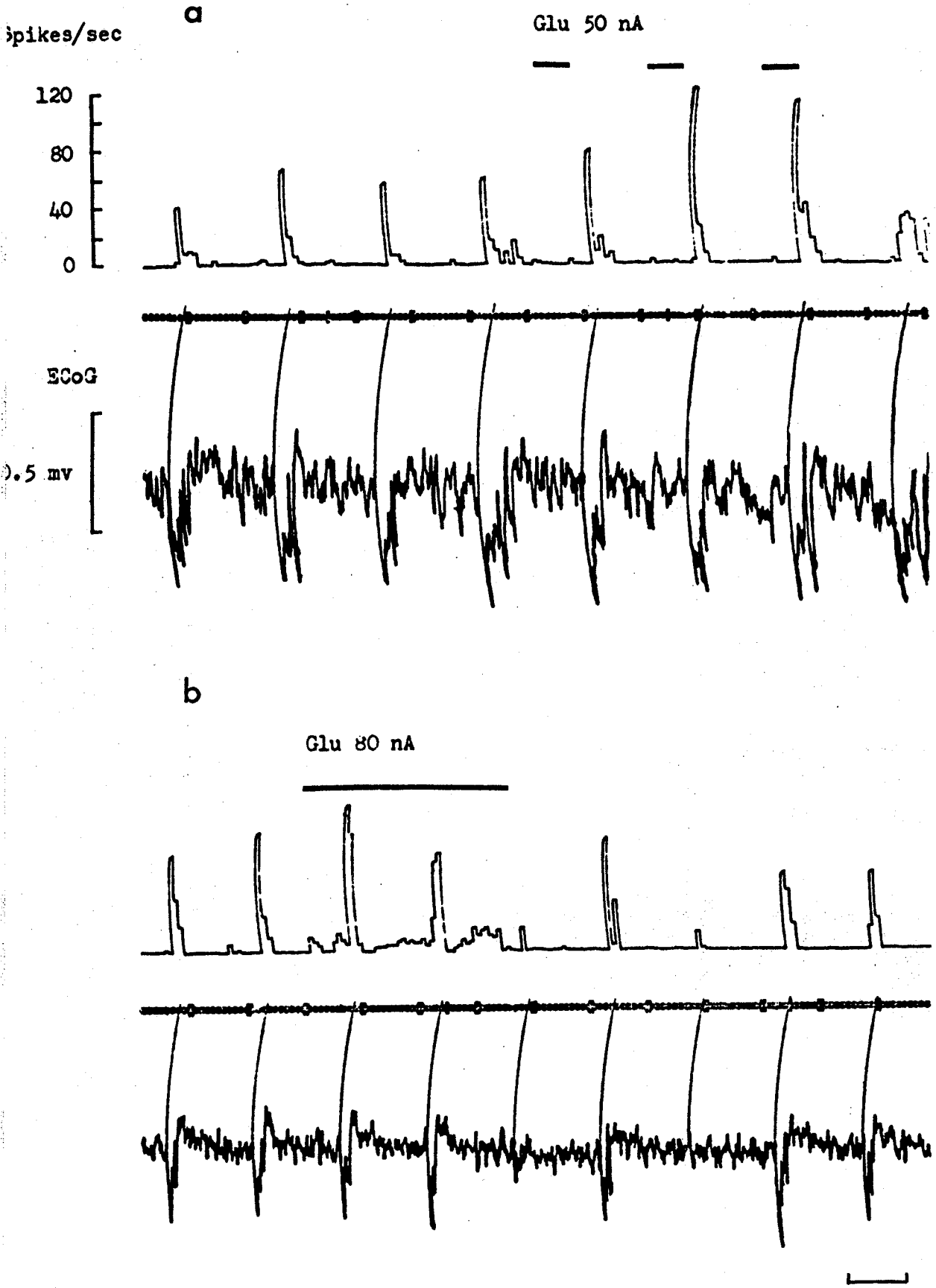


Fig. 44. Failure of glutamate current to evoke activity in units which fire during the SPBR. Records a) and b) were obtained from different units in the same cortical slab. Calibrations are the same for both records.

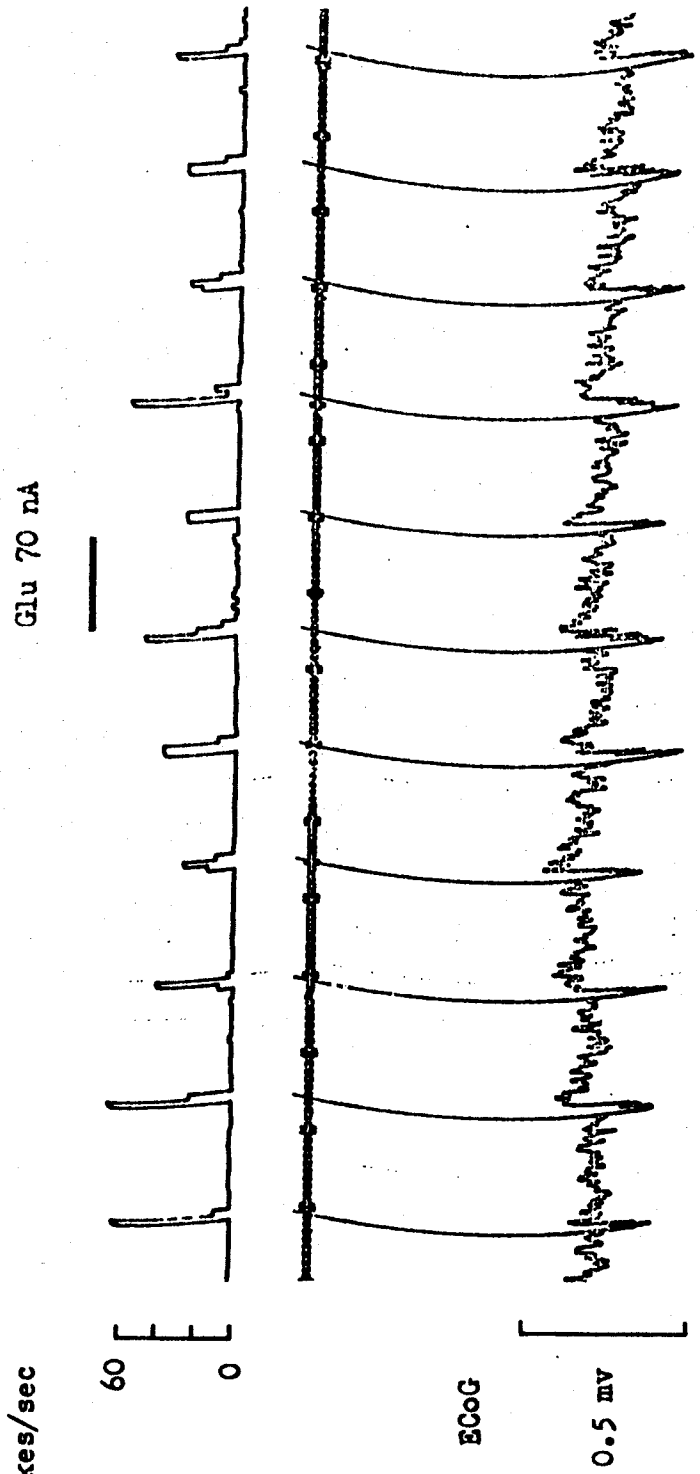


Fig. 45. Failure of glutamate current to evoke activity in a unit which fires during the SPBR.

such recordings, that electrode movement might occur while the activity of a single unit was being examined, particularly during the period of hyperpnea induced by CO_2. This could produce an artifactual change in the firing rate through a change in the concentration of glutamate reaching the responsive structure. However, there are several means of detecting such electrode movements. First, the recorded spike amplitude is generally regarded as a sensitive measure of the distance separating the electrode from the unit (Mountcastle, Davies & Berman, 1957). Second, it is considered likely that electrode movement is much less likely to occur during the period of quiet breathing which follows withdrawal of the gas, than during the period of CO_2 inhalation. Failure of the activity to return to the preinhalation level would indicate that electrode movement had occurred during the period of CO_2 inhalation. Therefore, units whose firing rate failed after withdrawal of the gas to return to the preinhalation level were excluded from analysis, as were those which exhibited an obvious change of spike amplitude.

Many of the firing patterns studied did not show a homogeneous distribution of spike amplitude. Such recordings were regarded as representing the activity of more than one neuronal structure. A single population of spikes could on occasion be selected by adjusting the DC level applied to the gated trigger circuit at the input of the electronic counter. Nevertheless, many firing patterns were studied which without doubt represented activity from several neuronal structures lying at various distances from the electrode tip. Such units have been included in this study because it is supposed that movement of the electrode would be less likely to alter artifactually the firing rate in the aggregate,

and that omission of this data would not have altered the relationships discerned from the examination of single-unit activity. The results presented are from those recordings where the photographic evidence indicates homogeneous spikes having fairly constant height for the duration of the recording period. Samples of these photographs are included in the figures.

A total of sixty units in five slabs in five cats were successfully tested for the effects of CO_2. Statistical comparisons of the pretreatment and treatment periods permitted grouping of the units according to the type of effect, for the purpose of averaging of the data.

In eleven units, there was an initial short-lived increase of the firing rate, followed by a variable decrease (See Fig. 46, 47). Upon withdrawal of the gas mixture the firing rate occasionally overshot the control level.

In thirteen units there was an enduring increase of the firing rate during the gas inhalation. This usually developed shortly after beginning the inhalation, (Fig. 48, 49) but on occasion was considerably delayed in onset. Upon withdrawal the firing rate occasionally fell below the control level.

