A STUDY OF A MARSUPIAL OLFACTORY SYSTEM; ITS ELECTRICAL ACTIVITY AND MODIFICATION BY DRUGS

A thesis submitted for the degree of
Doctor of Philosophy
by

Peter Robert Wilson, M.B., B.S.
Department of Human Physiology
University of Adelaide
Australia

October 1974

TABLE OF CONTENTS

SUMMARY OF THIS THESIS

DECLARATION

ACKNOWLEDGEMENTS

Chapter		Page
1	MARSUPIAL OLFACTORY SYSTEM	
	Summary	
	Introduction	1-1
	Structure of olfactory system	1-4
	Connexions within olfactory system	1-20
	Discussion	1-24
2	CONNEXIONS OF PHALANGER OLFACTORY BULB	
	Summary	
	Introduction	2-1
	Methods	2-2
	Anaesthetic technique	2-3
	Histological techniques	2-4
	Photographic techniques	2-5
	Evoked potential techniques	2-5
	Results	2-7
	Evoked potentials	2-7
	Centripetal bulbar connexions	2-8
	Centrifugal projections to bulb	2-9
	Anterior commissure	2-10
	Discussion	2-11
	Bulbar evoked potentials	2-11
	Mapping with evoked potentials	2-15
	Comparison with other studies	
	Adey (1953)	2-21
	Putnam & Cone (1966)	2-24
	Heath & Jones (1971)	2-25
	Conclusions	2-29
3	OLFACTORY INDUCED WAVES IN PHALANGER	
	Summary	
	Introduction	3-1
	Methods	3-9
	Anaesthesia	3-9
	Recording	3-10
	Stimulation	3-11
	Bulbar isolation	3-12
	Results	3-14
	Control experiments	3-15
	Induced waves	3-15
	Induced waves: olfactory cortex	3-17
	Induced waves: isolated bulb	3-18
	Alerting response	3-19
	Discussion	3-21
	Conclusions	3-31

Chapter		Page
4	EFFECTS OF ANAESTHETICS ON OLFACTORY ELECTRICAL ACTIVITY	
	Summary	
	Introduction	4-1
	Induced activity: drug effects	4-2
	Evoked activity: drug effects	4-8
	Unit activity: drug effects	4-11
	Methods	4-14
	Results and Discussion	
	Nitrous oxide	4-16
	Chloroform	4-20
	Halothane	4-23
	Barbiturates	4-27
	Propanidid	4-34
	Diazepam	4-36
	Conclusions	4-39
5	EFFECTS OF ANALEPTICS ON OLFACTORY INDUCED ACTIVITY	
	Summary	
	Introduction	5-1
	Induced waves: analeptic effects	5-2
	Evoked potentials: analeptic effects	5-7
	Unit activity: analeptic effects	5-8
	Isolated slabs: analeptic effects	5-11
	Histochemistry	5-11
	Methods	5-12
	Results and Discussion	
	Lignocaine	5-14
	Strychnine	5-17
	Picrotoxin	5-20
	Bicuculline	5-23
	Tetramine	5-25
	General Discussion	5-28
	Conclusions	5-30
6	OVERVIEW	
	Neuroanatomy	6-1
	Evoked potentials	6-3
	Induced waves	6-5
	Anaesthetics	6-7
	Analeptics	6-8
A	APPENDIX	
	Data collection and analysis	A-1
B	BIBLIOGRAPHY	B-1

MARSUPIAL OLFACTORY SYSTEM; ELECTRICAL ACTIVITY AND ITS MODIFICATION BY DRUGS

S U M M A R Y

The effects have been investigated of anaesthetic and analeptic agents on the electrical activity of a marsupial olfactory system. The phalanger, *Trichosurus vulpecula*, was chosen because of the accessibility of its large olfactory system to stereotaxic techniques.

Before undertaking the pharmacological study, it was necessary to investigate the connexions and normal electrical activity of its olfactory system.

Chapter 1 is an introductory review of current concepts of the gross anatomical structure of the vertebrate olfactory system, with particular reference to that in marsupials. The knowledge of its fine structure is reviewed, because of some important implications in the generation and modification of its electrical activity. Certain aspects of the structure of the olfactory system in the phalanger are discussed in detail.

Chapter 2 is an evoked potential study of the connexions of the olfactory bulb of the phalanger. Good correlation is shown between this method and previously-published degeneration studies. Some differences are shown. Delineation of primary olfactory cortex is clarified.

Chapter 3 reports the investigation of brain electrical activity induced by olfactory stimulation. Records were made from olfactory bulbs and cortex. The olfactory induced waves are described for the first time in the phalanger, and compared with those reported in other marsupials and mammals. The limitations of the method and changes caused by the experimental conditions are discussed.

Chapter 4 describes the results of experiments in which a number of central nervous system depressant drugs were given to modify this electrical activity. The agents used were nitrous oxide, chloroform, halothane, pentobarbitone, thiopentone, propanidid and diazepam. Differences were noted between the effects; no common structure-action relationships emerged.

Chapter 5 reports similar experiments in which analeptic agents were used. Strychnine, pictrotoxin, lignocaine, bicuculline and tetramine were used. Previously undescribed effects were noted.

Chapter 6 is an overview of the whole thesis, and contains conclusions and suggestions for further research.

An appendix makes brief mention of methods of data acquisition and analysis used in the studies. It illustrates some results of spectral analysis of induced waves.

DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any university, and that, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

P. R. WILSON

ACKNOWLEDGEMENTS

I cannot hope to express adequately my appreciation for the contributions of my supervisor, Dr D. I. B. Kerr, upon whose knowledge and wisdom I relied so heavily, and whose genial guidance and assistance was given with unlimited patience.

Dr Barbara Dennis taught me the basic experimental techniques: I am very grateful for this and the valued discussions, particularly of neuroanatomical and histological problems.

Dr Peter Polson gave freely of his time and experience in conceptual, computational and electrical matters: I am highly appreciative of this help.

Mr Reg Coutts willingly assisted me with problems of data analysis: I am indebted to him for this.

Mr Max King gave invaluable advice and assistance in histochemical and organic chemical matters: for this also I am deeply indebted.

Miss Julie Hagel provided competent and cheerful technical assistance for which I am most thankful.

Mrs Lilian Kingston has overcome many difficulties and frustrations in typing assorted drafts and the final copy of this thesis. I am very pleased to acknowledge her invaluable assistance.

This research was carried out with the aid of a National Health and Medical Research Council Medical Postgraduate Research Scholarship, and the Reginald Walker Research Fellowship, University of Adelaide.

Permission to use phalangers in this study was given by the Department of Environment and Conservation, South Australian Government.

To my wife Libby, without whose support, encouragement, forebearance and sacrifices this was not possible.

Our ignorance is boundless and overwhelming. Every new bit of knowledge we acquire serves to open our eyes further to the vastness of our ignorance.

K. R. Popper

Second thesis

1963

CHAPTER ONE

SUMMARY

1. Marsupials possess a prominent olfactory system, which can be readily studied in the phalanger (*Trichosurus vulpecula*).

2. The general organization of the phalanger olfactory system is similar to that in mammals, and contains the same components.

3. The detailed anatomy of the olfactory system of the phalanger is described, and the present work gives the first account of its anterior olfactory nucleus.

4. Divisions of the primary olfactory cortex are discussed and amended for the sake of simplicity.

5. Subcortical olfactory areas are described and discussed (nucleus of the diagonal band, olfactory tubercle, and amygdaloid nuclei).

6. Connexions of and within the olfactory bulb are described and discussed.

THE MARSUPIAL OLFACTORY SYSTEM

INTRODUCTION

'Truly, smell memories are all as wonderful as sight memories. For every one there is some faint, subtle, undefinable scent which can bring back whole scenes, whole periods of life, joyous or sad, and recall pictures difficult to evoke in the absence of odour itself. It is the indescribable, undefinable scents which are so wonderful for their memory power. I feel certain that no dog, were he turned anatomist, would credit me with my complex smell memories and associations, and that behoves me to be more careful how I deny him some things which I possess, but for which I cannot find in him great evidences of anatomical specialization.' So wrote Frederic Wood Jones in 1924, pointing out two of the least understood of the brain functions, even half a century later, smell and memory. He also pointed out the difficulty of structural and functional correlation within the brain. It is with a small aspect of this problem that this thesis is concerned, using electrical and pharmacological manipulations of the olfactory system as the tools of research.

There is a triad of methods in neurobiology, referred to by Adey (1970), stimulation, ablation and electrical recording, to which may be added a fourth one, neurochemistry.

Early brain research relied on observations of the gross and fine structure of neural tissue. Golgi's (1873) contribution of the chrome-silver stain contributed the greatest single advance in this field. Although only staining an occasional neurone, it does so completely, enabling workers (e.g. Cajal, 1909) to describe neuronal projections and imply functions. Degeneration studies were also able to contribute much to the understanding of neural connexions. The major contribution to nervous system function was the discovery by H. H. Dale & H. W. Dudley (1929) of chemical transmission at synapses in the peripheral nervous system, and by induction later, transmission in the central nervous system.

In 1875, Richard Caton demonstrated spontaneous fluctuations in electrical activity of the rabbit brain, and also changes related to sensory stimulation, setting the stage for the continuing investigation of brain function by this method.

Information from the external and internal environment is gleaned by specialized receptors. The electrical output from these various receptors is transmitted to "higher centres", where it is modified and

dealt with in an appropriate way, often manifested by somatic or visceral motor actions. The impulses from all sensory modalities were assumed to reach the brain itself, which would then initiate the behavioural changes necessary. However, in 1954, Hagbarth and Kerr showed that descending influences could modify the incoming sensory information at the spinal cord, at the first synapse. The following year they showed that a similar efferent system existed in the olfactory system, confirming Cajal's deductions from observations on the structure of that system. These major contributions to the understanding of brain function stimulated the search for such systems in other modalities. This culminated in the olfactory system with the demonstration of widespread influences on olfactory function by Dennis & Kerr (1968).

It is with a further comparative study of these aspects of the olfactory system that this thesis is concerned.

Classification of the mammals

As this thesis deals with olfaction in marsupials, it is useful to review the phylogenetic position of this sub-class.

The class mammalia embraces types of animals which may differ by considerable degrees, causing difficulty in orderly and rational classification. Three sub-classes were first described by de Blainville in 1816, using characteristics of the female reproductive system as the criteria for estimating evolutionary rank. The first sub-class had a reproductive system reminiscent of that seen in birds and therefore was named *Ornithodelphes*. An essentially bilateral internal genital system characterised the second sub-class, *Didelphes*, while the fused midline reproductive structures of the third group led to the term *Monodelphes*. By 1880, Huxley had introduced the terms *Prototheria, Metatheria* and *Eutheria* to describe these three sub-classes. Jones (1924a) retained the former classification in his descriptions of the mammals of South Australia.

The sub-class *Ornithodelphes (Prototheria)* is represented by two families only, *Echidnae*, the spiny ant-eater, and *Ornithorynchidae*, the platypus. Both are egg-laying mammals which suckle their young in the pouch (incubatorium) from mammary glands without nipples.

Sub-class II, *Didelphia, Marsupialia* or *Metatheria*, contains a varied assortment of animals of numerous species, including the tiny marsupial mouse (*Sminthopsis crassicaudata*), the anophthalmic marsupial moles (*Notoryctidae*), native cats (*Dasyurinae*), phalangers, and the

well-known kangaroos and wallabies. Parturition in this sub-class occurs at an early stage in development of the embryo and growth and differentiation continues in the pouch (marsupium).

There is an invariable neuroanatomical characteristic of these latter two sub-classes which sets them apart from *Monodelphes* (Eutheria); there is no corpus callosum. This implies that inter-hemispheric transfer of information must be mainly via the anterior commissure.

Satisfactory classification of the Didelphia is difficult. Marsupials may be divided into two main groups using the structure of the *pes* as the criterion. The *Didactyla* retain the primitive arrangement of the digits, each being separate and independent of the other. *Syndactyla* have the second and third pedal digits bound together over the greater part of their length, appearing to be a single toe with two toenails.

Although dietary classification itself cannot be precise, a division of the sub-class into two groups can be made by a study of the dentition. Carnivorous or insectivorous marsupials possess numerous front teeth and are named the *Polyprotodontia*. On the other hand, the predominantly vegetarian group has a reduced number of front teeth; the *Diprotodontia*. In most cases, the *Polyprotodontia* are also *Didactyla*, and the *Diprotodontia* are *Syndactyla*.

Jones (1924a) classified the order Marsupialia into two sub-orders, *Didactyla* and *Syndactyla*, the latter having two sections, *Syndactyla protodontia* and *Syndactyla diprodontia*. He also comments on the difference in habits. Didactyla are *'as a rule, alert, intelligent creatures preying upon almost any living thing they can overpower and kill'*, while the *Syndactylous diprotodontia* are *'for the most part rather markedly unintelligent, and many distinctly phylogenetically senile. They are the end products of Didelphian development, the most advanced phase of specialisation of the Marsupial stock'*.

This logical classification of the marsupials is complicated by neuroanatomical evidence colligated by Smith (1902). Part of the anterior commissure of the Diprotodontia enters the internal capsule as the *fasciculus aberrans*. This arrangement is not seen in the *Polyprotodontia*, in which the anterior commissure continues laterally to enter the external capsule, the usual mammalian arrangement.

Possums

There are animals in three families known by the common name

"possum". The sub-order Didactyla contains the family *Didelphidae*. The members of this family are the carnivorous marsupials confined to the Americas and commonly known as "Opossums". The section Diprotodontia of the sub-order Syndactyla contains the family *Phalangeridae*, which has sub-families *Phascolarctinae* and *Phalangerinae*, each defined dentally. The ring-tailed possum belongs to the genus *Pseudochirus* of the former sub-family, and the brush-tailed possum to the genus *Trichosurus* of the latter.

To avoid semantic confusion in this thesis, *Didelphidae* (e.g. *Didelphis virginiana*) will be referred to as "opossum", and *Trichosurus vulpecula* as "phalanger". The flying opossums, flying phalangers, and ring-tailed possum will not be considered in subsequent discussions.

The brush-tailed phalanger is an arboreal, crepuscular and nocturnal, herbivorous marsupial, the mature adult weighing about 2 kg. An inoffensive and stupid simplicity is the keynote of its psychology, but it will bite and scratch, and defend itself stoutly (Jones, 1924*a*). In the natural state it is fiercely territorial, such territory being marked in the olfactory domain both with urine and secretions from sub-mental chest and anal glands (Thomson & Pears, 1962). Secretions from the paracloacal glands provide olfactory cues in the reproductive process (Winter, 1971).

Because this thesis is concerned with normal and altered olfactory function, and the study of drug effects on the brain, using the olfactory system as a model, it is desirable to review the knowledge of the neuroanatomy of the marsupial forebrain. It will be necessary to use knowledge derived from other species to augment this. Such extrapolations will be justified where possible.

MARSUPIAL OLFACTORY SYSTEM

The olfactory system of mammals and marsupials has the simplest structural arrangement of the special senses, as the Table 1-1 shows (adapted from Pigache, 1970). It would seem that it would be an ideal system to examine because of its apparent anatomical simplicity and ease of access by conventional stereotaxic techniques. However, it is beset by certain problems. In the other special senses the stimulus can be fairly easily quantitated, e.g. vision. The basic stimuli for the olfactory transducers are unknown, and it is difficult, if not impossible, to quantitate the input.

The arrangements described above refer to the afferent component of

TABLE 1-1

(Adapted from Pigache, 1970.) A comparison of neuronal chaining in primary sensory systems.

System	Link 1	Link 2	Link 3	Link 4	Link 5	Link 6
Olfactory	Olfactory receptor in mucosa	Mitral neuron and tufted neuron	Primary olfactory cortex	-	-	-
Somato-sensory	Cell of dorsal root ganglion	Neuron of dorsal horn or dorsal column	Thalamic neuron (ventro-lateral nucleus)	Sensori-motor cortex	-	-
Visual	Rod or cone of retina	Bipolar neuron of retina	Retinal ganglion neuron	Thalamic neuron (lateral geniculate nucleus)	Visual cortex	-
Auditory	Hair cell of organ of Corti	Bipolar neuron of spiral ganglion	Neuron of cochlear nucleus	Neuron of either trapezoid body nucleus or superior olivary nucleus	Thalamic neuron (medial geniculate nucleus)	Auditory cortex

the systems, but the normal functioning of these must depend on their interaction with the efferent systems. The central connexions of the afferent components, and disposition of the efferent systems, are proving to be more complicated than suggested by early workers. The olfactory system is closely connected with reproductive functions and communication, and its function may be altered by circulating hormones, normally not regarded as neurohumors. These aspects have been discussed by (*inter alii*) Wenzel & Sieck (1966), Moulton (1967), Ralls (1971), Schneider (1971), and Eisenberg & Kleiman (1972).

'The olfactory cortex culminates in marsupials' (C. J. Herrick, 1924, his p. 355), but comparatively little attention has been paid to the neurobiology of olfaction in this order. C. L. Herrick described *'the cerebrum and olfactories of the opossum'* in 1892. Definitive anatomical studies of the olfactory system of this animal have been made since then by Johnston (1923*a*), Gray (1924), C. Judson Herrick (1924), van der Sprenkel (1926), and Loo (1931). The following summary of the neuroanatomy of the marsupial olfactory system is based largely on these papers. Other relevant contributions to this field have been made by G. Elliot Smith (1895), McCotter (1912), Obenchain (1925), who refers to 24 of Smith's papers on marsupials, Abbie (1942), and Putnam & Cone (1966).

Other neuroanatomical contributions to knowledge of forebrain and commissural systems of marsupials include those of C. L. Herrick (1893), Smith (1894, 1902), Johnston (1913, 1923*b*), Abbie (1939), Crosby & Humphrey (1939), Adey (1953), Pigache (1970), Ebner (1969), and Heath & Jones (1971).

Adey (1953) has published the only anatomical studies on the central olfactory connexions in the phalanger. The electrophysiological mapping experiments in Chapter 2 will be discussed bearing his work in mind.

External appearance of phalanger cerebrum
(Figures 1-1, 1-2)

The phalanger cerebrum is in the shape of a flattened pear some 35-40 mm long and 22-25 mm deep. It is similar in external appearance to that of the rabbit and opossum. The sessile olfactory bulbs, 10 mm long, are inclined upwards in front of the frontal poles of the cerebrum and separated from them by a deep fissure.

Each cortical surface is divided into an upper and lower part by the rhinal fissure, which curves on the lateral aspect from the bulbar

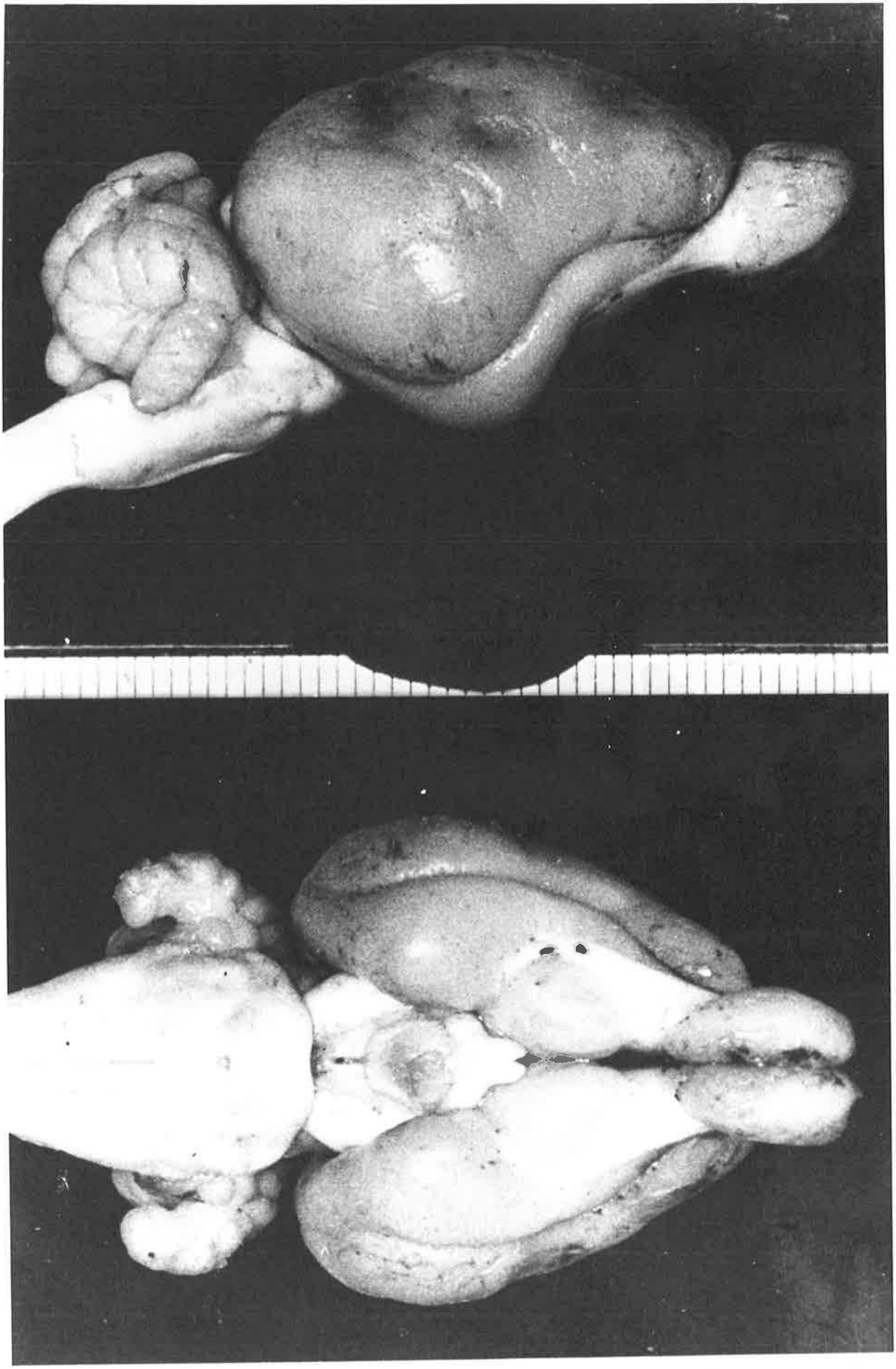


Fig. 1-1 Lateral and ventral views of the phalanger brain from an animal weighing 1830 g. Scale mm. A diagram showing the main features appears in Fig. 1-2.

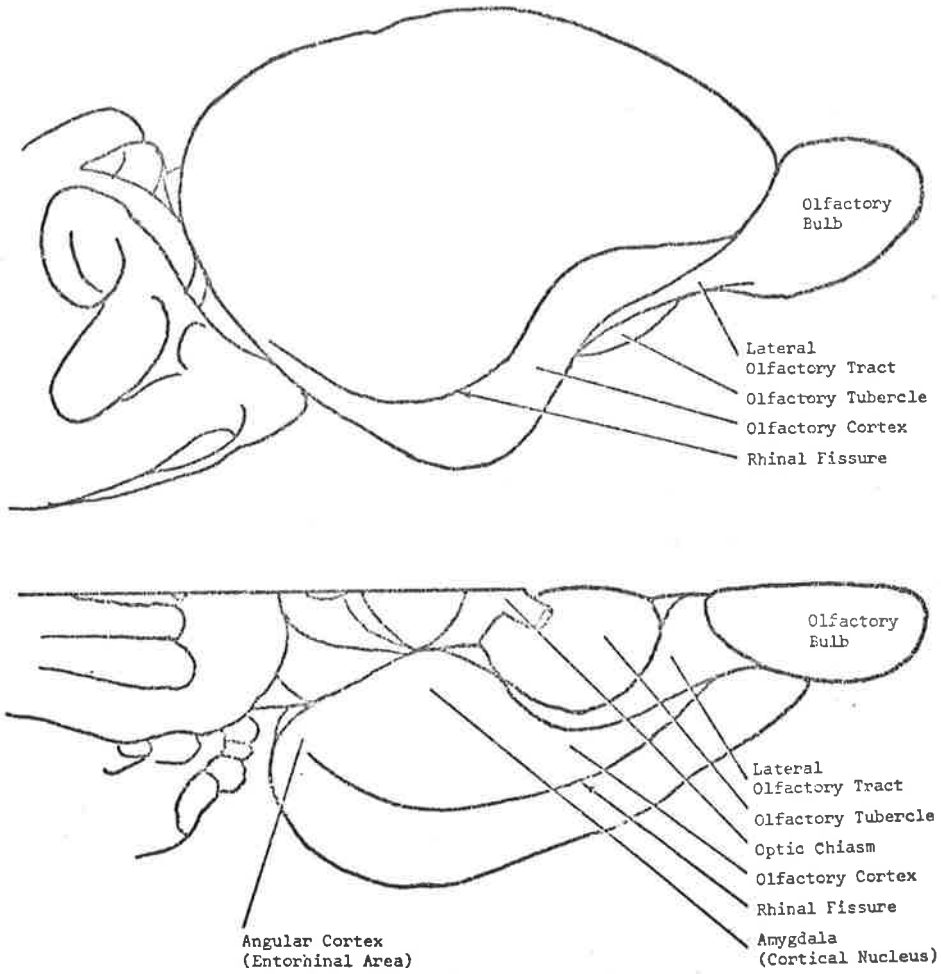


Fig. 1-2 Diagrammatic views of the lateral and ventral surfaces of the phalanger brain. The ventral surface is apparently foreshortened because it was photographed from a slightly rostral viewpoint. Parallax errors were reduced by having an object-camera distance of 1.2m.

fissure to the caudal extremity of the hemisphere. The lateral and dorsal surface of the cerebrum of the brain, delimited laterally by the rhinal fissure, comprises the rhinencephalon, which is also remarkably smooth.

The lateral olfactory tract emerges from the ventro-lateral part of the bulb as a white tract 4 mm wide. During its course over the surface it becomes narrower as it curves around the lateral side of the prominent olfactory tubercle. It disappears behind this structure at the level of the optic chiasm, 5 mm from the midline. Caudal to the tubercle is a groove which separates it from the large, nearly hemispherical amygdaloid complex, which is apparently continuous with the surrounding basal cortex. This cortex extends through the caudal aspect of the hemisphere, medial to the rhinal fissure. There is no obvious postero-superior extent of the basal rhinencephalon.

The olfactory bulb

C. L. Herrick (1892) was the first to describe the olfactory bulb of the opossum, and enumerated four concentric layers. The outer layer (1) included olfactory nerve fibres, blood vessels and glial cells. The 'glomerulary' layer (2) was thought to manufacture myelin sheaths. The external neuroglia layer (3) contained pyramidal cells, with strong apical processes entering layer 2, lateral anastomoses, and ganglion cells. The laminated granular layer (4) was thought to be the origin of the radix pedis lateralis (lateral olfactory tract) and the radix entalis (anterior part of the anterior commissure).

The bulb of marsupials was compared with that of other animals by several workers (e.g. McCotter, 1912; C. J. Herrick, 1924; Rose, 1926; reviewed by Nieuwenhuys, 1967) and shown to resemble that of other mammals. The subject has also been recently reviewed by Shepherd (1972). Morest & Morest (1966) have described the lamination in the opossum bulb and noted the similarity with other small mammals. Morest (1970) studied neurogenesis in the forebrain of opossum pouch young, and his findings were similar to those of Hinds (1968*a*, *b*; 1972*a*, *b*) in the mouse.

The following description of the cells and connexions of the olfactory bulb is based largely on the work of the Oxford school, using the rat as the experimental animal (Price & Powell, 1970*a*, *b*, *c*, *d*, *e*, *f*; 1971; Pinching & Powell, 1971*a*, *b*, *c*). Price & Powell's description (1970*a*) of the lamination of the bulb will be followed. They enumerated the following seven layers:

1. Olfactory nerve layer
2. Glomerular layer
3. External plexiform layer
4. Mitral cell layer
5. Internal plexiform layer
6. Granule cell layer
7. Periventricular layer

This lamination is present in the olfactory bulb of the phalanger,* illustrated in Figures 1-3 and 1-4A.

1. Olfactory nerve layer

Thin, unmyelinated axons from the primary receptors in the olfactory mucosa emerge from the cribriform plate and cover the surface of the bulb in interwoven bundles. In the rabbit there is partial topographical relationship between the dorsal nasal recess and the dorsal surface of the bulb (Land, Eager & Shepherd, 1970; Land, 1973; Freeman, 1974). There is a total of 50-100 million fibres to each bulb in the rabbit (Allison, 1953). The numbers and arrangements of these fibres have not been investigated in the phalanger. These afferent fibres turn inwards and form the glomeruli of the second layer.

2. Glomerular layer

The glomeruli are granulo-fibrillary ball-like regions 100-200 μ in diameter, embedded in cellular elements, forming the second layer of the bulb.

The relay of the information carried by the olfactory nerves to the next transmission stage (the mitral cell) occurs in this layer. Allison & Warwick (1949) estimated that in the rabbit, every glomerulus received impulses from about 26,000 olfactory receptors, and passed the information to 24 mitral and 68 tufted cells. Allison (1950) estimated that there were 2000 glomeruli in each olfactory bulb of the rabbit and rat (implying some 50,000 mitral and 150,000 tufted cells in each). Bulbar organization has been clarified by the work of Reese & Brightman (1965; 1970), Hinds (1968*a*, *b*; 1970; 1972*a*, *b*), Pinching & Powell (1971*a*, *b*, *c*, *d*; 1972*a*, *b*, *c*) and White (1972*a*, *b*; 1973).

The olfactory nerves terminate only in the glomeruli, and mostly enter from the superficial aspect. They divide to give tortuous and

---

* Dr R. S. Tulsi, of the Department of Anatomy, University of Adelaide, has kindly examined two specimens of olfactory bulbs of phalangers for me with electron microscopy. The components of the bulb appeared similar to those in other species, but a formal study has not been undertaken.

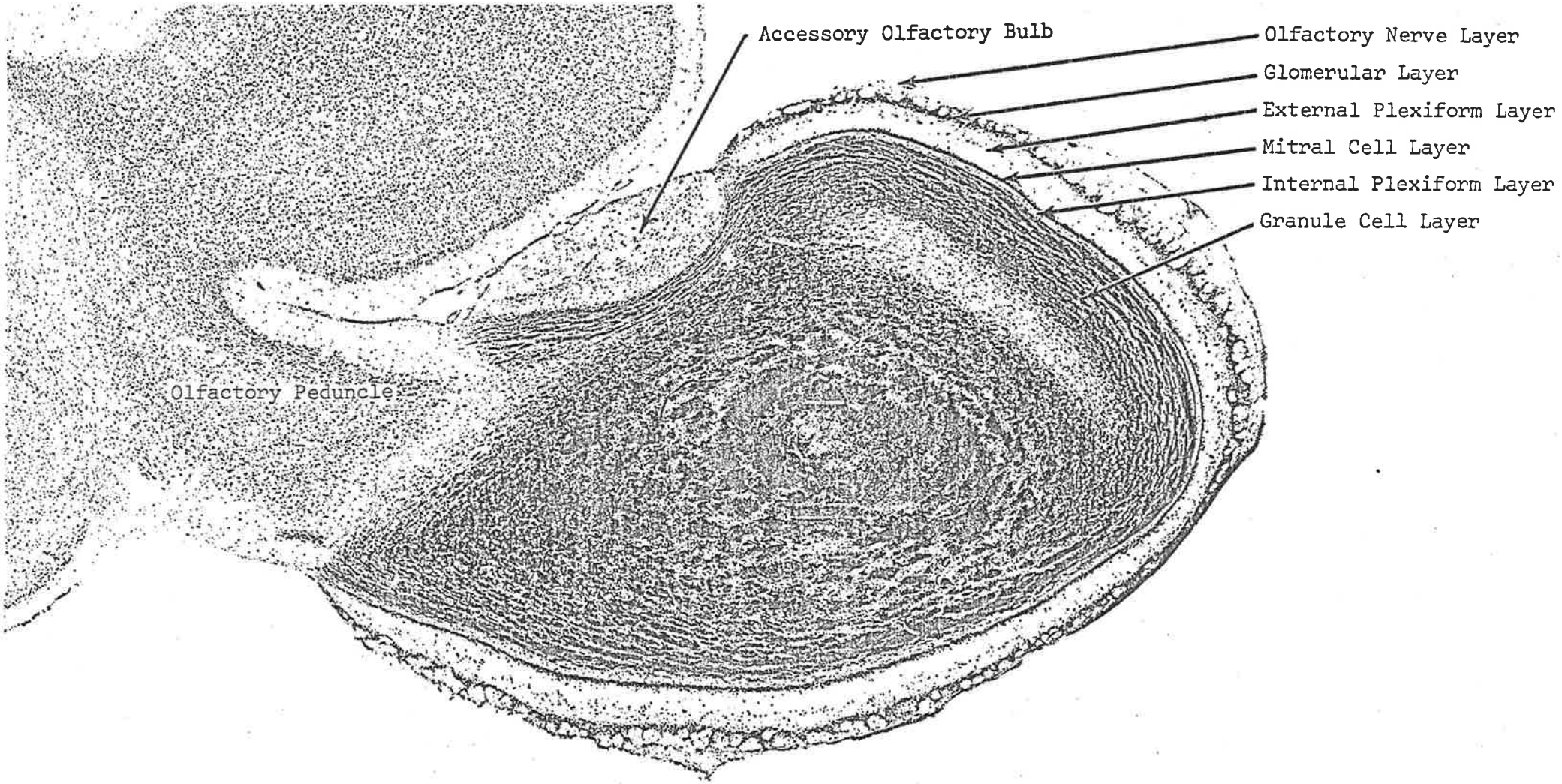


Fig. 1-3 Sagittal 50u section of the olfactory bulb of the phalanger, 2mm from the midline, stained with neutral red for cellular detail.
Note:(1) Position of the accessory bulb relative to the frontal pole. Transection of the bulb (referred to in Chapter 3) is made in front of the accessory bulb.
(2) Tufted cells are more numerous in the outer half of the external plexiform layer.

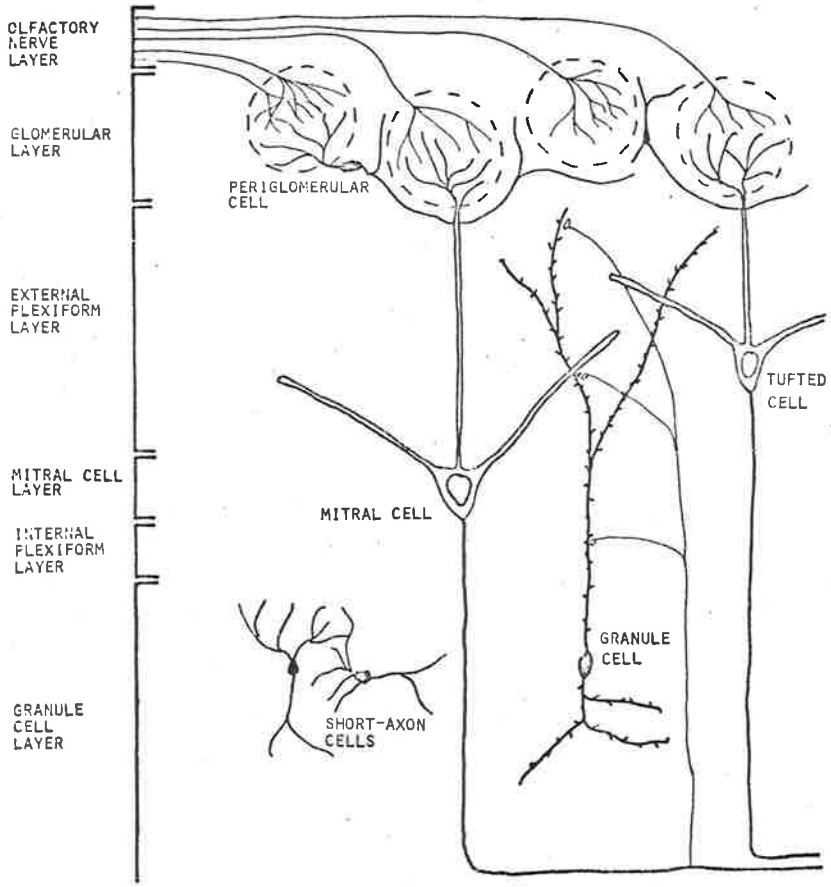


FIG. 1-4A DIAGRAM SHOWING THE MAIN CELL TYPES AND LAMINATION OF THE PHALANGER OLFACTORY BULB. (AFTER SHEPHERD, 1972). CENTRIFUGAL FIBRES ARE NOT SHOWN.

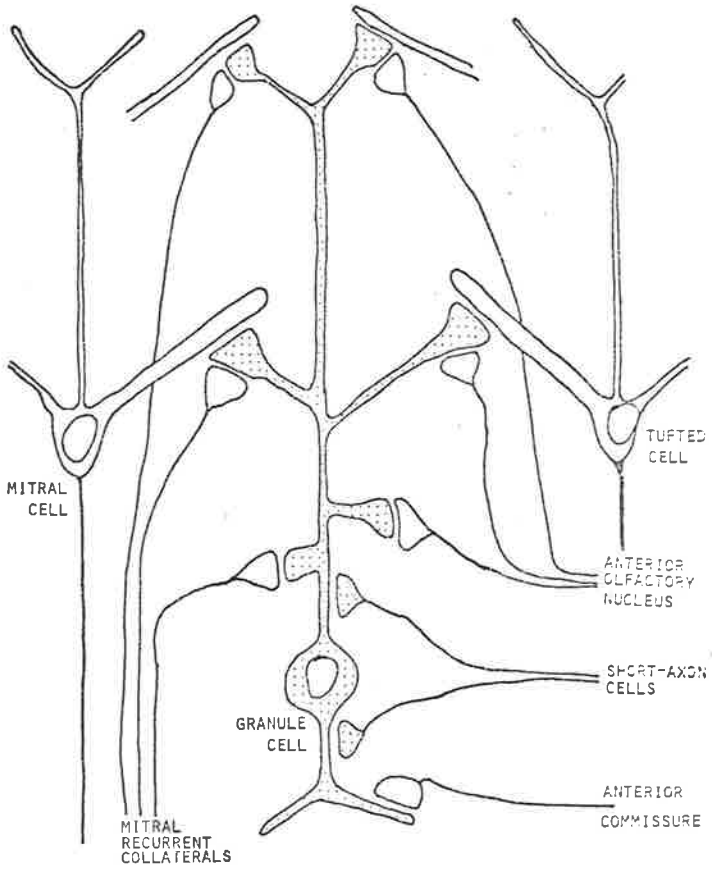


FIG. 1-4B DIAGRAM SHOWING THE MAIN CONNEXIONS OF THE MAMMALIAN GRANULE CELL. INHIBITORY SYNAPSES ARE STIPPLED. (AFTER SHEPHERD, 1972, PRICE & POWELL, 1970)

irregular branches, which fill the interstices between the larger mitral and tufted cell primary dendrites on which they terminate. The axon terminals are electron-dense and form asymmetrical synapses. No synapses have been seen ending on these axon terminals. There are few cells in the glomeruli.

There are three types of cells in this layer surrounding each glomerulus, described in the rat, rabbit, cat and monkey (Pinching & Powell, 1971*a*; their p. 322).

(a) External tufted cells are similar in structure but smaller than the middle or internal tufted cells elsewhere in the bulb. The fusiform or ovoid cell body has a large primary dendrite which enters a nearby glomerulus. Secondary dendrites are seen rarely. The primary dendrite forms a relatively simple arborization in its glomerulus and receives synapses from the olfactory nerve terminals. Their axons and recurrent collateral axons make contact with spines and gemmules of peri-glomerular cells up to 12 glomeruli distant. These external tufted cells are most common in the outer half of the external plexiform layer of the phalanger, forming an indistinct layer.

(b) Periglomerular cells are small (5-8 μ diameter) spherical cells, being the most common type in this layer. Pinching & Powell (1971*b*) and White (1973*a*, *b*) have described the synaptic connexions of these cells. Olfactory receptor cell axons synapse onto mitral and tufted cell primary dendrites. These dendrites are pre- and post-synaptic to periglomerular cell dendrites in approximately the same ratio. Olfactory nerve terminals also synapse onto periglomerular cell dendrites, except in the case of the Balb/c mouse. Synapses exist between periglomerular cell dendrites. There are no data on the connexions in the phalanger, but the arrangement described here applies to the rat (unspecified strain, Price & Powell; Sprague-Dawley, White), dwarf Netherlands rabbit and *Mus musculus* molossinus.

The periglomerular cell dendrites also receive symmetrical synapses from short-axon cells, and asymmetrical synapses from external tufted cell recurrent collateral axons, and centrifugal fibres (Price & Powell, 1970*c*). The periglomerular cell axons terminate up to four glomeruli distant on mitral and tufted cell primary dendrites, near their first branching. They also form synapses onto the somata, dendrites and axon initial segments of periglomerular and short-axon cells, and on the spines and gemmules of periglomerular cells. These periglomerular axon terminals do not receive synapses. Pinching & Powell

proposed 3 synaptic patterns involving the periglomerular cell: (a) reciprocal + symmetrical to the periglomerular cell; (b) reciprocal + asymmetrical to the periglomerular cell; (c) asymmetrical + symmetrical to the periglomerular cell. White (1973*b*) noted that some 20% of synapses between mitral and periglomerular dendrites were reciprocal.

(c) Short-axon cells have irregular spherical bodies (5-8 μ diam.) and short dendrites extending over about two glomeruli, with the few branches confined to the periglomerular region. These dendrites receive synapses from periglomerular, tufted recurrent and centrifugal axons, and do not form reciprocal synapses. The short axons are distributed for a distance of one to two glomeruli, and end on cells (all sites), and occasionally on another short axon cell. They do not terminate in relation to mitral or tufted cells or processes. Pinching & Powell (1971*a, b, c*) proposed two classes of dendrites in the glomerulus from their electron-microscopic studies. Class A only received synapses and Class B received and gave off synapses. This is a different approach from that of White (1972*a, b*) who postulated an hierarchical arrangement. From his electron-microscopic reconstructions (in which he saw only one type of presynaptic vesicle, spherical, 50 nm in diameter) he suggested that Stage 1 dendrites were postsynaptic to afferent neurones. These were shown to be mitral and tufted primary dendrites, and were never interconnected. They were presynaptic, post-synaptic, or reciprocally synaptic with Stage 2 dendrites. These latter ones were never postsynaptic to afferent (olfactory nerve) axons in Balb/c mice, but could be simultaneously pre- and post- synaptic with Stage 1 dendrites in other species.

The structure and relationships of the glomerular layer have not been resolved fully, but it is obvious from its complex arrangement that modulation of the signal could occur at this stage. Shepherd (1971) has produced electrophysiological evidence for dendrodendritic synaptic interactions by pairing electrical stimuli to the lateral olfactory tract (conditioning-test response), and recording with a micro-electrode deep in the glomerular layer. He concluded that inhibition of the mitral primary dendrite occurred through the periglomerular reciprocal synapse (similar to granule cell inhibition in the external plexiform layer), a conclusion also reached by White (1973*a, b*).

3. External plexiform layer

External plexiform layer is a relatively cell-free zone about 1 mm

thick beneath the glomerular layer. It contains the dendrites and recurrent collateral axons of mitral and tufted cells, arborizations of the peripheral processes of granule cells, a few fibres of short-axon cells, and centrifugal fibres. Tufted cells are scattered throughout, and in the phalanger form an indistinct band in the outer half of this layer (Fig. 1-3).

The problem of the tufted cells is unresolved. The deepest (internal tufted) cells are similar in structure and connexions to mitral cells (Pinching & Powell, 1971*a*), although Valverde (1965) claims that their axons do not leave the bulb. The middle tufted cell-bodies (in the external plexiform layer) become smaller, but their structure is probably the same. However, the external tufted cells in the glomerular layer are different in distribution, all their processes being confined to that layer. Each has the usual primary dendrite which enters a glomerulus, and occasionally has secondary dendrites. The axons and axon collaterals probably terminate on the periglomerular cell gemmule.

Pinching & Brooke (1973), using Golgi impregnation and electron-microscopy, reassessed "middle" tufted cells of young rats. They described new synaptic relationships in the external plexiform layer. Combined dendro-axonic and axodendritic synapses were in a reciprocal arrangement. Furthermore, their Fig. 9 shows a mitral gemmule in reciprocal arrangement with a tufted cell axon initial segment. This study illustrates the incompleteness of knowledge about the synaptic connexions of the bulb.

4. Mitral cell layer

The mitral cells are the largest cells of the bulb (20-30 μm) and take their name from the fancied similarity to a bishop's mitre (peaks directed centrally). They form a distinct layer about 2 cell-bodies in thickness centrally of the external plexiform layer. Each cell has a single primary dendrite which travels radially outwards through the external plexiform layer to terminate in an arborization in a glomerulus. It also has a small number of secondary dendrites (2-4) which are directed outwards and horizontally into the external plexiform layer where they branch a few times. These dendrites are never related to glomeruli and have no particular orientation (Price & Powell, 1970*d*). Shepherd (1966) had suggested that there was an antero-posterior bias in the orientation of the secondary dendrites. All primary and secondary mitral and tufted cell dendrites examined by Price & Powell (1970*d*)

were seen to make reciprocal synapses with gemmules. Within the glomeruli the gemmules were of periglomerular cell dendrites, and in the external plexiform layer they were of granule cell peripheral processes. All synapses onto the mitral cell soma (Ramon-Moliner, 1973), axon hillock and dendrites are reciprocal, except those from the olfactory nerve axon terminals. Mitral cell axons form the lateral olfactory tract, after giving off recurrent collateral axons.

These latter axons synapse with granule cell peripheral processes in the granule cell layer, and also are distributed to the anterior olfactory nucleus.

This layer also contains some granule cells.

5. Internal plexiform layer

Internal plexiform layer is deep to the mitral cell layer. It is a narrow fibrous zone which contains a few granule, mitral and tufted, and short-axon cells. This indistinct layer contains many of the recurrent axon collaterals of the mitral and deep tufted cells, and the peripheral processes of the granule cells. The mitral and tufted axons pass through this layer.

6. Granule cell layer

Granule cell layer is comprised mainly of granule cells, which are small (6-10 μ diam.) oval or rounded cells characterised by the absence of a true axon (cf the amacrine cells of the retina). They form a thick layer central to the internal plexiform layer, which is broken into irregular lamellae by a reticulation of myelinated and non-myelinated fibres. This layer also contains deep short-axon cells of two types, Golgi (horizontally oriented) and Cajal (radially oriented).

Granule cells have peripheral processes which are directed radially outwards, branching and becoming contorted in the external plexiform layer. Short dendrites are directed centrally; the total radial length of the cell is fairly constant. Spines are present on all parts of the cell processes, but in the external plexiform layer they are modified to form the "gemmules" (Rall, Shepherd, Reese & Brightman, 1966). The gemmules contain the only efferent synapse from the granule cells as part of the reciprocal synapse with the mitral cell secondary dendrite. Gemmules are also postsynaptic to axon terminals of mitral recurrent collateral axons, and centrifugal fibres.

Price & Powell (1970*b*) describe three main types of synapse involving the granule cells, using synaptic membrane configuration and vesicle size and shape to divide them.

(a) Asymmetrical synaptic thickenings onto dendritic spines are invariable, but occur rarely on the somata, shafts of peripheral processes or gemmules. All extrinsic fibres to the bulb make this type of contact, which is thought to be excitatory (Gray type I).

(b) Symmetrical synaptic thickenings are complementary to the asymmetrical ones. They are present on the shafts of the peripheral processes and dendrites, and have not been seen in relation to gemmules. They are thought to be from axons intrinsic to the bulb (mitral and tufted axon collaterals, and short-axon cell axons), and to be inhibitory (Gray type II).

(c) Reciprocal synapses occur where gemmules make contact with mitral and tufted secondary dendrites. The mitral-to-granule component is asymmetrical (excitatory), and the synapse in the reverse direction is symmetrical (inhibitory).

The main connexions of the granule cells are shown diagrammatically in Fig. 1-4B.

7. Periventricular layer

Fibres from mitral (and tufted) cells and centrifugal fibres form this layer, which also contains a few cells. It is difficult to determine the rostral extent of the anterior olfactory nucleus, and these cells may form part of that nucleus (Lohman & Mentink, 1969).

Accessory olfactory bulb

The accessory bulb has attracted relatively little attention. Ziehen (1897) described it as being wedged between the main bulb and peduncle in *Pseudochirus* (ring-tailed possum). It is shown in Fig. 1-3. McCotter (1912) described the vomeronasal organ projecting to the accessory bulb in the opossum and several other species. There has been a revival in interest in the accessory bulb, and it is apparent that it does not have the same projections as the main bulb.

Heimer (1969) reported on the central connexions of the accessory bulb in the tegu lizard. Winans & Scalia (1970) studied degenerative changes in rabbit brains after ablation of either main or accessory bulbs. They showed that the accessory bulb projected to the surface of the cortical and medial amygdaloid nuclei (their Figs. 2 and 3). Raisman (1972) made lesions in the stria terminalis and fimbria of young Wistar rats. He showed that the accessory bulb was reciprocally related to the cortico-medial amygdaloid nuclei via the lateral olfactory tract and stria terminalis (his Fig. 12). Similar conclusions were drawn: the accessory bulb has different projections to the main

bulb. Halpern (1973) noted different projections of each bulb in the snake.

Anterior olfactory nucleus

Anterior olfactory nucleus is the term introduced by C. J. Herrick (1910; his p. 494) and defined as "undifferentiated olfactory tissue of the second order, usually closely associated with the bulbus, the two often being represented in the terminal swelling commonly called the bulbus, and extending backwards a longer and shorter distance between the true bulbus (i.e. formatio bulbaris) and the more specialised parts to be enumerated". His paper (1924) on the anterior olfactory nucleus of the opossum was the basis for much of the subsequent work on this subject. Crosby & Humphrey (1939) have made comparative studies of olfactory bulbs and anterior olfactory nuclei. Morrison (1969) reviewed the literature in his Ph.D. dissertation, and made a comparative study of this structure in rodents and lagomorphs. Pigache (1970) reviewed the terminology and structure of the grey matter of the olfactory peduncle. He concluded that this area was insufficiently elaborate to be described as 'cortex' and that the original term was better. The caudal limit of the anterior olfactory nucleus was defined by the change to a three-layered cortex.

The cells of this nucleus make contact with secondary olfactory fibres in the plexiform layer via axon collaterals of the lateral olfactory tract. They appear to act as a bed nucleus for the anterior commissure, to which the olfactory bulbs do not contribute directly. Lohman (1963) made the connexions and nomenclature of this complex area clear, and proved for the first time that this nucleus was indeed the 'bed nucleus' of the anterior commissure which received no mitral or tufted axons. Dennis & Kerr (1968), Heimer (1968), Lohman & Mentink (1969), and Morrison (1969) have contributed to the understanding of this area.

Herrick (1924; his p. 331) divided this structure of the opossum into seven parts. *"A bulbar part anteriorly, entirely enclosed within the formatio bulbaris; within the olfactory crus lateral, dorsal, medial and ventral parts, and an external part closely associated with the lateral olfactory tract; behind the olfactory crus a posterior part which merges into the septal nuclei in the medial wall of the hemisphere and the piriform and amygdaloid fields laterally."* Later in the paper he mentions "pars cruralis". He commented that the limits between the nucleus and the adjacent cortical fields were, at best,

arbitrary.

Nomenclature of this nucleus has been formalized (for example, see Lohman & Mentink, 1969), and the terms to be used subsequently will be:

1. Pars rostralis
2. Pars lateralis
3. Pars dorsalis
4. Pars medialis
5. Pars ventralis
6. Pars posterior
7. Pars externa

The disposition of the anterior olfactory nucleus in the phalanger is similar to that in opossum and is depicted in Fig. 1-5. *Pars rostralis* extends forwards on the lateral side of the olfactory ventricle to the level of the middle of the accessory bulb. Passing caudally, it spreads out to envelop the olfactory ventricle and anterior limb of the anterior commissure, the parts then being named in relation to those structures (*dorsal, medial, lateral, ventral*). There is a *posterior* prolongation of the ventro-medial part of this cuff which merges into the olfactory tubercle. The lateral part merges into the primary olfactory cortex beneath the lateral olfactory tract. The dorso-medial part merges with the anterior extension of the hippocampal and septal nuclei. A thin, dense concentric plate of cells lies between pars lateralis and the lateral olfactory tract: this is *pars externa* of the nucleus. In the phalanger it has a dorsal and ventral division.

The connexions of the rodent anterior olfactory nucleus have been studied in some detail (Lohman, 1963; Lohman & Lammers, 1967; Lohman & Mentink, 1969; Heimer, 1968; Ferrer, 1969). Heimer (1968) recognized that fibres from the olfactory bulb terminated in the superficial part of the plexiform layer of the anterior olfactory nucleus, and those from the pyriform cortex ended in the deep half. Other fibres to this nucleus have been described from the medial forebrain bundle (Price & Powell, 1970*e*).

Axons from the anterior olfactory nucleus form the anterior limb of the anterior commissure (Lohman, 1963) and collaterals of these enter the ipsilateral bulb (Valverde, 1965). Price & Powell (1970*e*) included these fibres with the other afferents, and claimed that they ended on granule cells (peripheral processes, soma and deep dendrites). On the other hand, the axons from the contralateral anterior olfactory nucleus

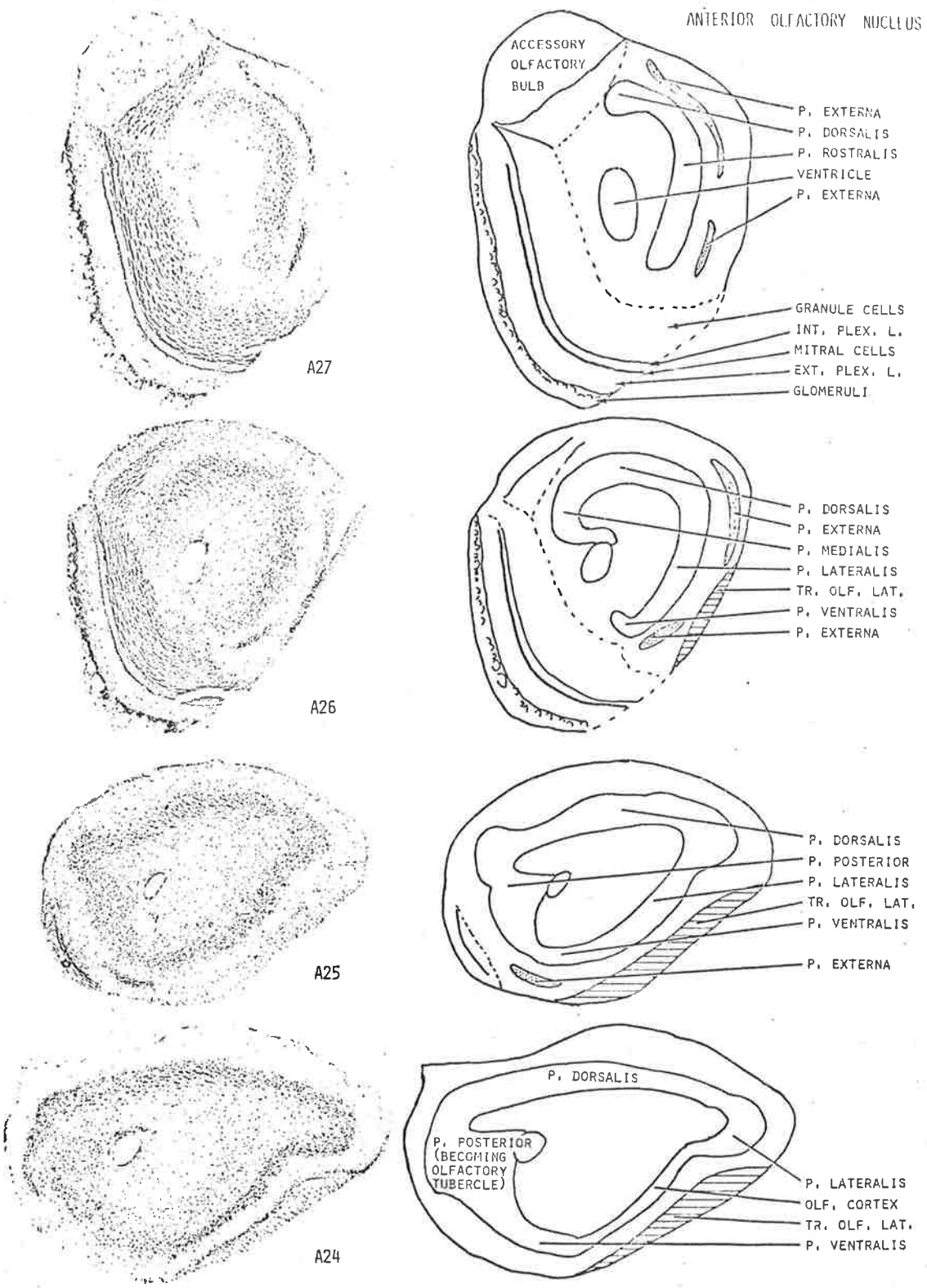


FIG. 1-5 ANTERIOR OLFACTORY NUCLEUS OF THE PHALANGER IN TRANSVERSE SECTION AT ONE MILLIMETER INTERVALS (STEREOTAXIC A-P LEVELS ARE INDICATED). NOTE: (1) PARS EXTERNA IS IN TWO PARTS (2) PARS LATERALIS BECOMES OLFACTORY CORTEX (3) PARS POSTERIOR BECOMES OLFACTORY TUBERCLE.

(via the anterior limb of the anterior commissure) ended on the granule cell somata and dendrites (not peripheral processes) and short-axon cell dendrites. These relationships are shown diagrammatically in Fig. 1-5.

Ferrer (1969) showed degenerating fibres from ablated rostral and medial parts of the anterior nucleus in the granule and mitral cell layers of the same side, lateral two-thirds of the olfactory tubercle, prepyriform* and periamygdala cortices, and cortical and medial amygdaloid nuclei. He also described degenerating fibres in the dorso-medial nucleus of the thalamus, lateral habenular nucleus, lateral hypothalamic area and supraoptic area of the same side.

Because of its site, indistinct nature and small size, there have been no electrophysiological studies specifically on this area and its function is unknown at present. However, its structure suggests that it has an integrative function, for it is interesting to note that all its known bulbopetal connexions are with the inhibitory interneurone, the granule cell.

Primary olfactory cortex

Another field in which nomenclature is prolific is that termed by O'Leary (1937) *'Primary olfactory cortex, the first cortical station in the olfactory pathway'*. Numerous subdivisions have been made using cytoarchitectonic parameters, reviewed in great detail by Pigache (1970). The following discussion will be restricted to comments on the work of Gray (1924), Abbie (1942) and Adey (1953).

Gray (1924) described the cortical lamination pattern of the pyriform lobe of opossum, and divided it into eight areas according to myelo- or cytoarchitectonic criteria.

(i) *Area perirhinalis* separated the temporal and insular areas from the pyriform lobe. The pyriform lobe itself was divided into seven approximately longitudinal zones.

(ii) *Area pyriformis dorsalis* was ventro-lateral to area perirhinalis.

(iii) *Area pyriformis fissuralis* was in and ventral to the rhinal fissure. The major area of the lateral surface was divided into

(iv) *Area pyriformis anterior* "confined to the interior of the

---

* The spelling of pyriform/piriform (pear-shaped) depends on whether the Greek or Latin derivation is adopted. For consistency within this thesis, the form *pyriform* will be used, regardless of the spelling used by the authors of the papers cited, or of the apparent derivation.

bulb" (his p. 248) (probably the anterior olfactory nucleus pars lateralis, see his Fig. 4), merging insensibly into

(v) *Area pyriformis medialis* (his p. 250), the largest area, between the rhinal fissure and endorhinal-amygdaloid fissure extending over the anterior two-thirds of the hemisphere, caudally becoming the four layered

(vi) *Area pyriformis posterior*, with no direct lateral olfactory tract projection (his p. 252).

(vii) *Area pyriformis ventralis* and (viii) *Area subpyriformis* were in the dorsal wall and floor, respectively, of the endorhinal-amygdaloid fissure. He did not include the amygdaloid complex, the olfactory tubercle or the diagonal band in his description of the cortex.

Abbie (1942), using his own previously-described (1940) cortical classification, divided the pyriform cortex of *Perameles nasuta* (the long-nosed bandicoot, a polyprotodont marsupial) into three areas.

(i) *The anterior pyriform area* lay under the posterior two-thirds of the ventral part of the lateral olfactory tract, gradually disappearing into the nucleus of that tract.

(ii) *The intermediate pyriform area* extended back below the rhinal fissure to about the junction of the middle and posterior thirds of the hemisphere, and extended ventrally to the anterior part of the amygdaloid fissure.

(iii) *The posterior pyriform area* (the posterior one-third of the hemisphere) was characterized by absence of lateral olfactory tract fibres.

Adey (1953) makes the simplest division possible of the rhinencephalon of the phalanger. Using the presence of degenerating fibres or terminals after olfactory bulb destruction as the criterion, he recognized only two areas:

(i) *The anterior (prepyriform) region* which contained evidence of degeneration.

(ii) *The posterior (entorhinal) region* which did not contain evidence of the degenerating lateral olfactory tract fibres.

Adey's basic classification will be used in this study and his anterior region will be called *primary olfactory cortex*. However, the primary olfactory cortex (Adey's anterior region) can be easily and justifiably divided into two areas on histological grounds. This will be discussed in Chapter 2.

Subcortical areas

Pigache (1970) defined cortex as a region of brain clearly divisible into a minimum of three tangential layers, the most superficial being plexiform, connected by a regular lattice of neuronal processes organized radially and tangentially. On these criteria, he considered the olfactory tubercle, diagonal band (and its nucleus), olfactory bulb and peduncle, and amygdaloid complex not to be cortex. However, these areas are indubitably part of the olfactory system.

1. Nucleus of diagonal band

'The nucleus of the diagonal band is not sharply distinguished from the medial parolfactory (septal) nucleus' (Johnston, 1923). These are regarded as part of his primitive olfactory nuclei which connect the medial and lateral olfactory areas. The nucleus of the diagonal band in the opossum fills the broad space beneath the internal capsule and anterior commissure and merges with the medial amygdaloid nucleus behind it. Price (1968*a*; 1969*a*), and Price & Powell (1970*e*, *f*) have shown in the rat that the nucleus of the diagonal band is the source of the efferent fibres to the bulb which run in and deep to the lateral olfactory tract. They described fibres leaving the nucleus, passing around the posterior edge of the olfactory tubercle to the caudal end of the tract. These fibres were said to be in the substance of, and deep to, the tract, and they fanned out into a cone as they approached the bulb. Degenerating fibres and terminals from lesions of this nucleus, or of the stated pathway, were found in the granule cell layer, external plexiform layer, and in the glomerular layer. They were presynaptic to gemmules in this area of wide distribution. Electrophysiological evidence concerning the function of this nucleus will be discussed in Chapter 2 of this thesis.

2. The olfactory tubercle

Johnston (1923) and Crosby & Humphrey (1941) reviewed the structure and divisions of the olfactory tubercle in the opossum, comparing it with other species. Johnston (1923) described this tubercle as being divided into a medial parolfactory area, and a lateral part forming part of the head of the caudate nucleus (bed nucleus of stria terminalis).

The medial part of the tubercle is continuous with the anterior olfactory nucleus rostrally, and the nucleus of the diagonal band caudally. Island formation is seen in the cells of this region (as in the amygdaloid nuclei). The tubercle shows the three layers necessary

for cortex, but the disorganization of the cells excludes it from this category (Pigache, 1970). Its role in the mechanism of olfaction is unknown. In the phalanger it receives fibres from the lateral olfactory tract as in other species, demonstrated both by anatomical (Adey, 1953) and evoked potential studies (this thesis). However, the extent of the projection is not clearly determined. The above two reports suggest that the projection is to the antero-lateral part of the tubercle, as do many others (e.g. Le Gros Clark & Meyer, 1947 - rabbit; Lohman, 1963 - guinea pig; Powell, Cowan & Raisman, 1965 - rat; Dennis & Kerr, 1968 - cat). The degeneration studies of White (1965), Heimer (1968), Price & Powell (1971), and Cowan, Gottlieb, Hendrickson, Price & Woolsey (1972) suggest that there may be some topographical projection of the bulb to the tubercle in the rat. The dorso-lateral part of the bulb projected to the antero-lateral part of the tubercle, and the ventral part of the bulb to the postero-medial area. Price & Powell (1971) also showed that the ipsilateral and contralateral anterior olfactory nuclei and posterior pyriform areas projected to the tubercle.

De Olmos (1972) described connexions from amygdala via stria terminalis to the medial part of the tubercle. Ferrer (1972) described the connexions of the olfactory tubercle of the golden hamster. These included the ipsilateral olfactory bulb and anterior olfactory nucleus, nucleus of the lateral olfactory tract, basolateral, cortical and medial amygdaloid nuclei, medial septal areas, medial forebrain bundle to lateral hypothalamus, pyriform cortex, contralateral tubercle via the anterior commissure, the preoptic area, and lateral habenular nuclei, but with none to hippocampus.

Anderson & Westrum (1972) have described the ultrastructure of three types of synapse in the olfactory tubercle of the rat. Their types A and B were asymmetric, with round vesicles, while type C was symmetric with flattened vesicles. Hosoya (1973) suggested that reciprocal synapses are present in the tubercle.

As with the anterior olfactory nucleus, its complexity and connexions suggest an integrative function. It is different from the rest of the olfactory system in the phalanger in that it stains very densely for cholinesterase.

3. Amygdaloid nuclei

The amygdaloid complex is an aggregation of cells described by Johnston (1923) in the opossum and reviewed by Oswaldo-Cruz &

Rocha-Miranda (1968).

The neurobiology of the amygdala has been reviewed by various authors. Cowan, Raisman & Powell (1965) described connexions of the amygdala of the rat after lesions. Diencephalic input to the amygdala was via stria terminalis and medial forebrain bundle. Olfactory input was via the lateral olfactory tract and also indirectly from prepyriform (olfactory) cortex. Outflow was described to preoptic and hypothalamic areas, and to the stria terminalis. These findings were largely confirmed by Leonard & Scott (1971). Hall (1972) described a similar arrangement in the amygdala of the cat.

A recent review containing papers by various authors appeared in Advances in Behavioral Biology, Vol. 2, 1972.

(a) The central nucleus forms part of the origin of the stria terminalis. It is continuous forwards with the nucleus of the diagonal band, medially with the medial nucleus, and close to the tail of the caudate laterally. (Johnston (1923) believed that the olfactory projection tract of Cajal (1909) began here.)

(b) Cortical nucleus, visible on the surface, may be divided into an anterior and posterior part.

(c) Accessory basal nucleus, first described by Johnston, is recognized in the opossum and other marsupials (e.g. Obenchain, 1925) but not in other mammals.

(d) Basal nuclei (magno- and parvocellular) contain large stellate cells and lie between the cortical and central nuclei.

(e) Medial nucleus is on the medial side of the basal nuclei, and is apparently continuous with the nucleus of the diagonal band rostrally.

(f) Lateral nucleus is separated from the rest of the amygdala by lying above and internal to the internal capsule, and is lateral to the rest of the nuclei.

(g) Nucleus of the lateral olfactory tract is an indistinct aggregation of cells at the rostral end of the amygdaloid complex.

In his summary of the amygdaloid nuclei Johnston (1923) divided them into two phylogenetic groups: (i) the primitive central, medial, cortical, and nucleus of the lateral olfactory tract; and (ii) new basal and lateral nuclei. The nucleus of the lateral olfactory tract is a remnant of the primitive lateral olfactory area of fishes, and connected with its contralateral fellow through the anterior commissure.

Oswaldo-Cruz & Rocha-Miranda (1968) define three other areas:

anterior and posterior amygdaloid areas (part of Johnston's basal and lateral areas) and an intercalated nucleus.

The connexions of this complex structure are not entirely clear, but it is known that the olfactory bulb projects to the cortical, and part of the medial, nuclei. This has been shown by degeneration studies by Adey (1953, phalanger), Lohman (1963, guinea pig), Powell *et al.* (1965, rat), White (1965, rat), and Heimer (1968, rat). Electrical connexions have also been shown by Dennis & Kerr (1968, cat), Morrison (1969, guinea pig), Cain & Bindra (1972, rat), Hughes, Andy, Hendrik, Wang, Wetzel & Peeler (1972, human), and in the phalanger in this study.

There is no doubt that the amygdala, forming part of the limbic system, is closely associated with many types of behaviour (for examples, see De France & Hutchinson, 1972; Rolls, 1972) and arousal (Pagano & Gault, 1964). It is likely that the olfactory connexions of the amygdala are related particularly to hedonic, sexual, territorial and aggressive behaviour.

CONNEXIONS WITHIN THE OLFACTORY SYSTEM

While there has been some eventual general agreement on the structure of the olfactory system, Pigache (1970) and Shepherd (1972) being the arbiters, the same cannot be said of the assumed functional arrangements of the various components. From the time of Golgi (1875) there has been speculation and assertion on the connexions within the olfactory system, and of this system with the rest of the central nervous system.

Original studies were based on selective staining methods and were followed by degeneration and electrical techniques. The connexions within the system were grouped by Loo (1931) under the general heading of "Fiber systems of the forebrain".

It is not intended to undertake a comprehensive review of Loo's work, but only to comment on significant aspects in the light of experimental results in this and other species.

Connexions of the olfactory bulb

Loo (1931) described afferent fibres as second order, arising from mitral cells or other elements of the formatio bulbaris, and third-order, arising behind the bulbar formation. (Afferent and efferent are in relation to central structures, not the bulb itself, as suggested by Allison (1953).) Loo pointed out that demonstrable fibre tracts may

contain both second- and third-order neurons, as well as an efferent component. He enumerated ten tracts, which are listed below. References were made to the 1924 paper of Herrick. The connexions of these tracts have not all been confirmed in the phalanger.

1. *Tractus olfactorius lateralis*, the major outflow from the main and accessory olfactory bulbs.

(a) Pars dorsalis

(b) Pars intermedia, the massive portion extending over the olfactory crus to the nucleus of the lateral olfactory tract, whose connexions define the primary olfactory cortex.

(c) Pars ventralis, said to be distributed to the antero-ventral amygdaloid nuclei.

2. *Tractus olfactorius ventralis*, over the external surface of the olfactory tubercle to end largely in the medial septal nucleus and anterior hippocampal cortex.

3. *Tractus olfacto-hypothalamicus ventralis*, a component of the medial forebrain bundle, containing some mitral cell axons, passing through the olfactory tubercle an unknown distance backwards.

4. *Tractus olfactorius medialis*, joined by fibres from the anterior olfactory nucleus and tubercle, with a similar distribution to 2. above.

5. *Tractus olfactorius dorsomedialis*, of two layers:

(a) Deep, myelinated

(b) Superficial, from the accessory bulb, distributed to the anterior hippocampal and overlying frontal cortices.

6. *Tractus olfacto-frontalis*, probably arising from the anterior olfactory nucleus, connecting with the neopallium.

7. *Tractus olfacto-corticalis*, arising from the anterior olfactory nucleus, connecting with the anterior hippocampus, with a small component entering the post-commissural hippocampus.

8. *Tractus olfacto-corticalis lateralis*, from olfactory bulb and the anterior olfactory nucleus accompany the anterior limb of the anterior commissure, to be distributed to the primary olfactory cortex.

9. *Pars olfactoria commissurae anterioris*, well-myelinated fibres which "assemble from the deepest part of the bulbar formation close to the lateral ependyma of the olfactory ventricle" and receive extensive additions from the anterior olfactory nucleus during their passage through it.

10. *Tractus olfacto-caudatus*, from the anterior olfactory nucleus, through the nucleus accumbens to the medial aspect of the caudate nucleus.

Fibre tracts of the septum

Loo admitted the complexity of this system and divided it into three groups: medial, intermediate and lateral. His list is presented with comments.

1. Medial group

This group comprises eight pathways, apparently connecting bulb with frontal cortex, septum, and olfactory tubercle.

2. Intermediate group

(a) Fasciculus of Zuckerkandl, the anterior part containing fibres ascending from the hypothalamic or preoptic areas, terminating in the medial septal nucleus.

(b) Precommissural fornix.

(c) Tract of diagonal band of Broca, a subcortical connexion between the medial septal area and the rostral end of the lateral olfactory area (central nucleus of the amygdala and nucleus of the lateral olfactory tract).

3. Lateral group

(a) Tractus cortico-septalis, between the alveus and lateral septal nucleus (dorsal portion).

(b) Tractus septo-hypothalamicus.

(c) Tractus cortico-caudatus medialis and *septo-caudatus.*

(d) Stria terminalis, components 3 and 4. Loo referred the reader to Johnston's (1923) paper, where the 5 components of this structure were illustrated in the latter's Fig. 20.

Fornix system

Loo divided this into two components, pre- and post-commissural.

Olfactory projection tracts

The great olfactory projection tract of Cajal was labelled ST1 by Johnston (1923).

1. *Dorsal olfactory projection tract* (Loo), arising from the antero-medial part of the amygdaloid complex (and possibly the overlying pyriform cortex), entering the stria terminalis and passing to the principal preoptic nucleus in the medial forebrain bundle.

2. *Ventral olfactory projection tract*, arising from the lateral amygdaloid nucleus, nucleus endopyriformis, and deep layer of the posterior pyriform area, and travelling via the external capsule, between medial and lateral forebrain bundles to end in the subthalamus and hypothalamus.

3. *Frontal olfactory projection tract*, arising from the anterior

pyriform cortex, nucleus endopyriformis, and the posterior part of the anterior olfactory nucleus and entering the medial forebrain bundle, for unknown destinations.

Medial forebrain bundle

In his Fig. 25, Loo illustrated the components and connexions of this tract, which, he said, is concerned with olfacto-visceral reflexes. (He reviewed Tsai's 1925 contribution.) He said that the most rostral fibres arise in the olfactory bulb and anterior olfactory nucleus, but did not comment on the cell of origin (or termination). Fibres were also said to arise from the septum, olfactory tubercle, head of caudate nucleus, lateral olfactory area, preoptic area, amygdala, fornix system, and deep layer of posterior pyriform area. It connects the olfactory cortical and subcortical centres, hypothalamus and mid-brain (including tegmentum and interpeduncular nuclei).

Hippocampal commissure

The dorsal commissure of the lamina terminalis, containing the following components:

(i) *True hippocampal commissural fibres.*

(ii) *Fornix fibres.*

(iii) *Cortico-habenular fibres.*

(iv) *Neocortical fibres.* (This last statement was based on a 1913 report by Johnston, but in the light of the subsequent work of Abbie (1939) and Heath & Jones (1971), it may be regarded as incorrect.)

Anterior commissure

Five parts of the massive anterior commissure were defined by Loo. These parts were depicted in his Figs. 70 (p. 85), 81 (p. 105) and 102 (p. 137). The commissure was divided into two limbs, anterior (olfactory) and temporal. It must be remembered that the arrangement of this structure distinguishes diprotodont and polyprotodont marsupials (Smith, 1902). The anterior commissure of the phalanger is shown in Figs. 1-6 and 1-7 of brain sections from the evoked potential study.

1. Pars anterior

Loo stated that the olfactory component arose from all parts of the olfactory bulb, and referred the reader to Herrick's (1924) Figs. 1 to 6. Examination of these shows the anterior commissure closely related to parts of the anterior olfactory nucleus, and separate from the lateral olfactory tract. They were said to terminate in the opposite olfactory bulb, a view held by Adey (1953), who demonstrated degeneration in the contralateral antero-dorsal glomeruli. However,

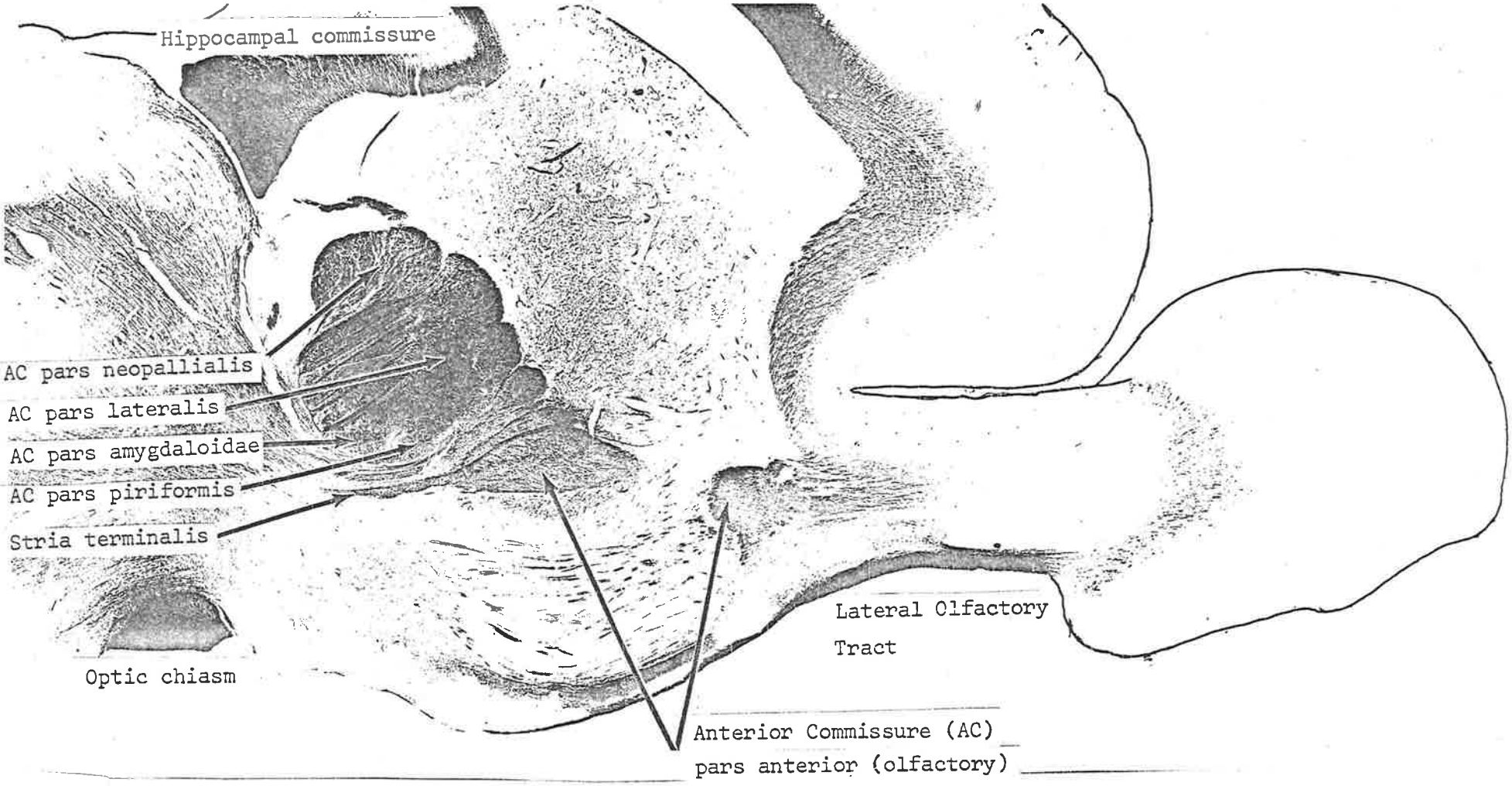


Fig. 1-6 Sagittal 50μ section of the phalanger brain, 3mm from the midline, stained with haematoxylin to show myelinated fibres. The outline of the bulb and frontal pole has been drawn for clarity.