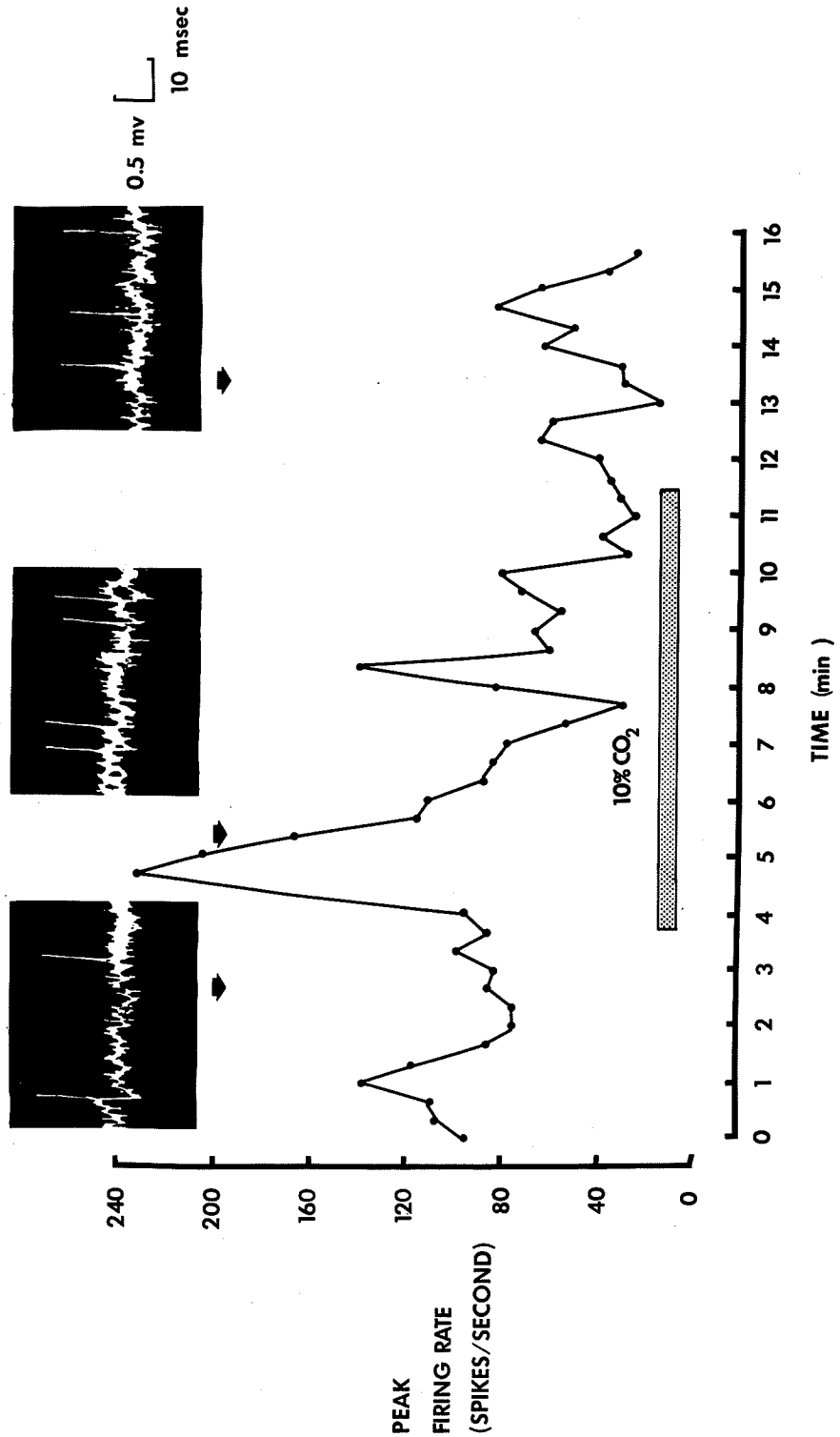
In nineteen units there was depression of unit activity. This usually persisted for the duration of the gas inhalation (Fig. 50, 51) but partial recovery would sometimes occur before the gas was withdrawn (Fig. 52). The firing rate occasionally was observed to overshoot the control level upon withdrawal.

In seventeen units no effect was observed.

The units lay at depths in the cortex ranging from 154 to

(facing p. 121)

Fig. 46. Facilitation followed by depression of glutamate-evoked unit activity by CO_2. The constancy of the spike height in the inset oscilloscope photographs indicates that the distance between the microelectrode and the source of the activity is unchanging.


0.5 mv
10 msec
240
200
160
120
80
40
0
PEAK
FIRING RATE
(SPIKES/SECOND)
10% CO_2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
TIME (min)

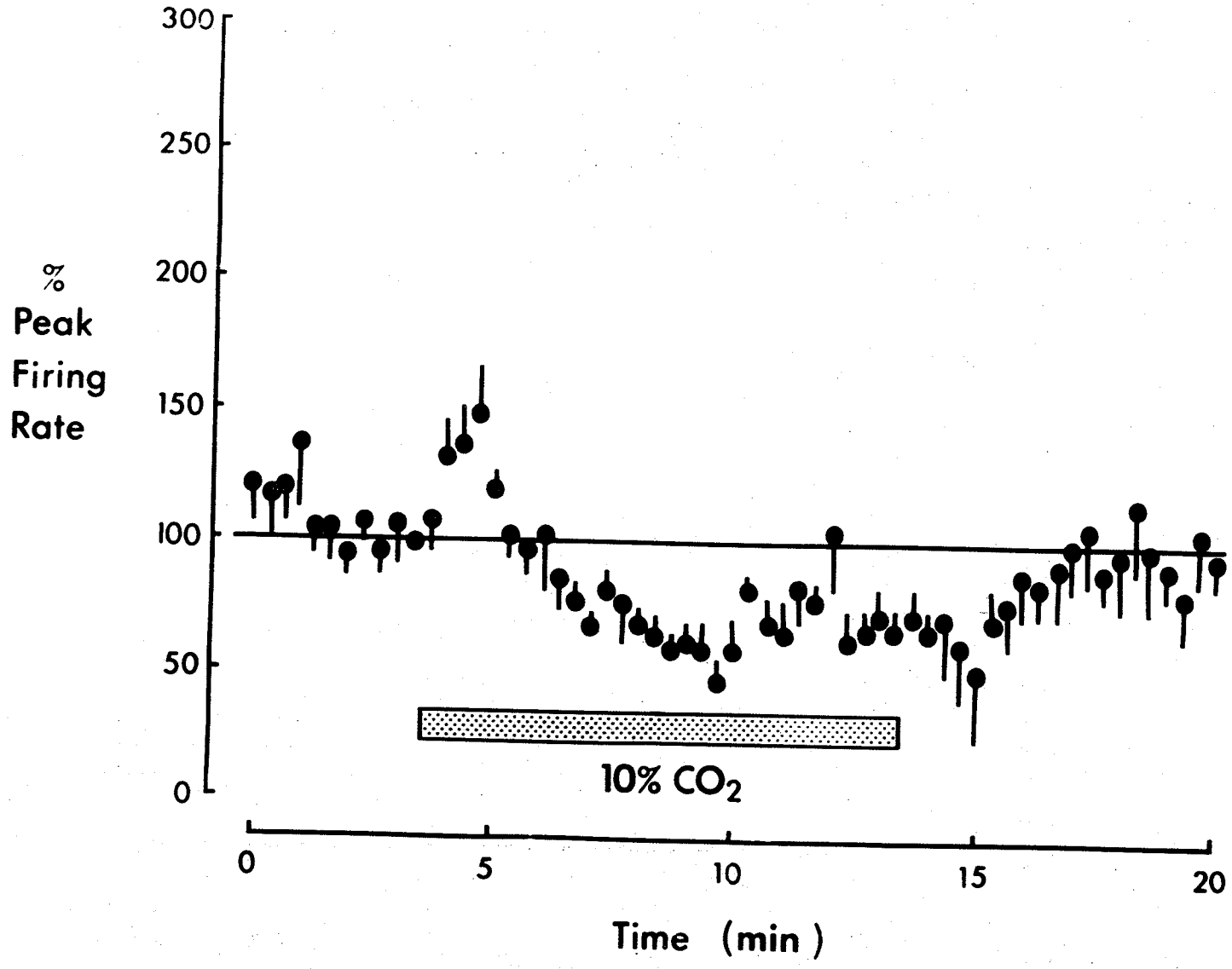
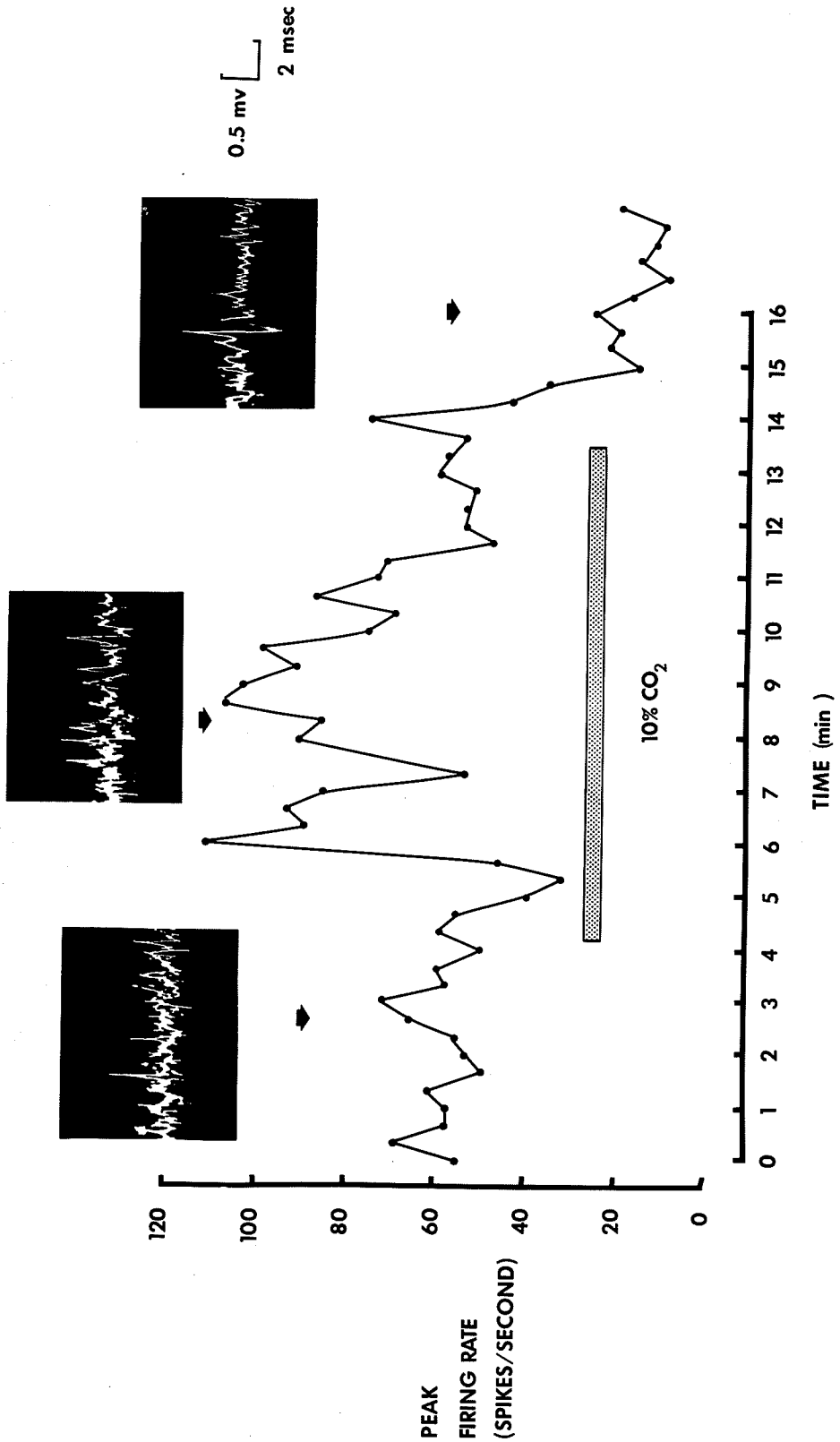


Fig. 47. Facilitation followed by depression of glutamate-evoked unit activity by CO_2. Averaged firing rate of 11 units. In this and in subsequent figures, upward-going standard error bars indicate a mean which differs statistically from 100% at the $p < 0.05$ level.

(facing p. 123)

Fig. 48. Facilitation of glutamate-evoked unit activity by CO_2.


0.5 mv
2 msec
PEAK
FIRING RATE
(SPIKES/SECOND)
120
100
80
60
40
20
0
10% CO_2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
TIME (min)

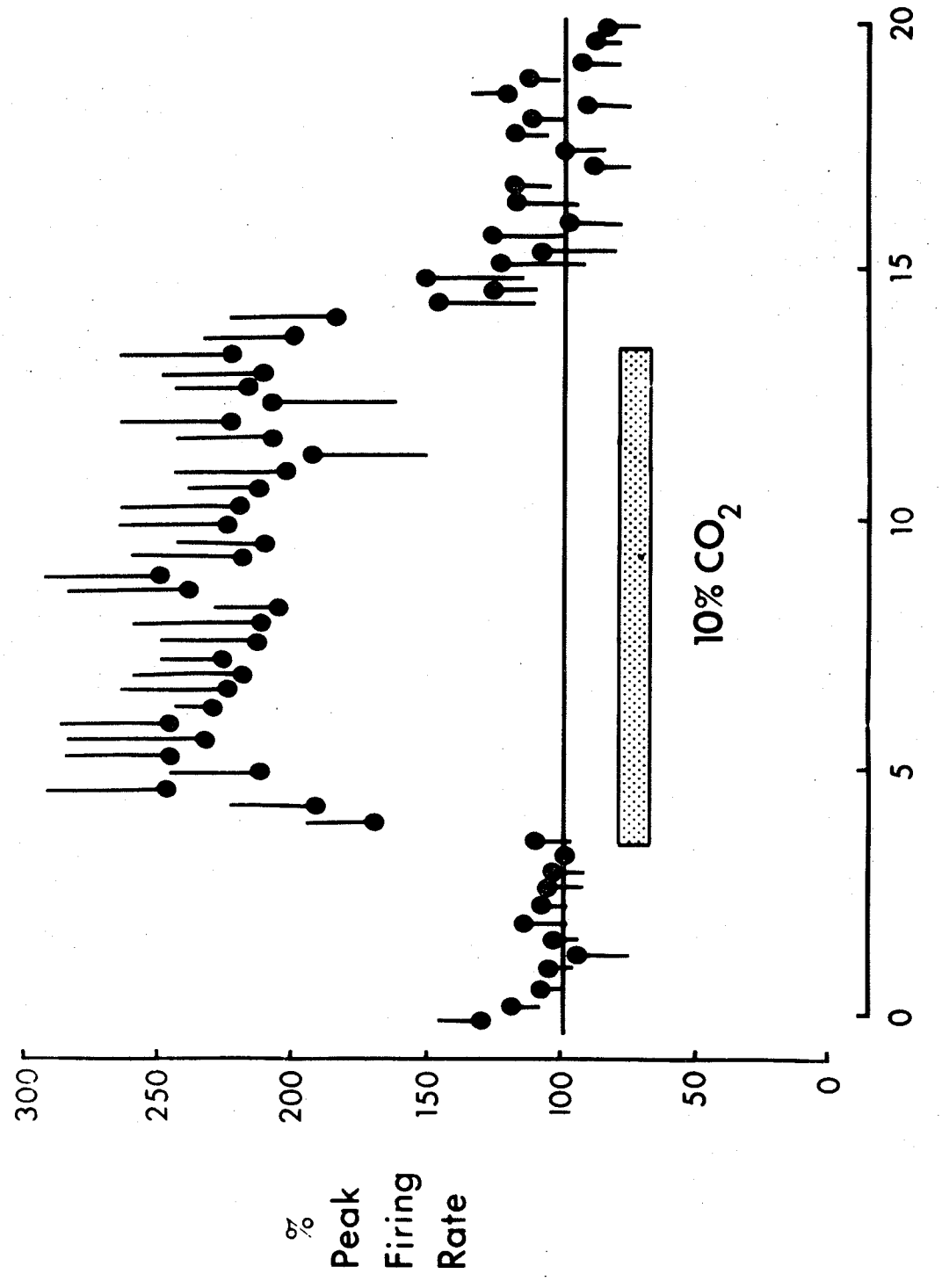
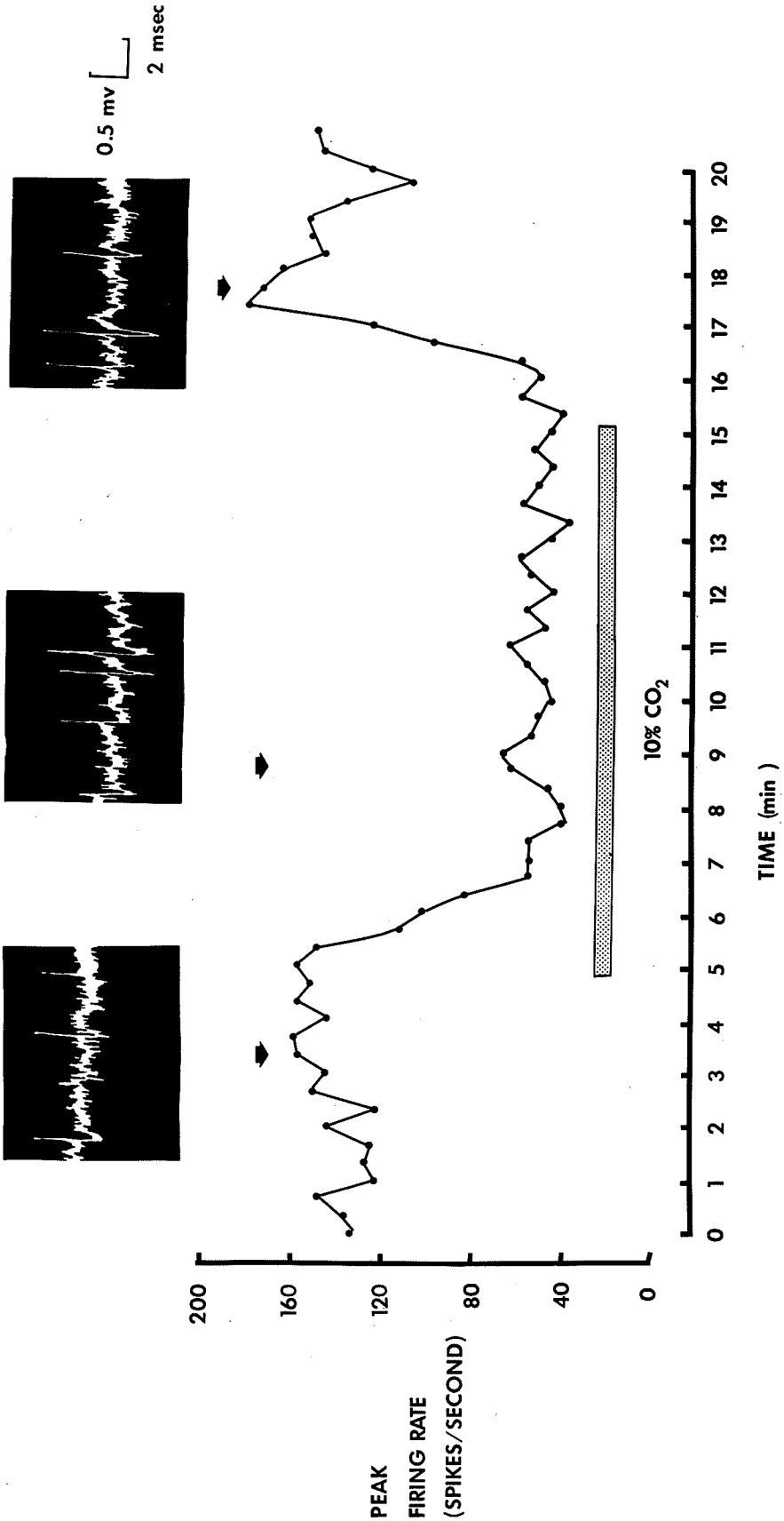


Fig. 49. Facilitation of glutamate-evoked unit activity by CO_2. Averaged firing rate of 13 units.

(facing p. 125)

Fig. 50. Depression of glutamate-evoked unit activity by CO_2.