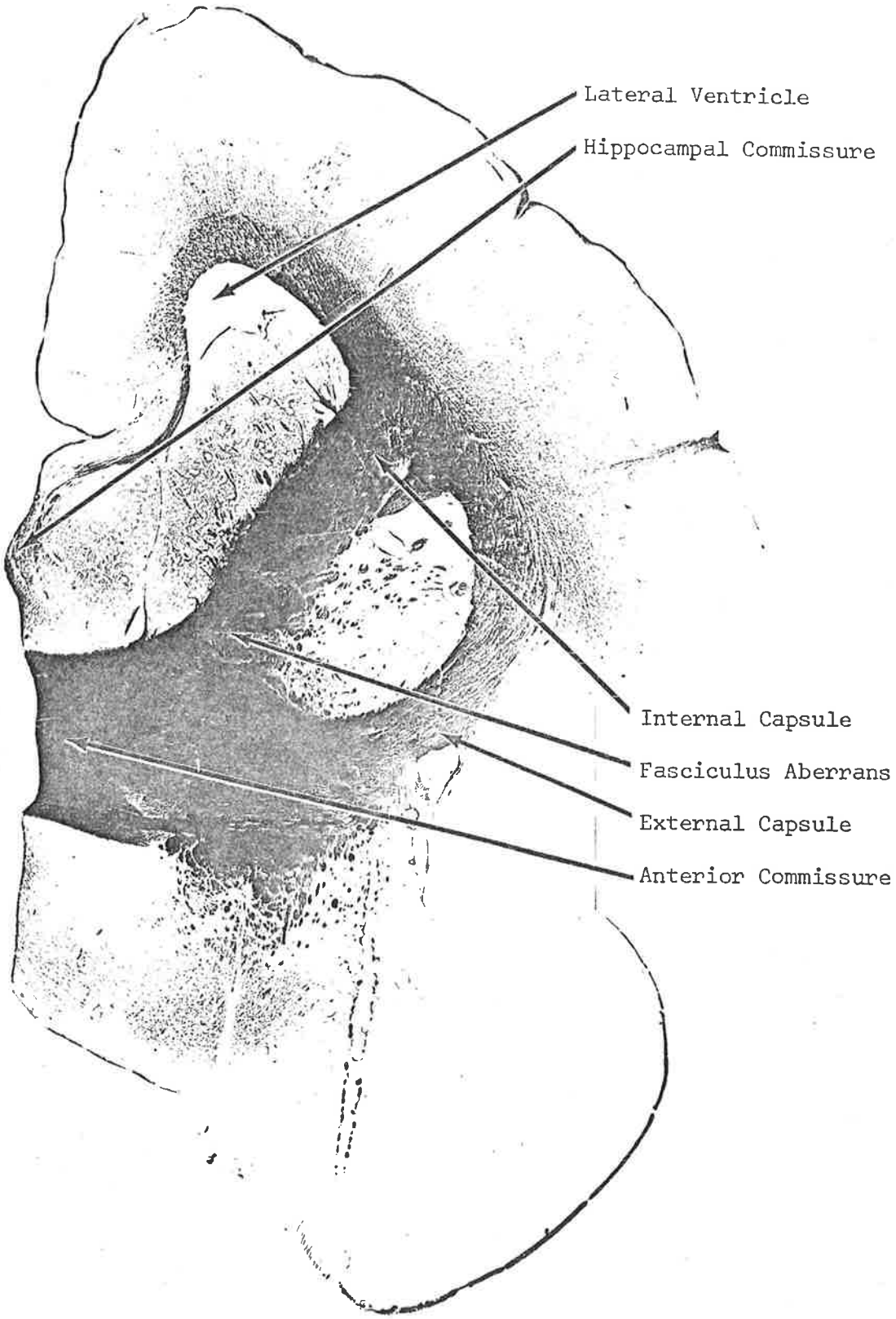


Fig. 1-7

Sagittal 50μ section of phalanger brain stained with haemotoxylin to show myelinated fibres. The large anterior commissure splits into the internal and external capsules. Two electrode tracks are visible. AP coordinate of this section A 18.

Lohman (1963) proved in the guinea pig that the anterior commissure arose from the rostral and dorsal parts of the anterior olfactory nucleus only, with no direct connexion to the contralateral bulb.

2. *Pars lateralis*

Lightly myelinated, or unmyelinated fibres, spread through the nucleus intermedius striati, nucleus endopyriformis, and possible anterior olfactory nucleus and head of the caudate nucleus.

3. *Pars neopallialis*

The largest component, which in the opossum passes into the external capsule, but in the phalanger splits, part to the internal (fasciculus aberrans) and part to the external capsule.

4. *Pars pyriformis*

Lightly myelinated fibres pass from the medial (Gray) and posterior pyriform areas through the posteroventral portion of the commissure.

5. *Pars amygdaloidea*

Contains fibres from component 1 of the stria terminalis (Johnston) in the posteroventral aspect of the anterior commissure. They were said to connect the nuclei of the lateral olfactory tract and give off collaterals to the bed nucleus of the commissure and median preoptic nucleus.

DISCUSSION

In view of the importance of the functional connexions of the olfactory system, an attempt is made below to summarize the current concepts, referring both to anatomical and electrophysiological studies.

The first experimental anatomical investigation into the olfactory projection was probably made by Gudden in 1870, and many have contributed since. Significant new concepts or reviews of knowledge have been contributed by Adey (1953), Allison (1953), Hagbarth & Kerr (1954), Adey (1957), Lohman (1963), Dennis (1965), Moulton & Beidler (1967), Ottoson & Shepherd (1967), Dennis & Kerr (1968), Scalia (1968), Morrison (1969), Westrum (1969), Adey (1970), Pigache (1970), Price & Powell (1970*a-e*), MacLeod (1971), Polson (1971), and Shepherd (1972). This list is not exhaustive.

The connexions will be discussed under the following headings as an overview of this introduction, relating to the phalanger where possible, and to the main bulb only.

1. Afferent connexions of the olfactory bulb:

(a) Olfactory nerves

(b) Lateral olfactory tract

(c) Anterior olfactory nucleus

2. Efferent connexions of the bulb:

(a) Anterior commissure

(b) Lateral olfactory tract

(c) Anterior olfactory nucleus

3. Intrinsic connexions of the bulb.

Afferent connexions of the olfactory bulb

1. Olfactory nerves

The olfactory nerve bundles pass through the cribriform plate of the ethmoid bone to cover the bulb. They turn inwards to end in tufts, only in the glomeruli. They form asymmetrical synapses with primary dendrites of mitral and tufted cells. There are no presynaptic terminals onto the olfactory axons.

The structure and function of the peripheral olfactory system (olfactory epithelium, receptors, nerves) has been reviewed by Moulton & Beidler (1967; 300 references cited), but the scope of that review is broader than required here. It is sufficient to note that there is a single axon from each receptor cell, and that the electro-olfactogram recorded from the surface of the mucosa does not necessarily parallel that of the averaged neural activity in olfactory nerves recorded simultaneously (Mozell, 1962). Likewise, the theories of odour discrimination are outside the scope of this thesis.

2. Lateral olfactory tract

The afferent component of the lateral olfactory tract is comprised of axons of the ipsilateral mitral and deep tufted cells. These axons pass from their cells of origin deeper into the bulb after becoming myelinated and giving off recurrent collaterals. They form this tract as they emerge in a bundle on the lateral side of the bulb, and remain on the surface of the brain. The tract curves around the lateral side of the olfactory tubercle, and ends at the rostral end of the amygdaloid complex in the nucleus of the lateral olfactory tract. Primary olfactory (prepyriform) cortex is that which receives lateral olfactory tract fibres, and it is the purpose of the first series of experiments reported in Chapter 2 to establish the distribution of this tract. The terminals end in the molecular layer of the cortex on the distal third of the dendrites of superficial neurones (White, 1965; Powell *et al.*, 1965; Biedenbach & Stevens, 1969; Stevens, 1969; Westrum, 1969).

In addition, lateral olfactory tract fibres also project to areas not regarded as cortex by Pigache (1970); olfactory tubercle, amygdala and nucleus of the lateral olfactory tract.

3. Anterior olfactory nucleus

In addition to the projections described above, the mitral and tufted cells send collaterals to the anterior olfactory nucleus, partly via the lateral olfactory tract. MacLeod (1971) and Polson (1971) have reviewed the information on this, and admit the complexity and confusion surrounding this nucleus.

Efferent (centrifugal) connexions

Fibres running from central brain structures to the bulb had been suspected for a considerable period. It was not until the classical experiments of Kerr & Hagbarth (1955) that central control of bulbar activity was shown to exist. Dennis & Kerr (1968) used an evoked potential method to map the extent of the centripetal (afferent) and centrifugal (efferent) connexions of the olfactory bulb of the cat. They described ipsilateral centrifugal systems in relation to all brain regions having lateral olfactory tract afferent projections, as well as certain other regions. Although the lateral olfactory tract carried centrifugal fibres, section of this tract did not interrupt all such fibres. However, they were unable to determine certainly whether the centrifugal effects they described were mono- or polysynaptic. Contralateral projections were mediated through the anterior commissure.

Price (1969*a*) and Price & Powell (1970*e*) used degeneration techniques in the rat to describe the origin and course of a centrifugal fibre system to the olfactory bulb. They concluded that the centrifugal fibres which formed part of the lateral olfactory tract arose in the nucleus of the horizontal limb of the diagonal band. They did not find degenerating terminals *in the bulb* after lesions of the primary olfactory cortex, olfactory tubercle, amygdala. However, fibres from these areas were said to travel to the anterior olfactory nucleus in the medial forebrain bundle. Collateral axons from this nucleus run forwards into the ipsilateral bulb.

They concluded that there were three efferent systems to the bulb itself:

1. *Anterior commissure* axons from the contralateral anterior olfactory nucleus terminate in the outer part of the granule cell layer, on spines and varicosities of the deep dendrites, and somatic spines of the granule cells (Price & Powell, 1970*c*; their Fig. 3).

2. *Lateral olfactory tract* fibres from the nucleus of the diagonal band terminate on gemmules throughout the bulb.
3. *Anterior olfactory nucleus* collateral axons to the ipsilateral bulb terminate throughout the bulb, including the peripheral processes of granule cells. The axons from the contralateral anterior olfactory nucleus terminate in a similar fashion, except on the granule cell peripheral processes.

The implication of this assertion is that all other efferent systems function via the anterior olfactory nucleus and do not directly influence any of the bulbar elements, either pre- or postsynaptically. All the extrinsic fibres form asymmetrical synapses and are presumed to be excitatory. However, they all act on gemmules or gemmule-bearing cells, which function as inhibitory interneurones. Physiological evidence for these systems will be discussed in Chapter 2.

Intrinsic connexions

The studies of Price & Powell (1970*a-e*), Pinching & Powell (1971*a-c*) and White (1972) have clarified the intrinsic connexions of the bulb, and this subject has been reviewed by Shepherd (1972). These intrinsic connexions are based on the connexions of the granule and periglomerular cells, which are the final common path for inhibition of the mitral and tufted cells, through the reciprocal synapses. There is no evidence of presynaptic inhibition of the olfactory nerves.

There is anatomical evidence for three intrinsic sources of axonal synaptic input to the granule cells: the axon collaterals of the mitral and tufted cells, and the short-axon cells of the granular and periglomerular layers. This evidence is supported by electrophysiological studies to be discussed later (Yamamoto *et al.*, 1963; Dennis & Kerr, 1968; Rall & Shepherd, 1968; and Polson, 1971).

From the foregoing it can be seen that there are at least five ipsilateral loops in the olfactory system:

1. *Dendrodendritic synapses* on mitral/tufted secondary dendrites. The granule-to-mitral part of this synapse appears to be the only type of inhibitory synapse in the olfactory bulb.
2. *Recurrent collateral axons of mitral/tufted cells* probably arise in the internal plexiform layer and are distributed to granule cell layer and external plexiform layer.
3. *Recurrent collateral axons from the lateral olfactory tract* pass to the anterior olfactory nucleus, which supplies its own bulb.
4. *Primary olfactory cortex* projects to the ipsilateral anterior

olfactory nucleus.

5. *Centrifugal fibres from the nucleus of the diagonal band* terminate on gemmules, and relate the olfactory with the non-olfactory part of the brain.

The most important single aspect of olfactory system function is that all inhibition in the bulb is mediated through a single type of synapse, and by a single transmitter. It is this feature, in addition to the relative anatomical simplicity, which makes the olfactory bulb a good model on which to study normal electrical activity of a neural system. This will be discussed in the next two chapters. The single final inhibitory transmitter also facilitates pharmacological studies, which will be discussed in Chapters 4 and 5.

CHAPTER TWO

SUMMARY

1. Potentials were evoked in the olfactory bulbs of the phalanger by electrical stimulation of more central brain areas.

2. Such potentials were similar in form to those recorded in other species.

3. The extent of the lateral olfactory tract (mitral cell axon) projection was defined by this method. The tract projected to the olfactory cortex, olfactory tubercle, nucleus of the lateral olfactory tract, and cortical and medial amygdaloid nuclei.

4. The extent of the brain areas having centrifugal influences on the bulb was also defined. These areas included olfactory cortex, anterior olfactory nucleus, olfactory tubercle, nucleus of the lateral olfactory tract, cortical and medial amygdaloid nuclei, and parts of the hippocampus.

5. These connexions are similar to those reported in the cat, guinea pig, rat and rabbit.

6. Two parts of the olfactory cortex have been defined, and the extent of angular (entorhinal) cortex more precisely delimited.

7. Comparison with the anatomical study of Adey (1953) shows reasonable agreement between the electrical and degeneration methods in defining olfactory bulbar connexions.

CONNEXIONS OF THE OLFACTORY BULB OF *TRICHOSURUS VULPECULA*

An evoked potential study of centripetal and centrifugal connexions of the olfactory bulb of the marsupial phalanger (*Trichosurus vulpecula*)

INTRODUCTION

Connexions of central neural systems may be determined in three main ways: by tracing the course of appropriately demarcated, normal or degenerating axons, by determining the extent of degenerating nerve terminals, or by mapping the extent of areas which have measurable electrical connexions. This third approach is the one to be used in this thesis.

Centrifugal effects on normal olfactory bulbar activity can be elicited from an extensive portion of the basal rhinencephalon, first demonstrated by Kerr & Hagbarth (1955) in the immobilized cat. Depressive influences on bulbar activity were caused by electrical stimulation of the prepyriform cortex, cortical amygdaloid nucleus, olfactory tubercle, and anterior commissure. Consequent studies by Dennis (1965), and Dennis & Kerr (1968) showed that the extent of both afferent and efferent connexions of the olfactory bulb could be determined by systematic electrical stimulation throughout the rhinencephalon. Morrison (1969) used a similar technique in his comparative study of rodent (rat and guinea pig) and lagomorph (rabbit) olfactory systems.

Electrical stimulation of the lateral olfactory tract evokes a triphasic response in the granule cell layer of the olfactory bulb.

1. An initial short-latency small negative potential corresponds to antidromic depolarization of the mitral cell axon hillock-initial segment.
2. A large positive potential which follows is predominantly due to depolarization of granule cell peripheral processes in the external plexiform layer via the dendrodendritic synapses between the granule cell gemmules and mitral cell secondary dendrites.
3. A late negative potential which follows has been interpreted as involving depolarization of deep dendrites and/or granule cell bodies deep to the mitral cell layer via a centrifugal efferent system.

If one component of this response is evoked in the bulb, it may be assumed that the area stimulated contains the appropriate bulbar

connexion (mitral cell axon or a centrifugal projection reaching the granule cell layer). (Dennis & Kerr, 1968; Polson, 1971).

There have been no previous studies using this technique of the extent of the olfactory connexions in the phalanger, or any other marsupial. Adey (1953) used degeneration techniques to map the extent of the olfactory bulb connexions in the phalanger. Adey, Sunderland & Dunlop (1957) and Adey, Dunlop & Sunderland (1958) used electrophysiological methods to study the 'rhinencephalic' connexions with the brainstem. However, the structures they stimulated (their posterior pyriform cortex and dorsal hippocampus) are not included in the present definition of rhinencephalon. It will be shown that these structures have no direct olfactory bulbar connexions.

Shepherd & Haberly (1970) used the opossum as the experimental animal in their study of the partial activation of its olfactory bulb, and the topographical projection of the bulb to the lateral olfactory tract. They did not map the extent of this projection.

Haberly (1973*a*, *b*) and Haberly & Shepherd (1973) continued to use the opossum as the experimental animal in studies on prepyriform cortical evoked potentials.

The first paper of the series (Haberly, 1973*a*) concluded that the synaptic organization of the opossum cortex was similar to that of the cat (Biedenbach & Stevens, 1969*a*, *b*). It should be remembered that all these studies were on animals anaesthetized with pentobarbitone. The second (Haberly, 1973*b*) plotted latencies of the cortical response to lateral olfactory tract stimulation. This was done on the surface and during penetration of known prepyriform cortex in the vicinity of the tract (his Figs. 3, 4 & 5). No mention was made of the extent of the lateral olfactory tract projection.

Current flow through the cortical layers was described in the third report (Haberly & Shepherd, 1973). A functional arrangement of the prepyriform cortex was suggested (their Fig. 11).

The hypotheses to be tested by this part of the project are (1) that the connexions of the olfactory system of the phalanger are similar to those of other mammals, and (2) that Adey's anatomical study of these connexions can be verified by an electrophysiological method.

METHODS

Fifteen phalangers were used primarily for the evoked potential studies which were carried out also on eight others following induced

wave experiments. The animals had been captured in the Adelaide area, and their obvious good health was assumed to be indicative of normal olfactory function.

Anaesthetic technique - Evoked potential studies

The unpremedicated animal was placed in a wooden box of about 10 litres capacity and known weight, with a sliding glass lid. After re-weighing to determine the animal's weight, the box was flushed with 100% oxygen for 3 minutes. A gas mixture of nitrous oxide (N_2O, 6.0 l/min) and oxygen (O_2, 2.0 l/min) was introduced from a Boyle anaesthetic machine (Commonwealth Industrial Gases). Chloroform ($CHC1_3$) was added from a vapourizer in series with the gas flow, taking advantage of the 'second gas effect' (Stoelting & Eger, 1969) to achieve rapid induction of anaesthesia. Once unconscious, the animal was removed to the operating table, anaesthesia then being maintained with the same gas mixture via a facemask. After cannulation of the trachea the anaesthetic circuit was changed to type 2(b) Ayre's T-piece (Harrison, 1964). The gas flow was then able to be reduced, but maintaining the oxygen concentration at 20-25%. The cephalic vein was cannulated, gaseous anaesthesia discontinued, and urethane 50% aqueous solution infused slowly (3-5 min) until respiration ceased (about 500 mg/kg). Artificial ventilation was maintained with a Palmer Respiration Pump. Colonic temperature was maintained in the normal range for this marsupial of $35^{\circ}C \pm 0.5^{\circ}$ (personal unpublished observations; also Dawson, 1969) by a thermistor-controlled heating pad.

Construction of stereotaxic atlas

A stereotaxic atlas was constructed of the cerebrum of the phalanger. Six animals were used for this initial neuroanatomical study before the evoked potential studies. The head of the anaesthetized animal was held in the Horsley-Clarke plane in a Labtronics stereotaxic cat frame. Earbars with removable curved tips directed ventrally and rostrally were inserted into the external auditory meati and adjusted to hold the head centrally in the frame. Similar earbars were used by Warner (1971) in a similar project. The infraorbital margins were held against eyebars of local manufacture by a transverse rod held against the alveus behind the incisor teeth. Later in the study it became apparent that the head could be held securely with standard cat earbars, with the eyebars adjusted to maintain the Horsley-Clarke plane. This method was then used for the rest of the study, making appropriate adjustments to the co-ordinates. The head was held 6 mm higher and

6 mm posterior by the straight bars. Fig. 2-1 shows a skull held in the frame with straight earbars.

Scalp skin and masseter muscles were reflected, and the sagittal suture checked for alignment in the midline of the frame. The appropriate part of the calvarium was removed, the dura being left intact. A fine needle (23 SWG) held in a micromanipulator attached to the stereotaxic frame was inserted vertically into the brain through small incisions in the dura at several known stereotaxic co-ordinates. The needle was also passed in the horizontal plane from behind, a known vertical and lateral distance from the zero point between the straight earbars, through a burrhole in the occipital bone.

Unless otherwise indicated, photographs and drawings of brain sections and surface markings will be from animal W14, an adult which weighed 1830 g*. Stereotaxic co-ordinates in the following figures are given in relation to the straight earbars.

Histological techniques

At the conclusion of an experiment, the animal was killed with an overdose of anaesthetic and the electrodes were removed. With the animal still held in the stereotaxic frame, fixation of the brain was achieved by pressure-perfusion for 20 min through the left ventricle with phosphate-buffered 1% glutaraldehyde/1% formaldehyde solution (pH 7.2, 320 mOsm/l). The calvarium was removed, and the brain cut across in the frontal plane at an appropriate level by a scalpel blade held in a micromanipulator. The brain was then carefully removed, photographed, and transferred to the fixative. Cryogenic cellular damage was prevented by storing the brain in 30% sucrose/1% glutaraldehyde/1% formaldehyde solution, and deferring further manipulation until it had sunk in this solution, usually 24-48 hours later.

Serial transverse or sagittal frozen sections 50 µm thick were made, the block of tissue resting on its cut surface on the stage of the freezing microtome. These sections were transferred to fixative for at least 20 min and then mounted from distilled water. Alternate sections were stained with neutral red to show cellular detail, and haematoxylin to delineate myelinated fibres. Examination of these revealed the electrode tracks, position of recording tips, and position

---

* The mean weight of 65 animals used in the course of all experiments was 1790 g, S.D. 250, range 1400-2200 g.

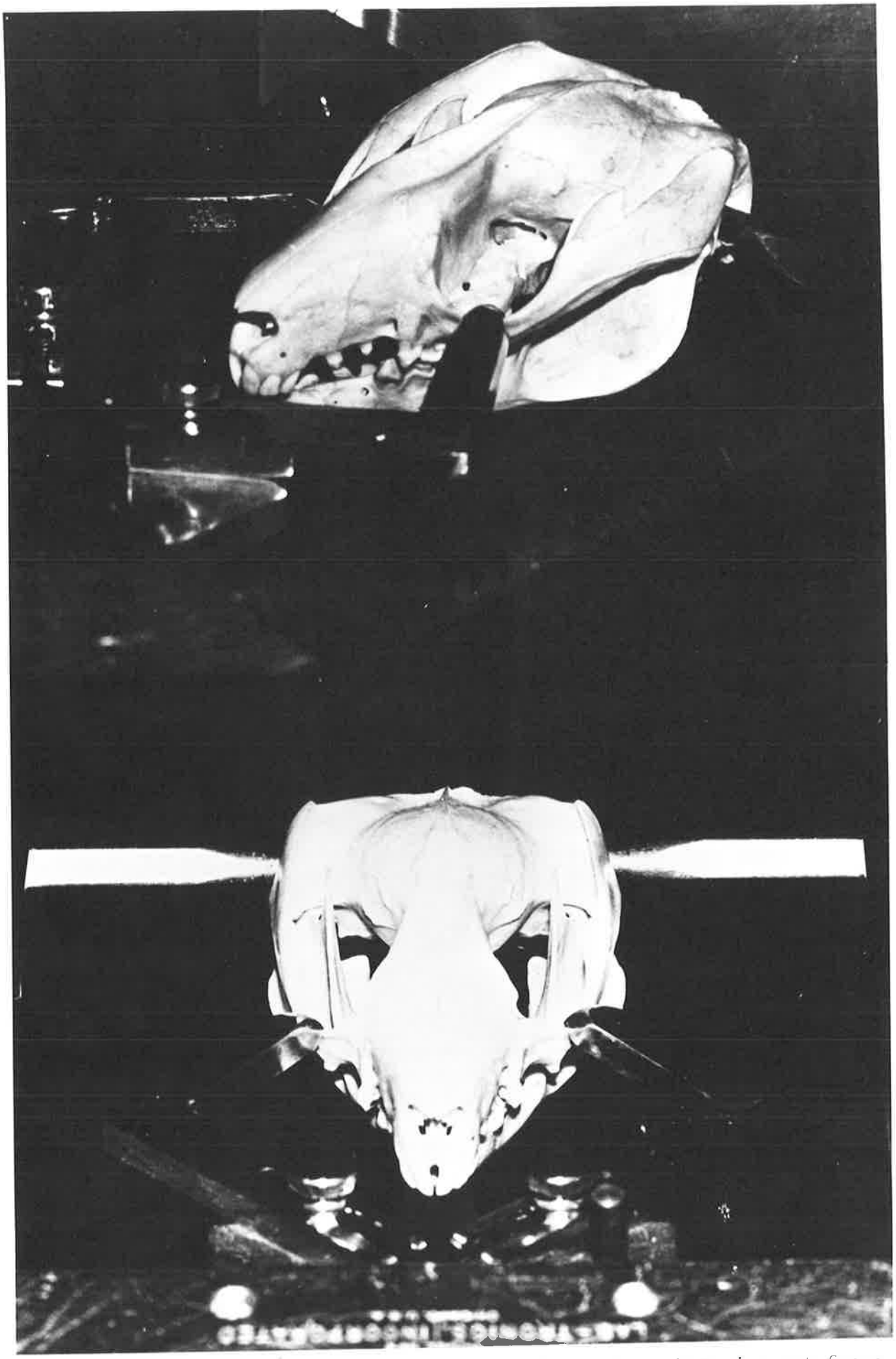


Fig. 2-1 Photographs of a phalanger skull held in the Labtronics cat frame with the straight cat ear bars and modified eye bars.

and extent of lesions.

Photographic techniques

Photographic records at a known magnification were made of appropriate histological sections. The microscope slide was placed in a locally-constructed holder in a photographic enlarger (Durst Laborator - 1000) fitted with a 50 mm Rodagon lens, green filter, and punctiform quartz-iodine light source, also of local manufacture. The magnification was calculated and adjusted by measuring the distance between two needle tracks in the projected image, knowing the distance between them *in vivo*. By this method, changes in brain size caused by fixation, dehydration, freezing and staining were corrected. This image was projected onto photographic plate producing a negative which could be used for contact prints or enlargements.

Formalith line film (Ilford SP 4-G4E.74) reproduced the best cellular detail, while fine grain ordinary film (Ilford N5-31) gave best reproduction of fibres. Both films were developed in Formalith Liquid Developer (Ilford).

Axes and scales were drawn on the final photographs, knowing the stereotaxic co-ordinates of the electrode tracks.

Stimulating and recording procedure - Evoked studies

The aim of these experiments was to deliver effective but discretely-localized electrical stimuli in known positions, recording any response evoked in the olfactory bulb. The technique of Dennis & Kerr (1968) was adopted. Stimulating electrodes were constructed from No. 00 stainless steel insect pins soldered into a shaft of stainless steel tubing (21 SWG), insulated with several thin layers of baked epoxylite. The tips were bared of insulation by stroking on a fine oilstone whilst observing with a dissecting microscope. The impedance to direct current of these tips was 30-40 KΩ. The recording electrodes were constructed in a similar manner, but the tips were bared over a larger area to reduce their impedance to 20-25 KΩ. Although some advantages may have been gained by plating the tips with noble metals (platinum, gold or rhodium; Polson, 1971), the tips were used in a freshly abraded state, having been mechanically cleaned before each experiment. The electrodes were held in micromanipulators calibrated in the Horsley-Clarke planes.

Stimulation was by square-wave-pulses 0.1 - 2.0 msec duration and 0.1 - 12 volts according to the electrode impedance and brain site stimulated. Supra-threshold stimuli were used. These pulses were

given singly or in trains of 5, 5 msec apart. Positioning of recording and indifferent electrodes was carried out in the manner described by Dennis & Kerr (1968). Stimuli were isolated from ground by stimulus isolation units (Devices Ltd).

The indifferent electrode for the recording and stimulating electrodes were clipped to the scalp wound margin and adjusted in position to minimize the stimulus artefact. The stereotaxic frame was earthed.

The position of the recording electrodes was adjusted so that stimulation of the lateral olfactory tract evoked the typical positive-negative response (see Dennis & Kerr, 1968; their Fig. 1). In these experiments the recording electrodes were introduced 2.0 - 2.5 mm below the dorsal surface of the bulb, and shown to be in the granule cell layer.

Two Digitimers (Devices Ltd) were used to control the repetitive electrical stimuli (0.5 - 1/sec), and the display and recording equipment. The evoked responses from both olfactory bulbs were preamplified (Grass P5A Preamplifiers, bandwidth 0.1 Hz - 10 KHz). The preamplifier gain was adjusted so that the responses could be conveniently displayed on a dual-beam oscilloscope (Tektronix 502A), positive downwards, and photographed (Grass kymograph camera). The evoked response from the same side as the stimulus was displayed by the lower beam.

All experiments and recording were carried out in an electrically-shielded room.

At the conclusion of each experiment the fixation and subsequent histological procedures described above were carried out. Examination of the serial sections located the electrode tracks and final tip positions. Camera lucida drawings were made of relevant sections and correlated with the recorded responses at the respective positions on each electrode penetration. The composite results were finally represented in graphic form on tracings of brain sections at various planes.

In a few cases, the evoked responses were averaged with a Mnemetron Computer of Average Transients (CAT-400C) and plotted (Bryans 2200 XY plotter). Many of the responses were photographed with a Grass camera, but all were recorded on protocol sheets in a simple shorthand showing the type of response and the stereotaxic co-ordinates.

All electrode positions were verified by subsequent microscopic

examination of stained serial sections.

RESULTS

Evoked potentials

The six types of bulbar granule cell layer response demonstrated by Dennis & Kerr (1968) and Polson (1971; his Chapter 3) have been seen in this series. They are discussed briefly below.

1. Late "cortical" negativity

A negative potential was evoked in the ipsilateral bulb after stimulation of the primary olfactory cortex (deep to the cellular layers) and some other areas. It had a long latency (5 - 20 msec) and high threshold. This response usually heralded the onset of the next type of response as the stimulating electrode passed through the brain.

2. Early monophasic positivity

An early positive potential in the ipsilateral bulb was the diagnostic feature of antidromic activity in the lateral olfactory tract fibres (mitral cell axons). It was usually associated with a type (1) response, to which it appeared to add, forming a type (3) response. This positivity was sometimes preceded by a short-latency small negative potential. As the electrode was inserted further (i.e. it approached the cortical surface), the late cortical negativity either persisted or became smaller as the positive potential was apparently added to this response (see Fig. 2-5).

3. "LOT/cortex" positive-negative response

A complex response occurred after stimulation of the lateral olfactory tract itself, or to cortical areas it supplied. It appeared to be a complex wave formed by the addition of the late cortical negativity and the early positivity.

4. Anterior commissure negativity

Invariably bilateral negative responses were evoked by stimulation of the anterior limb of the anterior commissure. These responses were sometimes 'humped' (see Fig. 2-5).

5. Late contralateral negativity

A response similar to, and accompanying *1.* above, appeared after stimulation of certain parts of the olfactory cortex and hippocampus. It sometimes appeared on its own.

6. Other responses

Other responses, not fitting into the previous categories, occasionally arose after stimulation of the claustrum, hippocampus,

stria terminalis or medial forebrain bundle.

Any positive potential, latency to 30 msec, recorded in the granule cell layer was assumed to have arisen from a projection of the lateral olfactory tract. Such potentials were always ipsilateral. Any negative potential with latency of this order was assumed to have arisen in a centrifugal system. All evoked potentials from any part of the brain were noted.

Centripetal bulbar projections

Correlation of histological sections with recorded responses enabled a composite diagram to be drawn of the extent of the lateral olfactory tract projections. A series of diagrammatic transverse sections was prepared from photomicrographs. A black dot marks those areas from which a positive bulbar response was produced. A qualitative indication of the extent of the lateral olfactory tract fibres was obtained in this way., (Fig. 2-2).

Positive bulbar potentials were evoked from the lateral olfactory tract itself, the primary olfactory cortex, olfactory tubercle, and cortico-medial amygdaloid nuclei. These potentials were evoked as the stimulating electrode passed through the superficial layer of cortical cells to the superficial plexiform layer.

1. Lateral olfactory tract

Stimulation of the tract itself produced the shortest latency and largest positive responses, with lowest stimulus threshold.

2. Primary olfactory (prepyriform) cortex

Primary olfactory cortex has been defined as that which receives lateral olfactory tract fibres. It has the same characteristic structure in the phalanger as in other mammals of an outer plexiform layer of lateral olfactory tract fibres and pyramidal cell dendrites, and two layers of pyramidal cells. In its anterior part (rostral and lateral to the amygdaloid complex) the cellular layers are thick and well-defined. They gradually thin out towards the caudal extent of the lateral olfactory tract projection. Positive potentials could still be recorded from this thin layer in the transitional zone between olfactory and angular (posterior pyriform) areas.

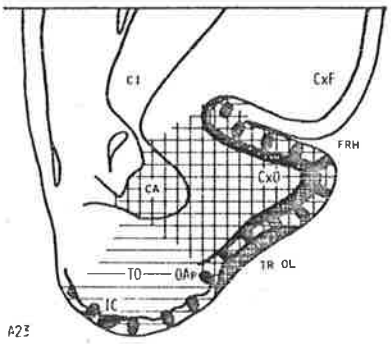
Fig. 2-3 shows a sagittal section of phalanger brain, 8mm from the midline, stained with neutral red. Four electrode tracks are seen, 2mm apart. The length of the olfactory cortex is shown.

The responses from antero-posterior stereotaxic levels A 15, 13, and 11 show the positive potentials evoked by stimulation of lateral

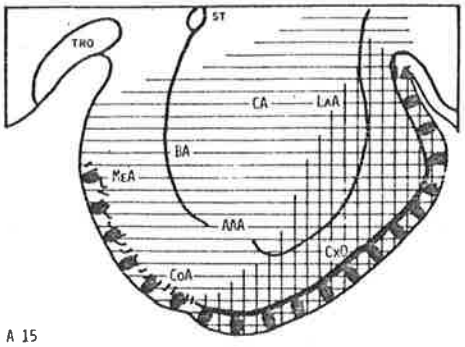
Fig. 2-2 Tracings of transverse sections of phalanger brain, at the stereotaxic coordinates indicated. Electrical stimulation of areas indicated by the large black dots evoked positive potentials in the ipsilateral bulb.
Ipsilateral negative potentials were evoked from areas indicated by horizontal line shading.
Negative potentials in the contralateral bulb were evoked from areas indicated by vertical line shading.