0.5 mv
2 msec
PEAK
FIRING RATE
(SPIKES/SECOND)
200
160
120
80
40
0
10% CO_2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
TIME (min)

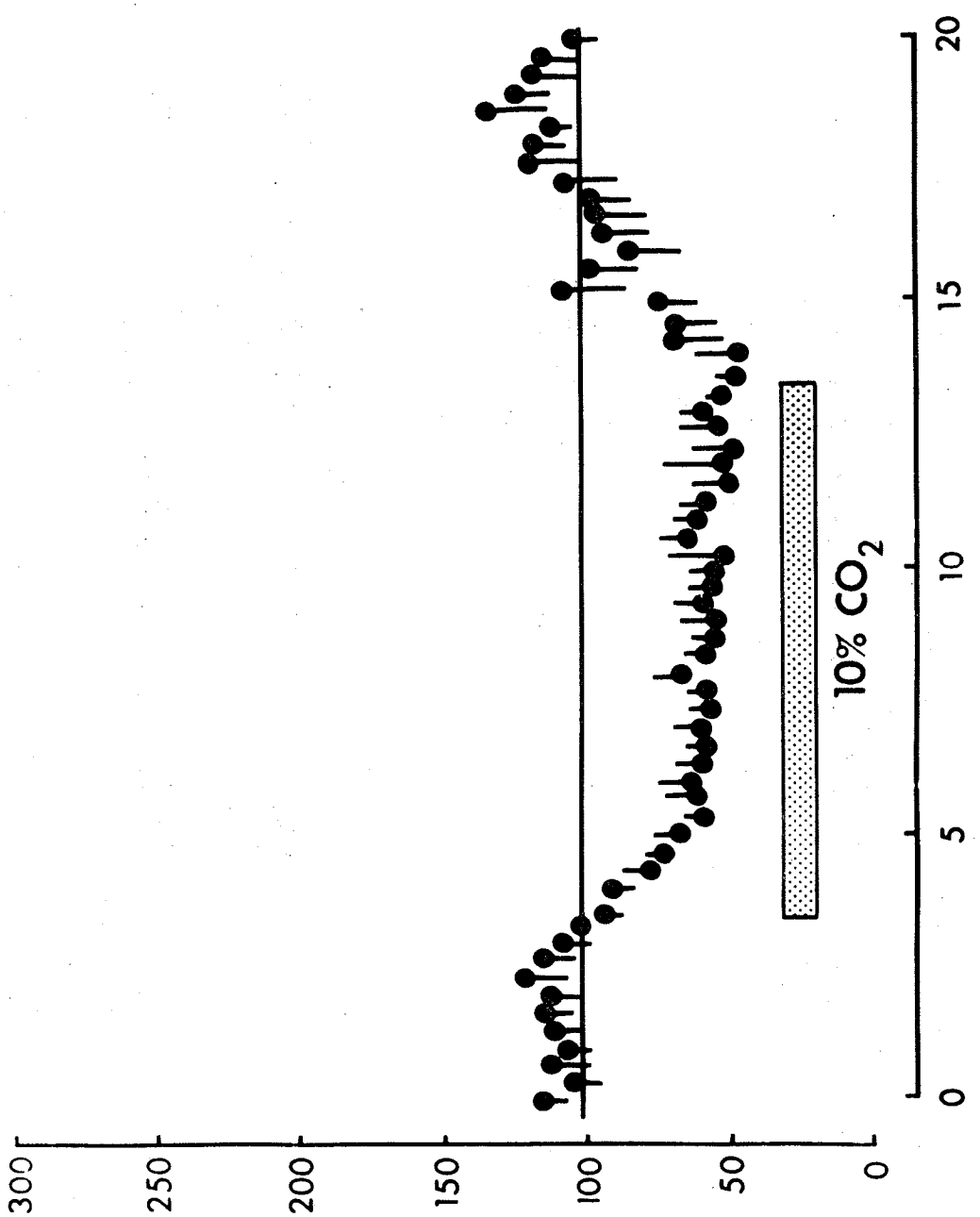
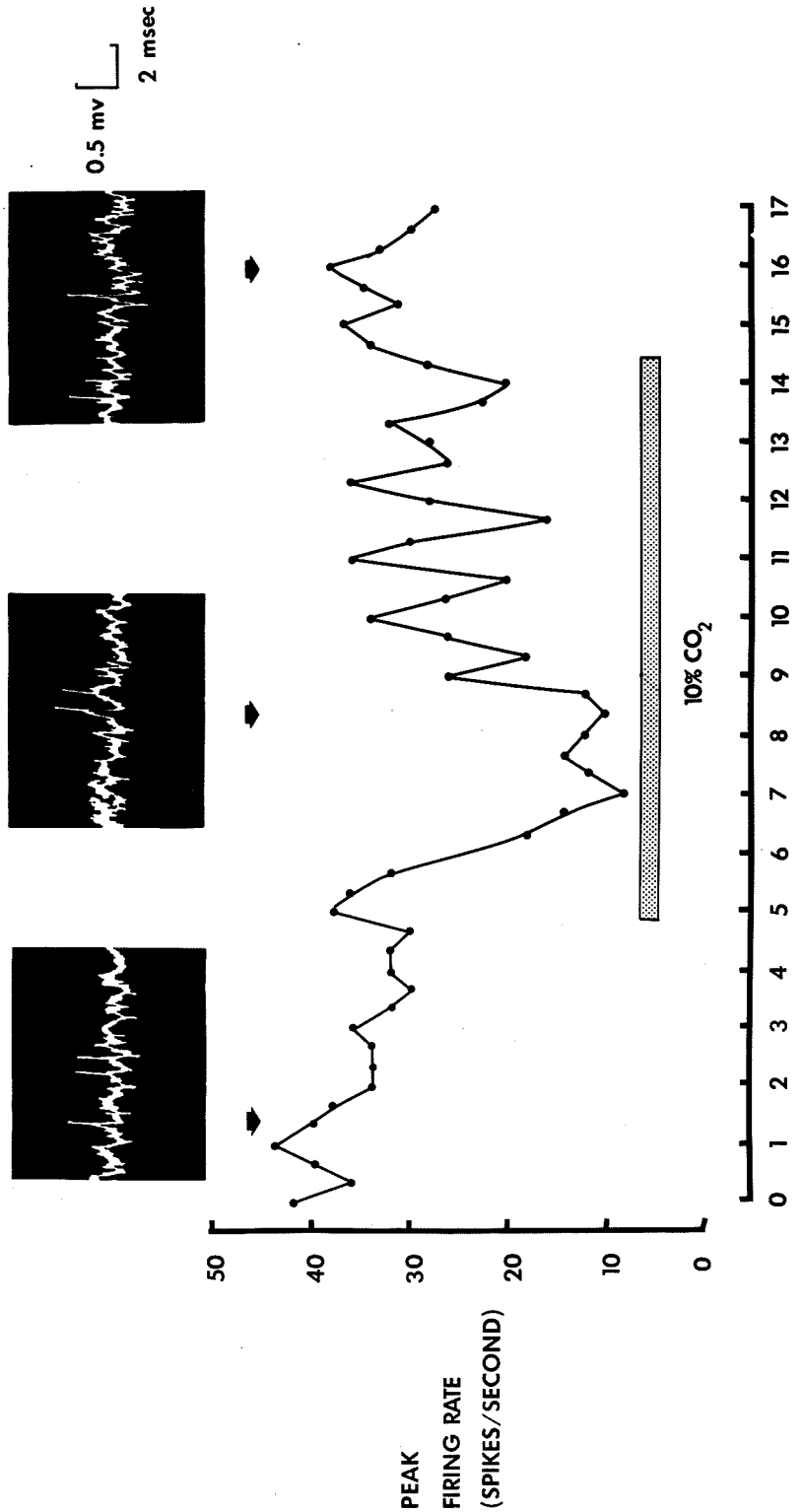


Fig. 51. Depression of glutamate-evoked unit activity by CO_2. Averaged firing rate of 19 units.

(facing p. 127)

Fig. 52. Short-lived depression of glutamate-evoked unit activity by CO_2.


0.5 mv
2 msec
PEAK
FIRING RATE
(SPIKES/SECOND)
50
40
30
20
10
0
10% CO_2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
TIME (min)

1628 μm below the pia mater. There were no apparent differences among the distributions of the depths of the units grouped according to the type of effect exerted by CO_2, as is shown in Table 2.

3. Effects of 10% CO_2 inhalation upon unit activity coinciding with the SPBR:

In early experiments, units which fired during iontophoresis of glutamate were tested to see whether they also fired during the SPBR. It was found that a surprisingly low proportion of such glutamate-sensitive units did so.

A less time-consuming method for finding units which fired during the SPBR was to advance the electrode through the cortex in steps of 10-20 μm, while evoking SPBR's at ten-second intervals with supra-threshold stimuli applied to the surface of the cortex. Thirty-seven units found in this manner were studied in five slabs in five cats.

In 26 units no effect was discernible.

In four units there appeared to be an initial short-lived facilitation of activity which was followed by depression (Fig. 53). Averaging was not practical with this small number of observations.

In seven units there was an enduring facilitation of activity (Fig. 54, 55).

In no unit was depression observed solely.

4. Effects of 10% CO_2 inhalation upon the latency-to-onset of SPBR-associated neuronal unit activity:

In recordings from thirteen units in two cats, the photographed oscilloscope tracings were complete enough to permit examination of the effect of CO_2 upon the latency-to-onset of unit activity. It was found

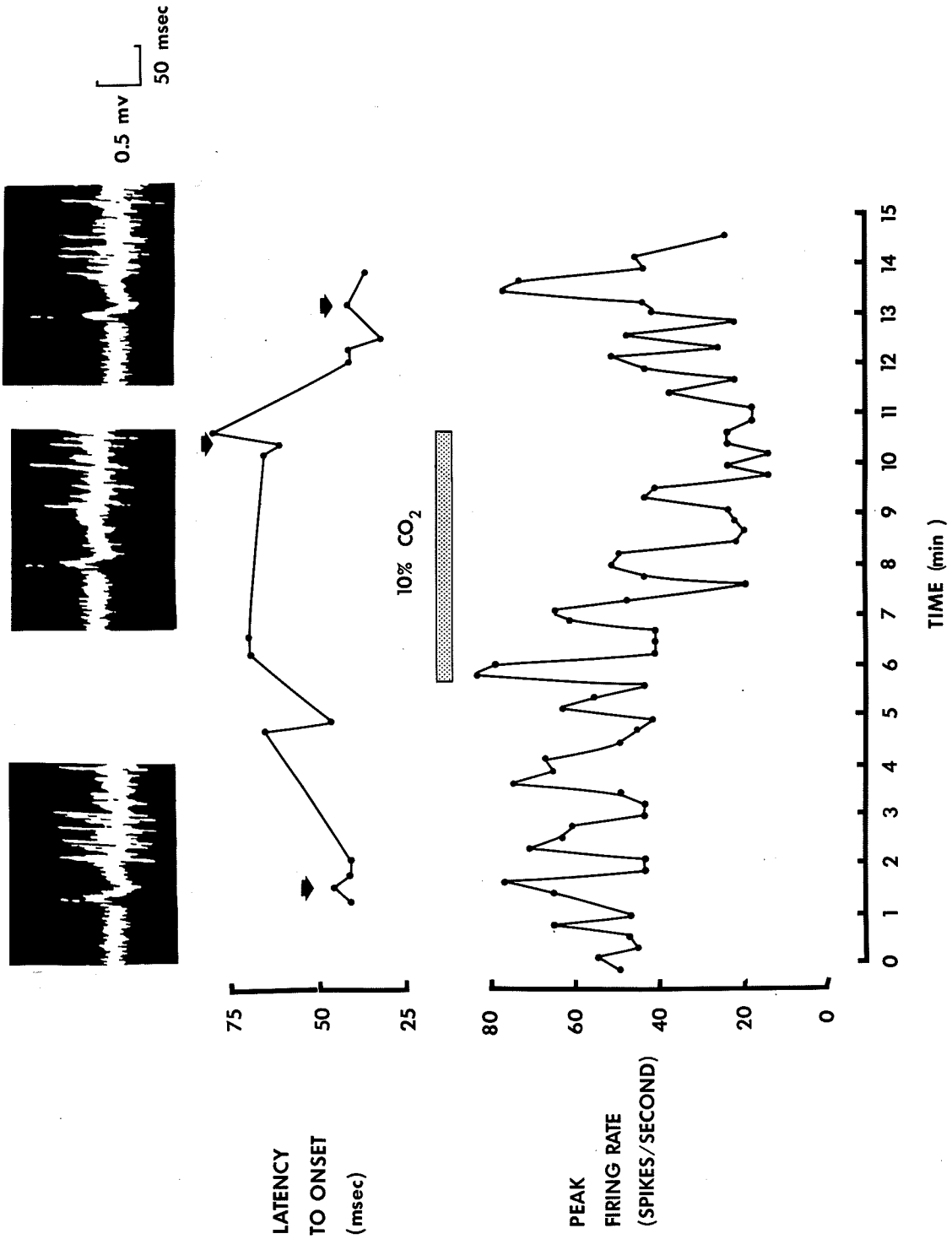
TABLE 2

Relation between the effect of 10% CO_2 upon glutamate-evoked neuronal unit activity and the depth in the cortex at which the corresponding activity was recorded.

Effect:	Stimulation followed by depression	Stimulation	Depression	n.e.
Depth:				
mean	810.5	775.2	943.6	748.7
s.e.	104.7	88.1	92.3	69.8
Number of units	11	13	19	17

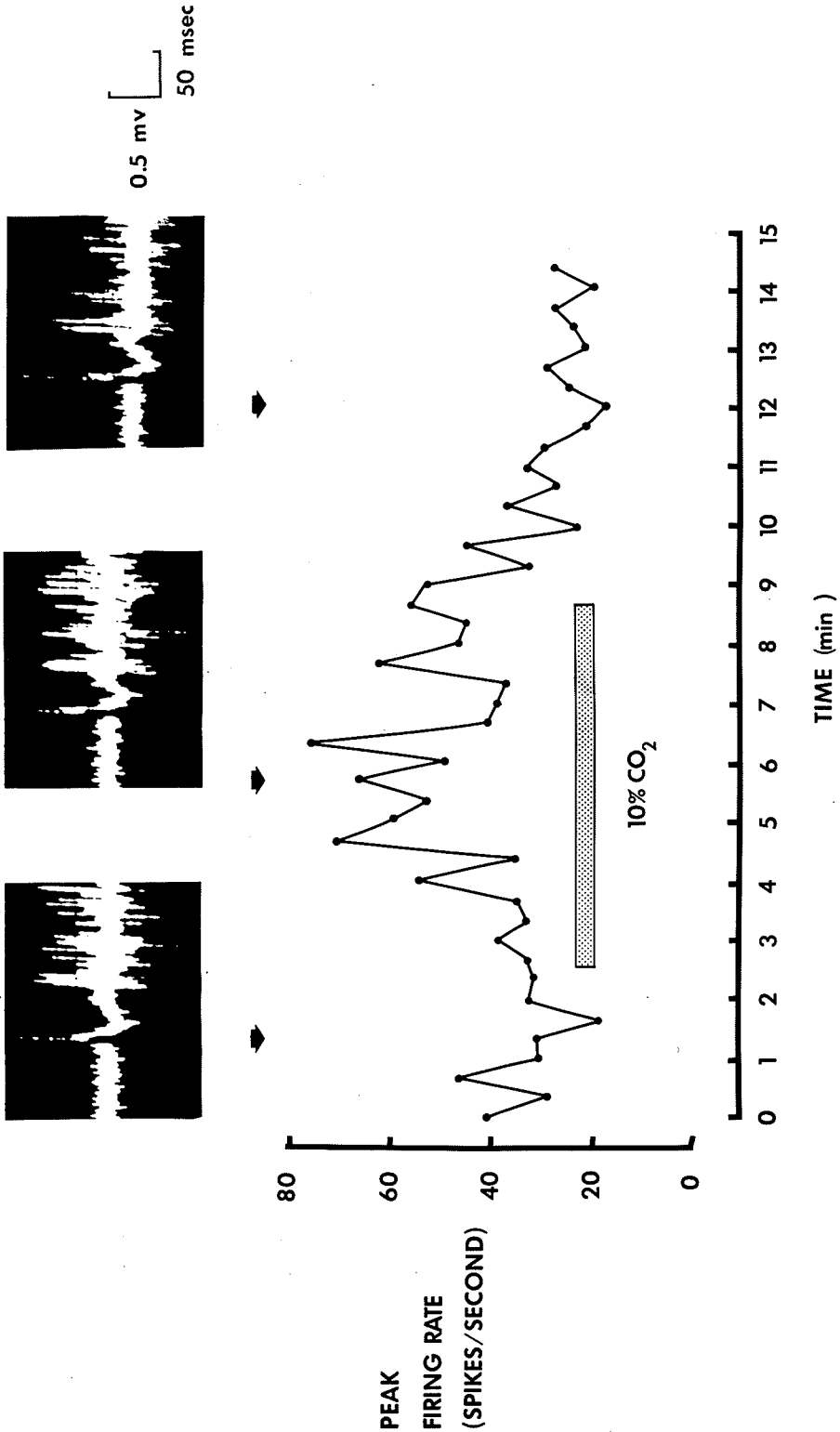
(facing p. 130)

Fig. 53. Short-lived facilitation of SPBR-associated unit activity by CO_2, and its tendency to increase the latency-to-onset of the activity.


0.5 mv
50 msec
10% CO_2
LATENCY
TO ONSET
(msec)
75
50
25
PEAK
FIRING RATE
(SPIKES/SECOND)
80
60
40
20
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (min)

(facing p. 131)

Fig. 54. Facilitation of SPBR-associated unit activity by CO_2.


0.5 mv
50 msec
PEAK
FIRING RATE
(SPIKES/SECOND)
80
60
40
20
0
10% CO_2
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15
TIME (min)

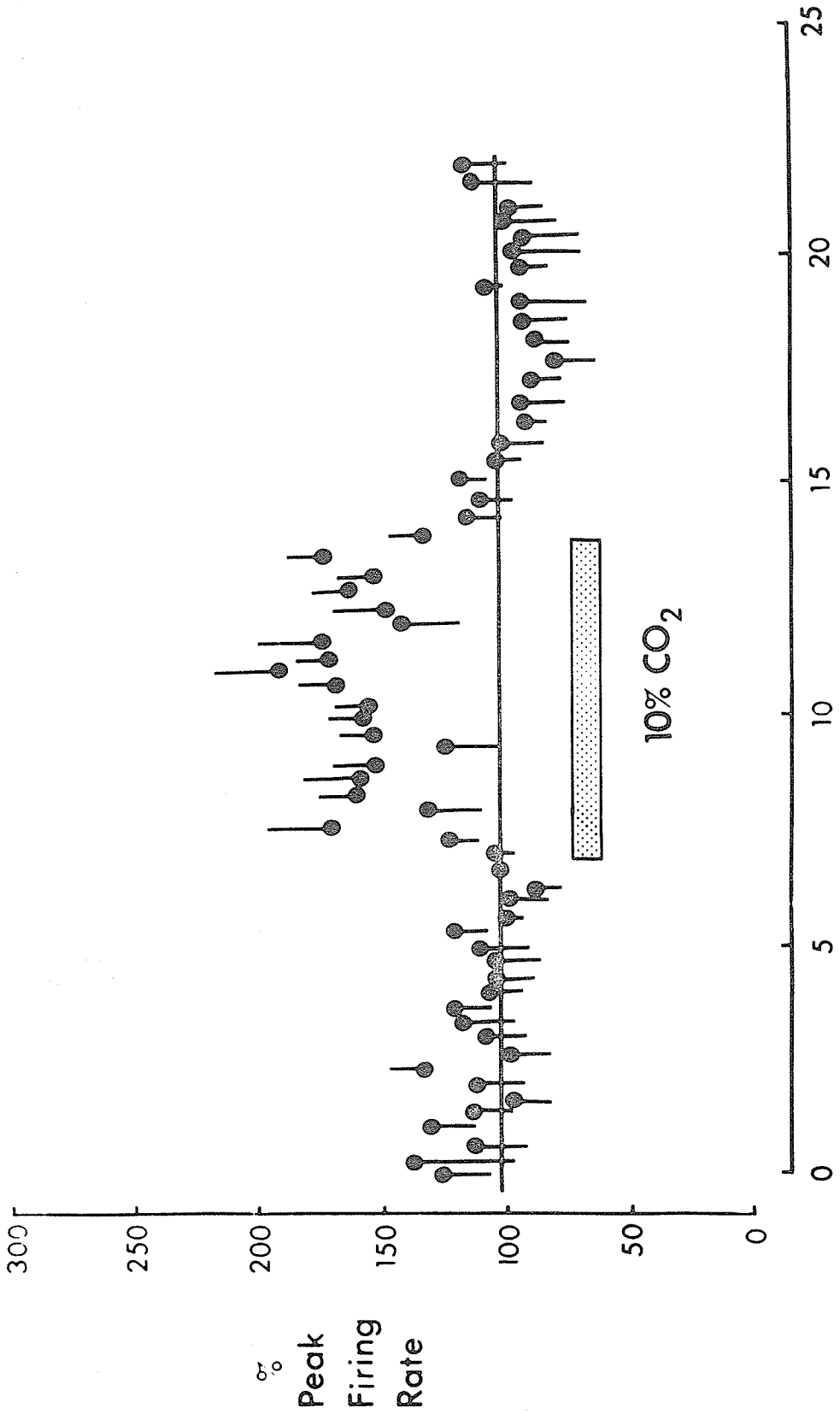


Fig. 55. Facilitation of SPBR-associated unit activity by CO_2. Averaged firing rate of 7 units.

that a statistically significant increase of latency occurred in six instances. The cortical depth at which these units lay did not differ from the depth of those units whose latency was unaltered. In four of the six there was no change in the firing rate during the gas inhalation, but in two instances there was initial facilitation followed by depression (e.g. Fig. 53).

5. Relation between SPBR-associated unit activity and SPBR amplitude:

In recordings from twenty units in four cats, the level of background noise in the ECoG tracing was sufficiently low to permit satisfactory measurement of the SPBR amplitude. Analysis of correlation was made of the peak firing rate and the SPBR peak amplitude, with a minimum of fifteen data pairs for each neuronal unit.