Abbreviations :

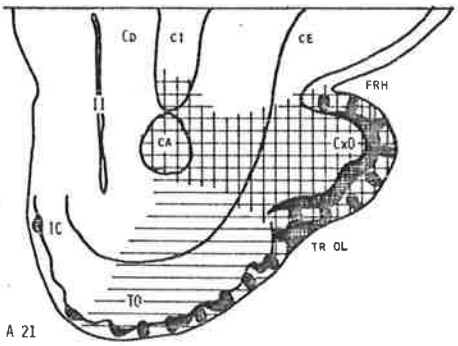
AAA	area anterior amygdalae
APA	area posterior amygdalae
BAA	nucleus basalis amygdalae accessorius
BA	nucleus basalis amygdalae
CA	nucleus centralis amygdalae
CA	commissura anterior
CD	nucleus caudatus
CE	capsula externa
CI	capsula interna
CoA	nucleus corticalis amygdalae
CxA	cortex Ammonis
CxF	cortex frontalis
CxO	cortex olfactorius
CxRp	cortex retrosplenialis: pars posterior
DB	nucleus fasciculi diagonalis Brocae
FD	fascia dentata hippocampi
FHIP	fissura hippocampi
FRH	fissura rhinalis
IC	insula Callejae
LaA	nucleus lateralis amygdalae
MeA	nucleus medialis amygdalae
OAp	nucleus olfactorius anterior: pars posterior
SB	subiculum
ST	stria terminalis
TO	tuberculum olfactorium
TRO	tractus opticus
TROL	tractus olfactorius lateralis
TrOL	nucleus tractus olfactorius lateralis
II	ventriculus lateralis



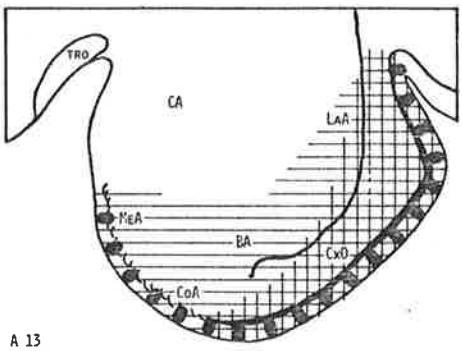
A23



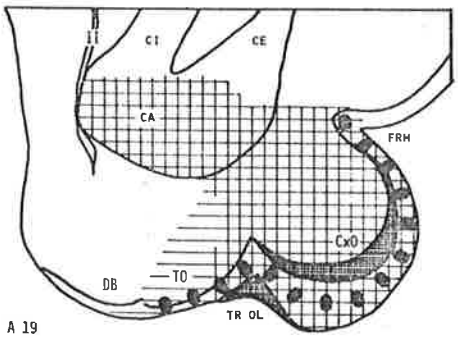
A 15



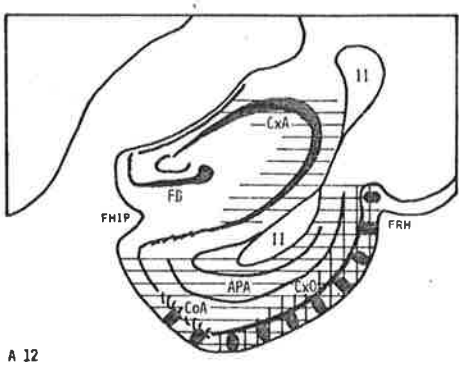
A 21



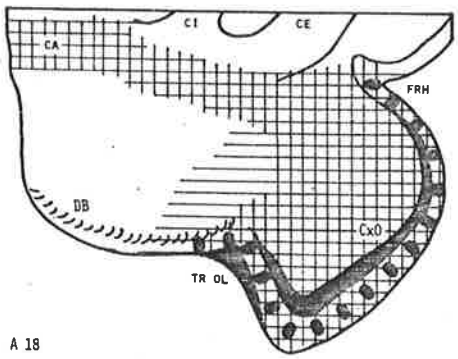
A 13



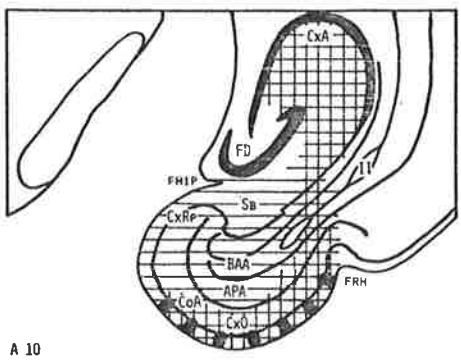
A 19



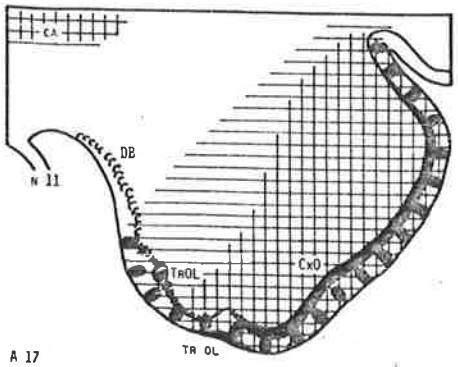
A 12



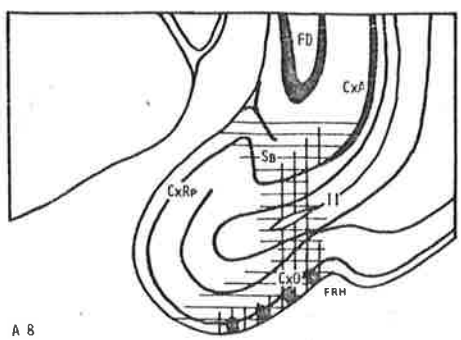
A 18



A 10



A 17



A 8

Fig. 2-2

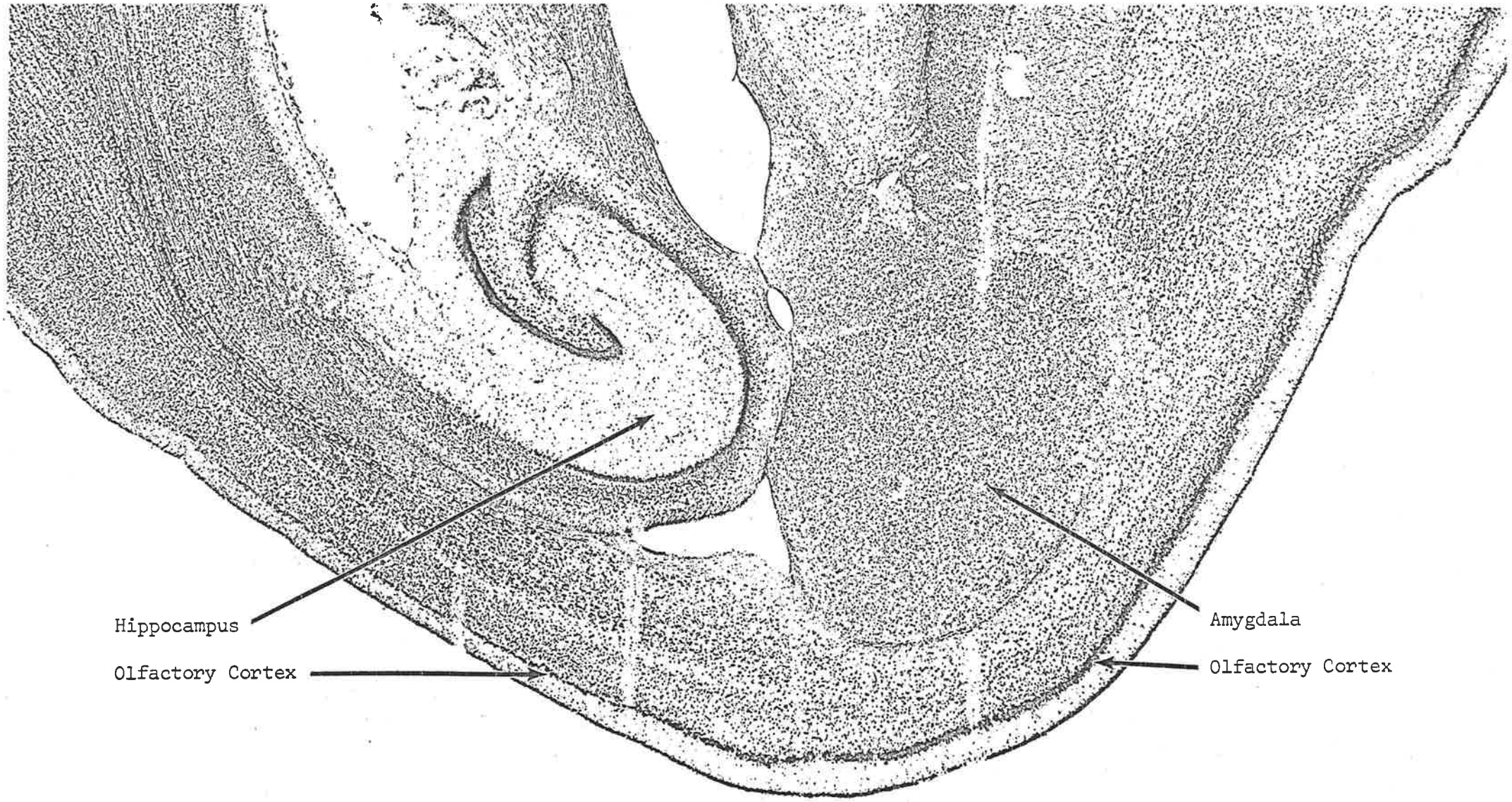


Fig. 2-3 Sagittal section of phalanger brain, 8mm from the mid line, stained for cellular detail, showing the olfactory cortex thinning from its rostral to its caudal extent. Four electrode tracks are seen.

olfactory tract fibres (Fig. 2-4). At the depths and stimulus strengths used here, there were minimal negative potentials evoked in the contralateral bulb. At A9, there was only a very small positive potential evoked in the ipsilateral bulb (checked by averaging) and the bilateral negative potential.

The lateral extent of the lateral olfactory tract projection was always the depth of the rhinal fissure. The medial extent of its cortical projection was not as well defined. On its medial aspect, the cortex is continuous with the olfactory tubercle, diagonal band, and amygdala, progressing caudally. These also receive lateral olfactory tract fibres, and there is no way of determining electrically the transition between true cortex and these other areas.

3. Olfactory tubercle

This structure, not regarded as cortex, was the source of evoked positive potentials throughout its length. In these experiments, recording in the dorsal part of the granule cell layer, the lateral olfactory tract projection extended furthest medially in the rostral part of the tubercle.

4. Amygdaloid nuclei

Stimulation of the cortical amygdaloid nucleus (superficial plexiform layer) throughout its antero-posterior extent evoked positive bulbar potentials. The anterior part of the medial nucleus was also the source of these positives. The late ipsilateral negative potential was evoked from this region. No contralateral negatives were seen.

5. Nucleus of lateral olfactory tract

This structure was also the source of evoked positive potentials. Like the amygdaloid nuclei, it was the source of ipsilateral negative potentials also.

Centrifugal projections to the olfactory bulb

The negative potential evoked in the granule cell layer by stimulation of central areas implies activation of a centrifugal inhibitory mechanism (Dennis & Kerr, 1968; Polson, 1971). It was not the purpose of this study to determine whether there was a direct connexion with the olfactory bulb, or whether the activity was mediated through the anterior olfactory nucleus.

Areas from which negative potentials were evoked in the ipsilateral bulb are indicated in Fig. 2-2 by horizontal line shading. Areas producing contralateral negative potentials are shown with vertical line shading. As before, the major interest was in the qualitative

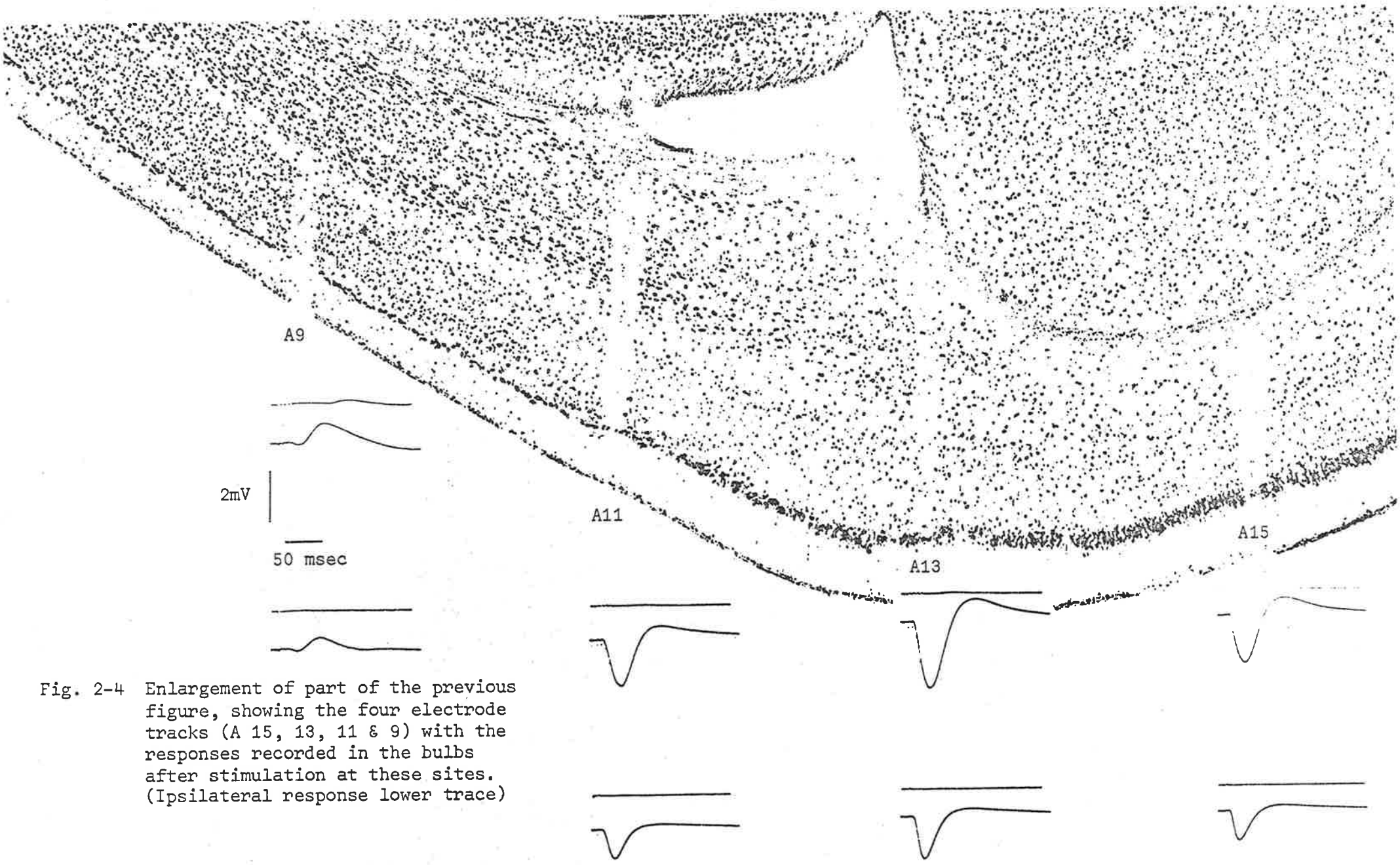


Fig. 2-4 Enlargement of part of the previous figure, showing the four electrode tracks (A 15, 13, 11 & 9) with the responses recorded in the bulbs after stimulation at these sites. (Ipsilateral response lower trace)

presence of negative potentials, with less importance being placed on threshold, latency, size or shape. Three main types of late negative response have been described (Polson, 1971; types 1, 4 & 5).

The early negative response, with latency of 1.0 - 2.6 msec (Rall & Shepherd, 1968, period 1; Nicoll, 1969, N_1 wave; Westecker, 1970*c*, component 1) has not been emphasized in the results. The recording conditions (relatively low gain and CRO sweep speed, relatively large recording electrodes) were not optimal for recording this part of the evoked potential. The positive potential described above is recorded only after stimulation of lateral olfactory tract fibres, and is a more convenient parameter to use in this type of experiment. It is often increased by repetitive stimulation (in these experiments, trains of 4 or 5 stimuli, 5 msec apart).

1. Ipsilateral negativity

Long-latency (10 - 30 msec) ipsilateral negative potentials were evoked from all the areas producing positive potentials, and a short distance medially and caudally from these. Strictly ipsilateral responses (i.e. without a contemporaneous contralateral negative) were evoked from the areas of the basal rhinencephalon producing positive responses, but which are not considered to be true cortex. These include the olfactory tubercle (all anterior and postero-lateral parts in this study), cortical and anterior part of the medial nuclei of the amygdala and the sub-commissural area, including the diagonal band. In all cases, these negative potentials were evoked for a distance of several millimeters below the surface of the brain. Parts of the ventral hippocampus (CA1) and subiculum also gave rise to these potentials.

2. Anterior commissure negativity

Bilateral, short-latency negative potentials were evoked when the anterior limb of the anterior commissure was stimulated. These had a low threshold, and were often 'humped', apparently having two components. They were restricted to the anatomical extent of the commissure, appearing and disappearing when the stimulating electrode was moved 0.25 mm from this structure in the vertical plane. However, this bilateral projection fanned out laterally, rostrally and ventrally, in continuity with the responses of the olfactory cortex.

Fig. 2-5 shows a transverse section of phalanger brain stained for fibres. An electrode track is marked, and responses evoked at three depths are shown.

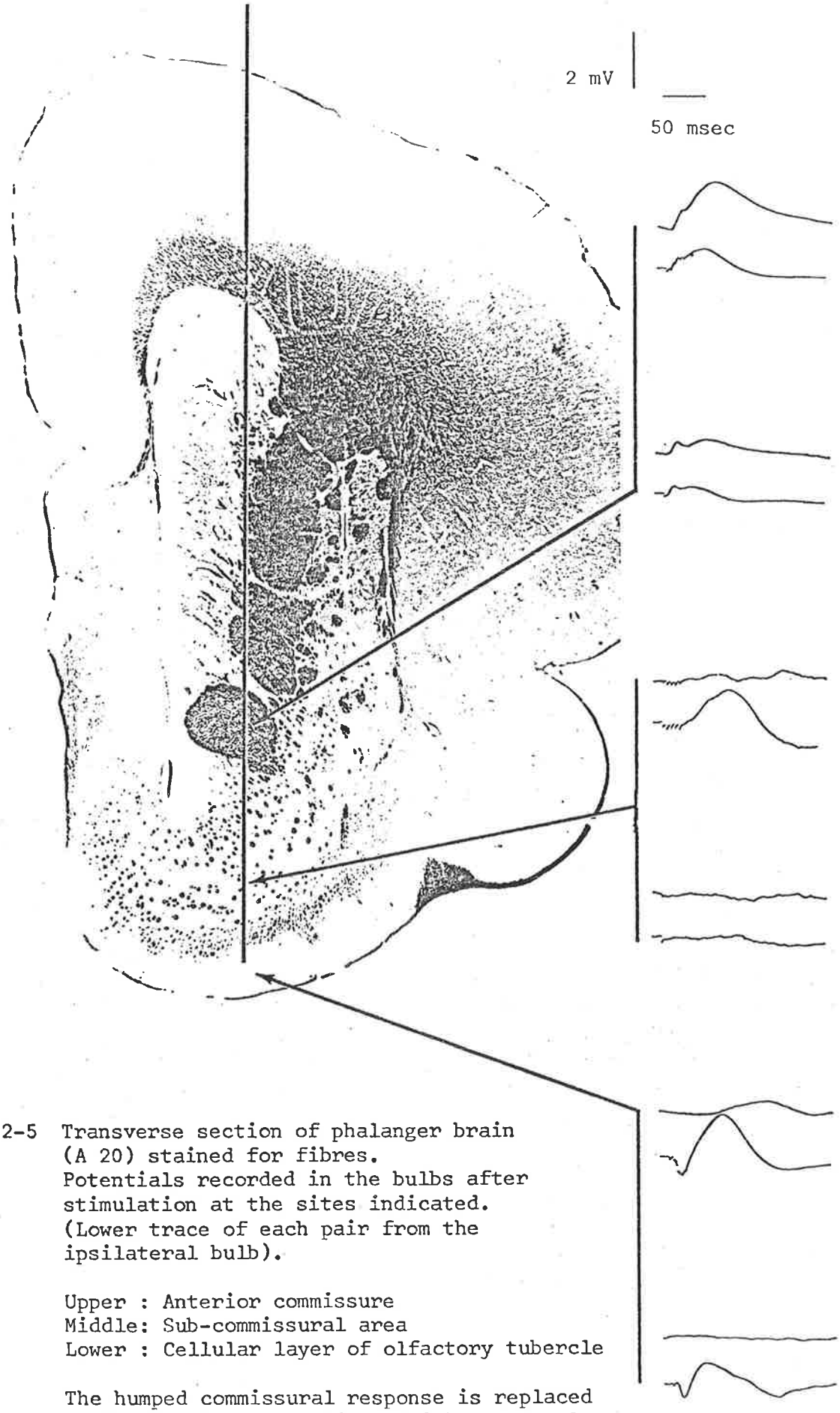


Fig. 2-5 Transverse section of phalanger brain (A 20) stained for fibres.
Potentials recorded in the bulbs after stimulation at the sites indicated.
(Lower trace of each pair from the ipsilateral bulb).

Upper : Anterior commissure
Middle: Sub-commissural area
Lower : Cellular layer of olfactory tubercle

The humped commissural response is replaced by the bilateral negative, which apparently has the positive component added to it in the superficial layer stimulation.

3. Bilateral negativity

Stimulation of olfactory cortex evoked both the ipsilateral positive-negative ("LOT/cortex") response and a simultaneous contralateral negative response. In its anterior extent, this response could be evoked from the volume of tissue lying between the anterior commissure and the olfactory cortex. In the posterior part of the brain, behind the nucleus of the lateral olfactory tract, this bilateral negative response was evoked from a decreasing amount of tissue on the lateral part of the olfactory cortex. At the caudal extremity, only the cortex immediately medial to the rhinal fissure was the source of this bilateral negative potential.

The threshold for evoking the negative potentials was higher than that for the positive potentials. In some of the illustrations in this chapter, contralateral negative potentials indicated in Fig. 2-2 may not be seen because stimuli were inadequate.

4. Other responses

Positive responses were never evoked from sites other than those described above. However, negative responses were sometimes evoked from elsewhere. Stimulation of the posterior part of the dorsal hippocampus often evoked late (>50 msec) bilateral negative responses. Late ipsilateral negatives were evoked from the transition zone between olfactory and angular cortices. Stimulation of the nucleus accumbens, claustrum, putamen, diagonal band, internal and external capsule, and ventral hippocampus has evoked negative responses in one or both bulbs. On one occasion, a 'humped' ipsilateral response was evoked from the rostral part of the frontal lobe, close to the fissure.

DISCUSSION

Bulbar evoked potentials

The nature of the bulbar potentials evoked by stimulation of the lateral olfactory tract has been the subject of much speculation. However, with the structural arrangement of the bulb clarified, it is now possible to interpret electrophysiological findings with more certainty.

The key to bulbar function appears to be the gemmules which form dendrodendritic synapses with mitral and tufted cell secondary dendrites. These gemmules contain the only inhibitory synapse onto the mitral and tufted cells, and therefore are the final common pathway of all negative feedback. For the purposes of this discussion, only

mitral cells will be named as contributors to the lateral olfactory tract, although Nicoll (1970*b*) has shown that tufted cells also contribute axons to this tract.

The theoretical and experimental studies of Rall *et al.* (1966), Rall & Shepherd (1968), Nicoll (1969; 1970*a*, *b*; 1972), Westecker (1969; 1970*a*, *b*, *c*), Polson (1971), Haberly (1973*a*, *b*), and Haberly & Shepherd (1973) have given some insight into the electrical events which occur after the lateral olfactory tract is stimulated electrically.

Stimulation of the lateral olfactory tract (with the forebrain intact {most workers} or isolated {Nicoll, 1969}) causes complex extracellular potentials in the bulb. The nature of the recorded response depends on the site of recording (for example, see Rall & Shepherd, 1968, their Figs. 3 & 4; Westecker, 1970*c*, her Fig. 1; Dennis & Kerr, 1968, their Fig. 1; Nicoll, 1969, his Figs. 4 & 5; Polson's, 1971, field plots of current sources and sinks). All agree that the response recorded deep to the mitral cell layer is roughly the mirror-image of that recorded superficial to that layer (e.g. Polson (1971), his Fig. 70*). This is not surprising when the sites of the sources and sinks of the extracellular current are considered. In the experiments reported in the phalanger, only the granule cell layer response was recorded, and comparisons can be made between these and other data.

Polson's (1971) source-sink distributions in the guinea pig olfactory bulb have been summarized in his Fig. 78**. He showed that there was a high degree of hemispherical symmetry of the fields, with his plane 2 as the hemispherical base. His curve numbers (36, 44, 61 & 180) refer to storage locations in the Computer of Average Transients, and represent the potential plotted against radial location at certain instants of time. (In my studies, potential is plotted against time, with radial position constant.)

Curve 36 =	Peak initial negativity	at 2.65 msec
44 =	Beginning of deep positivity	at 3.90 msec
61 =	Peak deep positivity	at 6.55 msec
180 =	Peak late negativity	at 25.12 msec

His curve 36, at 2.65 msec, after lateral olfactory tract stimulus onset, indicates an extracellular sink just external to the datum line.

---

* Reproduced, with permission, as Fig. 2-6.
** Polson's Fig. 78 is reproduced as Fig. 2-7

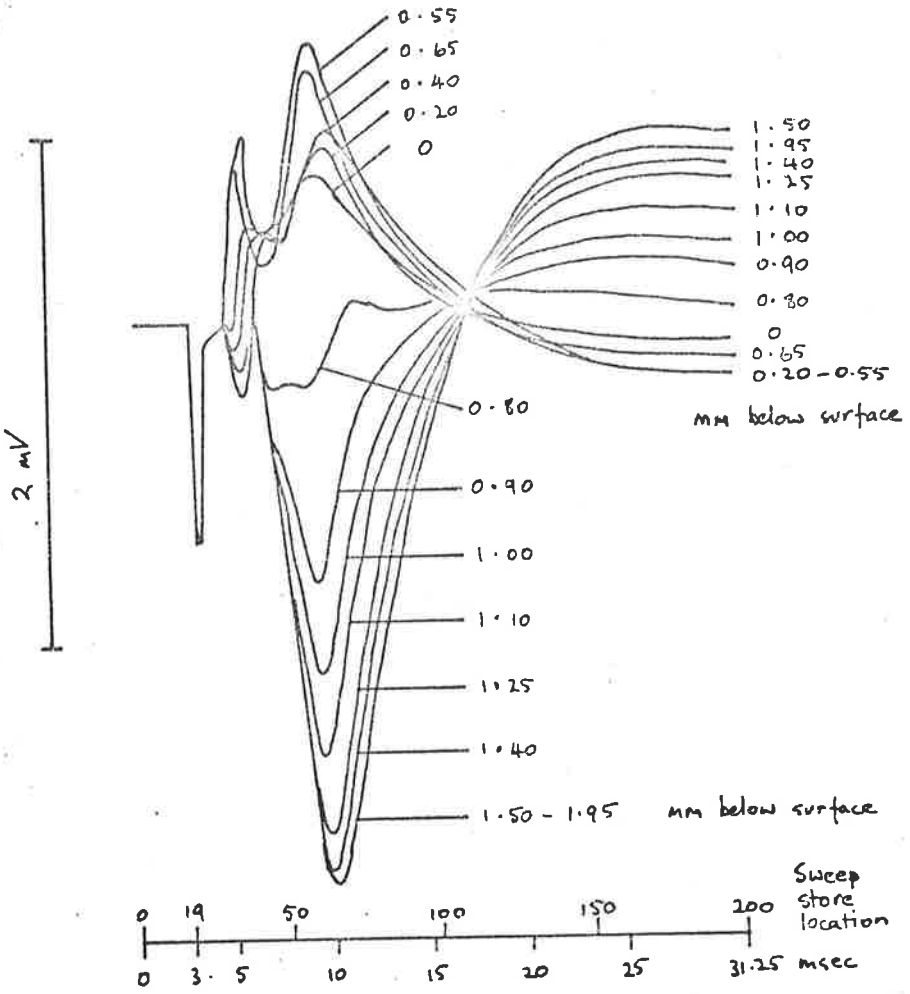


Fig. 2-6 Superimposed tracings of "LOT/cortex" average evoked responses obtained during diametral penetration of a guinea pig olfactory bulb. the distance of the recording electrode tip below the bulbar surface is indicated. It can be seen that the "turn-over" occurs between 0.65 and 0.80 mm below the surface, i.e. in the mitral cell layer.
(Redrawn with permission from Polson's (1971) Fig. 70)

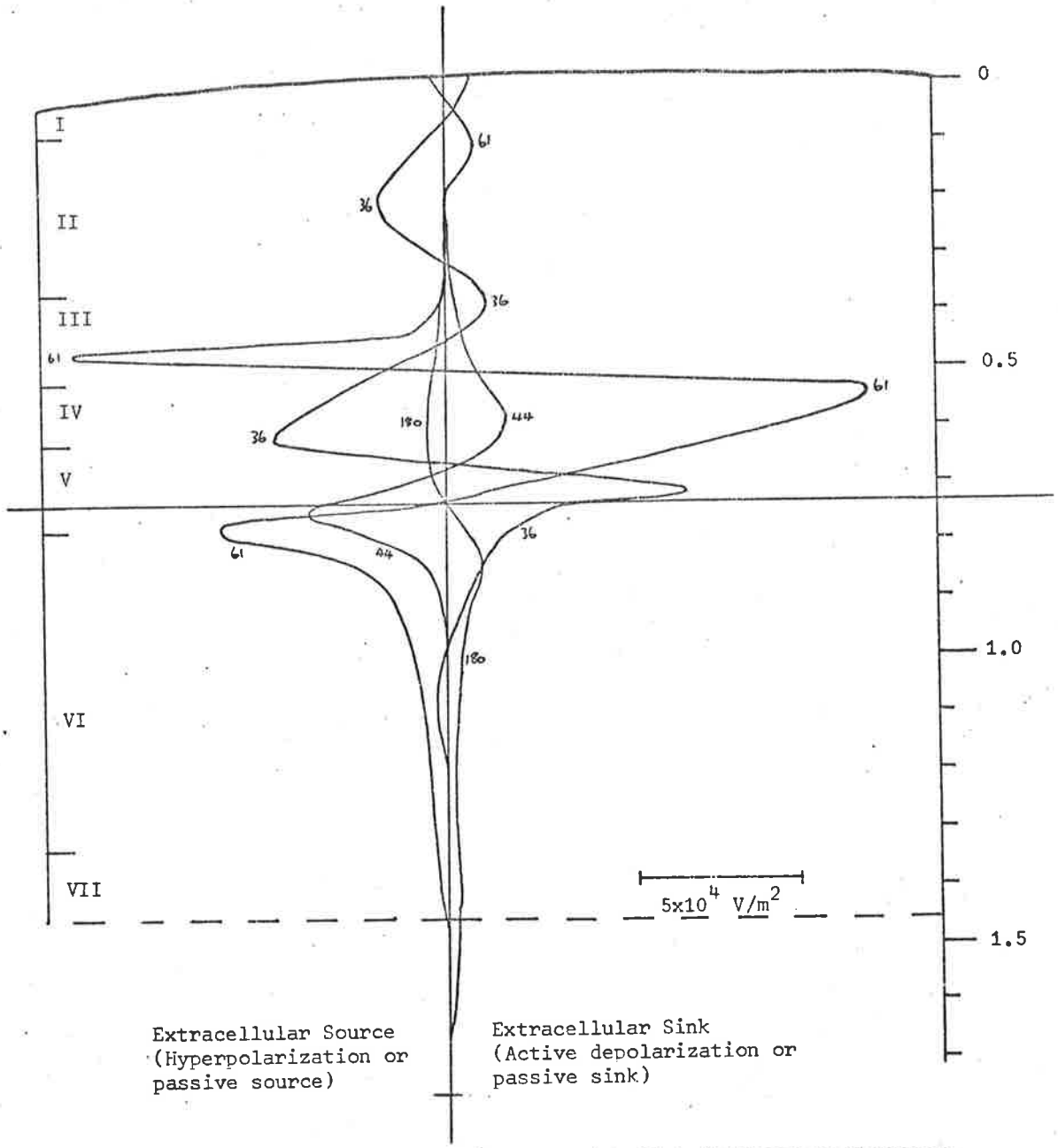


Fig. 2.7. Superimposed plots of current source-sink distributions at different times during a LOT/cortex evoked response in the olfactory bulb of a guinea pig. The distance below the surface and lamination (Valverde (1965) Fig. 25) are shown. The curves represent the values stored in the four numbered locations in the Computer of Average Transients at each 50μ step during penetration of the bulb.

Curve	Time	Description
36	5.6 msec	Peak of initial negativity.
44	6.9 msec	Commencement of positivity.
61	9.5 msec	Peak of positivity.
180	27.1 msec	Peak of late negativity.

(Redrawn with permission from Polson's (1971) Fig. 78).

The datum line is at the level of evoked potential turnover, as the electrode penetrates the bulb, and is located at the internal plexiform layer.

About 0.1 mm further out is an extracellular source, with another sink, and sources 0.2 mm and 0.4 mm further out, respectively. If this curve is superimposed on a diagram of the bulb to the same scale (Polson referred to Valverde, 1965), the deep extracellular sink is at the mitral cell axon hillock level, with the datum line (turnover point of the evoked potential) located at the internal plexiform layer. (This turnover point was located by Polson within 0.1 mm in his experiments.)

The curve 44, 3.9 msec at the commencement of the positivity, therefore represents the repolarization of the mitral cell axon hillock/initial segment region, with complementary passive sources and sinks in the dendrites of the external plexiform layer. It is an approximate mirror-image of curve 36, and corresponds with the beginning of Rall & Shepherd's period III (not period II, as Polson suggests, his p. 6-16).

At the time of the peak of the deep positivity (curve 61, 6.25 msec after lateral olfactory tract stimulus onset) there is an intense source in the external plexiform layer, confirming the opinion of Rall & Shepherd (1968) that dendrodendritic synaptic activation is occurring (depolarization of gemmules). Another source in the granule cell layer suggests that there could be activation, hyperpolarization (and inhibition - Polson, his p. 6-13) of the deep dendrites of the granule cells. It is known that the mitral cells are inhibited during this period (Phillips, Powell & Shepherd, 1963), which adds to the evidence for dendrodendritic activation in the external plexiform layer, via the dendrodendritic synapse.

Polson's curve 180, at 25.1 msec, during the late negativity (beyond Rall & Shepherd's calculations) is the complement of his curve 61, and represents repolarization of depolarized elements (granule cells). There are low current flows and a long time-course.

In contrast with the lateral olfactory tract response discussed above, the anterior commissure response is entirely negative. Polson (1971, his p. 6-19) discusses a 'humped' response, with peaks at 8 and 22 msec after stimulus onset. He shows that the anterior commissure potential distribution has all the characteristics of a slow negative wavefront building up and spreading radially outwards from a region

corresponding with the deep boundary of the internal granule layer. This is reminiscent of the model of spike and wave propagation illustrated by Petsche (1970, his Fig. 13).

Polson concluded that the LOT/cortex response was able to be resolved into two components: (1) a positive component identified with activation of the gemmules by way of antidromic invasion of the mitral cells causing an action potential which spread to the secondary dendrite (Rall & Shepherd, 1968; their p. 895); (2) a contemporaneous, barbiturate-sensitive negative component identified with the deep centrifugal pathway associated with the lateral olfactory tract. Applying this knowledge to his field-plots, it was concluded that the positive potential arises from dendrodendritic excitation of granule cell peripheral processes by mitral cell secondary dendrites in the external plexiform layer after stimulation of mitral axons. The negative potential was due to excitation of granule cell somata and deep dendrites by an efferent system. The calculations and discussion have depended on radial symmetry of the bulb and its complete activation. It implies that there is no topographical projection of the mitral cell axons into the lateral olfactory tract. Polson was unable to detect significant differences between the fields caused by 'prepyriform' or 'periamygdalar' cortical stimulation. Topographical differences might be expected to show subtle field differences, either on addition or subtraction of the individual response, but none was seen.

However, Shepherd & Haberly (1970) had demonstrated a topographic arrangement during partial activation of the bulb in the nembutal-anaesthetized opossum. They used focal stimulation to the lateral olfactory tract where it emerged from the bulb, and recorded the evoked fields in the bulb with microelectrodes. They concluded that the dorsal bulb projected to the dorsal part of the tract, and the ventral bulb to the ventral part of the tract. The medial part of the bulb projected to the dorsal and ventral parts of the tract. The tract projection in its dorso-ventral axis was thought to represent the bulb in cross section. The medial projection of the bulb did not lie under the lateral bulb projection. It was thought possible that longitudinal segments of the bulb project in the form of elongated strips running through most of the tract. However, no conclusions could be made regarding possible topographic projection to the cortex.

Freeman has described the characteristics and distribution of potentials recorded in the bulbs of anaesthetized cats and rabbits

following electrical stimulation of primary olfactory nerves or the lateral olfactory tract (Freeman, 1972a,b,c,d; 1974). He suggested that in certain circumstances the average evoked potential may be generated by the granule cell population. Field plots were used to arrive at this conclusion, in a similar method to that of Polson (1971).

Mapping with evoked potentials

It can be seen from the preceding discussion that the method used by Dennis (1965), Dennis & Kerr (1968), and Morrison (1969) is valid for determining the extent of the mitral cell axons (lateral olfactory tract) and the afferent systems of the olfactory bulb. This method was used to study the connexions of the olfactory bulb of the phalanger in this thesis.

The types of the evoked bulbar potentials are similar in this animal to those in the cat (Dennis & Kerr, 1968), guinea pig (Morrison, 1969; Polson, 1971), rat and rabbit (Morrison, 1969). The general arrangement of the olfactory system is also similar to the cat, rabbit, rat and guinea pig, when the details are compared.

Olfactory cortex

In order for an area to be classified as primary olfactory cortex, it must possess two attributes: (1) the histological arrangements necessary for cortex (defined by Pigache (1970) as having a minimum of three tangential layers, the most superficial being plexiform, connected by radial and tangential processes), and (2) terminals of lateral olfactory tract axons. Unless both are present, it cannot be described as olfactory cortex.

In the phalanger, olfactory cortex extends from the retrobulbar area rostrally to the level of the caudal end of the amygdaloid complex caudally; from the rhinal fissure on the lateral side, to the olfactory tubercle, diagonal band, and amygdala medially. This cortex has its greatest depth of pyramidal cells (layers 2 and 3) at its rostral extremity, and it thins out to layers 1 - 2 cells thick at the caudal part, but still as a continuous sheet. Stimulation of the plexiform layer of this area evokes the "LOT/cortex" response; the positive potential and contemporaneous negative potential in the granule cell layer of the ipsilateral bulb (see Fig. 2-3).

There is an additional electrophysiological characteristic of this area. Stimulation of the cortex itself just medial to the rhinal fissure, and the tissue lying between this and the external capsule or the anterior commissure, evokes a negative potential in the contralateral

bulb (the crossed negative, Polson type 5) as well as the ipsilateral negative. This crossed negative is characterized by a longer latency, higher threshold, lower voltage, and absence of after-positivity.

The olfactory cortex in the cat was divided into five by Dennis & Kerr (1968): Prepyriform (PP) 1, 2 & 3, Periamygdaloid and Parahippocampal areas. The same could be done in the phalanger, but the divisions would be less clear. PP1 would lie between the lateral olfactory tract and rhinal fissure, and is the thickest and best-defined of the areas. PP2 would be the narrow strip beneath the tract itself. It is the transitional zone between the clear PP1 and the olfactory tubercle or diagonal band behind. PP3 would be the posterior extension of PP1 behind the nucleus of the lateral olfactory tract, and is the continuous layer of cortical cells superficial and lateral to the external capsule. This layer becomes somewhat fragmented medially as it merges with the cortical and medial amygdaloid nuclei, and the subiculum/hippocampal complex. There would be some justification in calling these areas Periamygdaloid (PAM) and Parahippocampal (PH) as Dennis & Kerr did. These latter two areas were not the source of contralateral evoked potentials. However, the resolution achieved with this method may be insufficient to determine the precise boundaries. There may be functional as well as structural differences within the olfactory cortex, but this point will require further investigation. It is sufficient to refer to olfactory cortex and recognize that there are two main areas. The anterior part (rostral to the nucleus of the lateral olfactory tract) is thick and well-defined. The posterior part is a thinner layer of pyramidal cells than the anterior part. There is no clear electrophysiological distinction between the two, but latency of the positive evoked potential is longer in the posterior part.

Olfactory tubercle

This subcortical area has a similar histological appearance to the same structure in other species, with the superficial layers of cells forming 'scallops'. It also contains cellular condensations - islands of Calleja - predominantly in the superficial zone. Positive potentials are evoked from the antero-lateral aspect of the tubercle. This area extends across the width (to within 2 mm of the midline) at the rostral end, and is reduced to a strip about 2 mm wide on the lateral aspect as it runs into the diagonal band. The "LOT/cortex" response without the crossed negative is evoked from the medial part of this area. There

may be crossed negative potentials evoked from the zone adjacent to the lateral olfactory tract from the fragmented cortical transition area, but this was not a constant finding.

On this evidence, it appears that the postero-medial part of the tubercle does not send or receive bulbar connexions. It must be remembered that the recording is from the dorsal granule cell layer of the bulb. There is electrophysiological evidence of topographical projection of the bulb to the lateral olfactory tract (Shepherd & Haberly, 1970) and anatomical evidence of differential projection of the bulb to the tubercle (Heimer, 1968). The study in this thesis has shown that lateral olfactory tract fibres project from the dorsal part of the olfactory bulb to the anterolateral part of the tubercle. As part of another experiment, I stained serial sections of phalanger brain to demonstrate cholinesterase, and no regional differences were seen within the tubercle. Further experiments will be needed to determine the presence of differential projection of the bulb to the tubercle.

Amygdala

Stimulation of the superficial plexiform layer of the cortical and medial amygdaloid nuclei evoked the "LOT/cortex" response. As with the tubercle, the lateral parts of this plexiform layer caused the "crossed negative" response. Again, the only apparent correlation between histological appearance and areas producing the crossed negative was that this response was evoked from the transition zone of fragmented cortex. More precise delineation was not possible with this technique.

Winans & Scalia (1970) showed in the rabbit that the accessory bulb was the main origin of the projection to the cortical and medial nuclei. Raisman (1972) has shown that after section of the stria terminalis and fimbria in the rat, degenerating terminals (5% of all counted) were found in electronmicrographs of the ipsilateral accessory olfactory bulb. (A total of nearly 13,000 synapses was counted in the study.)

It will require more electrophysiological experiments to determine whether the accessory bulb was being activated by stimulation of the amygdala. Field plots of the caudal part of the bulb after such stimulation would be valuable in locating sources and sinks of electrical activity, and the hemispherical symmetry of the rostral part of the bulb would be distorted if the accessory bulb made a significant contribution. However, in the studies reported here, responses evoked

in the main bulb by stimulation of the amygdala were similar to the "LOT/cortex" response, but without the contralateral negative component evoked from the cortex. The olfactory tubercle does not receive a projection from the accessory bulb. Therefore, within the experimental limitations, it was assumed that the cortical and medial amygdaloid nuclei received axons from the main bulb via the lateral olfactory tract.

The nucleus of the lateral olfactory tract

The nucleus of the lateral olfactory tract gave rise to a "LOT/cortex" response, with bilateral negative potentials being evoked deep to it, and an ipsilateral positive from this nucleus itself. It should be remembered that this structure was thought to be connected with its fellow via the anterior commissure. Stimulation of this nucleus evoked no unusual potentials in the contralateral olfactory bulb.

Efferent systems

Lateral olfactory tract

Apart from its afferent component, the lateral olfactory tract transmits efferent fibres in the phalanger, demonstrated by the evoking of bilateral negative potentials from it. However, Dennis & Kerr (1968) sectioned the tract on the surface of their area PP2 in the cat, and the ipsilateral bulbar negatives responses were still evoked by stimulation behind the lesion. They also interrupted the anterior limb of the anterior commissure without effect on the ipsilateral bulbar negatives. They concluded that an efferent system reached the bulb by a pathway not in anterior limb of the anterior commissure or lateral olfactory tract, but were unable to identify it. There were no lesions made in the phalangers in this study, so it is only possible to say that efferent fibres travel in the lateral olfactory tract and are present in the area deep to the olfactory cortex.

Olfactory cortex

In addition to the positive potentials, negative potentials were evoked in the bulb by stimulation of the olfactory cortex. This implies inhibition in the bulb, but, as suggested above, the precise origin (cell or nucleus type), course or termination of this system could not be determined by this method without interrupting putative pathways.

Olfactory tubercle

The olfactory tubercle is also the source of ipsilateral evoked

bulbar negative potentials, but, again, this method cannot determine whether a system arises in the tubercle or if fibres from elsewhere are stimulated as they pass through the structure. Price & Powell (1970*c*, *e*) described their efferent system passing through the posterior extremity of the tubercle, before entering the lateral olfactory tract. However, this was unable to be confirmed in this study.

Amygdala

Stimulation of cortical and medial nuclei, and the superficial parts of the basal nucleus and anterior amygdaloid area caused ipsilateral negative potentials. These centrifugal effects could be transmitted by two main routes: the medial forebrain bundle or via amygdalar connexions with the olfactory cortex. The area from which evoked negative responses were obtained was much greater than that for positive responses, which has been the usual observation in other species (Dennis & Kerr, 1968; Morrison, 1969).

Diagonal band

Stimulation of the ventral part of the diagonal band (nucleus of the horizontal limb - Price & Powell, 1970*c*, *e*) in its lateral part occasionally evoked a small negative potential in the ipsilateral bulb. The relative electrical silence is surprising in view of the importance vested in this area by these authors. They showed by anatomical methods that an efferent system arose in this nucleus, passed through the granule cell layer, and ended in the glomerular layer of the bulb. They did not comment on the glomerular layer terminals. It is possible that the evoked potential technique used here is inadequate for detecting such a system. It does identify the anterior commissure (contralateral anterior olfactory nucleus) efferents which end on the granule cells, and the Dennis & Kerr efferent system, which runs neither in the lateral olfactory tract (the Price & Powell system), nor in the anterior limb of the anterior commissure.

Because of the superficial termination of the Price & Powell (1970) diagonal band system, it may be necessary to investigate it by other methods. Recording with micro or macro-electrodes in the glomerular layer itself during central stimulation (stimulating the horizontal limb of the diagonal band electrically or chemically) might prove fruitful. Attempting to modify induced or spontaneous activity via this system would also indicate its functional role. The study in the cat of Dennis & Kerr (before Price & Powell's work) also failed to show effects of stimulation of the horizontal limb of the diagonal band.

Anterior commissure

Stimulation of the anterior commissure (bulbar component) or anterior olfactory nucleus evokes a characteristic bilateral short-latency 'humped' negative. It has been shown that anterior commissural fibres arise in the anterior olfactory nucleus and are distributed to the contralateral granule cell layer. The shape of the potential could imply that other factors are operating: (1) two fibre systems, with distinct populations of fibres to the bulb, are being activated; for example, axons to one bulb, and recurrent collaterals to the other; (2) there is a direct inhibition and one mediated via synapse(s); or (3) the anterior commissure ends on spatially separated parts of the bulb. It would need a different series of experiments to answer this question.

Penetration of the anterior commissure with the stimulating electrode demonstrated the well-defined extent of the anterior commissure and the small amount of stimulus spread. This response appeared and disappeared with movements of the stimulating electrode as small as 0.25 mm as the commissure was penetrated.

The anterior commissure also transmits fibres from the olfactory cortex to the opposite side (Valverde, 1965; Dennis & Kerr, 1968), but the course and termination of these in the phalanger has not been determined. Such fibres are presumably the origin of the contralateral negative potential evoked by stimulation of the olfactory cortex and underlying tissue. Whether they end in the anterior olfactory nucleus or the bulb itself has not been determined.

The simple experiments reported here do not allow conclusions to be drawn about the function of this negative potential or the anatomical components of the system. Dennis & Kerr used over 100 animals in their study and were able to clarify certain points about the negative potentials they observed. They showed that section of the lateral olfactory tract did not influence the ipsilateral bulbar negative responses. Lesions of the anterior limb of the anterior commissure likewise had no effect on this response. This indicated that centrifugal fibres reach the bulb by some other route from the ipsilateral cortex. However, a lesion which interrupted the anterior commissure, or its contralateral anterior limb, prevented the contralateral negative from being evoked after cortical stimulation. This observation showed that the anterior commissure is the route for the crossed negative, but whether from cortex to the contralateral

anterior olfactory nucleus, or via the ipsilateral nucleus, is not clear from these experiments. The suggestion from the degeneration studies of Price & Powell and Pinching & Powell suggest that the pathway is prepyriform cortex - ipsilateral anterior olfactory nucleus - anterior commissure - contralateral granule cell layer. The experiments in this section could not clarify the problem, either by measurement of latency, inspection of the waveform, or effects of drugs.

The other areas from which negative responses were evoked showed that a large number of structures may influence the olfactory system. The amygdala has both diencephalic and olfactory connexions (Cowan, Raisman & Powell, 1965), and it is possible that stimulation of non-olfactory areas could influence bulbar activity through this complex area.

Comparison with other studies

A comparison can be made with results and conclusions from other workers on the extent of the olfactory connexions in the phalanger (Adey, 1953; Heath & Jones, 1971; Putnam & Cone, 1966, opossum) and cat (Guevara-Aquilar, Aquilar-Baturoni, Aréchiga & Alcocer-Cuarón, 1973).

Adey (1953)

Adey carried out studies of the degeneration following unilateral olfactory bulbar ablation, using his own modification of the Glees method for impregnating degenerating fibres and terminals. He concluded that:

(1) The lateral olfactory tract terminated in the lateral part of the olfactory tubercle only.

(2) Degeneration in the pyriform lobe was restricted to the anterior (prepyriform, olfactory) area. None was detected in the entorhinal area (angular cortex) or hippocampal formation.

(3) Degeneration in the amygdaloid complex was restricted to the nucleus of the lateral olfactory tract, the cortical and medial nuclei, and the bed nucleus of the stria terminalis. Very little degeneration was found in the central nucleus, and none in the basal or lateral nuclei.

(4) No degeneration was detected in the septal area.

(5) Degenerating fascicles were present in the anterior limb of the anterior commissure.

(6) Degeneration was found in the anterodorsal glomeruli of the contralateral olfactory bulb, but not the granule cell layer.

Examination of Adey's figures enables correlations to be made between my electrophysiological and his neuroanatomical findings. Adey's Figs. 1 to 6 are transverse; 7, 8 and 10 sagittal; and 9 is oblique.

It is now relevant to discuss Adey's figures in the light of the present study, for which purpose reference may be made to Fig. 2-2 of this chapter.

Adey's Fig. 1 corresponding with my A23, is at the level of the caudal end of the anterior olfactory nucleus and olfactory ventricle. Degeneration was shown by Adey to be present in the olfactory cortex beneath the lateral olfactory tract, and superficial to the anterior olfactory nucleus and anterior hippocampal nucleus, extending ventrally on the medial side to the same horizontal level as on the lateral side (rhinal fissure). It would be expected, therefore, that all this area would produce similar evoked potentials. The study in this chapter has shown that positive potentials were not evoked 1 mm from the midline, but a bilateral negative response occurred in the region of the anterior olfactory nucleus, pars posterior. These observations demonstrate that the lateral olfactory tract does not project as far medially as Adey had suggested. Dennis & Kerr (1968) made similar conclusions in the cat (their Fig. 5A). Adey's observations could be explained by changes following damage to the anterior parts of the anterior olfactory nucleus, although he himself refuted this possibility.

Adey's Fig. 2, at the level of the olfactory tubercle and septal region, corresponds to my section A21. Neither Adey's studies nor the present electrophysiological evidence clearly define the medial extent of the projection of the lateral olfactory tract to the tubercle. This point of anatomical detail awaits further study.

Adey's Fig. 3, a transverse section at the level of the anterior commissure and posterior part of tubercle, corresponds with my A18. At this level, there is good agreement between the anatomical and electrical methods, except for the bed nucleus of the stria terminalis. Adey's results imply a direct connexion between the bed nucleus and the olfactory bulb, not <u>via</u> the stria terminalis. This contention has been refuted by the present studies. The nucleus of the diagonal band was unremarkable in either study, with no degeneration being seen in it, and no potentials able to be evoked from it. This level (A18) corresponds with Dennis & Kerr's (1968) Fig. 5D, which showed that the medial extent of the projection is not as great in the cat as in the phalanger.

Adey's Fig. 4 is at the level of the nucleus of the lateral olfactory tract (my A17). He showed degeneration in the olfactory cortex, nucleus of the lateral olfactory tract, and cortical amygdaloid nucleus. The present evoked potential studies confirm the presence of direct connexions between these structures and the olfactory bulb. Adey also described degeneration in the hypothalamic bundle of the stria terminalis, its bed nucleus, and in the periventricular olfactory-habenular tract. There were no positive bulbar potentials evoked by stimulation of these structures. The implication from this disparity is that there is no direct projection from these structures to the bulb.

Adey's Fig. 5 is at the middle of the amygdaloid complex, the same level as A14 of my Fig. 2-2. Adey acknowledged Johnston's nomenclature of this structure, and showed degeneration in the medial, cortical and central nuclei in decreasing amounts. None was seen in the basal or lateral nuclei. In the present investigation, positive responses were evoked only from the superficial parts of the cortical and medial nuclei. This implies that the lateral olfactory tract fibres travel over the surface of the amygdala (and olfactory tubercle), and penetrate for a distance of 0.25 mm or so. It was noticed in this study that this response evoked from the amygdala could be distinguished from the response evoked by olfactory cortical stimulation because of the absence of a contralateral bulbar negative response. Negative responses in the ipsilateral bulb were evoked from most of the amygdaloid complex. This implies an inhibitory action of this complex on bulbar function. If this were via the anterior olfactory nucleus, and Adey had damaged it in his study, the present results would corroborate his findings.

Adey's Fig. 6 is at the caudal part of the amygdala, with the anterior part of the ventral hippocampus appearing, A12 in this study. Degenerating fascicles were described from the rhinal fissure to the amygdaloid fissure, and in the superficial layers of the cortical and medial amygdaloid nuclei. The electrophysiological findings are compatible with this description.

Adey's Figs. 7 & 8 are sagittal sections which show the posterior limit of degeneration after bulbar ablation. They correspond with Figs. 2-3 & 2-4 of this chapter. It is not clear from Adey's descriptions or figures where the posterior extent of the degeneration lies, and therefore the extent of his entorhinal area. He implies that it is the posterior pyriform area with radial shading in his figures. Figs. 2-3 and 2-4 of this chapter are taken from an experiment (W14) which clarified this point. Four electrode tracks are shown,

and the corresponding evoked responses are illustrated. Positive bulbar potentials were evoked by stimulation at the deepest point at each location. (The most posterior one, A8, was checked with the Computer of Average Transients). These results indicate that the histological structure of the olfactory cortex is not a good indication of the specific projection of lateral olfactory tract fibres which are more precisely delineated by electrophysiological techniques. The thick layer of pyramidal cells superficial to the amygdaloid complex becomes attenuated as it runs caudally, until it consists of only one layer of cells. From this part of the cortex caudally, fine radial myelinated fibres become more numerous (this point has been unable to be conveniently illustrated in this thesis, because of the lack of suitable photomicrographic definition of these fibres with the haematoxylin stain). The presence of these fibres does not correlate with the absence of lateral olfactory tract fibres. On this question of the extent of the angular cortex (angular ganglion of Cajal, entorhinal area), the most reliable anatomical criterion of the transition seems to be the lamina diseccans (Rose, 1912). It was described in the mouse as a broad, cell-free layer deep to the pyriform cells of the posterior part of the olfactory cortex. The present study has shown that this lamina is also present in the phalanger. It becomes narrower and more sharply defined where the thick layer of olfactory cortical cells splits for a short distance beneath the ventral hippocampus (at AP level A9.5 in Figs. 2-3 and 2-4). Both lamina diseccans and the attenuated layer of olfactory cortical cells end at a shallow fissure (Figs. 2-3 and 2-4). The electrophysiological evidence here presented serves to confirm that this marks the true extent of olfactory cortex. Further caudally, radial orientation and circumferential lamination of small pyramidal cells define the angular cortex (entorhinal area), which merges with occipital cortex above and behind it.

In the light of the evidence presented here, it is difficult to know from his descriptions what Adey included in his entorhinal area. He stated (1957) that some 7 mm of its length was able to be explored with a vertical electrode. This statement and his 1953 diagram imply that he considered it to be larger than the present evidence would suggest.

Putnam & Cone (1966) reported similar findings in the opossum to those of Adey (1953) in the phalanger. They removed one or both bulbs, and after an appropriate period, used the Nauta method to detect degeneration.

They made the following conclusions:

(1) The lateral olfactory tract was distributed to the ipsilateral crural, anterior and medial parts of the pyriform cortex, the cortico-medial group of the amygdaloid nuclei, the olfactory tubercle and anterior olfactory nucleus.

(2) The olfactory part of the anterior commissure was distributed to the contralateral olfactory bulb, anterior olfactory nucleus, cortical and medial amygdaloid nuclei and bed nucleus of stria terminalis on both sides.

(3) No degeneration was present in the septal area or hippocampus.

(4) Only two tracts existed, the lateral olfactory tract and anterior commissure.

Unfortunately, this abstract was unillustrated, and no mention was made of bulbar projections to the olfactory tubercle or to the caudal extent of the projection.

They interpreted their findings as confirmation of Adey's contention that the olfactory bulb has direct projections to the contralateral cortical and medial amygdaloid nuclei. The electrophysiological evidence presented here would indicate that in the phalanger, at least, the only projection is the ipsilateral nuclei.

Heath & Jones (1971) have made a definitive study of interhemispheric connexions in the phalanger using degeneration techniques employing the Nauta & Gygax (1954) and Fink & Heimer (1967) silver methods. They divided whole or part of the commissures in the first series, and destroyed unilateral areas of the cortex in their second group, the animals being allowed to survive from 4 to 8 days. After complete transection of the anterior commissure they described degenerating fibres and terminal degeneration throughout most of the caudate nucleus and putamen, with maximal terminal degeneration in the dorsolateral aspects of the head of the caudate nucleus. They noted dense terminal degeneration in the deep layers of the whole "entorhinal" cortex, and sparse degeneration in the molecular layer of the pyriform cortex, and in the basolateral nuclei of the amygdala. In the one brain sectioned horizontally, degenerating terminals were visible in the granule cell layer of the olfactory bulb, but very few encroached upon the more superficial layers. In their discussion they commented that the commissural connexions of the hippocampal formation, olfactory bulb and basal forebrain structures appeared to be identical to those described for other mammals, and referred the reader to Blackstad (1956) and Raisman et al (1965); they did not think it necessary to discuss

the details. It is curious to note that they did not refer directly to work on the same animal by Adey (1953) or Adey, Merrillees & Sunderland (1956). They did not make any comments on the papers of Johnston (1923) or Loo (1931), who had described the commissures of the opossum.

Study of Fig. 2 of Heath and Jones, and comparison with similar sections of my material, suggests that there is also degeneration which they do not describe in the anterior part of the olfactory tubercle (their sections 2 and 3), nucleus of the lateral olfactory tract (the isolated dot in their section 4), anterior amygdaloid area (the isolated dot deep to olfactory cortex in their section 5), and the medial and cortical amygdaloid nuclei (their sections 7 and 8).

Adey (1953) reported degeneration in the contralateral medial and cortical amygdaloid nuclei (his Fig. 9), and it is reasonable to assume that this was the result of anterior olfactory nuclear damage. These fibres travel in the anterior commissure. Heath and Jones did not describe degeneration in the amygdaloid nuclei after anterior commissural damage, a finding at variance with Adey's. They also did not show any degeneration in the anterior limb of the anterior commissure (their sections 1, 2 and 3) which should have occurred if their observations were consistent with their experimental technique.

My observations were not particularly directed towards the details of interhemispheric connexions. Heath and Jones' Fig. 2 shows areas of degeneration to which they make no reference in their text. Further study of their sections 2 and 3, the isolated dot in their section 4, the isolated dot deep to olfactory cortex deep to section 5, and their sections 7 and 8, would seem to provide evidence of a commissural connexion from the anterior olfactory nucleus to the amygdaloid area.

In the period since my own experiments were completed, electrophysiological techniques have been used to explore the connexions between olfactory and respiratory areas. Guevara-Aquilar, Aquilar-Baturoni, Aréchiga & Alcocer-Cuarón (1973) used an evoked potential technique to define the connexions between olfactory and respiratory areas of the brain of the cat. They stimulated the floor of the fourth ventricle and recorded from olfactory (prepyriform) cortex, olfactory tubercle, lateral olfactory tract and olfactory bulb. They recorded potentials evoked in these areas, in addition to the anterior commissure and mesencephalic reticular formation. No evoked responses were recorded from the diagonal band, fornix, mammillary peduncles or frontal cortex. Responses in the bulb were maximal at the surface, not in its depth.

The potentials evoked in the olfactory areas consisted of a negative-positive-negative response (designated N1, P1, N2). Latencies were measured to the peak of N1 in order to determine the anatomical course of the pathway. Table 2-1 (from their Table 1) shows their findings, after single stimuli of 0.1-1.5 V intensity and duration 0.1-0.5 msec. (Average latency (msec), Standard error of mean).

TABLE 2-1

Structure	Component	Latency msec (Average, S.E.)
Olfactory bulb	N1	5.5±0.68
	P1	6.5±3.20
Prepyriform cortex	N1	8.5±0.40
	P1	18.5±2.00
Olfactory tubercle	N1	4.9±0.30
	P1	9.8±0.91
Lateral olfactory tract	N1	5.2±0.14
	P1	13.8±1.61

They concluded that there existed a dual and bilateral projection from the brainstem reticular formation to olfactory structures. On the basis of N1 latency, they suggested that there was a common path to the tubercle. From this structure, two branches arose, one to prepyriform cortex and one to the lateral olfactory tract. The tubercle was said to be the final common path for efferent impulses to the bulb, since it also received efferent connexions of the prepyriform cortex. Section of the lateral olfactory tract abolished the evoked responses in the bulb, and it was concluded that both efferent pathways ran in the tract. Blocking (with procaine) or section of the olfactory tubercle had the same effect as tract section in abolishing the bulbar evoked response.

Several points are not raised in their paper:

(1) There is no mention of the postulated efferent system arising in the nucleus of the horizontal limb of the diagonal band (Price & Powell). They do comment that no activity was evoked from the diagonal band.

(2) There is no mention of the Dennis & Kerr (1968) efferent system, deep to the lateral olfactory tract. However, they show (their Fig. 2) that the appearance of the evoked tract potential depends on the depth of the recording electrode in the tract. (These records also appear to have multiple stimulus artefacts - possibly trains of stimuli). The most prominent P1 wave is in their Fig. 2A, the deepest site of recording, therefore closest to the surface.

(3) There is no comment on the significance of the P1 wave (see Table 2-1). Its latency suggests that the sequence of its generation/ transmission is olfactory bulb-tubercle-tract-prepyriform cortex. This sequence is not in accord with any described single pathway, and no comment about it is made in the paper. However, if a dual system were present, the sequence could be bulb-tract-cortex, and the other, bulb-tubercle.

(4) The evoked potential itself is of the order of 40 μV, compared with the range 1-4 mV of the present study, despite similar stimulus parameters. Such a small evoked potential would have been ignored in the present study. A possibility exists that artefacts may be present, for example, antidromic stimulation of 5th cranial nerve fibres.

(5) The effects of barbiturates were noted. Low doses (2mg/kg IV) affected the prepyriform responses most severely, increasing the latency and depressing the amplitude of the potential, particularly N2. (There was an occasional transient increase in amplitude.) No explanations were given.

(6) Connexions between the bulb are said to be directly between tufted cells and the contralateral granule cells.

(7) The reasons for the maximal bulbar response being at the surface were not discussed.

Despite these objections, the paper shows that there is a need to re-evaluate the connexions within the olfactory system. Their method could be modified to obtain similar data for the known efferent systems. Their conclusion that there is a mesencephalic origin for an efferent system can be accepted, but there was evidence of this previously (Hernández-Peón, Lavin, Alcocer-Cuarón & Marcelin, 1960; Lavin, Alcocer-Cuarón & Hernández-Peón, 1959). The course of this system has been defined, but they state, "It is possible that other routes must not be overlooked."

They claimed that two types of modulatory neural action of the olfactory system seemed to exist: an intra-olfactory one arising from the contralateral homologous structure (no mention of anterior olfactory nucleus) and an efferent extra-olfactory one that reaches the olfactory bulb through the lateral olfactory tract, receiving fibres from various origins, brain stem reticular formation, mesencephalic reticular formation, hypothalamus and medial forebrain bundle, "and probably other non-olfactory structures such as the amygdalas".

The salient conclusion from this study is the implication that the olfactory tubercle functions as a common pathway of efferent traffic to the bulb. My own work provides no evidence to support or refute this contention, which will be resolved only by future studies.

CONCLUSIONS

Connexions within the olfactory system of the phalanger have been studied with an evoked potential method. Potentials were recorded from the granule cell layer of the olfactory bulbs after systematic stimulation of brain areas. Six types of evoked potential have been described, similar to those recorded in other species.

These studies present the first clear evidence that the marsupial olfactory bulb mitral cell axons project to the ipsilateral olfactory cortex, anterior olfactory nucleus, olfactory tubercle, nucleus of the lateral olfactory tract, and the cortical and medial amygdaloid nuclei. These connexions are similar to those in other species.

Efferent influences on the olfactory bulbs have been shown to arise in or to be transmitted by the anterior olfactory nuclei and anterior commissure, olfactory cortex, lateral olfactory tract, olfactory tubercle, nucleus of the lateral olfactory tract, cortical and medial amygdaloid nuclei, and hippocampus.

The functional connexions have been compared with the anatomical ones described by Adey (1953). Although there is generally good agreement between the findings from different techniques, discrepancies have been revealed in the angular cortex. The present study has clarified the extent and connexions of this area with respect to olfactory function. Evidence is presented that the electrophysiological technique is superior to a degeneration method in producing a precise delineation of true functional connexions.

CHAPTER THREE

SUMMARY

1. Induced waves following standardized olfactory stimulation were recorded from intact and isolated olfactory bulbs of phalangers.

2. These induced waves were similar to those recorded from other marsupials and mammals. They began with a frequency of 50-60 Hz, had a duration of about 1 sec, and finished with a frequency of 20-30 Hz, slowing uniformly throughout their duration.

3. Induced waves were recorded also from the olfactory cortex of these phalangers. Such waves appeared to consist of two components, a high frequency similar to the bulbar frequency, and a sub harmonic of this.

4. Experiments are described which show that the factors controlling fundamental frequency, duration, and amplitude of bulbar induced waves, are intrinsic to the bulb.

5. The following factors did not alter the appearance of the induced waves: 100% oxygen, 5% carbon dioxide, non-depolarizing neuromuscular blocking agents, mid-brain transection and mild hypothermia.

INDUCED ELECTRICAL ACTIVITY IN THE OLFACTORY SYSTEM OF THE PHALANGER

INTRODUCTION

Richard Caton demonstrated in 1875 that electrical activity occurred in mammalian cerebral cortex, and recorded both spontaneous voltage changes and changes related to peripheral sensory stimulation. These observations were the foundation of the science of electroencephalography.

Adrian (1942) recorded large sinusoidal bursts of electrical activity from the olfactory bulbs and pyriform (olfactory) cortex of hedgehogs, and lateral olfactory tract of cats, during stimulation of the olfactory mucosa by puffs of odorous air. He found that the frequency of these waves increased as the force of the air blown or sucked through the nose increased. He also noted that these waves slowed in frequency throughout their duration, often beginning at 30 Hz and ending at 20 Hz (his Figs. 10B and 12). This observation has often been ignored by subsequent workers. Odour added to the air (clove oil or asafoetida) produced small irregular waves with a frequency of 50 Hz. He concluded that the olfactory organ could be stimulated mechanically as well as chemically, and that a familiar smell was recognized by the specific pattern which it aroused in the brain.

He subsequently investigated these findings more thoroughly in the rabbit as well (Adrian, 1950), and described two kinds of electrical activity in the olfactory bulb; 'induced waves' and 'intrinsic waves'. The induced waves were set up during strong olfactory stimuli, while the intrinsic waves represented the on-going, spontaneous activity of the cells of the bulb. These intrinsic waves persisted despite destruction of the olfactory mucosa or transverse section of the olfactory peduncle. There was no comment on the effects of these procedures on the induced waves. The mitral cell dendrites were postulated to be the source of the induced waves, while the intrinsic activity originated in the "cells with short axons", being suppressed by the induced wave. Adrian also examined the effects of barbiturate and urethane anaesthesia on these waves*.

Ottoson (1954) used a different preamplifier from Adrian (direct-coupled instead of resistance-capacitance coupled) and recorded induced olfactory activity in rabbits. He showed that the bulbar induced waves

---

* Discussion of drug effects recorded in this and other papers to be cited will be adjourned until Chapter 4.

were superimposed on slow surface-positive potentials of 0.5-1.0 mV. These "slow waves" were found to depend on the presence of odour in air blown over the olfactory mucosa, and were not produced by purified air alone. In his study each slow wave began at the onset of the stimulus, and reached its peak within 250 msec. The potential declined gradually during the remainder of the stimulus, falling rapidly to its resting level at the cessation of the stimulus. The amplitude of the slow wave seemed to depend on the stimulus intensity. Ottoson concluded that this wave was an olfactory receptor (generator)potential. He also studied the effects of anaesthetics on this slow wave.

Kerr & Hagbarth (1955) showed that the olfactory bulbar induced waves could be influenced by electrical stimulation of certain basal rhinencephalic structures. These observations proved the functional existence of an efferent olfactory system suspected on histological grounds (Cajal, 1911). In this way they confirmed in another somatic system their earlier radical hypothesis (Hagbarth & Kerr, 1954), that central activity could modify afferent somatic information at the first relays. The induced waves were thought to arise from synchronized activity of the mitral and tufted cells. Centrifugal effects were thought to be mediated by the granule cells acting as interneurones. However, recurrent collateral fibres of mitral axons were postulated not to have an interneuron, predicting the discovery of the gemmule.

Kerr subsequently (1960) showed that strong olfactory stimulation of one bulb depressed afferent induced activity in the contralateral bulb through the anterior limb of the anterior commissure.

Freeman (1959) recorded induced waves from various parts of the olfactory cortex of cats. They had predominant frequencies of 34-36 Hz, but variations were noted with side (right or left) and position of recording electrodes in the cortex. Sleep was shown to abolish the induced waves (his Fig. 4). Pentobarbitone was said to cause changes comparable with those of sleep, with spindles.

Freeman (1960*a*) again recorded induced waves from the prepyriform cortex of cats. He described sinusoidal wave of 38 Hz with a variable amplitude to 2 mV as the major component of the induced wave.

In a subsequent paper (Freeman, 1960*b*), he attempted to correlate the olfactory cortical electrical activity with hedonic behaviour in the cat. He described three types of waves in the prepyriform cortex; the slow waves of sleep, the "truncated alternating wave associated with respiration" and the "sinusoidal wave associated with behavioral activity". His Fig. 1

shows the alterations in the induced wave recorded from various parts of the olfactory cortex. Line A is the bulbar record, and shows the expected induced waves at the high frequency (38 Hz) with no subharmonics. As this induced wave was recorded further caudally (B-E), it began to show an increasing contribution of a low-frequency component. Line E, recorded from 17 mm behind the electrode in the bulb (line A), has induced waves of the low frequency, with little visible high-frequency contribution.

He was able to relate only two of the four parameters of the prepyriform activity to behaviour. The frequency of respiratory wave occurrence indicated changes in respiratory rate and depth. The amplitude of the induced waves was said to be related to resting, anticipatory and active behaviour. The wave form and frequencies within the bursts could not be correlated with behaviour. It must be remembered that respiratory movements were measured with a pneumograph, and no measurements were made of nasal air flow. This makes interpretation of records difficult. For example, in his Fig. 6, two episodes of sniffing are recorded. The bulbar records and pneumograph records are markedly different for the same observed behaviour. This paper served to show some of the difficulties in recording and interpreting induced waves in the intact, alert and mobile animal.

Attempts to correlate activity unit activity in the bulb with the induced waves were made independently by von Baumgarten, Green & Mancia (1962*a*), Moulton (1963), and Freeman (1963). The first group, using rabbits, concluded that there were at least two generators involved in the production of the induced waves, one above and one below the mitral cell layer. They also showed that action potentials of mitral, tufted and granule (sic) cells were correlated with the negative-going phase of the induced waves recorded from the same micro-electrode. The induced wave was shown to invert in phase below the mitral cell layer.

Moulton (1963), also using rabbits, showed marked disconformities between the behaviour of the spike and wave activity of the bulb, unless the bulb was severed from its central connexions. In this case, with oil of carrot seed or coyote urine as the stimulus, spike discharges stood out clearly against the low amplitude background activity and summated strongly.

Freeman (1963) used a mathematical approach to define the characteristics of the spontaneous and evoked olfactory potentials recorded in the prepyriform cortex of cats. He concluded that spontaneous (induced) waves were regulated in their frequency and rate of decay by the intrinsic

characteristics of cortical cells, and in phase and amplitude by the number, velocity and degree of synchrony of unit activity in the tract. However, he did not consider the spontaneous (induced) waves of the bulb itself.

Boudreau (1963, 1964) also recorded electrical activity in the pre-pyriform cortex and olfactory bulbs of cats. He used the mathematical methods of analysis of Freeman (1963), also reported jointly by them (Boudreau & Freeman, 1963). One point to be remembered is their use of 10-second epochs for analysis. Records of bulbar induced waves were nearly identical with the simultaneous cortical ones (Boudreau, 1963, his Fig. 21). Another conclusion was that the signal from much of the pre-pyriform cortex led the bulbar signal (Boudreau, 1963, his p.123). Induced waves recorded both in bulb and cortex were shown to have a constant frequency of 40 Hz.

Mechelse & Lieuwens (1969) showed that bursting activity in cats, produced by blowing air into the nostrils, seemed similar to the spontaneous burst with regard to frequency, amplitude variations, and slow waves. They suggested that the frequency of the bursts depended on the localization of the electrodes in the bulb and not on the air-flow characteristics. They comment that the cominant frequencies in various levels of the bulb varied from 20-60 Hz. However, it is not easy to see this in their Figs. 3, 5 and 6.

Schwartze (1970*a*), described the changes burst amplitude with nasal airflow in tracheotomized rabbits. He showed a linear relationship between the two (his Fig. 2b). His Fig. 2a shows that the frequency within the bursts does not change with intensity of stimulation. Each burst begins at approximately 60 Hz, and slows to about 45 Hz by the end. He also showed that acoustic and tactile stimuli caused an increase in burst amplitude. Bending the toes of the hindfoot, a painful stimulus, caused a decrease in amplitude of the bursts.

His second paper (1970*b*) showed the effects of hypoxia (an initial increase in frequency) in rabbits anaesthetized with urethane.

All these workers were satisfied that odour blown over the olfactory mucosa was the stimulus for the induced waves. However, Hernández-Peón, Lavin, Alcocer-Cuarón & Marcelin (1960) postulated that the induced waves of the olfactory bulb of awake cats were caused by alertness or arousal. Any type of sensory stimulation which aroused the cat was said to evoke bursts of rhythmic activity in the bulb. The magnitude of this activity was said to be related to the degree of alertness. This theory is no

longer tenable, shown by more recent work (e.g. Domino & Ueki, 1960; Ueki & Domino, 1961; Gault & Leaton, 1963; Gault & Coustan, 1965; Pagano, 1966; Mechelse & Lieuwens, 1969, and the present studies). The elegant undercut cortex experiments of Becker & Freeman (1968) were particularly relevant in this respect. In these last-mentioned experiments, induced waves were recorded from a slab of olfactory cortex connected with the bulb via the lateral olfactory tract, but severed from central connexions.

Motokizawa & Furuya (1973) investigated the role of olfactory stimulation in EEG arousal. They used paralyzed de-afferented cats (midpontine pretrigeminal section or transection of spinal cord at C2 plus trigeminal nerve section). Olfactory stimulation was by odours (acetic acid, amyl acetate, clove oil and xylene) or electrical stimulation of the olfactory bulb directly (100/sec, 1 msec, 1-15 V). Their Fig. 1 shows an arousal reaction to odour. An induced wave is seen in bulb and prepyriform cortex records, and it is followed by a general desynchronization of all leads. Their Fig. 2 shows that the effect of electrical stimulation of the olfactory bulb was similar. However, this record shows what appear to be induced waves in the prepyriform cortex, one being suppressed by the olfactory bulbar stimulation. It is difficult to explain these in the paralyzed (and therefore artificially ventilated) animal. Such olfactory cortical bursts were never seen in the absence of effective olfactory stimuli in the present studies on the phalanger. They may correspond to the bursts of Penaloza-Rojas & Alcocer-Cuarón (1967) and Lavin et al.(1959). Fig. 3 of Motokizawa & Furuya's 1973 paper also shows prepyriform bursts every three seconds after Nembutal. These do not have the appearance of barbiturate spindles, being too high in frequency.

Their work showing the effects of section of fornix, stria terminalis and medial forebrain bundle does suggest that the medial forebrain bundle is the pathway for cortical and hippocampal arousal following olfactory stimuli.

In the introduction to their paper, they make the provocative statement that at present it cannot be concluded that the prepyriform cortex is the primary olfactory cortex. There is no reference to Pigache (1970) and this point has been discussed in Chapter 1. In their proposed scheme of the olfactory system (their Fig. 10), they show prepyriform cortex as a secondary olfactory area. They do not show the olfactory bulb to amygdala projection, and wisely do not include efferent systems. Although they propose a primary olfactory area on the cerebral cortex, they do not

indicate how it would fit into their scheme. They may imply that the broad stippled arrow of their Fig. 10 projects to it. This suggestion of another cortical olfactory area has never been supported by anatomical or electrical evidence including that presented in this thesis. However, their conclusion that the olfactory arousal response is produced by projection of olfactory impulses to the mesencephalic reticular formation through the medial forebrain bundle is probably valid. It would have been more convincing if there were no prepyriform burst in the stated absence of olfactory stimuli.

The basic purpose of research, in any field of sensory physiology, is to elucidate the neural mechanisms of information generation, transmission, storage and retrieval. There are certain landmarks mentioned above in the progress of knowledge in olfactory physiology. However, the basic questions about odour identification, neural coding and behavioural responses are still largely unanswered. The decreasing importance of olfaction with phylogenetic development is recognized - sex and alarm pheromones are essential for certain insect communication and survival. Anosmic man is able to survive well but with some impairment of aesthetic sensibilities (Wood Jones, 1924*a*; Schneider, 1971). Excellent work has been carried out in the field of olfaction and communication (for example, Eisenberg & Kleiman, 1972); much has been reported in symposia*. This aspect of olfaction is not considered in this thesis.

There has been some interest in the role of the trigeminal nerve in the process of olfaction (e.g. Tucker, 1963*a*,*b*; Stone, Carregal & Williams, 1966; Stone, Williams & Carregal, 1968; Tucker, 1971). This nerve is the major source of somatic sensation from the face, mouth and nasopharynx. Its function in various cardio-respiratory reflexes is clearly defined (Elsner, Franklin, van Citters & Kenney, 1966), and it is not surprising that it has been investigated in this other context. Olfaction normally relies on the conscious, complex respiratory act of sniffing to deliver the odour in question to the olfactory receptors. The trigeminal nerve has been shown to play a part in the central control of olfaction, and is important in aversive responses. This aspect of olfaction is not considered in this thesis either.

---

* For example, the Wenner-Gren International Symposium Series on Olfaction and Taste; 1, Ed. Y. Zotterman, 1963; 11, Ed. T.Hayashi, 1967; 111, Ed. C. Pfaffman, 1969; Gustation and Olfaction; An International Symposium, Eds. G. Ohloff & A.F. Thomas, London, Academic Press, 1971.

It is interesting to speculate on the role of olfaction in the birth of a marsupial. The blind, hairless neonate has rudimentary but functional forelimbs, but only hindlimb buds. It is able to make its way, without assistance, from the birth canal to the pouch, and find a nipple on which it remains until capable of independent existence. At birth, the olfactory system may have reached a sufficiently advanced stage of development to allow it to function (Morest, 1970; Ulinski, 1971). However, it would be reasonable to postulate that the trigeminal nerve also is involved in this process.