In 19 of the 20 units no statistically significant correlation occurred.

In one unit there was a correlation which was significant at the $p < 0.01$ level. This unit was located at a depth of 790 μm, and is the same as that from which Fig. 53 was derived.

6. Effect of inhaled 10% CO_2 upon spontaneous recurrent burst activity:

In some cats, usually those which suffered least from surgical trauma, spontaneous recurrent burst activity was present, often in both slabs, from the moment recording was commenced. This activity usually abated after several hours, but on occasion was quite prolonged. In some of these cats administration of 10% carbon dioxide arrested this activity, and removal of the gas was followed by its prompt return. Three cats in which such activity was present were studied in the experiments where neuronal unit activity was recorded. Neuronal units in these cats

were found to fire only during the spontaneous bursts or during evoked SPBRs.

As shown in Fig. 56 and 57 (obtained from the same unit), administration of the gas mixture arrested the spontaneous activity in both the surface and unit recordings. Both forms of activity could then be evoked by surface stimulation. In this example, the duration of inhalation was six minutes. Spontaneous activity tended to return during exposure to 10% CO_2 as its duration was increased, but the frequency of occurrence of spontaneous bursts during inhalation of the gas mixture never was found to approximate that seen in its absence.

Repeated inhalations of the gas (up to 20 times) never diminished the ability of such inhalation to abolish spontaneous activity.

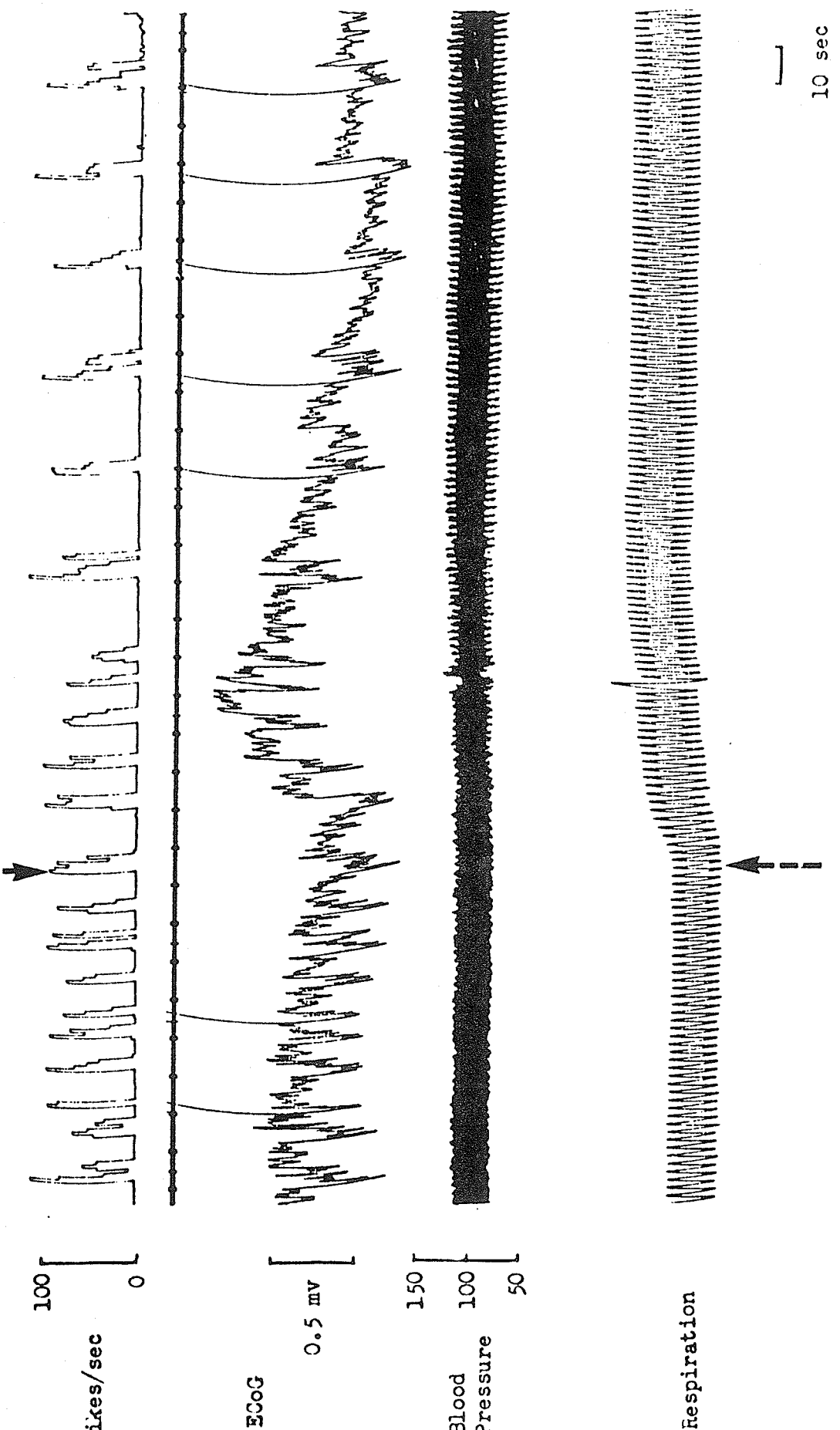


Fig. 56. Abolition of spontaneous burst activity and associated unit activity by CO_2. Evoked responses are indicated by the large stimulus artifact in the ECoG recording.

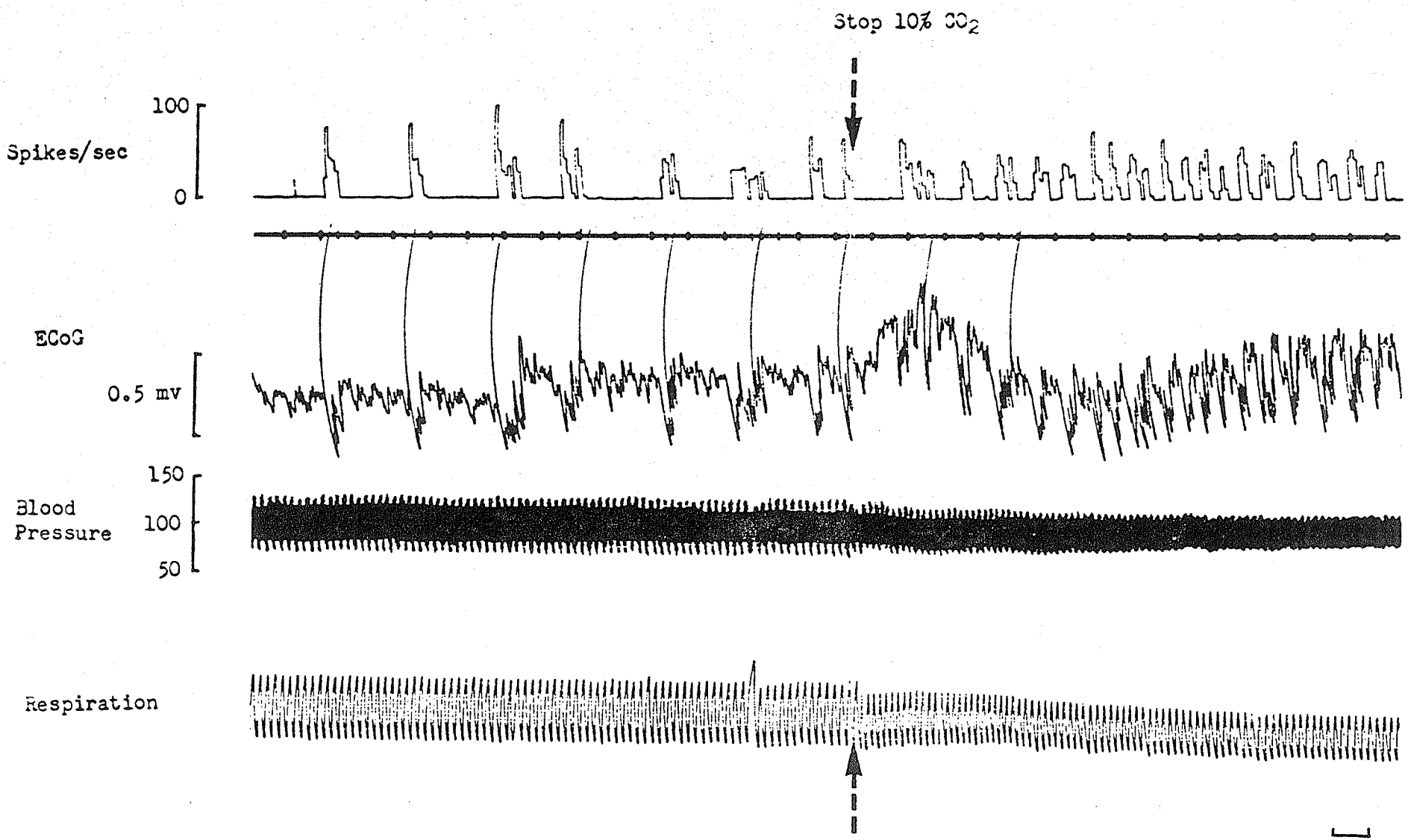


Fig. 57. Return of spontaneous burst activity and associated unit activity after withdrawal of CO_2. This record is a continuation of that shown in Fig. 56. A period of six minutes separates the commencement and the termination of the CO_2 inhalation.

DISCUSSION

I. Authenticity of SPBR threshold measurements.

The assumption has been made throughout this work that the amplitude of a pulse delivered from a constant-voltage (i.e., low impedance) source is a valid measure of the relative intensity of the effective stimulus at the neuronal membrane sites which must be excited in order to initiate the chain of events constituting the SPBR. It may be argued against this assumption that any alteration of the relative proportion of the cortex made up of inexcitable constituents (e.g. blood, blood vessels, and neuroglia) might result in altered delivery of stimulating current to the primary sites of excitation. This would lead to an apparent change in threshold and an artifactual assessment of this variable. An important consideration for the present study is that both hypoxia and hypercarbia produce increased blood flow through the cortex, with an associated increase of the blood volume (Risberg, Ancri & Ingvar, 1969). This would tend to lower the impedance of the tissue underlying the stimulating electrodes at the cortical surface. Such shunting would have no effect on the potential gradients produced by the stimulus pulse across the neuronal elements which lie in parallel with the inexcitable tissues, since the output impedance of the stimulus source used in this work was adequately low. However, at least some proportion of the inexcitable components must lie in series with the excitable components; impedance changes in the former would result in altered stimulus currents to the excitable sites.