Functional studies by Schwartze (1973) have suggested that the onset of olfactory activity measured by induced waves, depends on the state of development at birth. Induced waves were recorded from guinea pigs within an hour of birth, but were not recorded in kittens for several days. A similar question could be asked in the latter case - how do kittens find the nipple?

Induced activity related to olfactory stimulation has been recorded from the olfactory bulbs of many vertebrate species. There are similar general features in all cases. A slow wave, positive at the surface and negative in the granule cell layer, has the higher-frequency induced wave superimposed. Much attention has been paid to the high frequencies contained in the induced wave, and attempts to relate this frequency to the odour have been made (e.g. Hughes & Hendrix, 1967). In other papers different odours have been used to produce the induced wave, but no correlation has been attempted. Table 3-1 shows the dominant frequencies of the induced waves of various vertebrates. Some of these results have been derived by mathematicl analysis and are so indicated.

The only studies of marsupial olfactory induced waves have been those of Vaccarezza & Affanni (1964; 1966) and Affanni, Morita & Garcia Samartino (1968)* *Didelphis azarae* was the animal used in these studies, with macro-electrodes chronically implanted in the olfactory bulbs.

The first of these studies distinguished three kinds of waves in the electrical activity of the bulb: (1) spindles of large sinusoidal waves with frequencies of 45-50 Hz and 100-120 μV amplitude; (2) rapid, irregular waves of frequency 35-40 Hz and smaller amplitude (30-60 μV) than the former; and (3) slow waves related to respiration (ventilation).

---

* Invaluable assistance in the translation of these papers was given by Miss Sue Cadby.

TABLE 3-1

SPECIES	FREQUENCY OF INDUCED WAVES (Hz)	REFERENCE
Hedghog	15-52	Adrian, 1942
Rabbit	55-60	Adrian, 1950
	40	von Baumgarten *et al.*, 1962
	40-90*	Hughes & Mazurowski, 1964
	38-42	Fukuda, 1966
	45-125*	Hughes & Hendrix, 1967
	55-65	Khazan *et al.*, 1967
	50-60	Schwartze, 1970
Cat	34-38	Freeman, 1959
	34-48	Hernandez-Peon *et al.*, 1960
	40*	Boudreau & Freeman, 1963
	40	Gault & Leaton, 1963
	40	Peñaloza-Rojas *et al.*, 1967
	40	Becker & Freeman, 1968
Rhesus Monkey	20-50*	Hughes & Mazurowski, 1962*b*
Caiman	12-25	Huggins *et al.*, 1968
Pigeon	15-25	Sieck & Wenzel, 1969
OPOSSUM	35-40	Vaccarezza & Affanni, 1966
	40-60	Vaccarezza & Affanni, 1968
	35-40	Van Twyver & Allison, 1970
Echidna	18-23	Van Twyver & Allison, 1970

Table 3-1. Frequencies of induced waves recorded from the olfactory bulbs of various animals (an incomplete list). There is considerable variation between and within the species.
* mathematically derived frequencies.

These waves varied with the state of alertness of the animal, the large sinusoidal waves disappearing with the onset of sleep. Application of an olfactory stimulus, such as tobacco smoke (which also stimulated the trigeminal nerve), increased frequency and size of the sinusoidal waves. The sinusoidal activity and the slow waves remained after section of the olfactory peduncle. Normal sniffing was seen to occur at rates of 3-4/sec.

The second paper of Vaccarezza & Affanni (1966) is basically the same as the first, using the identical figures for illustration, and drawing the same conclusions.

Affanni, Morita & Garcia Samartino (1968) described the chronic effects of section of the olfactory peduncle and anterior commissure on the activity of the olfactory bulb of the same species. Base line records of bulbar and olfactory cortical activity were made during vigilance, slow-wave and rapid-eye-movement sleep. Olfactory stimulation was by tobacco smoke and rotten fish. Transection of the peduncle caused an increase in the amplitude of the induced waves, but no change in the previously-described frequency. This change persisted until the death of the animal. Transection of the anterior commissure in the mid-line produced a smaller increase in the amplitude of the induced burst. Subsequent transection of the olfactory peduncle increased the size of the burst to that expected after peduncle transection alone. They concluded that in *Didelphis azarae* there were at least two paths for tonic centrifugal influence on the olfactory bulbs: one coming through the anterior commissure, and the other from some other place. The moderating influence did not appear to originate in the contralateral olfactory bulb, but was thought to be transmitted along the "non-bulbar component of the anterior commissure".

Biedler & Tucker (1955) had previously recorded from olfactory nerves of the opossum.

There have been no previous studies of induced olfactory activity in the phalanger. Experiments described in this chapter were carried out to define the electrical activity of the olfactory system under standard conditions with semi-micro-electrodes.

It seemed desirable to confirm that so basic a biological function as olfaction is consistently represented in this phylogenetically primitive species. It was also considered desirable to confirm or refute the hypothesis that induced olfactory activity is dependent on arousal, not olfactory stimulation *per se*. Initial experiments to be described were intended to define the electrical activity under standard conditions.

Subsequently the following interventions were investigated: anaesthesia for preparation, hypoxia, hyperoxia, hypercapina, neuromuscular blockade, hypothermia, olfactory peduncular transection, and mid-brain (inter-collicular) transection.

METHODS

Adult phalangers of both sexes were used in this study, again assuming that their survival in the natural state indicated normal olfactory function.

Anaesthesia and Surgery

General anaesthesia was induced in the previously-described manner, except that halothane (Fluothane, I.C.I.) was used instead of chloroform with the nitrous oxide (75-80%) and oxygen. Cannulation of the trachea enabled the gaseous anaesthesia to be maintained until all surgery and positioning of electrodes was complete. A rubber catheter was passed through the upper cut end of the trachea into the nasopharynx, to allow free egress of air blown into the nostrils. A convenient vein was cannulated (usually the cephalic). Benzocaine (5%) ointment was applied to these wounds, which were sutured over the respective cannulae, and to the scalp incision.

Pressure points were infiltrated with 2% lignocaine (external auditory meati, infraorbital margins, tympanic membranes and chordae tympani) before placing the animal in the stereotaxic apparatus, and at two-hourly intervals thereafter. Each cornea was anaesthetized with 2% amethocaine eye-drops at the same intervals. The head of the animal was held in the Horsley-Clarke plane with standard cat earbars and modified eyebars as before. The tracheal cannula was fixed to a convenient part of the apparatus to prevent movement and consequent sensory stimulation. General anaesthesia was maintained, and the cranial vault was exposed from nasion to occiput by a longitudinal midline incision. Skin and masseter muscles were reflected. Burr holes were made 10 mm behind the nasion over each olfactory bulb and enlarged with fine rongeurs if necessary. Appropriate parts of the cranial vault were removed in the same way. When a mid-brain lesion was to be made, part of the occiput between cerebrum and cerebellum was removed, revealing the intercollicular region. Dura was left intact, except where penetrated by electrodes. Estimated blood loss was replaced with an electrolyte solution (Na^+, 140; K^+, 5; Ca^{++}, 5; Cl^-, 150 mEq/l). Exposed neural structures (including dura) were covered with cotton wool pledgets soaked in the same solution.

General anaesthesia was discontinued after completion of the surgery, satisfactory placement of the electrodes, and coating all surgical wounds with benzocaine ointment. Gallamine triethiodide (Flaxedil, May & Baker) was given in sufficient doses to allow artificial ventilation with a Palmer respiratory pump. Colonic temperature was maintained at 35 ± 1°C by a thermistor-controlled electric heating pad under the animal.

Recording procedure

A block diagram of the experimental arrangement appears in Fig. 3-1.

With the anaesthetized animal held securely in the stereotaxic apparatus, bipolar parallel electrodes were inserted symmetrically into the bulbs. The deep (active) electrode of the pair was placed in the granule cell layer, initially 2.5 mm below the surface. The inactive electrode was 2 mm superficial to this.

The bipolar cortical electrodes, used both for stimulating and recording, had the inner (active) component projecting 1 mm beyond the outer shaft. Electrode positioning was based on the evoked potential studies described above. The electrodes were placed with the inner component lying in the superficial plexiform layer of olfactory cortex. The optimal positions of the electrodes were achieved by using supra-threshold stimulation through the cortical electrodes to evoke responses in the bulb. Electrode positions were adjusted to yield the maximal positive bulbar evoked potential. In most experiments, electrodes were placed in the cortex of each side; in some cases, on one side only. The co-ordinates of these were AP 15mm, Lat 7 mm, unless stated otherwise. After optimal placement, the electrodes were connected for recording. All electrodes had a DC impedance of 30-40 KΩ.

Electrode positions were confirmed by post mortem histological examination.

Potentials recorded from the two bulbar and the two cortical electrodes were preamplified with two Grass P5A and two Tektronix 122 amplifiers. The band-pass was usually set at 7-100 Hz on the former type, and 8-250 Hz on the latter.

The gain of the Grass amplifiers was adjusted from 1000-10,000 according to the signal. However, the gain of the Tektronix amplifiers was fixed at 1000. The output signals of these four amplifiers were monitored on two 2-channel CRO displays.

These outputs were used as the inputs to a 4-channel F-M tape recorder (PI-6200), tape speed 3.75 in/sec (9.5 cm/sec). The outputs of the tape recorder were used as the inputs to a Grass 5D 4-channel polygraph

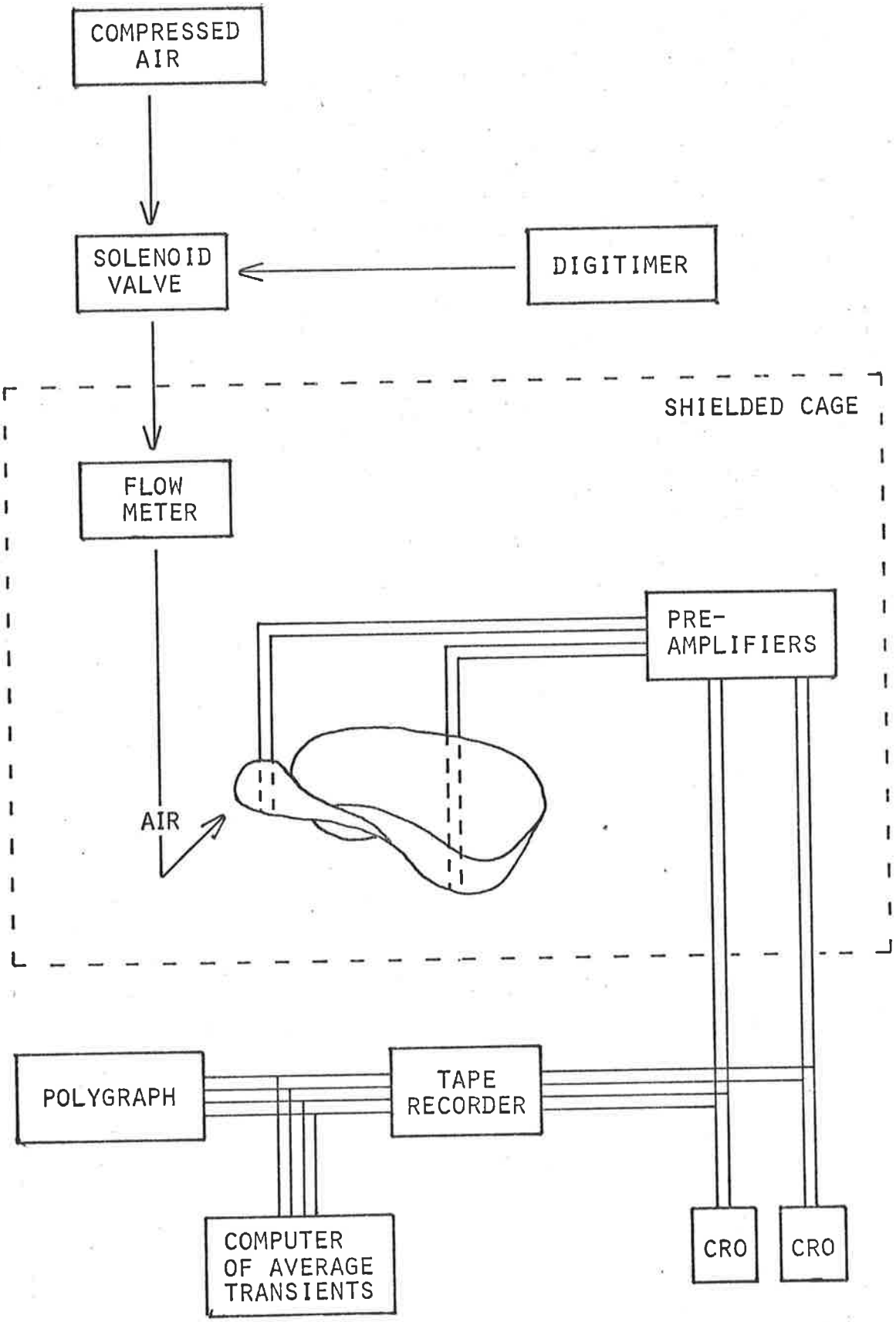


Fig. 3-1 Block diagram showing the experimental arrangement for recording induced waves. The output from the flowmeter is taken to the polygraph or tape recorder.

for producing a permanent record. They also served to monitor DC recording levels and any noise, and the function of the tape recorder itself, enabling appropriate adjustments to be made if necessary.

Subsequent retrieval of information stored in this way was achieved by reference to marker pulses on the tape, the accurate footage counter of the recorder, and contemporaneous notations on both polygraph record and protocol sheets. No data were lost during this process.

Most of the polygraph records illustrating this thesis have been obtained from tapes replayed at 0.375 in/sec (1/10 real time). The pens of the polygraph have a half-amplitude high frequency response of 60 Hz, close to the frequencies of interest, but replaying the tapes at 1/10 real time eliminated any mechanical distortion of the records caused by the inertia of the polygraph pens limiting the frequency response.

In most cases, estimates of the frequencies within bursts were made by direct measurement. With tape replaying at 1/10 recording speed, the polygraph record was made at several paper speeds. The optimal speed for illustrations was 10 mm/sec, but for frequency estimates, 25 or 50 mm/sec. Zero-crossing intervals were measured with a dial micrometer for each cycle of the burst. Assuming each cycle to approximate a sine-wave, an estimate of its frequency was thus made.

Some of the records have been recorded in real time, and this will be indicated by the time base.

Olfactory stimulation

In an attempt to simplify the experiments, and because the problems of odour coding were not to be considered, the olfactory mucosa was stimulated with puffs of air without deliberately-added odorant. The whole system would contain some inherent odorant qualities since no attempt to further purify the airstream was made, and even if this had been done, the red rubber catheter would have added odour to the air. Compressed, dry "medical air" (CIG) was led through pressure-reducing valves to an electro-pneumatic solenoid controlling the duration of air-flow. New materials were used in the construction of this apparatus (brass and nylon tubing, stainless steel valves).

A flowmeter was incorporated in the system just proximal to the soft rubber catheter inserted into the nostril of the animal. This flowmeter, also of local construction, employed the principle of generation of a pressure differential across a resistance in the air path. A small brass chamber, 7.5 cm long and 2.5 cm diameter, was fitted with two layers of fine stainless steel mesh (400 mesh). Pressure generated by air flow was

measured on either side of the mesh with arterial blood pressure transducers. The output of these was taken to a transducer mixer (Grass TM-1), and the resulting differential voltage used as the signal for a polygraph and the tape recorder.

The flowmeter was calibrated at a constant flow with a Rotameter (British Oxygen Company) flowmeter. Pulsatile flow calibration was achieved by collecting the air expelled with each cycle of a respiratory pump, measuring its volume directly by water displacement, and measuring the peak of the pressure wave and area under its curve. The usual setting of the flow controllers produced a peak flow of 3 l/min for a duration of 300 msec, repeated every 5-10 seconds.

If the olfactory mucosa of one side only was to be stimulated, the rubber catheter was inserted 1 cm into that nostril. The other nostril was occluded with a pledget of cotton wool impregnated with petroleum jelly. If stimuli were to be applied to both sides, the airstream was divided beyond the measuring chamber with a Y-piece, and two catheters were used, one for each nostril. The flowmeter had to be recalibrated whenever a change was made distal to it.

Olfactory bulb isolation

Isolation of the olfactory bulb was accomplished by sectioning the previously-exposed peduncle with a fine dural elevator passed vertically and transversely across it. The section was made in the circular fissure of the bulb in front of the rostral extremity of the frontal pole of the cerebral hemisphere. The plane of the section is shown in Fig. 3-2A & B. The minor bleeding usually stopped spontaneously, but absorbable gelatin sponge (Allen & Hanburys) was sometimes applied to the upper surface to expedite haemostasis.

The bulb on the side of the sectioned peduncle will be referred to as the "isolated bulb" and the cortex on that side as "de-afferented cortex". This latter term, in this context, implies nothing more than the section of its connexions with the ipsilateral bulb (after Becker & Freeman, 1968).

Midbrain section

Peripheral deafferentiation was achieved in some of the experiments by destruction of the intercollicular region of the brainstem. Two methods were used: (1) high frequency electrocoagulation between points of an array of electrodes under stereotaxic control, and (2) section of the midbrain in the same region with a flat stainless steel blade 8 mm wide, also with stereotaxic control. The latter method was usually

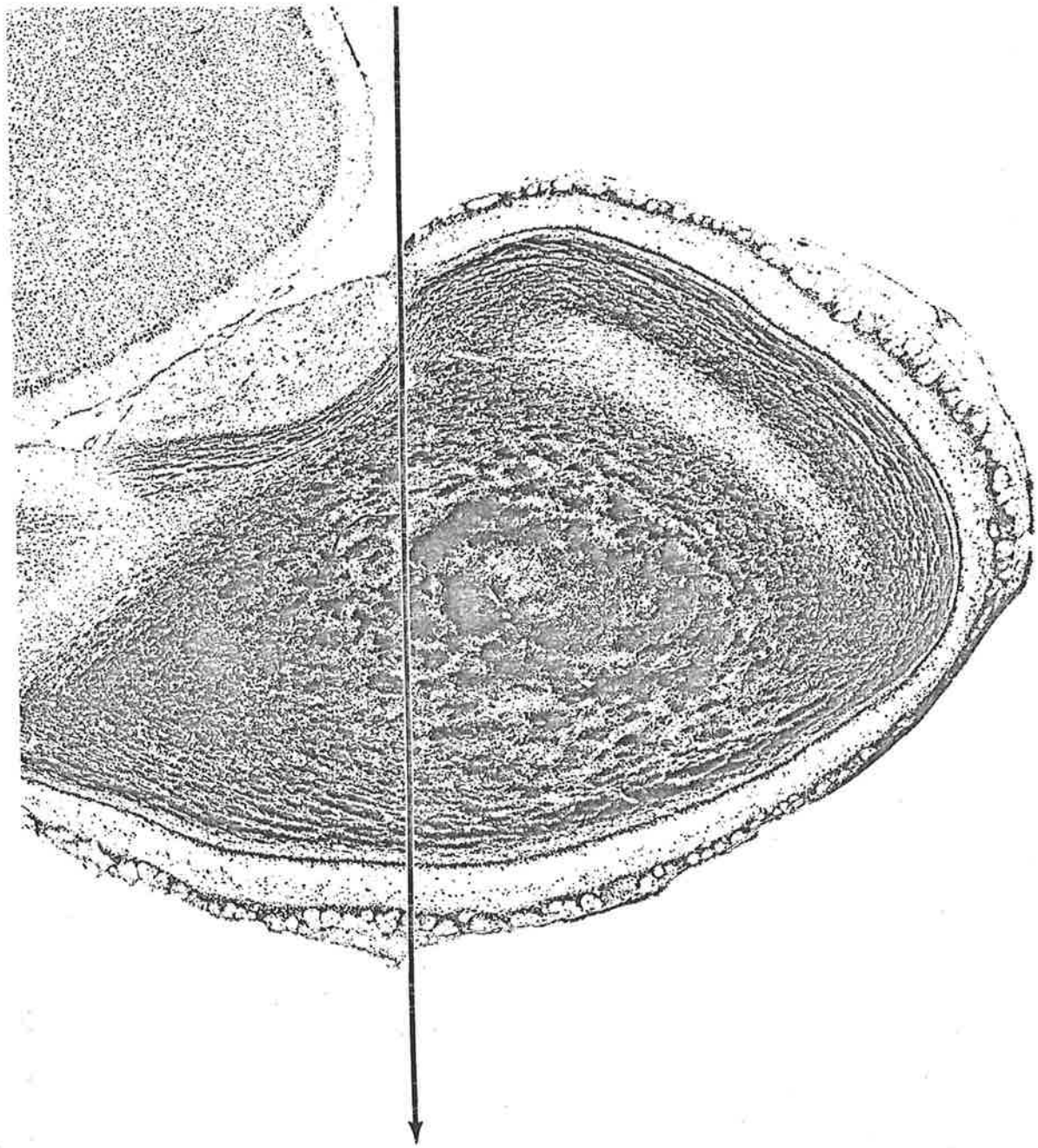


Fig. 3-2A Sagittal section of olfactory bulb and frontal pole of phalanger, stained with neutral red to show cellular detail, 2mm from the mid line. The plane of peduncular transection is shown by the the arrow. Note that it passes rostral to the accessory bulb. (It is from the same section as Fig. 1-3)

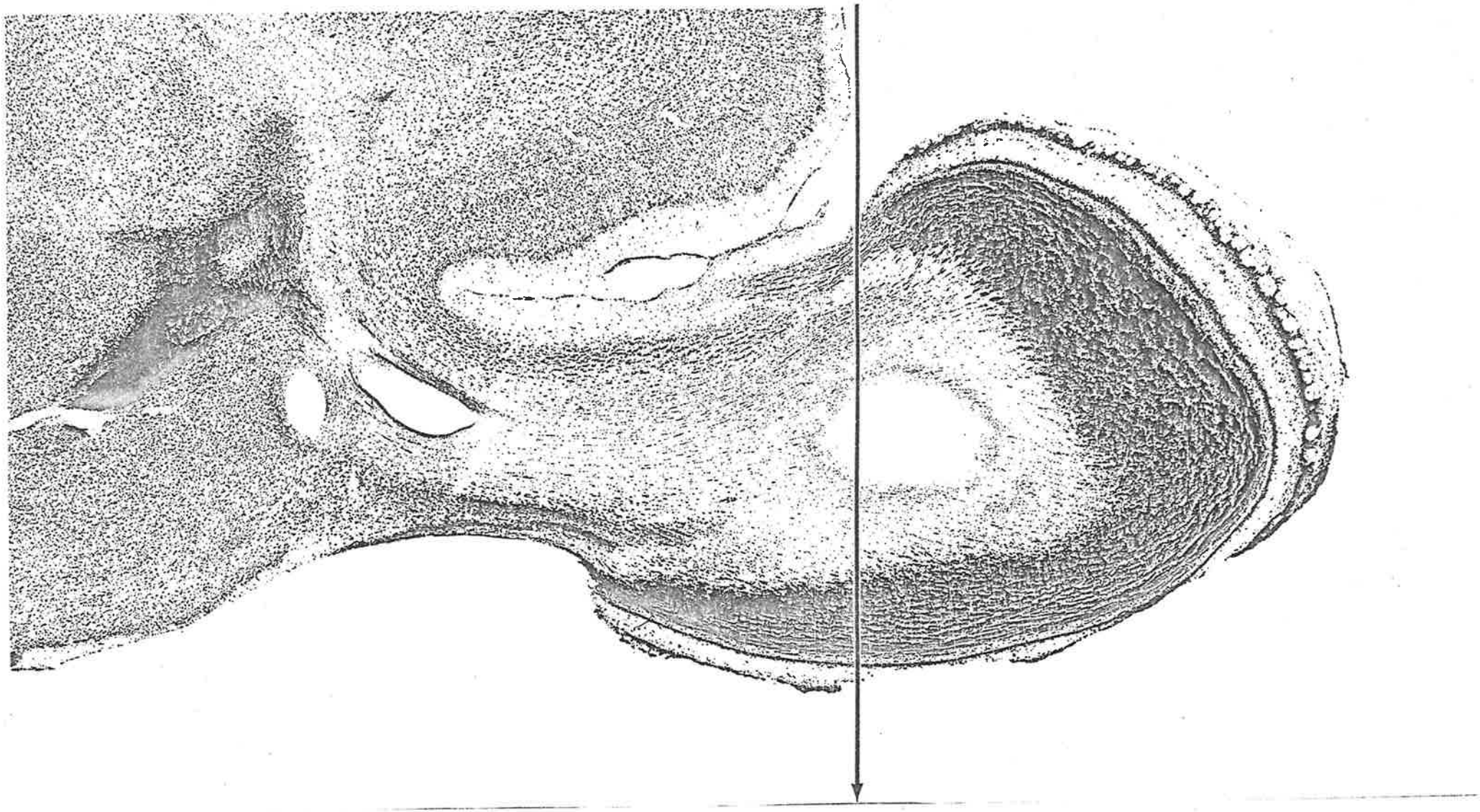


Fig. 3-2B Sagittal section of olfactory bulb and frontal pole of the phalanger, 3mm from the mid line, stained with neutral red for cellular detail. The plane of peduncular transection is shown by the arrow. Note that it passes rostral to the anterior olfactory nucleus.

adopted because it was quicker and produced less visible cerebral oedema, if any at all.

Experimental procedures

The aim of this series of experiments was to keep constant as many as possible of the parameters. A standardized anaesthetic and surgical technique was used, ventilation was adjusted according to the body weight of the animal, and the air blown over the olfactory mucosa had no added odorant, other than that inherent in the components of the stimulating set-up. Recordings of electrical activity were made with the animal still anaesthetized, during recovery from the initial anaesthetic, and during alterations in this state. Filters on preamplifiers and power amplifiers were altered to show different characteristics of the electrical activity.

1. *Control of experiments*

In each experiment, stability of the electrical records was allowed to occur before the next manipulation (for example, section of olfactory peduncle, administration of drug or further local anaesthetic). In this way, each animal acted as its own control.

On one occasion, preparation for recording was carried out as usual, but in addition a heparinized femoral arterial cannula was inserted with the usual local anaesthetic application to the region. This enabled the direct recording of arterial blood pressure and sampling of the arterial blood for analysis of oxygen and carbon dioxide partial pressures. On this occasion, and two others, samples of mixed expired air were collected for analysis with the Scholander (1947) 0.5 ml apparatus.

2. *Alerting response*

After a large increment of gallamine, the animal was not disturbed for at least 20 minutes. The shielded cage was darkened, the olfactory stimuli were not given, the animal was not touched, and noisy equipment (polygraph and air-conditioner) was turned off. At the end of this quiescent period, during which the animals often exhibited the EEG change of slow wave sleep, the tape recorder was started. A few minutes later the repetitive olfactory stimuli were begun again.

3. *100% Oxygen*

Oxygen from the anaesthetic machine was introduced at a high flow-rate into a reservoir at the inlet of the respiratory pump. The dead-space of the pump and connecting tubing of the inspiratory limb of the circuit was shown to be cleared by the new gas in 30 seconds for the average animal, so that the time of onset of the new conditions could be noted.

4. *Carbon dioxide*

A commercial mixture of 5% CO_2/95% O_2 (carbogen, C.I.G.) was introduced in the same manner as the oxygen.

5. *Hypothermia*

Records were made at intervals during cooling of the animal occasioned by disconnecting the heating pad, and during subsequent rewarming. Colonic temperature was monitored with a mercury-in-glass thermometer.

6. *Muscle relaxants*

The effects of gallamine or tubocurarine were investigated by giving a bolus injection equivalent to the initial dose when indicated clinically. Records were made of at least five minutes of electrical activity before and after the injection.

7. *Identification of data*

Data to be presented will have an identifying number of the segment of record. The initial digits indicate the experiment number, and the second digits the location of the data on its tape. For example, 38/0374 means that the segment of record was from experiment 38, found at 374 feet from the beginning of the tape used for that experiment.

Unless otherwise specified, records were made with the preamplifier band pass at 7-100 Hz (for the Grass) or 8-250 Hz (Tektronix), and calibration for amplitude and time will be shown.

Abbreviations for olfactory bulb and olfactory cortex will be OB and Cx, respectively. Left and right sides are indicated by L and R. Records from an isolated bulb and corresponding cortex will be indicated; all other records may be assumed to be from intact sites.

In the interests of temporal and financial economy, polygraph records in this thesis have been reduced in size and reproduced by xerography. There is some loss of definition within large areas of black (at slow polygraph paper speeds), but edges are still reproduced accurately, and show the relevant features (Ratliff, 1972).

RESULTS

One of the problems associated with these experiments was the quantity of data produced. Useful information could be recorded for extended periods. The longest experiment was some 19 hours' duration after recording began. At the end of this time, induced waves were still being produced, and these were similar in gross appearance to those recorded at the beginning of the experiment.

A typical experiment generated about 750 m of magnetic tape (recorded at 3.75 in/sec - 9.5 cm/sec) and 100 m of polygraph paper, both recorded intermittently. Retrieval and rewriting at 1/10 recording speed was tedious, and analogue-to-digital conversion of the data time-consuming. These points will be discussed in the appendix.

Unless stated otherwise, records in this chapter are taken from animals which had not had any drugs but the volatile and local anaesthetics, and which were paralyzed and being ventilated with air. At least 1½ hours had elapsed between cessation of general anaesthesia and recording.

1. Control Experiments.

Measurement of arterial blood pressure showed that it was of the order of 120 mm Hg systolic, 80 mm Hg diastolic. It remained at this level after gallamine triethiodide was given. However, it fell to levels of 60 mm Hg systolic, 30 mm Hg diastolic after a bolus injection of tubocurarine. This hypotension occurred within one minute of the injection, and slowly recovered during the next half hour. Despite this hypotension, there was no apparent change in the spontaneous or induced electrical activity of the bulb or cortex. The pulse rate was approximately 100/min in the anaesthetized animal. Gallamine caused a transient rise in pulse rate to 120/min.

Blood gases were estimated with a Radiometer Blood Gas Analyzer (PHM-71). It was not possible to obtain a sample of arterial blood from an unrestrained, unanaesthetized animal as a control.

Results from the experiment are presented in Table 3-2.

The samples of mixed expired air collected during artificial ventilation in the three experiments were shown to contain 16.9±0.5% oxygen and 3.35±0.25% carbon dioxide at one and four hours.

2. Induced Waves

Electrical activity in the olfactory bulb induced by stimulation of the olfactory mucosa is illustrated in Fig. 3-3. This record has been retrieved from tape replayed at 1/10 the recording speed. It shows two consecutive bulbar induced waves.

Channel 1 of each shows the air-flow record, and illustrates a puff of air, 300 msec duration, with a peak flowrate of 3 1/min. The same stimulus was used in all experiments and will not be shown in subsequent records.

Channels 2, 3 and 4 are records of the one olfactory bulbar signal which has been subjected to different electronic manipulations.

TABLE 3-2

	Time	Pa_{O_2}	Pa_{CO_2}
	hr.	mm Hg	mm Hg
Halothane anaesthesia Spontaneous ventilation	0.5	83	54
No anaesthesia Artificial ventilation	1.0	95	41
	4.0	101	41

Results of estimations of the partial pressures of oxygen and carbon dioxide of arterial blood in different experimental conditions.

Halothane anaesthesia caused the expected hypoventilation (lowered arterial oxygen and raised carbon dioxide tensions).

Intermittent positive pressure ventilation restored these parameters to normal mammalian values.

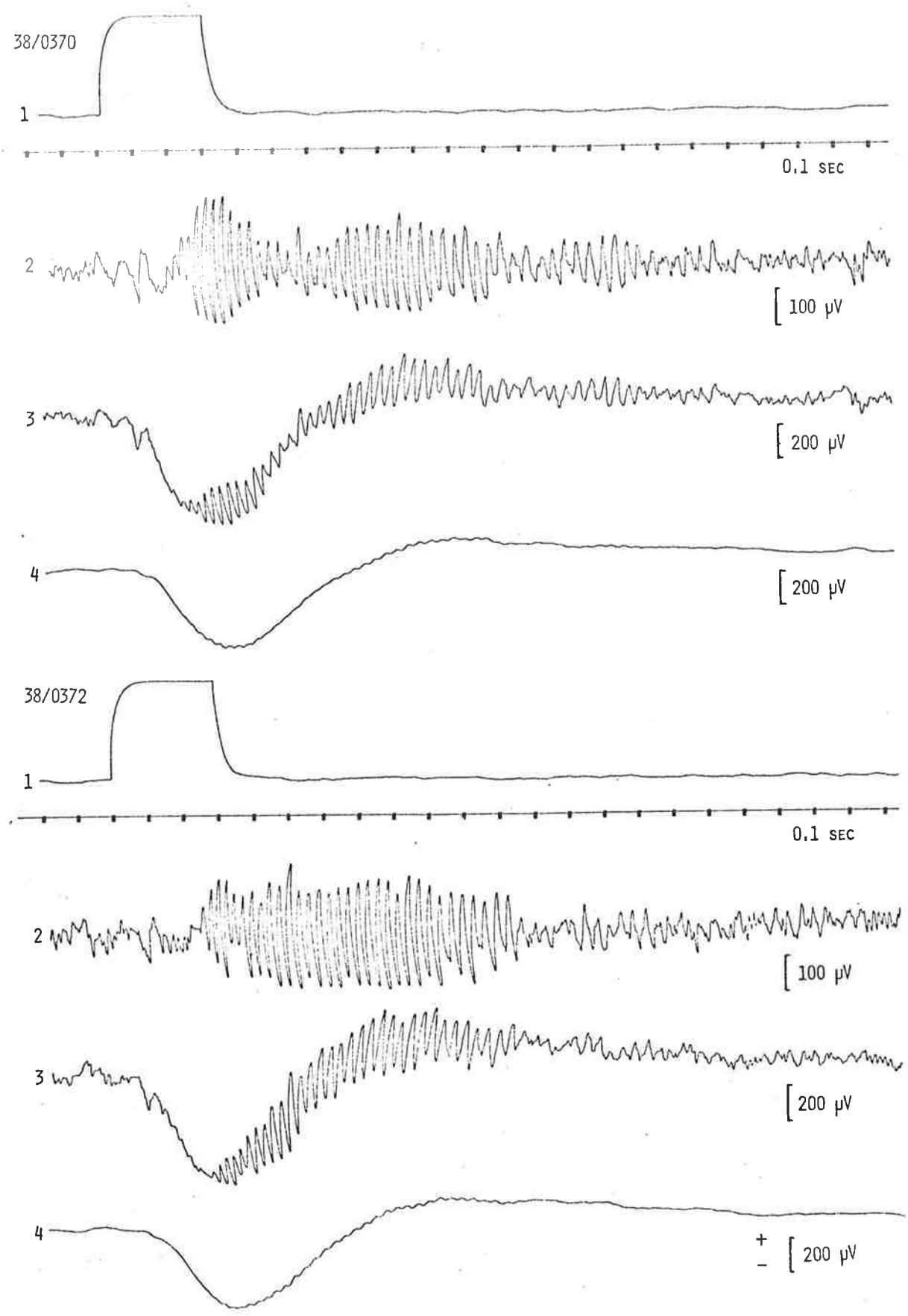


Fig. 3-3 Polygraph records of consecutive induced waves recorded from the olfactory bulb of an unanaesthetized, immobilized phalanger.
1. Airflow measured by differential pressure technique.
2. Preamplifier band pass 7-100 Hz.
3. Preamplifier band pass 0.1 Hz - 1 KHz.
4. As 3, but half amplitude high frequency pen response 0.1 Hz.

Channel 2 is the record of the electrical activity of the bulb recorded from the granule cell layer under standard conditions (pre-amplifier band-pass 7-100 Hz, polygraph pens set to maximum frequency response). The spontaneous activity (beginning of the segment) is low-voltage complex activity containing a high-frequency component of 75-90 Hz by crude measurement of peaks. Shortly after (50-100 msec) the onset of the airflow to the nasal mucosa there is a sharp negative deflexion followed by apparently sinusoidal activity of 45-50 Hz frequency. There is increasing voltage to a maximum at about 500 msec, and then a decrease to the resting levels after 0.8-1.3 sec. During this time, the frequency of the sinusoidal wave decreases to about 30 Hz. This induced wave terminates as its final low-voltage activity apparently merges with the spontaneous activity. There is no clear end-point of the burst.

Channel 3 is a record of the same signal at half the previous gain, and with the preamplifier filters set to pass frequencies between 0.1 Hz and 1 KHz. This method is able to demonstrate slow potential changes as well as high frequency activity. The induced wave is seen to consist of two contemporaneous components: (1) the sinusoidal burst described above, superimposed on (2) a slow potential shift, initially negative, and then with a positive overshoot.

Channel 4 is the same signal as before, with the same recording parameters as Channel 3. However, the driver amplifier of the polygraph has been adjusted to produce a high frequency half-amplitude pen response of 0.1 Hz, in contrast with the 60 Hz high frequency half-amplitude pen response in channels 2 and 3. This method produces a record of the slow potential changes (Ottoson's slow wave) without the high-frequency components.

It can be seen that the burst of activity persists for about one second after cessation of the stimulus, and terminates as it merges into the spontaneous bulbar activity. The slow negative deflexion continues for about the same time as the air flow. When the air flow stops, this negative wave returns to the base-line in a similar time (300 msec). There follows an overshoot which returns slowly to the base-line by the time the burst has finished.