It can be reasonably shown, nevertheless, that these series-pathway effects would be necessarily minute, and that they may be discounted also on the basis of the observed direction in which the assessed

threshold was altered by various treatments. The arguments for this view are presented in the following.

The net impedance resulting from a series network of neurons, neuroglia, and vascular tissue would be predominantly determined by the cellular elements since their impedance is known to be very much higher (Krnjevic & Schwartz, 1967a) than that of blood. Hence the current change induced by alteration of the low-impedance portion of the series system would be small, even with profound changes in that portion. In addition, the membrane impedance of neuroglia appears to be very stable (idem).

There is, also, experimental evidence from the present study to support the view that any alteration in SPBR threshold, as assessed by the method described herein, was a valid representation of cortical neuronal excitability. Hypercarbia, for example, is known to increase cortical circulation (vide supra), and the stimulus current in a series network would tend to increase from such a result if the previous arguments are fallacious. This should yield an apparent decrease in SPBR threshold when the series network is excited from a constant - voltage source. Such a result was not observed in this work; in fact the opposite has been noted (See Fig. 17,18,21,22). Additionally, it can be noted that the various treatments studied in the present thesis were in most cases found to produce short-lived changes of SPBR threshold, whereas adaptation of cerebral blood flow to hypercarbia and to hypoxia is known to require days to develop (Betz, 1965).

The foregoing considerations appear to justify, therefore, the use of the constant-voltage source method used in this study to initiate

the SPBR and to assess the excitability of the responding elements.

II. Stability of the SPBR.

The present results demonstrate that impairment of the circulation through the cerebral cortex produces depression of its excitability, as measured by the SPBR threshold. This depression is often preceded by a brief period of facilitation. It appears that hypoxia and mild hypercarbia can each independently be implicated as the proximate cause of the effects of ischemia, since the SPBR threshold changes observed with ischemia were essentially duplicated by the experimental induction of either condition. However, when hypoxia produced abolition of the response, this occurred usually with an abruptness not seen with the abolition produced by hypercarbia (except at relatively high concentrations of carbon dioxide). This abruptness approximated that seen with ischemia.

The most uniformly remarkable feature of these studies has been the stability of the SPBR threshold, as evidenced by its consistent tendency to accommodate to the actions of the various treatments. Threshold values almost always returned to their pretreatment values while the treatment was still being applied, whenever the treatment had altered the response threshold without rendering the response unobtainable. This was seen with the breathing of 10% oxygen (see Fig. 15, 16), of 3% carbon dioxide (Fig. 34), 10% carbon dioxide (Fig. 17, 18, 24, 25), and with the injection of acetazolamide (Fig. 35, 37), and of INPEA (Fig. 42).

In the present studies on the breathing of 10% CO_2, it was found that accommodation was complete in approximately thirteen minutes (Fig. 25). It is difficult to state categorically whether or not accommodation to the

depressant action of CO_2 can occur in other CNS preparations, because exposures of sufficient duration have not been carried out in many instances. Furthermore, many of the studies available are not explicit with regard to the duration of the period during which observations were made. Of the ones that are, the durations of application or of observation were often much shorter than those in the present study. However, some relevant comparisons are possible and these are provided in the following.

Woodbury et al. (1958) found no difference in the incidence of electroshock seizures in rats exposed to a given concentration of carbon dioxide for periods having durations in excess of five minutes. Ivanov (1963) observed the effects of various concentrations of carbon dioxide upon the EEG for up to ten minutes and did not report any accommodation to its actions. Maiti & Domino (1964) found that the thresholds for evoked afterdischarges in the amygdala and in isolated cerebral cortex were elevated by carbon dioxide (various concentrations) for the duration of a 15-minute inhalation. Metz (1971) studied the effect of 12% carbon dioxide inhalation upon release of acetylcholine from the cerebral cortex and the medulla oblongata of morphine-urethane anesthetized dogs, and reported enhancement of release, together with augmentation of the integrated electrical activity, for the duration of the 30-minute inhalation of the gas.

The possibility that the accommodation observed in the present study is related to an adaptation of the cerebral vasculature to the dilatory effects of carbon dioxide is opposed by the findings of Betz (1965; 1969). He observed that 5% CO_2 can be given to an animal to breathe for

a period of six hours daily, for several days, before the vasodilatory effect begins to abate (as alterations occur in the CSF bicarbonate).

Esplin & Rosenstein (1963) found, in the spinal cord, that 10% CO_2 depressed the monosynaptic reflex for the entire duration of a 10-minute inhalation, but 5% CO_2 allowed some accommodation to occur toward the end of the gas inhalation. Unfortunately, these workers did not discuss the latter finding, nor did they state whether it was reproducible.

The duration of the effects of acetazolamide produced in the CNS have been measured in terms of hours and days. These phenomena include depression of the monosynaptic spike of the spinal cord (Esplin & Rosenstein, 1963), protection against electroshock seizures (Gray, Maren, Sisson, & Smith, 1957; Gray & Rauh, 1967), depression of cerebral cortical pH (Meyer, Gotoh & Tazaki, 1961), augmentation of the CBF (Ehrenreich, Burns, Alman & Fazekas, 1961), and alteration of whole blood pH and P_{CO_2} (Gotoh, Meyer & Tomita, 1966). Possibly of some significance is an observation of Gotoh et al. (1966) on arterial blood gases after the intravenous injection of acetazolamide. The oxygen tension and saturation showed a small but statistically significant decline which reached a maximum at three minutes, and disappeared by about five minutes.

The cardiovascular effects of β-adrenergic blockade produced by INPEA are of relatively long duration (Somani & Lum, 1965; Almirante & Murmann, 1966). The currently available studies on the facilitating action of this drug on the CNS, however, permit almost nothing to be said concerning the time course of this action (Murmann, Almirante & Saccani-Guelfi, 1966a; 1966b), which is unrelated to β-adrenergic block-

ade (Murmann et al., 1966a).

In a comparison of the present finding of accommodation to the facilitatory action of hypoxia upon the SPBR threshold to the findings of studies in other CNS preparations, it appears that the phase of facilitation has not lent itself to examination independent of the phase of depression. In one study where monkeys were given 8% oxygen to breathe for 30 minutes (Massopust, Wolin, Kadoya & White, 1969), a brief phase of facilitation characterized by 30-Hz spindle-burst activity was succeeded by progressive depression with lengthening periods of electrical silence.

III. Possible mechanisms for the stability of the SPBR.

From the foregoing, it appears that accommodation to these treatments is a phenomenon peculiar to the isolated cerebral cortical slab. Since there is no concurrent change in either the duration or the amplitude of the SPBR, the locus in the cortex at which the threshold alterations are brought about would most likely lie in those structures which are responsible for initiating the response, rather than in those which sustain it. In agreement with this idea are experiments where there was ongoing spontaneous burst activity which was regularly and reversibly terminated by inhalation of carbon dioxide (See Fig. 56, 57). Also, the action of CO_2 to increase the latency to onset of the SPBR-associated neuronal unit activity, without altering the intensity of this activity (Fig. 53) points similarly to a locus of action in the structures where the SPBR is initiated.

The conservative assumption may be made that the regenerative activity which constitutes the SPBR requires for its initiation the

activation, by the brief stimulus current, of a minimum number of neuronal structures (Burns, 1951). The fact that the SPBR threshold resists changes, and recovers rapidly from them despite the persistence of the agents which produced them, would thus indicate that the extent of invasion of the stimulus impulse is modified by collateral inhibition which regulates the activity of the invaded structures to a constant level. In other words, if a depressant influence were to act upon both the responsive neurons and the inhibitory interneurons, although fewer responsive elements would be activated, their excitation would be opposed to a lesser extent by recurrent inhibition. In this way the "safety factor" of the fewer activated pathways would be enhanced in such a way as to cancel the effect of the reduction of the number of pathways. An end result would be a tendency of the stimulus current to activate a similar number of deep structures, and therefore for the apparent excitability of the cortex to remain unchanged.

A similar scheme of depressed inhibition (Miyahara, Esplin & Zablocka, 1966) may be at least in part responsible for the finding that carbon dioxide depresses the polysynaptic reflex of the spinal cord to a much greater extent than it does the monosynaptic reflex (Esplin & Rosenstein, 1963).

The fact that any change of SPBR threshold occurs at all could be attributed to the period of time taken by the depressant action to reach a steady level, during which the responsive neurons and the inhibitory interneurons could be unequally depressed. Along this line of thought, the time-to-peak for the SPBR threshold-elevating effect of carbon dioxide is in reasonable agreement with the reported time course of the change in cortical P_{CO_2} produced by inhalation of CO_2, as monitored

directly by CO_2 electrodes placed on the surface of the cortex (Krnjevic, Randic & Siesjo, 1965). Similarly, the time required for the stabilization of the effect of acetazolamide on blood gases and CO_2-carrying capacity (which are known fairly certainly to be the agencies for the CNS effects of carbonic anhydrase inhibitors (Giacobini, 1961; 1962; Leder, 1966; Gray & Rauh, 1967; 1968)) would result in a period of instability until the establishment of a new steady state P_{CO_2} in cortical tissue.

Carbon dioxide is known to have an important action upon the synthesis of acetylcholine (Tenney, 1960) and of γ-aminobutyric acid (GABA) (Woodbury & Vernadakis, 1958; Woodbury & Karler, 1960), and upon the activity of the sympathico-adrenal system whereby the level of circulating catecholamines is elevated (Sechzer et al., 1960; Tenney, 1960; Morris & Millar, 1962). Hypoxia also elevates brain GABA, mainly in the "free" fraction (Elliott & van Gelder, 1960; Lovell, Elliott & Elliott, 1963; Wood, 1967; Wood & Watson, 1969). The time course of these effects has not, unfortunately, been studied adequately to permit any conclusion concerning their involvement in the effects observed in the present study.

That inhibition occurs in the cerebral cortex has been known for some time. Hyperpolarizing potentials evoked by pyramidal and surface stimulation, having a delay suggestive of the presence of an inhibitory interneuron, were demonstrated in the very first reported intracellular recordings from Betz cells (Phillips, 1956a; 1956b), and subsequently by other workers (Branch & Martin, 1958; Li & Chou, 1962; Li, 1963; Pollen, 1964; Purpura & Shafer, 1964; Stefanis & Jasper, 1964;

Armstrong, 1965; Kubota, Sakata, Takahashi & Uno, 1965; Tsukahara, Fuller & Brooks, 1968; Biedenbach & Stevens, 1969). Inhibitory synapses have been demonstrated to occur on hippocampal pyramidal cell somata (Kandel, Spencer & Brinley, 1961; Andersen, Eccles & Loyning, 1963; 1964a; 1964b), and the inhibitory interneuron has been identified as the basket stellate cell. The observation that inhibitory synaptic potentials evoked by various routes almost always have a latency indicative of a di-synaptic linkage (Phillips, 1959; Pollen, 1964; Stefanis & Jasper, 1964; Armstrong, 1965; Kubota et al. 1965; Grampp & Oscarsson, 1968; Humphrey, 1968) has led to the conclusion that inhibitory internuncial neurons reside within the cerebral cortex (Grampp & Oscarsson, 1968). The suggestion has been made that the inhibitory transmitter substance in the cerebral cortex is acetylcholine, on the observation that depression of glutamic acid-evoked cortical unit activity by pyramidal tract stimulation is sensitive to atropine (Phillis & York, 1967). Basket cells have been demonstrated to occur in the cerebral cortex, in layers 3, 4, and 5 (Ramon y Cajal, 1952; Colonnier, 1966; Marin-Padilla, 1969). Further, cortical synaptic inhibition evoked by surface stimulation is readily detectable in isolated cortical slabs (Krnjevic, Randic & Straughan, 1966b). Unfortunately, the quantitative significance of inhibition in relation to excitation is difficult to evaluate since almost all of these studies were done with the use of general anesthesia which is known to depress excitation while having very little effect upon inhibition in the cerebral cortex (Krnjevic, Randic & Straughan, 1966c; Krnjevic & Schwartz, 1967 ; Crawford, 1970). However, the evidence certainly is not contrary to the explanation offered here for the stability of the

SPBR threshold.

The report that inhibition of hippocampal pyramidal cells can be countered by tetanic stimulation (Andersen & Lomo, 1968) is reminiscent of Adrian's finding (Adrian, 1936) that the deep response of the cerebral cortex appeared only after a period of repetitive stimulation. Both studies were done with barbiturate anesthesia. Taken together, these reports further suggest the involvement of inhibitory interneurons in determining the threshold for evoking the SPBR.

If a reduction of recurrent inhibition is involved in recovery of the SPBR threshold from the depressant action of inhaled 10% CO_2, the gradual development of accommodation produced by repeated brief inhalations of the gas (See Fig. 31) over a long period of time is best explained by an effect upon synthesis and/or storage of neurotransmitter substances, since it is unlikely that changes at excitable membranes or of ionic gradients could have such a long time course. If acetylcholine is an inhibitory transmitter in the cerebral cortex (Phillis & York, 1968a; 1968b) and is involved in recurrent inhibition (Phillis & York, 1967), then the impairment of its synthesis by CO_2 (Tenney, 1960) might intensify the depression of inhibition during repeated inhalations of the gas, thereby leading to accommodation of the response threshold to the depressant action. However, the failure of scolopamine to interact with inhaled 10% CO_2 (Fig. 40) leaves this question open.

The SPBR threshold-elevating action of acetazolamide is consistent with the hypothesis that the drug causes accumulation of endogenous carbon dioxide, since exogenous CO_2 has a similar effect and time course. Also in agreement is the observation that acetazolamide given during CO_2

inhalation (Fig. 38) prevents the occurrence of hyperexcitability upon withdrawal of the gas. What cannot be explained are 1) the failure of the drug to elevate the SPBR threshold when it is given while the animal is breathing 10% CO_2, since Meyer, Gotoh, & Tazaki (1961a) reported that acetazolamide given during CO_2 narcosis resulted in elevation of the brain P_{CO_2} and "worsening" (slowing and increased amplitude) of the EEG; and 2) the action of 10% CO_2 given after the administration of acetazolamide to lower the SPBR threshold (Fig. 35, 36). It could be argued that carbon dioxide given after the drug would fail to equilibrate with the extravascular space as rapidly as in the absence of the drug. But it would be expected, in view of the many reports that acetazolamide potentiates the depressant actions of carbon dioxide (Woodbury & Karler, 1960; Esplin & Rosenstein, 1963; Gray & Rauh, 1968), that inhalation of CO_2 after treatment with acetazolamide should further elevate the threshold. That it was instead lowered is beyond precise explanation, since 20% and 30% CO_2 were found to exert a more powerful depressant action than 10% CO_2 upon the SPBR threshold. It should be mentioned that in four of the five cats in which acetazolamide was studied, 3% CO_2 was tested before the administration of acetazolamide. In three cases the gas produced no effect, and in the fourth it raised the threshold slightly. These results tend to exclude the explanation that acetazolamide pretreatment reverses the action of 10% CO_2 inhalation by reducing the concentration which reaches the site of action.

IV. <u>The nature of the SPBR.</u>

As mentioned in the Introduction, Burns & Grafstein (1952) thought that the SPBR must be a "component of normal physiological

activity" of the cerebral cortex. Countering this view is the criticism that the SPBR represents a hypersynchronous condition of the cortical neurons which never occurs physiologically (Adrian, 1936; Sholl, 1956; Burns, 1958). From the present study, it is apparent that the intensity and duration of the response are not related to the condition of the cortex (insofar as the oxygen and carbon dioxide gas tensions are concerned). This contradicts the premise on which Burns & Grafstein based their conclusion, but does not necessarily exclude the validity of their concept.

The complete absence of a relation between the SPBR threshold and SPBR amplitude and duration suggests that different circumstances prevail for the initiation of the response, and for the extent of activation of the neural components whose activity constitutes the response. The same conclusion follows from the observation of Burns (1951) that the response amplitude is constant with increasing the stimulus intensity, although the transmission delay to onset of the response varies with changing the stimulus intensity (Brown & Pinsky, 1971). From the earlier discussion of the involvement of inhibitory synaptic phenomena in determining the excitability of cortical neurons, and from the suggestion by Andersen & Eccles (1962) that the rhythmic discharge of a neuron is strongly influenced by the interplay of excitation and recurrent inhibition, the lack of a relation between SPBR threshold and SPBR amplitude should not be surprising.

What is surprising, however, is the failure to find any relation between the SPBR amplitude and the coincident firing rate of single neuronal units (See p.133). This may indicate that the SPBR amplitude is a reflection solely of the number of activated neurons in the slab,

rather than of the intensity of activity in single neurons.

The presence of some neuronal units which would fire during the SPBR only in the presence of a continuous subliminal micro-electrophoretic current of glutamic acid is similar to a finding of Phillis & Ochs (1971). They observed that a small glutamate current would often allow a neuronal unit to fire in relation to the nDCR which otherwise would not. These workers concluded that the excitatory membrane currents generated in dendrites during the nDCR are often attenuated to an extent where they are inadequate by themselves to excite the soma. The same conclusion appears to be appropriate with regard to the present observation. A possible additional explanation is that inhibitory currents are generated as well. Glutamic acid iontophoresis might effectively augment the excitatory currents to the extent where inhibitory currents no longer predominate, since it is known that iontophoretically applied glutamate can overcome synaptically-evoked inhibitory hyperpolarization of cerebral cortical neurons (Krnjevic, Randic & Straughan, 1966b).

The observation that iontophoresis of glutamate ions may fail to excite units which fire in synchrony with the SPBR (See Fig. 44, 45) can be explained by supposing either that some neurons are insensitive to glutamic acid, or that such unresponding units lie beyond the range of diffusion of an effective concentration. It has been estimated (Curtis, 1964) that this distance is as small as 40 μm, while acceptable signal-to-noise characteristics for extracellular recording can be obtained up to a distance of 50 μm (idem). (In contrast, the results of Herz, Zieglgansberger & Farber (1969) indicate that iontophoresed glutamate ions can diffuse in concentrations which are effective beyond the

range of the recording electrode.)

A third possibility is that the glutamate ions ejected from a microelectrode tip do not diffuse freely in all directions, and may therefore fail to reach the structure responsible for the spike activity seen during the SPBR.

Several possibilities are suggested by the observed differences between the response to inhalation of 10% carbon dioxide in neurons whose activity had been evoked by iontophoretically applied glutamic acid and in neurons whose firing was the result of participation in an evoked SPBR. Inhalation of the gas mixture depressed the firing of a sizable proportion of the glutamate-sensitive units but depressed only a small proportion of those neurons which were activated during the SPBR. These findings may indicate that the population of neurons which are activated during the SPBR does not include many of the neurons that are directly depressed by CO_2. It is also possible, however, that the neurons whose glutamate-evoked activity is depressed by carbon dioxide are not inherently sensitive to the depressant effects of this gas but rather that they have had their sensitivity to glutamic acid altered by this agent. This would imply the lack of a physiological role for glutamic acid in the activation of neurons which occurs during the SPBR.

Another possible interpretation is that the finding of a group of neurons whose activity was depressed was due to the concentration of iontophoretically applied glutamic acid made available to the neurons being reduced by the enhancement of the cerebral cortical circulation by the inhalation of excess CO_2.

It is more tempting, however, to consider that there are essentially two populations of neurons, as indicated by the response to CO_2 of units. The more complex result seen with glutamate-activated neurons in response to CO_2 can then be explained, perhaps, partly by the various proposed mechanisms in the foregoing, and partly by the possibility that each glutamate-sensitive neuron has two receptive sites for that ion (e.g. the dendritic field and the cell body) and that these sites differ in their responsiveness to the interactions which can occur between CO_2 and glutamic acid at their receptive surfaces.

It is of interest, at least on philosophical and perhaps on pragmatic grounds as well, to speculate on the possible significance of the SPBR to the normal function of the cerebral cortex. It is difficult to deny that once the response has been initiated and has begun to spread across the isolated cortical slab, it must be associated with activity in the same neurons which subserve normal cortical function. Libet (1965) and Libet et al. (1964; 1967) have reported the stimulus parameters necessary for evoking "conscious experience" in unanesthetized unoperated human cerebral cortex as reported by the subjects. Single-pulse stimuli which evoked the nDCR applied to the cortical surface did not produce conscious experience. Rather, a train of stimuli was necessary. The minimum duration required was 0.5-1.5 seconds, for all pulse rates from 15/sec to 120/sec. It might be expected from Adrian's studies in anesthetized unoperated cat's cerebral cortex (Adrian, 1936) that a train of stimuli having such duration would lead to invasion of the deep layers of the cortex (i.e. evoke the "deep" response) while briefer durations

would not. It thus appears that the stimulation found necessary by Libet to evoke a reported conscious experience may correspond to that stimulation which evokes the deep response of unoperated cortex and to the neuronal activity of isolated cortex which is detected at the surface as the SPBR. If the activity which constitutes the SPBR is thus related to the cortical activity which consitutes conscious experience then the concept of Burns & Grafstein (1952) is to a large extent exonerated.

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