Not all induced waves have the same gross appearance. Fig. 3-4 shows the six successive responses which followed the first example recorded as above. The stimuli were 5.3 sec. apart. Intervening spontaneous activity and the air flow record (channel 1) have been

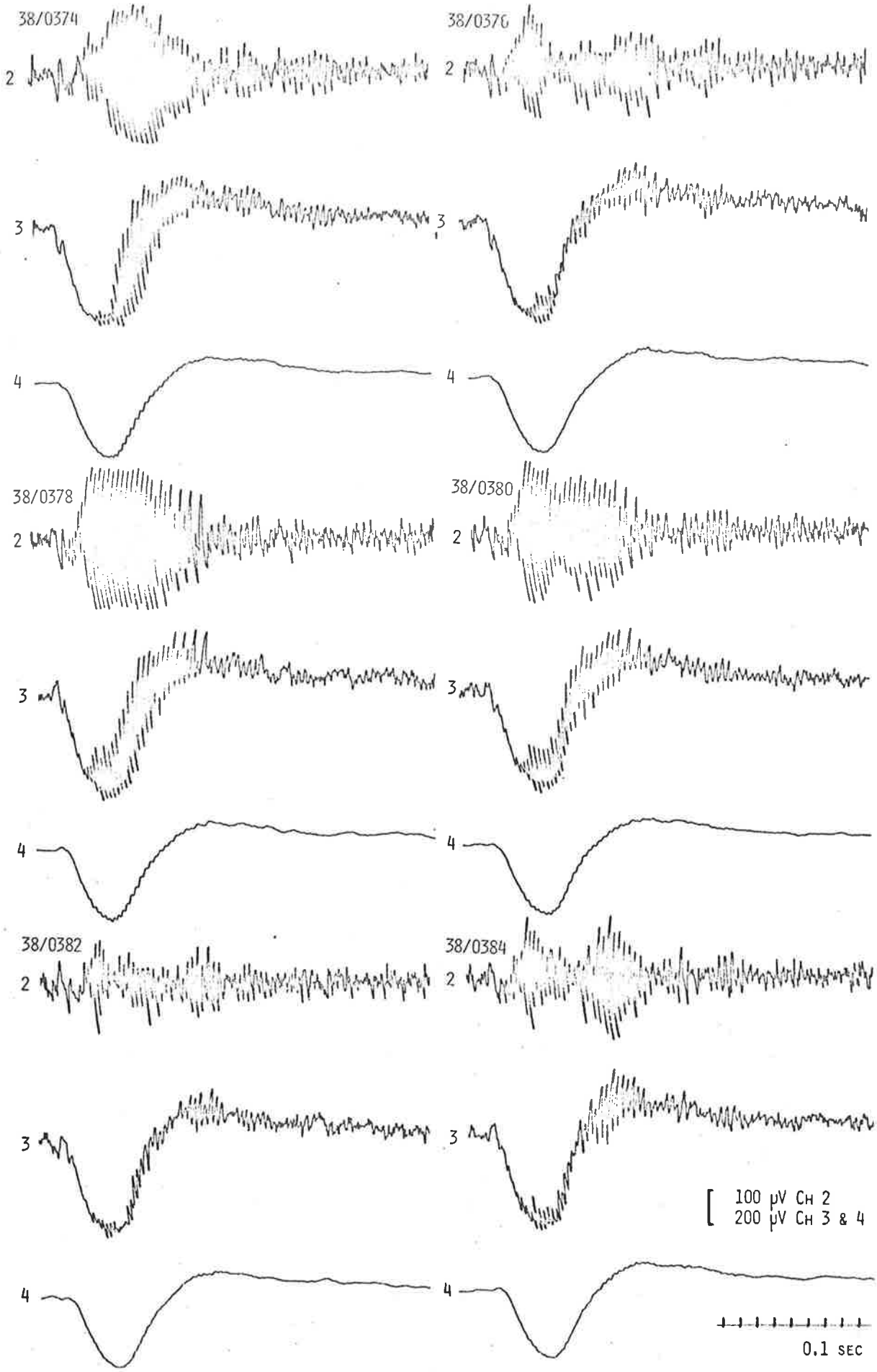


Fig. 3-4 The six induced waves following the two illustrated in the preceding figure. Recording and writeout parameters constant, except that the time base is halved.

removed for economy of space. It can be seen that there are marked differences in the shape and duration of the individual bursts. However, there are two common features: (1) all bursts begin with a frequency of approximately 45-50 Hz and finish with a frequency of about 30 Hz; (2) the slow-waves are all identical in shape and duration, regardless of the shape or apparent duration of the induced burst.

3. Induced waves: olfactory cortex

Simultaneous records were made of olfactory bulbar and cortical electrical activity in all experiments.

Fig. 3-5 is a real-time record of the electrical activity of left and right olfactory bulbs and their corresponding cortices (co-ordinates A15, L7). The standard stimulus was applied to both nostrils at intervals of ten seconds.

Induced waves are present in the olfactory bulb and cortex of each side. The waves recorded from one site differ in appearance from one to the next, as illustrated previously (Fig. 3-4). There are also apparent differences between those from either side, both bulbs, or both cortices.

Fig. 3-6 is the 1/10-speed record of the first burst shown in Fig. 3-5. The bulbar response to the stimulus begins in the usual way with a sharp negative deflexion followed by 45 Hz sinusoidal activity. However, the latter part of the burst is more complex than the first example. In the left bulbar record, there is low-frequency activity apparently added to the usual bulbar frequency. This produces a waveform similar to that obtained by the addition of a sine wave and its first harmonic.

Activity of the cortex is different from that of the bulb. There is a small initial negative deflexion followed by a larger biphasic one. This activity lasts about 100 msec. In the next 200-300 msec there is sinusoidal activity similar in frequency to the corresponding bulbar activity, but smaller in amplitude. There is an abrupt change to high-voltage activity of approximately half the bulbar frequency, with little evidence of the latter being present. This slower cortical activity becomes smaller, and ends by merging into spontaneous activity.

A section of simultaneous bulbar and ipsilateral cortical activity, the first 1.6 sec. of the induced wave, was digitized (CAT-400C) and written out on the X-Y plotter. The polygraph record and digitized plot are both shown in Fig. 3-7. This illustrates more clearly the general features and phase relationships of this simultaneous activity. In the bulb there is the usual high-frequency sinusoidal activity, followed

61/0224

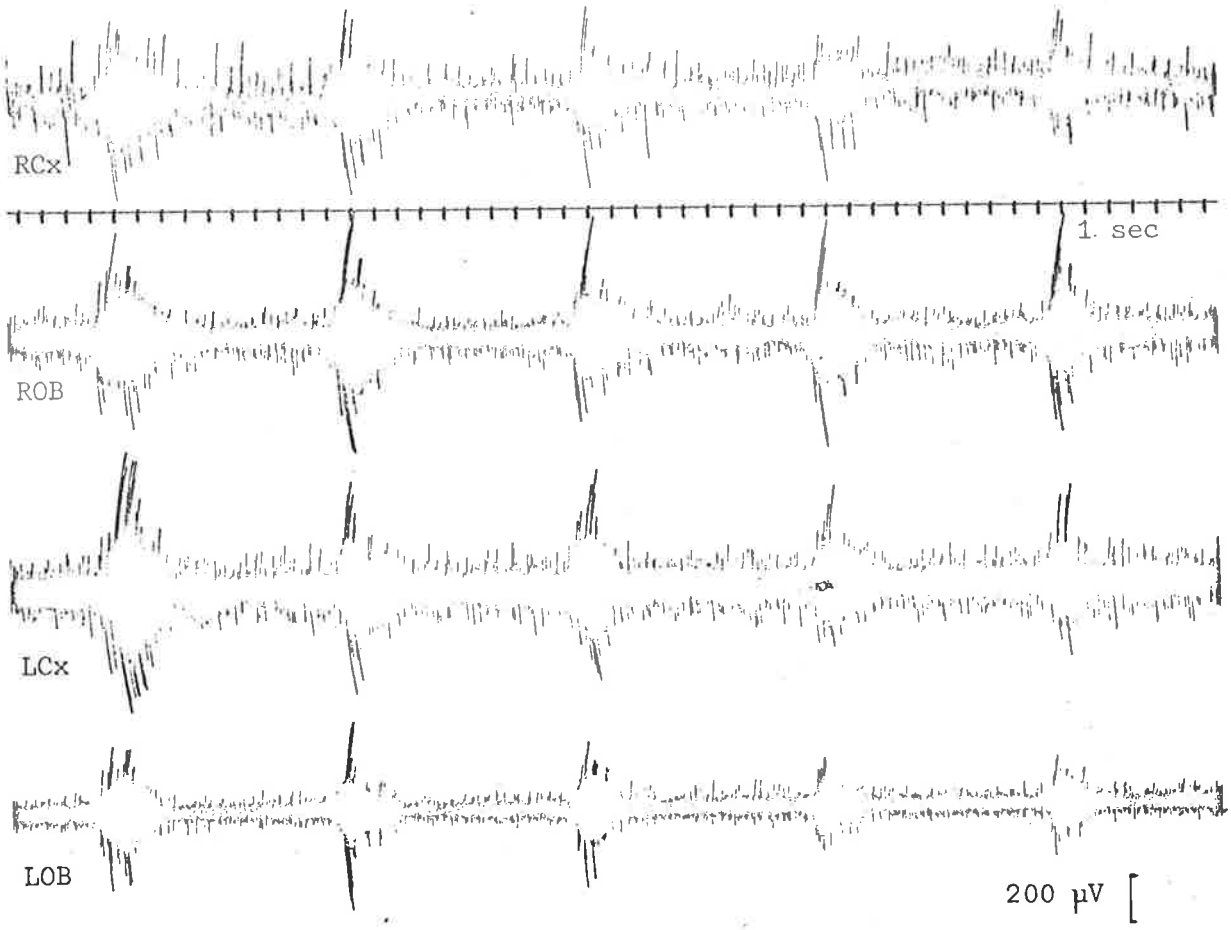


Fig. 3-5 Induced waves recorded from both olfactory bulbs (intact) and corresponding olfactory cortices of the immobilized phalanger. The standard olfactory stimulus was applied to both nostrils at 10-sec intervals. The record is in real time. In this, and subsequent illustrations, band pass of the preamplifier is 7-100 Hz, unless otherwise stated.
Notice that there are differences in the appearance of the induced waves recorded from a particular site. There are also differences when the effects of a particular stimulus are recorded from different sites.

61/0224

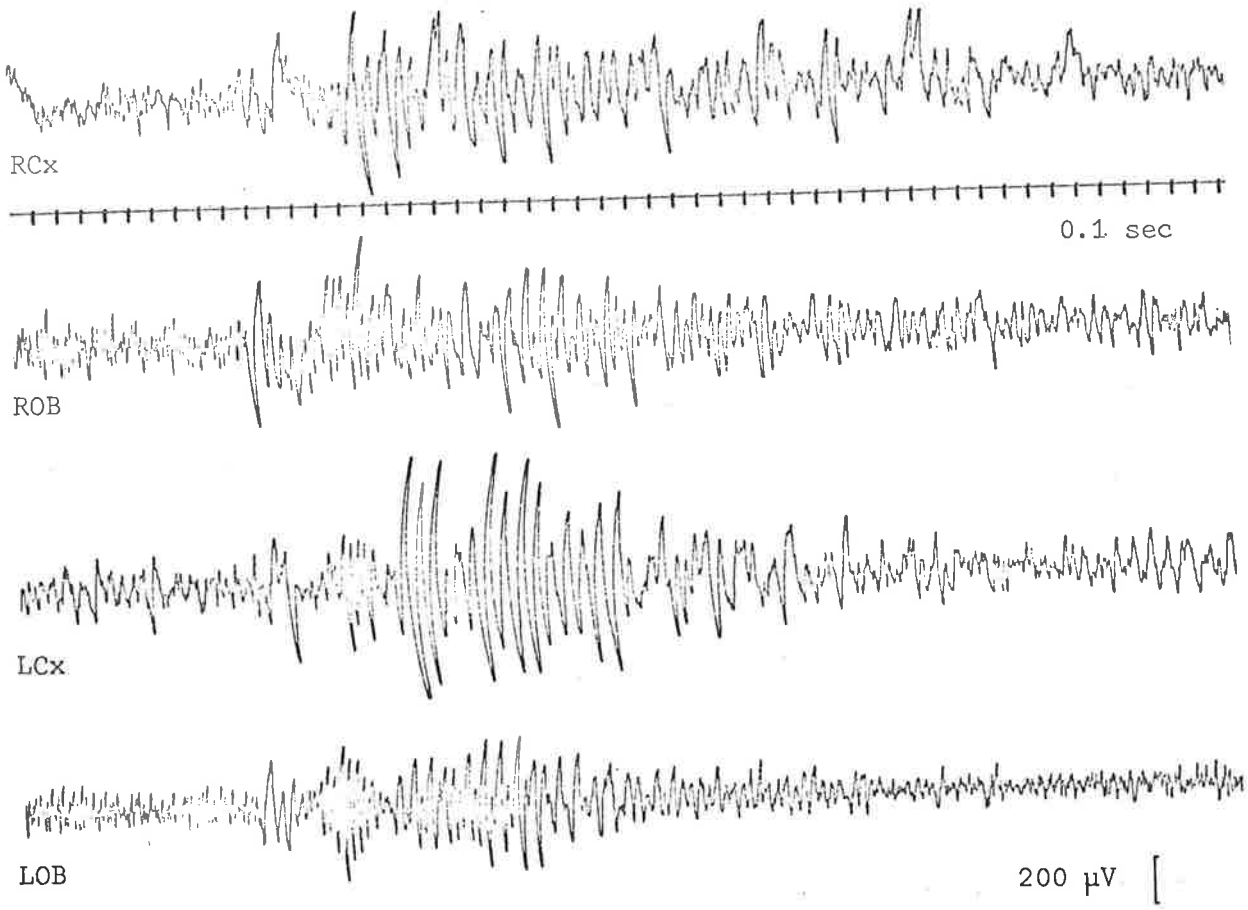


Fig. 3-6 The first induced wave of the preceding figure, retrieved from magnetic tape replayed at one-tenth recording speed. The bulbar induced waves (ROB & LOB) begin with high frequency, and in the later part apparently have the lower cortical frequency superimposed. The bulbar high frequency is not prominent in the cortical records. It is difficult to determine the precise end of the induced wave, as it merges into the ongoing intrinsic activity.

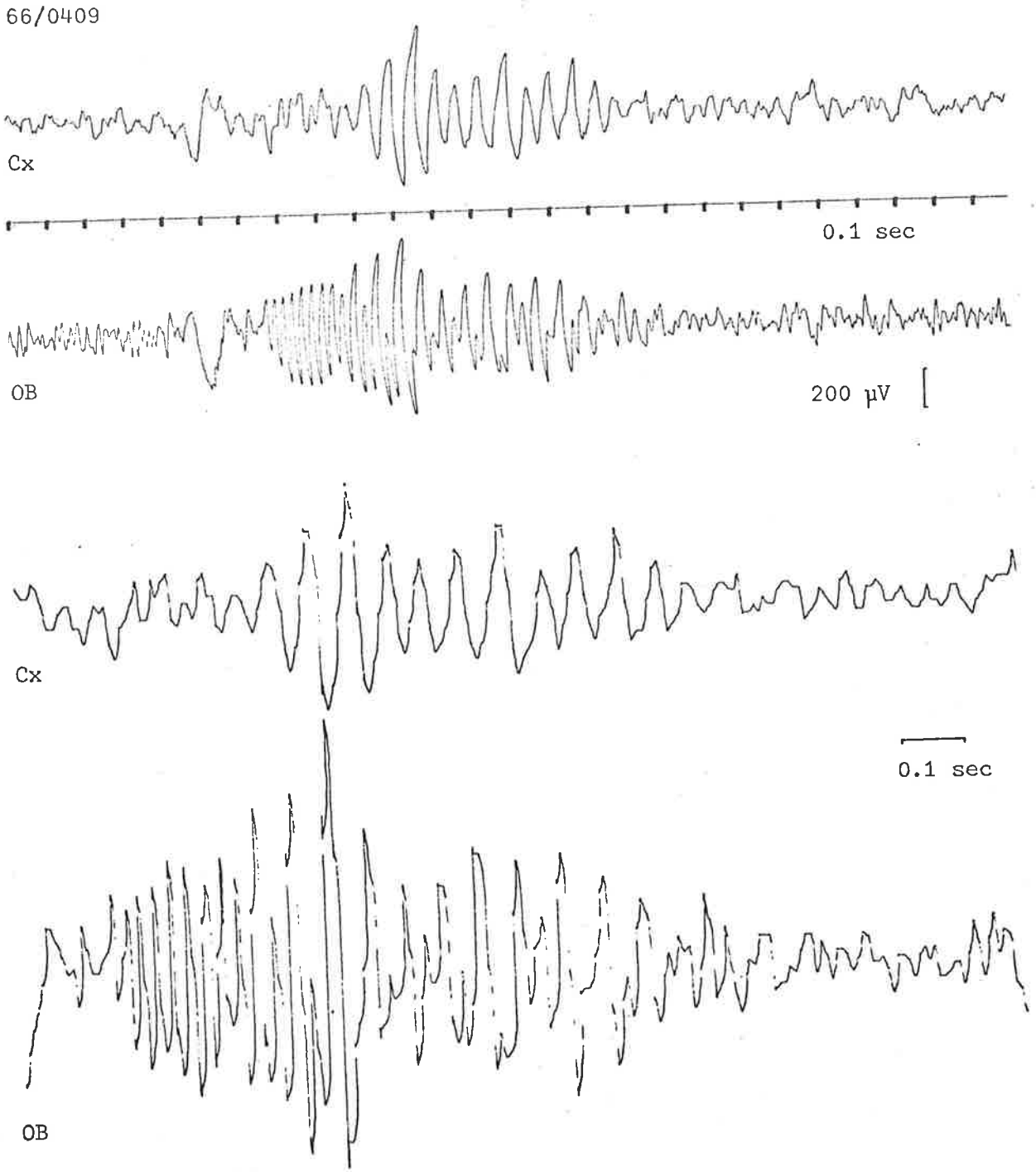


Fig. 3-7 Induced waves recorded simultaneously in the olfactory bulb and olfactory cortex of an immobilized phalanger. The upper two records are the analogue outputs from the magnetic tape replayed at 0.1 recording speed. They show the anticipated features of the bulbar and cortical induced waves.
The lower two records are the output of the Computer of Average Transients which had been used to digitize and store a simultaneous 1.6 sec segment of the bulbar and cortical record illustrated in the upper traces. It is not possible to determine in this way the phase relationships between bulb and cortex.

by complex activity of slower frequency. The initial high-frequency activity is not as obvious in the cortical record, but there is the delayed low frequency sinusoidal burst. It is not possible to tell which component leads using this method.

4. *Induced waves: isolated bulb*

Figs. 3-8 and 3-9 show the polygraph records of the induced wave immediately preceding (3-8), and the one immediately after (3-9) section of the left olfactory peduncle. (This lesion was later shown to be complete, except for a small area of tissue on the medial side, about $1mm^2$).

The response preceding the section has the usual appearance in bulb and cortex. It ends in the normal, irregular spontaneous activity, the bulbar frequency being higher than the cortical.

Immediately after section of the peduncle, the voltage of the ipsilateral bulbar and cortical spontaneous activity is reduced markedly.

The usual stimulus produces an induced wave in the isolated bulb, but not in the cortex of that side. This induced wave has the appearance of a sine wave which begins at a frequency of 50-60 Hz, and slows down during the next 0.8 seconds to about 35 Hz. Electrical activity in the intact bulb and cortex is essentially unaltered, and shows the features described previously.

Another example of induced waves before and after transection of the left olfactory peduncle is given in Fig. 3-10. Similar changes are seen; reduction of intrinsic activity in the deafferented cortex and removal of cortical influence to the now-isolated bulb.

Successive stimuli to the nostrils continue to induce bursts of activity in the isolated bulb. However, the resulting induced waves are not identical. Fig. 3-11 demonstrates as much variation in these induced waves as in the intact system. These successive waves have similar general features: high frequency at the beginning which slows uniformly, and a duration approximately the same as those of the intact side. There is no visual evidence of a subharmonic frequency in any of these bursts.

Some induced waves do not assume the classical spindle shape, but show "flares". The initial spindle is followed by an isoelectric pause (20-50 msec) succeeded by other spindles, each of lower voltage and duration than the preceding one. The frequencies contained in the flares is similar to those of the main spindle.

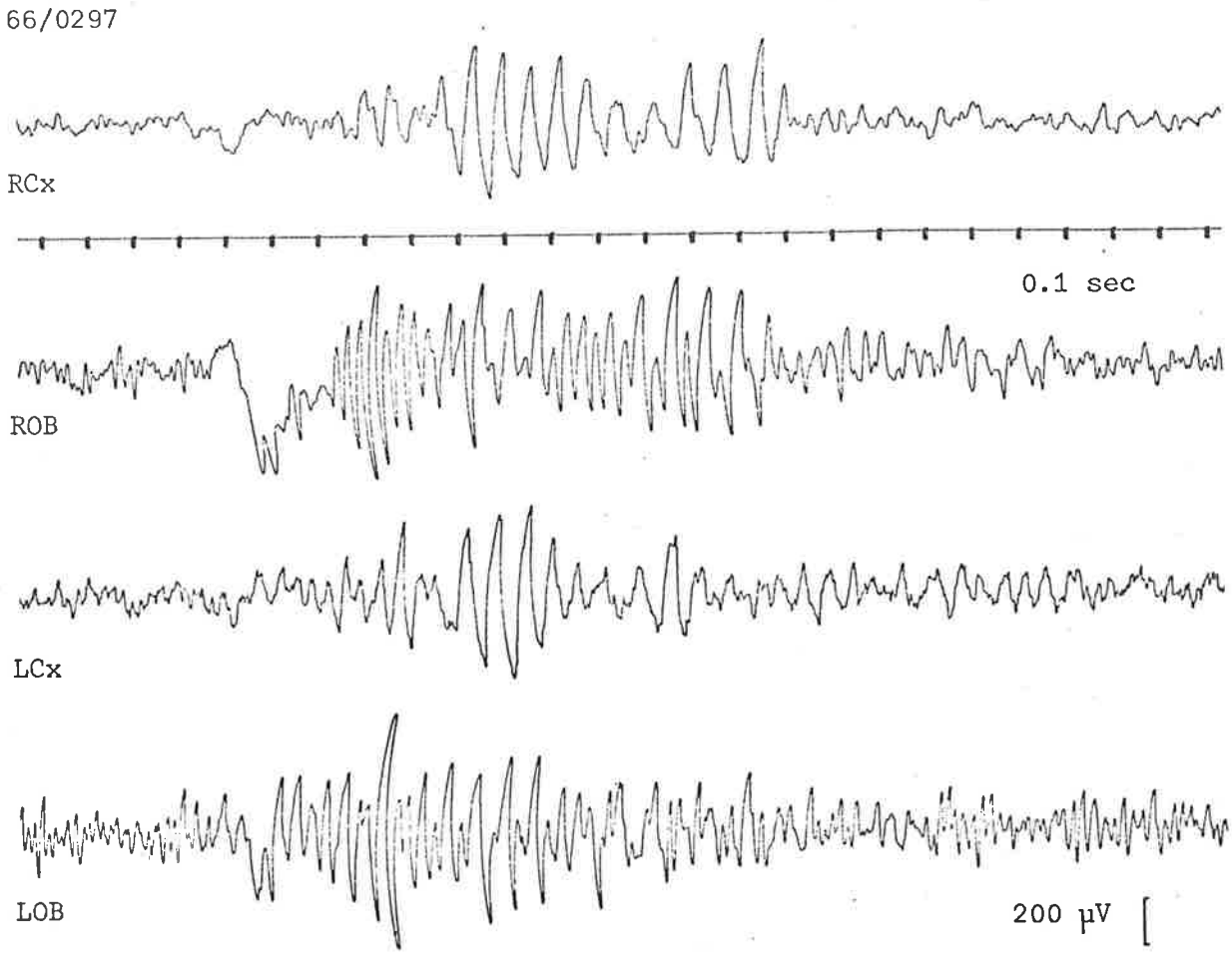


Fig. 3-8 Polygraph records of the induced waves resulting from bilateral olfactory stimulation in the intact phalanger. Records are from each intact bulb and its corresponding cortex. They are the induced waves immediately preceding section of the left olfactory peduncle.

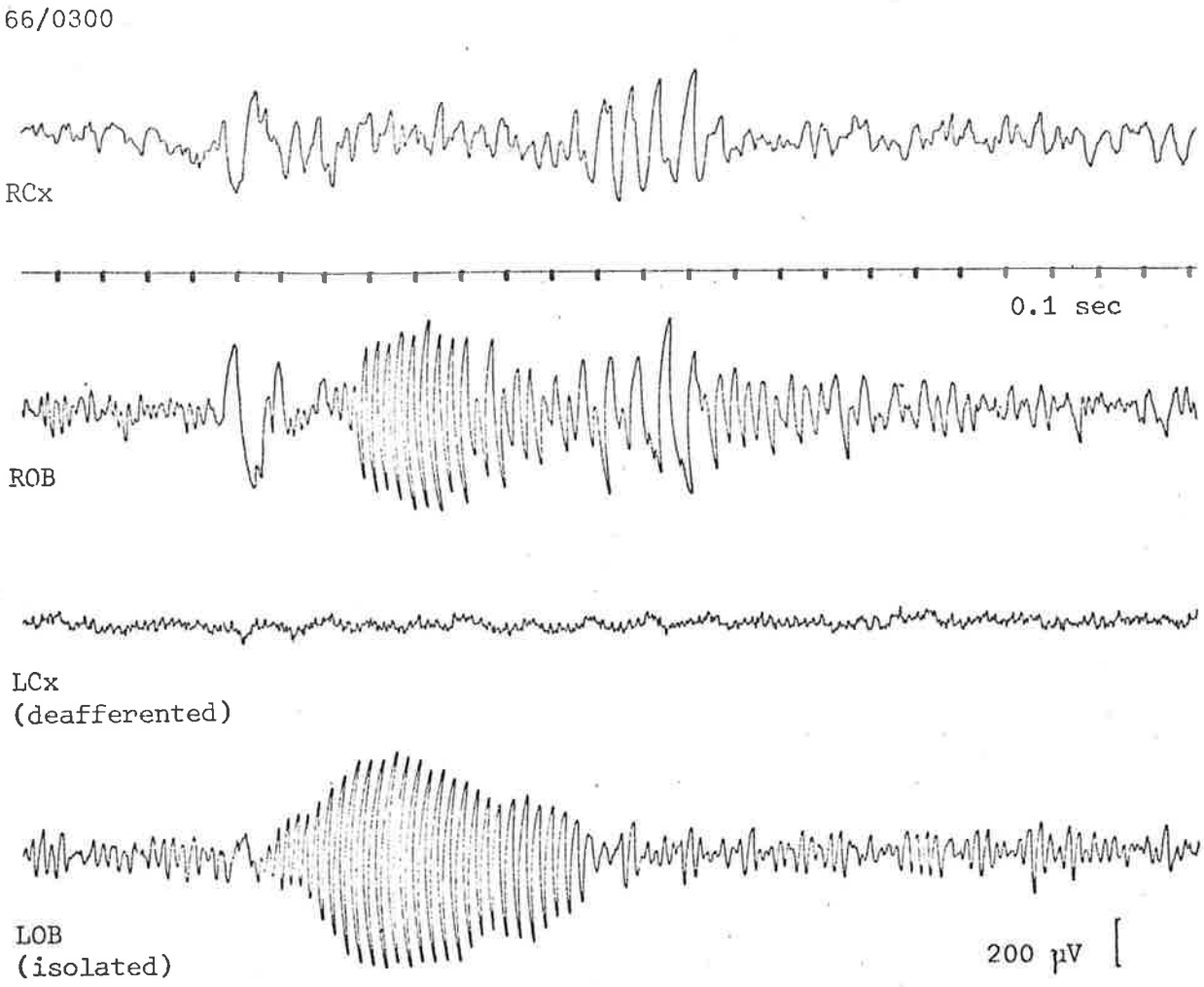


Fig. 3-9 Induced waves recorded from the same sites as the preceding example immediately after section of the left olfactory peduncle. Note the reduction in intrinsic activity of the left cortical record, and absence of induced wave. The activity in the left (isolated) bulb is "purer" than before, with no visible evidence of a cortical low-frequency component. The induced waves of the right bulb and cortex are essentially unchanged.

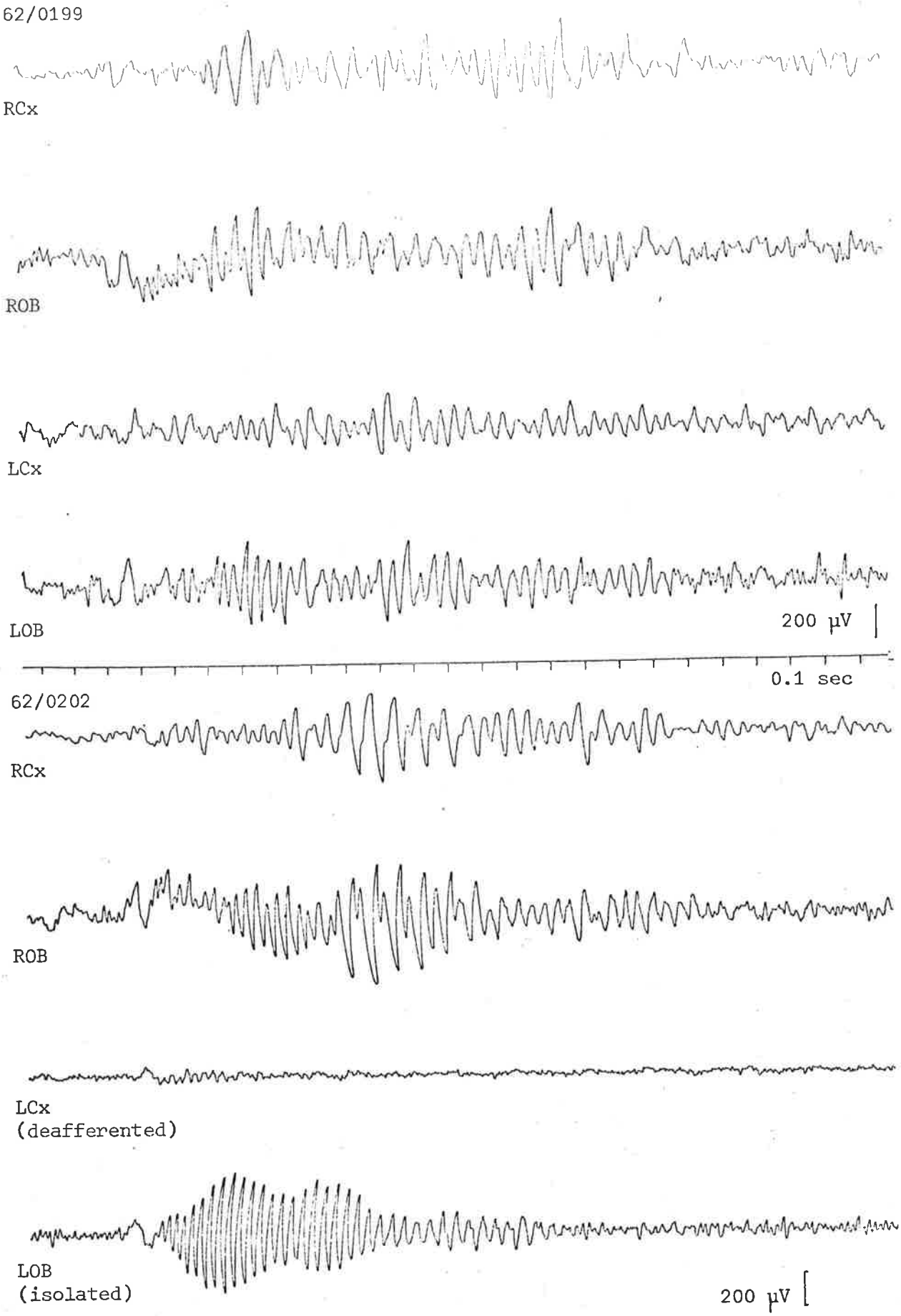


Fig. 3-10 Induced waves immediately before (upper) and after (lower) section of the left olfactory peduncle in another experiment. Similar changes to the preceding example are seen.

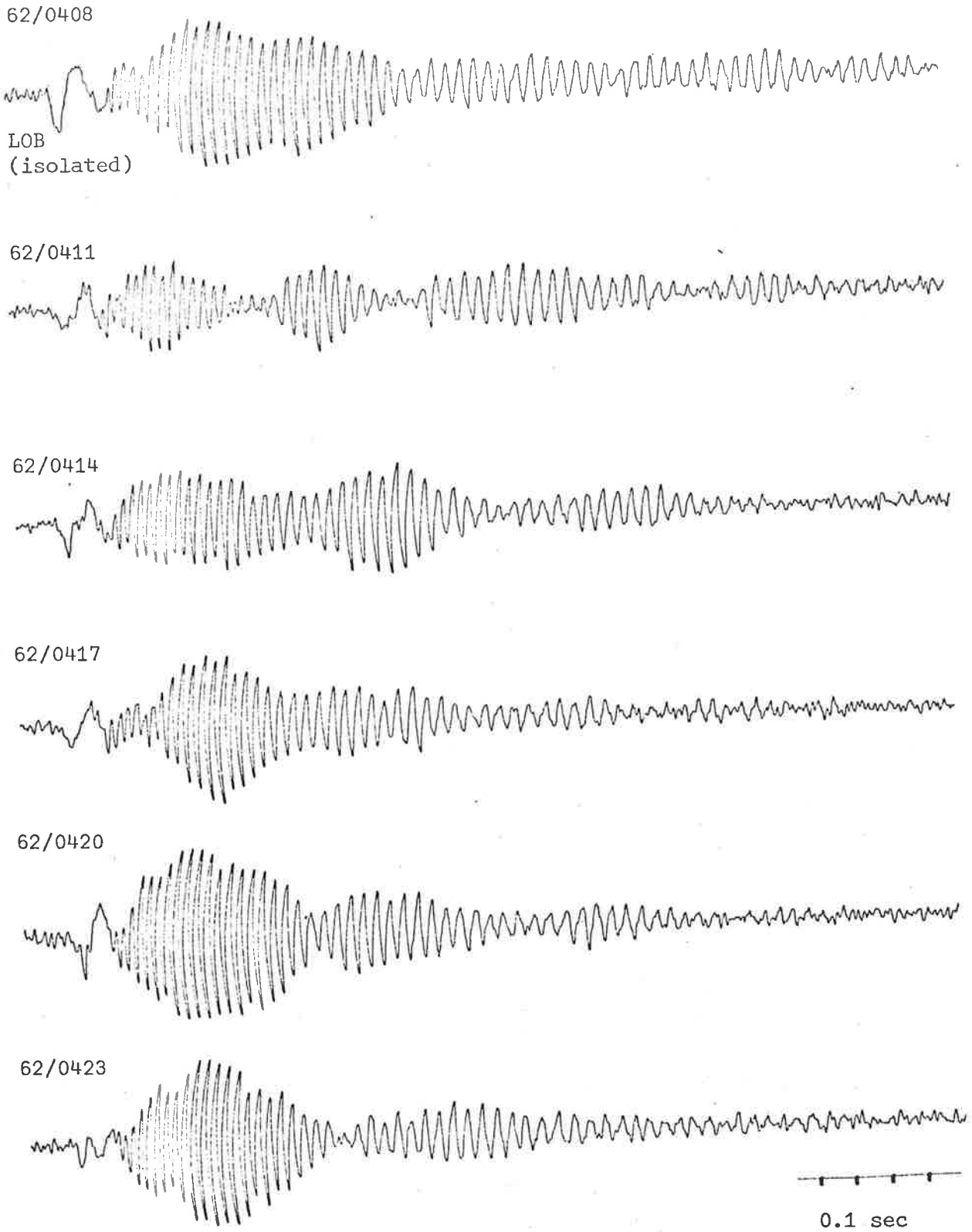


Fig. 3-11 Six successive induced waves recorded from the isolated bulb of a phalanger in response to the standard olfactory stimulus. They are similar in duration and contain similar frequencies, but have different envelopes, showing "flares".

The occurrence of these flares is not obviously related to airflow or odour. They may appear and disappear during an experiment, and have also been seen in the isolated bulb.

Fig. 3-12 shows the induced waves of the intact side corresponding to the first three induced waves illustrated in Fig. 3-11. They show the marked influence of the cortical activity.

5. *Alerting response*

All the examples of induced waves shown above have been from long sequences. The first few olfactory stimuli to an undisturbed animal often produce induced waves which are manifestly different from the succeeding ones.

Fig. 3-13 (upper part) shows an example of an alerting response. In this instance, the animal had been undisturbed for 30 minutes. The stimuli were to the left nostril, the right having been occluded previously. Recording was from both bulbs, and the left olfactory cortex.

The real-time record shows that the first induced waves (bulb and cortex) on the left side are larger and longer than usual. It also shows that there is an "induced wave" recorded in the right bulb which did not receive a stimulus. The responses of the left bulb and cortex assume the expected size and duration by the third stimulus. By the fifth, there was no "induced wave" recorded in the right bulb, either in real-time or one tenth time.

The lower part of Fig. 3-13 shows the first induced wave written out at 1/10 real-time. The induced wave of the left bulb begins in the usual way, with the negative deflexion followed by a burst of 0.5 sec. duration beginning with a frequency of about 45 Hz, slowing to 30 Hz. There is an abrupt change to a lower frequency (9-12 Hz) after this, with visual evidence of an insignificant high-frequency component. The duration of the burst is over three seconds.

The induced wave has the usual appearance in the left olfactory cortex, but is of longer duration than normal. There is the suggestion of bulbar high-frequency activity in the first 0.5 sec. This is followed by high-amplitude sinusoidal activity at 10-15 Hz, which continues for another 2.0 sec, with little evidence of a significant high-frequency component.

Spontaneous electrical activity in the right bulb (unstimulated) shows a slight reduction in amplitude for 0.8 sec after the onset of airflow to the left nostril. An "induced wave" of 10-15 Hz follows for the next 3.5 sec, to be followed by the spontaneous activity.

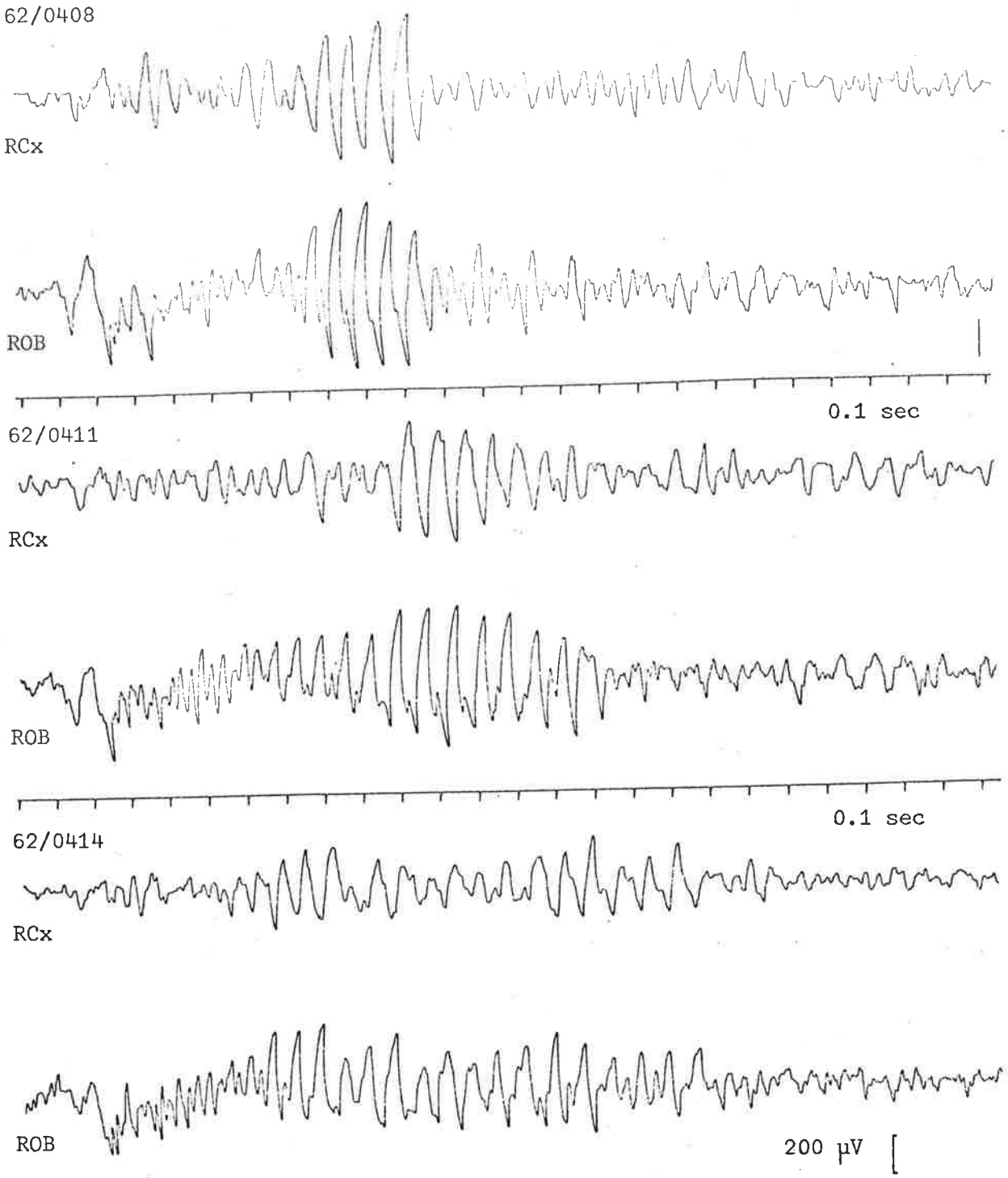


Fig. 3-12 Induced waves recorded from the intact olfactory bulb and cortex at the same time as the first three responses of Fig. 3-11. The marked influence of the cortex on the bulb is seen. (There is, of course, the possibility that another structure is influencing both bulb and cortex, but this will need further experiments to confirm or refute).

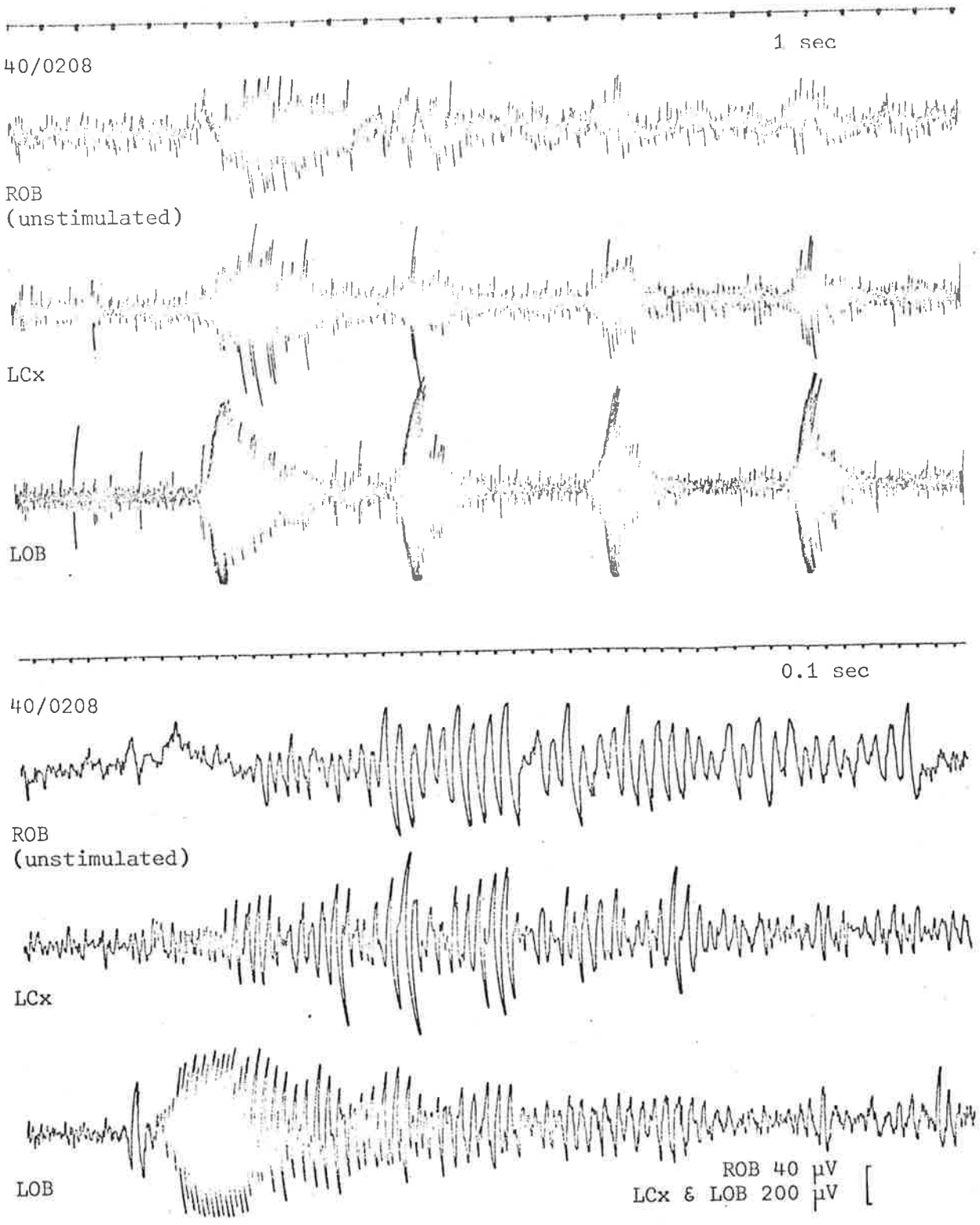


Fig. 3-13 "Alerting response" in the olfactory bulbs and cortex of the phalanger. The standard stimulus was applied to the left nostril only. The upper records are from the left bulb and cortex and the unstimulated right bulb. The first induced wave of the series is significantly longer than the subsequent ones. An "induced wave" is recorded from the unstimulated bulb after the first stimulus.
The lower records show the first of the three responses written out at 0.1 tape speed. It contains low frequencies only.
The animal had been undisturbed for 20 min before this stimulus.

A similar example from another experiment is given in Fig. 3-14. The olfactory stimulus was given to the left nostril, the right having been occluded previously. A decreasing amount of rhythmic activity was recorded in the right (unstimulated) bulb for three stimuli, after which none was discernible. The upper part of the figure is the real-time record; the lower part is the first response written out at 1/10 real-time. The same general features are seen; the usual bulbar and cortical records on the side of stimulation, and the delayed (0.8 sec) activity in the unstimulated bulb.

Fig. 3-15 is the record of the first five responses of the series illustrated in Fig. 3-14, written out in real-time, showing the disappearance of the activity in the unstimulated bulb, and return to usual appearance of the stimulated side.

6. Induced waves after mid-brain section

Peripheral de-afferentation by intercollicular transection of the brainstem has no effect on the induced waves. Fig. 3-16 shows the appearance of the sequence of induced waves before and after such a transection. However, there was sometimes gradual increase in spontaneous background activity, and the induced waves became lost in this. In the example given here, there is the impression of a reduction in background activity. Recording continued for another hour, and the induced waves were apparently unchanged.

7. Effect of 100% oxygen

After ventilation with 100% oxygen for one hour there was no change in the induced waves.

8. Effect of 5% carbon dioxide on induced waves

No gross changes in the induced waves were noticed in the induced waves after ventilation with 5% CO_2 - 95% O_2 for 20 minutes, compared with 100% O_2. There was the qualitative impression that cortical spontaneous activity was slightly depressed, and the cortical frequency was less apparent in the bulbar burst. An example is shown in Fig. 3-17. Quantitative analysis of activity before and after carbon dioxide administration has not been carried out.

9. Effect of different neuromuscular blocking agents

Neither gallamine nor tubocurarine had any obvious effect on the induced waves. An example of gallamine effect is shown in Fig. 3-18.

10. Effect of hypothermia

Despite a fall in core temperature to 28.5°C, there was no change in the appearance of the induced waves. An example is shown in Fig. 3-19.

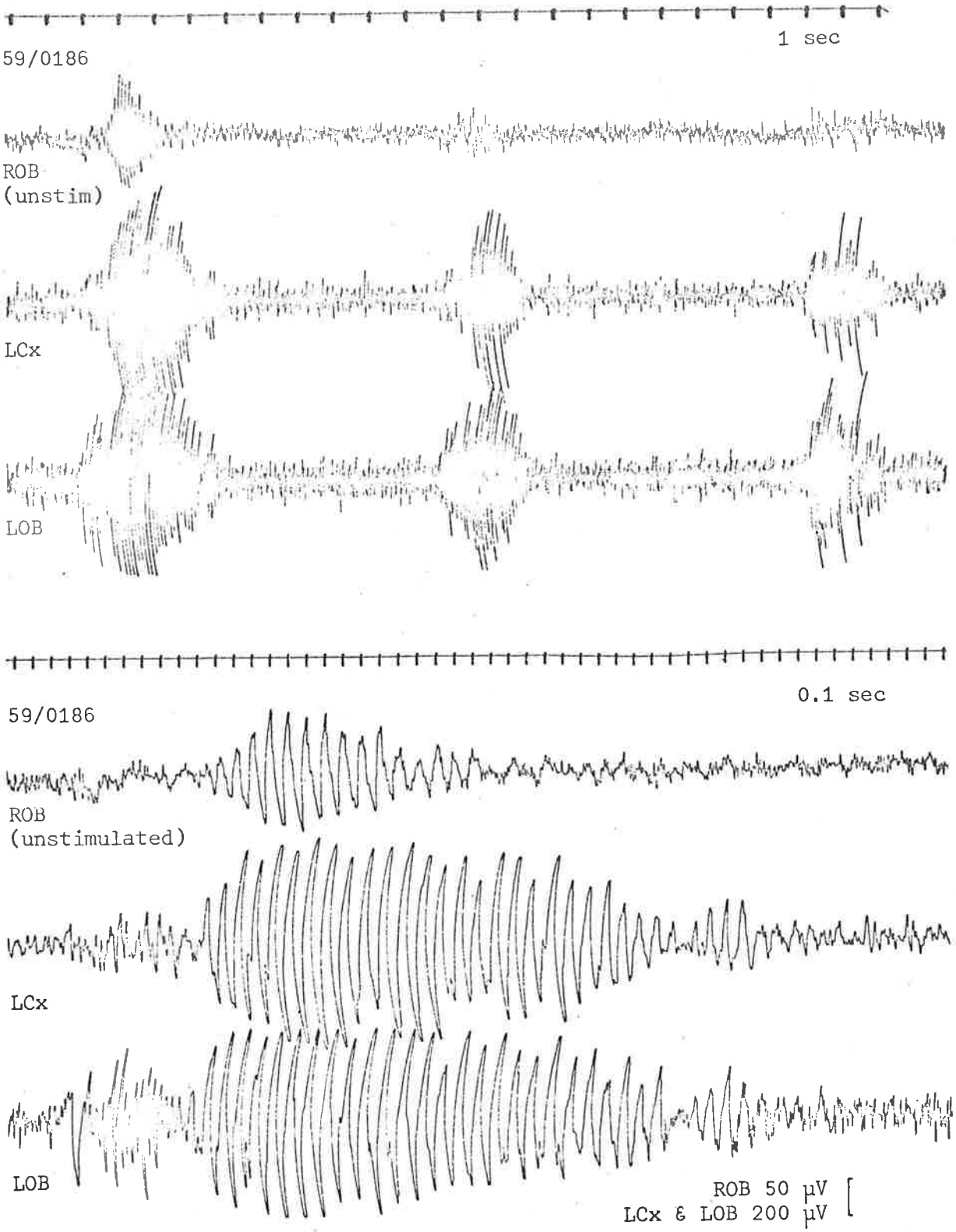


Fig. 3-14 Alerting response from another experiment. The upper part is a real-time record of the responses to repetitive olfactory stimuli to the left nostril only, after 20 min of no stimulation, recorded from the left bulb and cortex, and right (unstimulated) bulb. The "induced wave"of the right bulb has virtually disappeared by the third response.
The lower records show the first response written at 0.1 recording speed. The delay in onset of the right bulbar response and its low frequency are clearly seen.

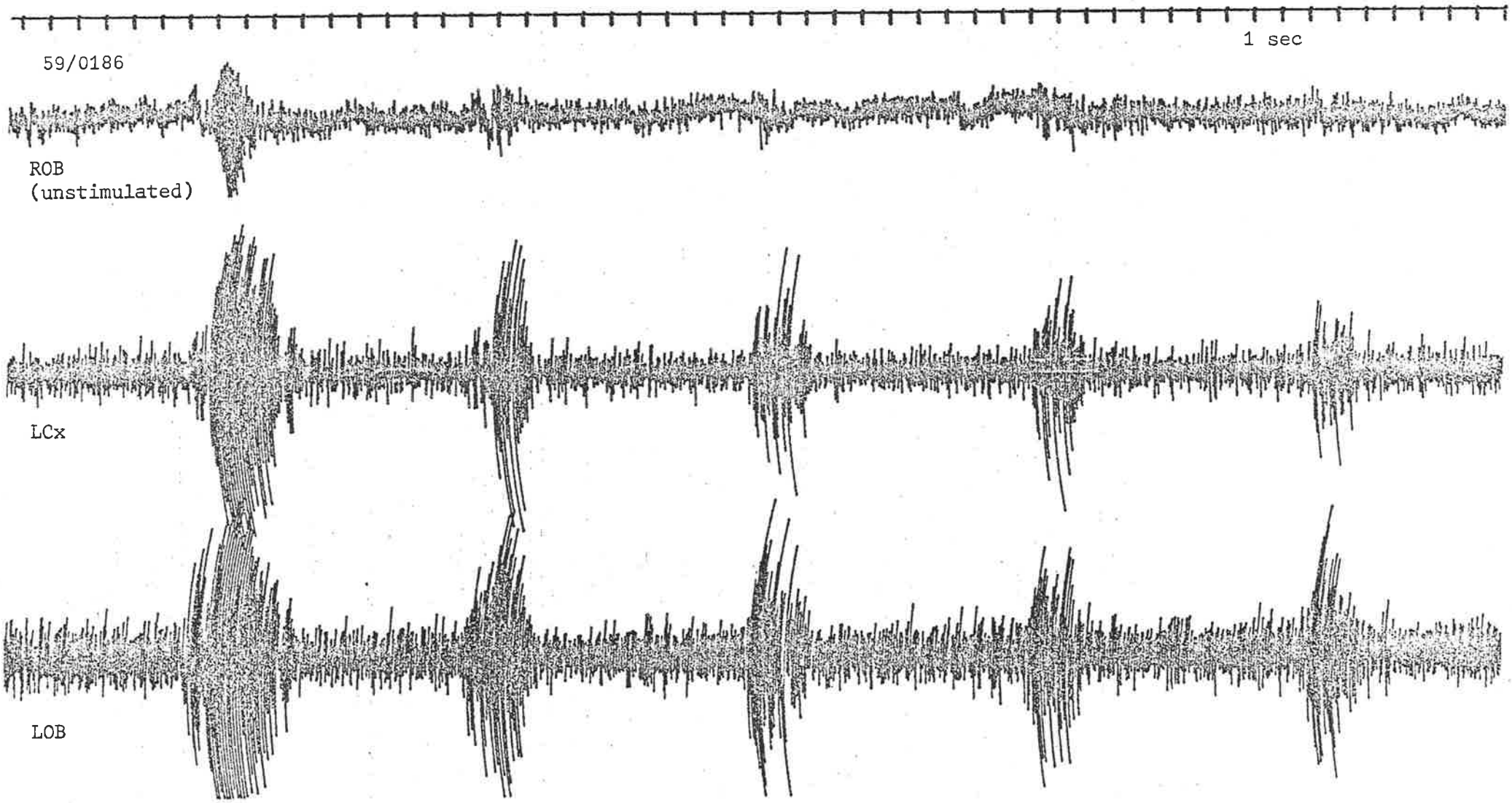


Fig. 3-15 The same sequence as the previous figure (3-14), showing the disappearance of the "induced wave" from the right bulbar record.

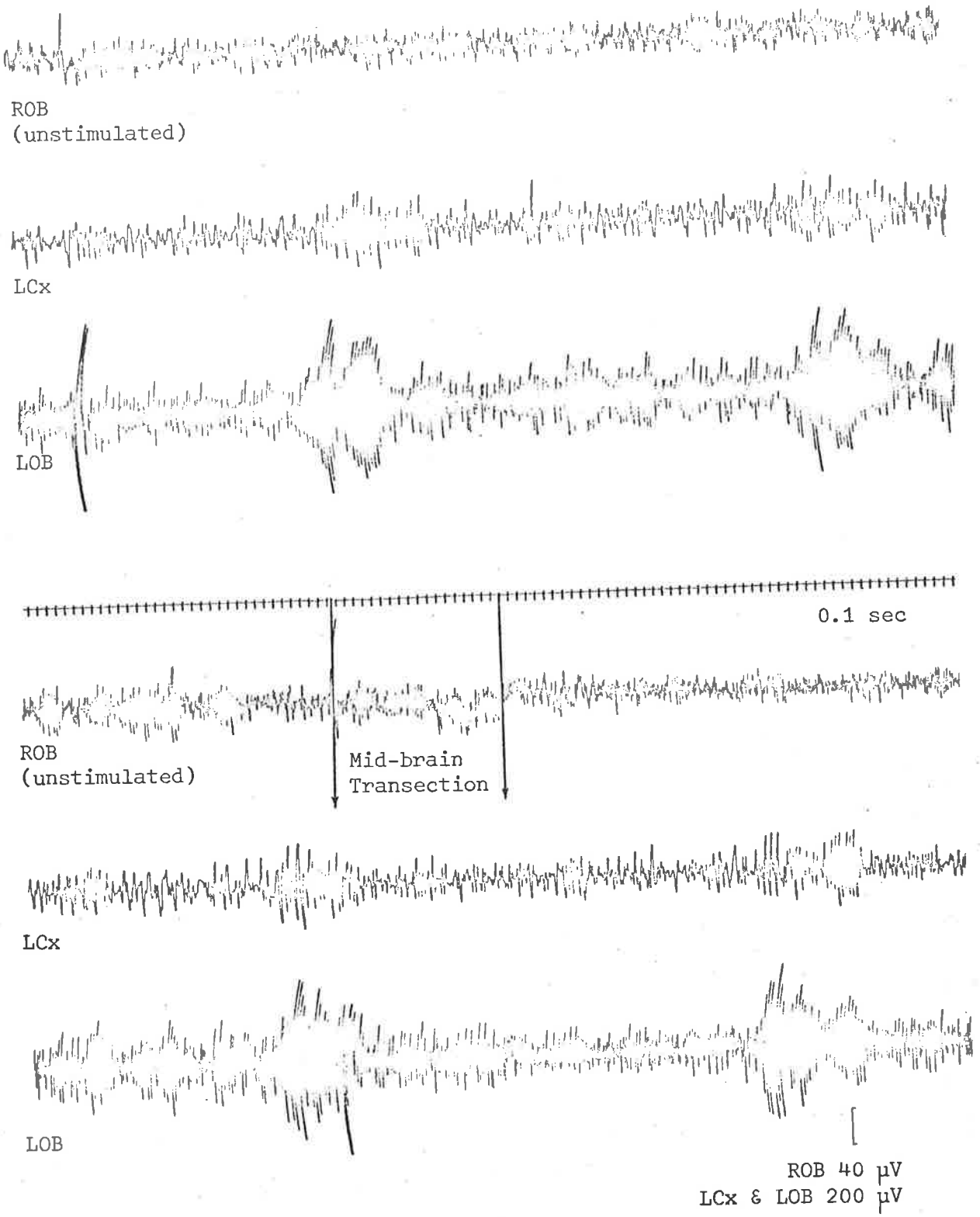


Fig. 3-16 Effects of intercollicular transection (between arrows) on the induced waves of the phalanger. There appears to be minimal reduction of intrinsic activity, and no effect on the induced waves. Olfactory stimuli to the left side only.

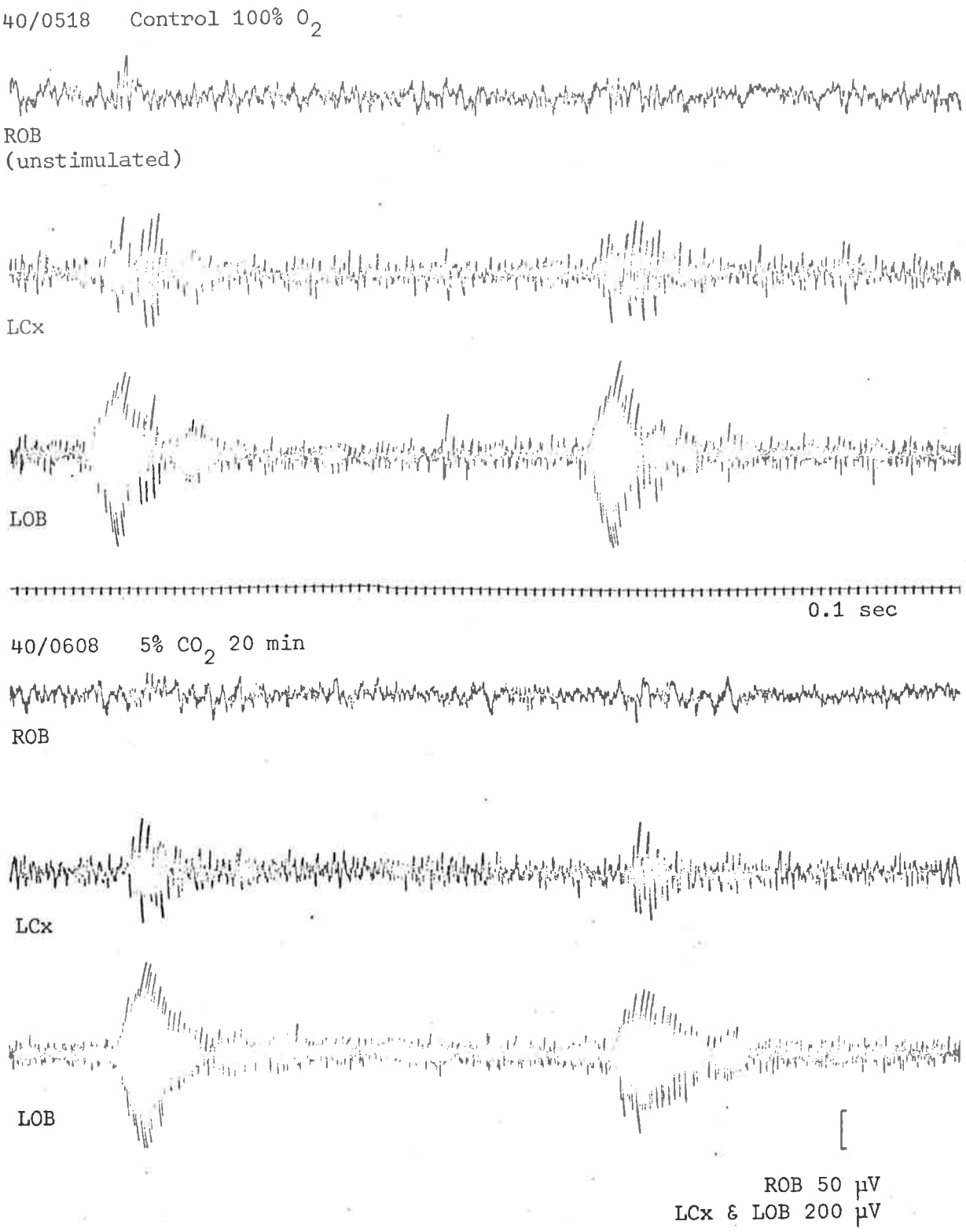


Fig. 3-17 Induced waves before (upper) and after (lower) 20 min ventilation with 5% CO_2 in O_2. Stimuli to left nostril only. There is no apparent change.

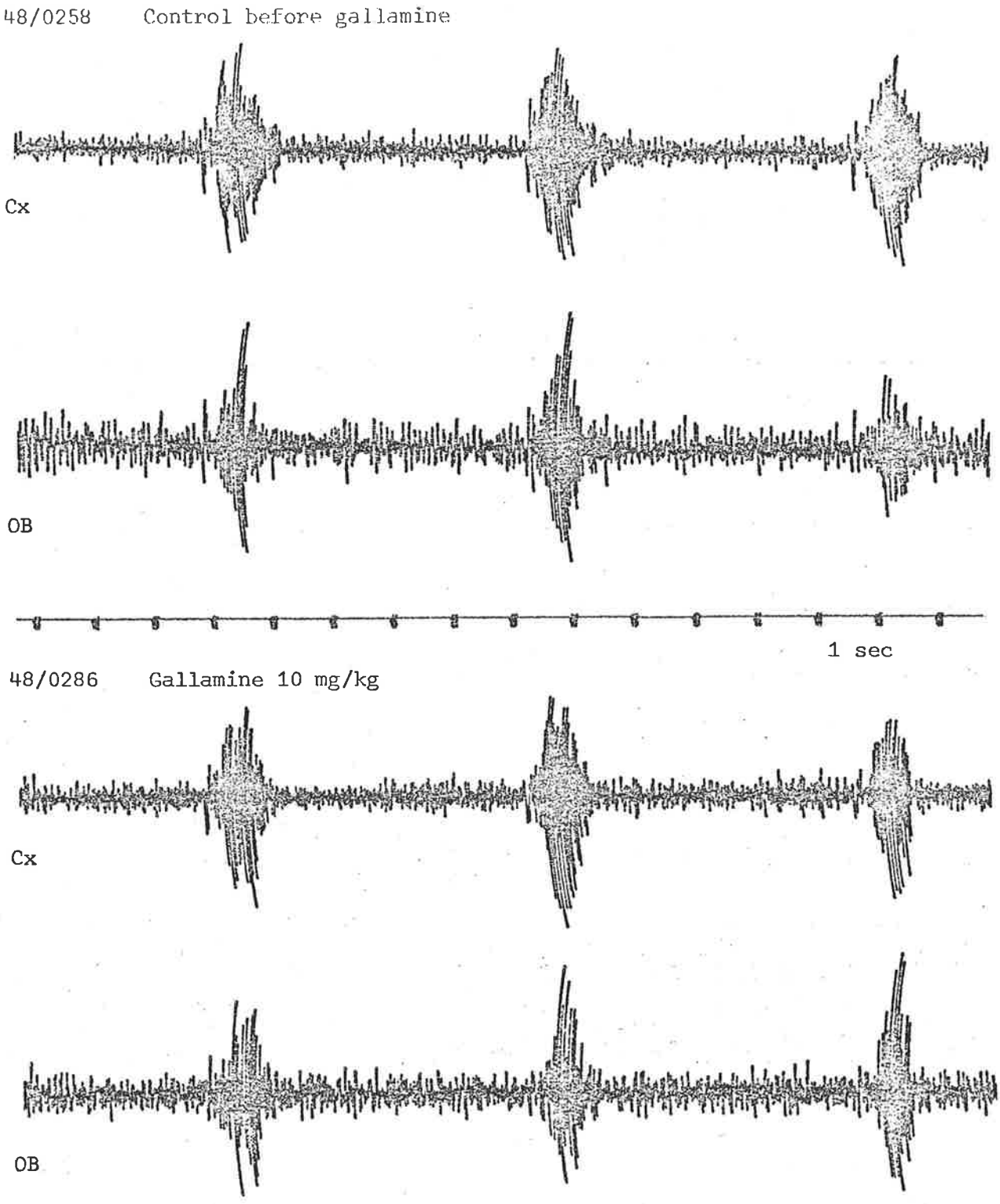


Fig. 3-18 Induced waves recorded from phalanger olfactory bulb and cortex before (upper) and after (lower) IV administration of gallamine, 10 mg/kg. No change is seen.

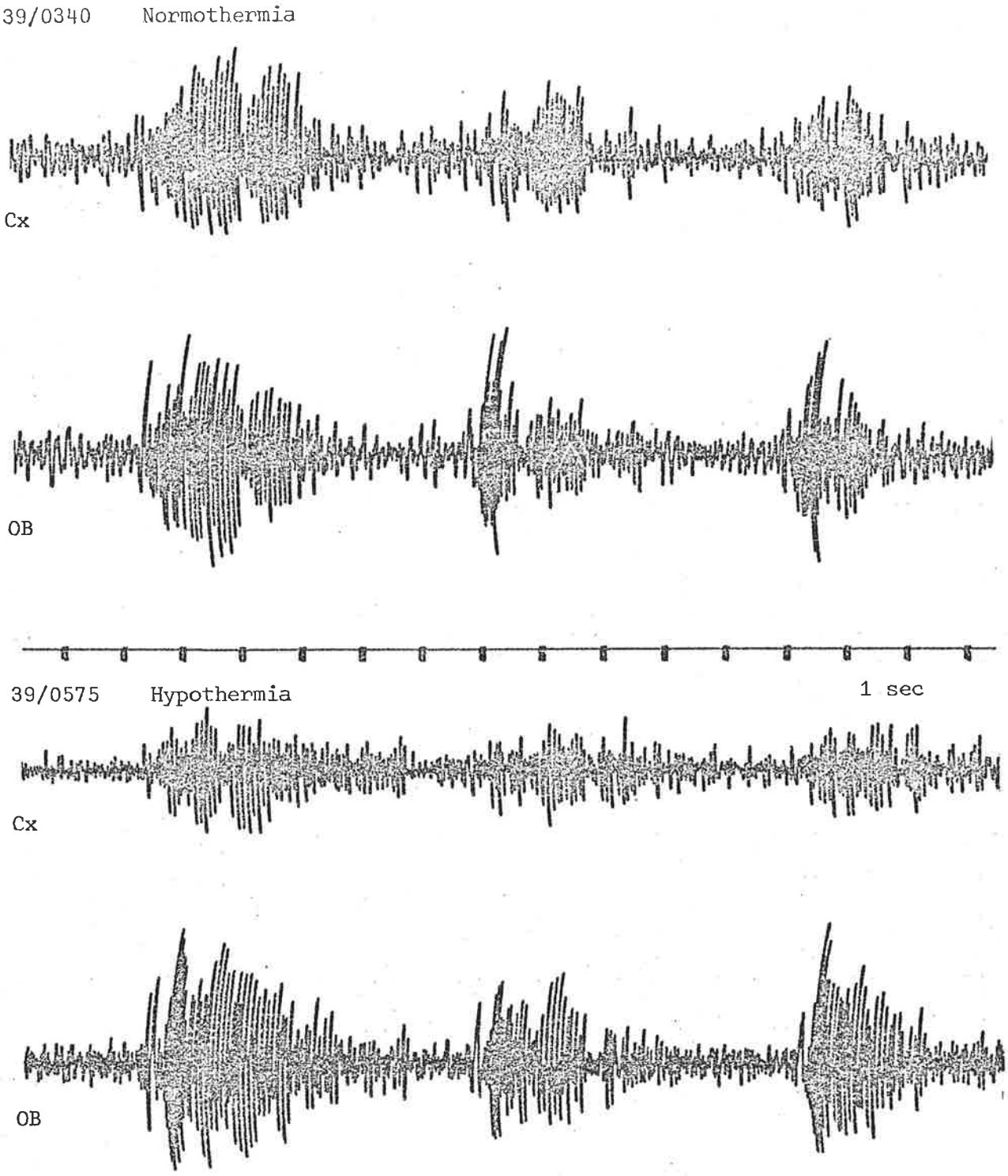


Fig. 3-19 Induced waves recorded from phalanger bulb and olfactory cortex in normothermia (upper) and hypothermia (lower) of 28.5'C. There is no apparent change.

DISCUSSION

This investigation has shown that induced olfactory electrical activity is similar in the phalanger to that in the opossum and in those mammals which have been studied. Such observations have not been made previously.

Discussion will be carried out under the following headings:

1a. *Anaesthesia and surgery*
1b. *Olfactory stimulation and recording*
2. *Induced waves: intact olfactory bulb*
3. *Induced waves: intact olfactory cortex*
4a. *Induced waves: isolated olfactory bulb*
4b. *Electrical activity of deafferented cortex*
5. *Alerting response*
6. *Induced waves after midbrain section*
7. *Oxygen effects*
8. *Carbon dioxide effects*
9. *Neuromuscular blockade*
10. *Hypothermia*
11. *Accessory bulb*

1a. *Anaesthesia and surgery*

This part of the study was designed to investigate the electrical activity induced in the olfactory system of the undrugged phalanger. Surgery and placement of electrodes was carried out under inhalational anaesthesia. Such anaesthesia relies on physical exchange of gases, not metabolism, for induction and recovery from anaesthesia (for a recent review, see Eger, 1971), so that complete recovery is assured.

Initial attempts to record induced activity were unsuccessful. The activity recorded from bulb and cortex consisted of high voltage spike-like activity, with no discernible alteration caused by the olfactory stimulus. Chloroform was the induction and maintenance agent used for the surgery, because it had been used successfully in the evoked studies. When halothane was substituted, however, induced activity was invariably recorded. There was no reason to try any other agent once satisfactory results were being obtained, despite the expense of halothane.

Diethyl ether, frequently used by other workers, was not used in this laboratory because of its high flammability and high blood-gas partition coefficient (Secher, 1971). The latter attribute increases its anaesthetic induction and recovery times; for example, Pagano (1966) allowed at least eight hours to elapse between termination of ether and

onset of recording or stimulation in his cat experiments. Huggins, Parsons & Pena (1968) showed that ether suppressed normal olfactory activity in the caiman for 24 hours. In my experiments, at least one and a half hours were allowed to elapse before recording, although induced activity usually became apparent within five minutes of removing the anaesthetic gases.

Nitrous oxide was used in this study as the "carrier" gas, to decrease anaesthetic induction time (second gas effect) and to reduce the amount of halothane required for surgical anaesthesia. Oxygen was added to maintain an inspired concentration of 20-25%. The T-piece technique was chosen for ease of single-handed use, enabling artificial ventilation to be instituted if required.

A previous study (largely unpublished, but see Fanning & Wilson, 1971) showed that the phalanger had similar respiratory parameters to other small mammals. Accordingly, minute volume of artificial ventilation was derived from its body weight. The empirical formula arrived at was: Tidal volume (ml) = BW (gm)/80, with respiratory rate 12.5/min. This respiratory rate was shown by intratracheal pressure studies to allow normal lung emptying and adequate venous return to the right side of the heart. The single study of arterial blood gases reported here showed that this formula produced acceptable respiratory conditions. This was corroborated by the two other cases where mixed expired air was collected and analysed. This gas was found to have the same composition as that from the control experiment. It would have been desirable to monitor the respiratory parameters (e.g. end-tidal CO_2) in all cases, but equipment and technical assistance for this was not available. Gordon (1971) suggested that it may be desirable to hyperventilate when brain lesions are present, but Yamaguchi, Regli & Waltz (1972) implied that hypocapnia had no protective effect during cerebral ischaemia or infarction.

After completion of surgery and satisfactory placement of electrodes, the likely sources of painful stimuli were eliminated with local anaesthesia. Muscle relaxation was produced with gallamine, and artificial ventilation with air was begun. Under these conditions, the animal was in no apparent discomfort. If the effect of the muscle relaxant was allowed to wear off, the animal being undisturbed, it would lie quietly, sometimes asleep, tolerating the artificial ventilation. However, if it were stimulated by the olfactory stimulus, or by lightly touching it, it would struggle until re-paralyzed. This method is acceptable for use in experimental animals. Indeed, there is some stereotaxic surgery performed

on humans with local anaesthesia only, and conscious patients can be artificially ventilated without discomfort *via* a recent tracheostomy, or endotracheal tube (for example see Spencer, 1971, his Fig. 40-10).

Gallamine was chosen as the neuromuscular blocking agent because of its lack of depressant effects on the cardiovascular system when compared with tubocurarine. It is a tri-quaternary ammonium compound, and such drugs do not cross the blood-brain barrier to significant degree (Andersen, Barstad & Laake, 1972). Straw (1969) has shown that it has no effect on the hippocampal and amygdaloid after-discharge. However, Halpern & Black (1968) showed augmentation of electrical after-discharge in the cerebral cortex of the cat. Their experiments compared bolus injections of gallamine and β-erythroidine with prolonged infusions of tubocurarine, succinylcholine and decamethonium. This method of comparison must be questioned, because of the different methods of drug administration. Induced waves in the phalanger were unaffected by bolus injections of gallamine, but an interval of at least 15 minutes was always allowed to elapse before giving other drugs, or recording.

Intercollicular (pretrigeminal) transection of the brainstem was used in some experiments. In a few of these cases, the electrical activity of the brain increased over the next 1-2 hours, with spiking and high-voltage fast activity, until the induced waves became indiscernible. This was not associated with macroscopic cerebral oedema.

1b. Olfactory Stimulation

A standard olfactory stimulus was used in all cases. Cylinders of compressed, dry, Medical Air (Commonwealth Industrial Gases) passed through nylon tubing and metal valves to the flow-meter. There was no added odorant. However, it has been shown by Tucker (1963a) that polyvinyl (PVC) tubing may contaminate the air and act as an olfactory stimulant. The problem of odour coding is outside the scope of this thesis, but it has been assumed that all olfactory stimuli were identical.

The puff of air into each nostril was 300 msec duration, at a peak flow rate of 3 l/min. These conditions were found by trial-and-error to produce adequate olfactory stimulation. Ueki & Domino (1961) found that nozzle size (therefore air flow velocity) influenced the induced waves. They determined a threshold flow of 12 l/min (dog and monkey), with the optimal flowrate being 3-4 l/min, with no nozzle size stated. No attempt was made to study the effects of "sniffing", or of prolonged air flow over the mucosa. Stimuli were given 5-10 sec apart to allow one response to finish before the next was induced.

It is not known whether the vomero-nasal organ was stimulated at the same time. This structure projects to the accessory bulb. It was assumed that the accessory bulb provided a negligible contribution to the induced activity recorded from the granule cell layer in the centre of the main bulb. This assumption is based on the disparity between the mass of tissue in each structure, and the appearance of the induced waves after section of the peduncle. It would require specific recording from the accessory bulb, with field studies, to determine its contribution to the main bulbar induced waves.

2. *Induced waves: intact olfactory bulb*

Recordings of induced waves in the intact olfactory bulb illustrated here indicate that they are similar to those of other species and, in particular, of the opossum. This study does not elucidate the structures of origin of either the slow wave or the induced wave. However, in the series presented in Fig. 3-4 it is apparent that the slow component is constant, and the induced wave varies. It was noted, but not illustrated here, that the size of the slow wave was related to the duration and flow-rate of the stimulus. The question of whether this slow wave represented "mucosal potential" or "generator potential" is unanswered.

There is little variation in the frequencies within each burst from one to the next. The initial dominant frequency is approximately 45-50 Hz by direct measurement of zero-crossing intervals. This decreases uniformly to the final value of 25-30 Hz. The possibility that each puff contains a slightly different odour can be discounted. Perhaps changes in the regional air-flow over the olfactory receptors may be caused by changing mucosal congestion or exudate in the nasopharynx. The most likely explanation is that there is stimulation of various receptors at different sites with each puff of air, and that the bulb is not activated uniformly. Field studies might help in elucidating this matter.

The most common type of induced wave recorded from the bulb is illustrated in Fig. 3-3. There is initial inhibition of the intrinsic activity, during a slow negative wave. This is followed by sinusoidal activity of about 50 Hz, increasing in amplitude for 150-300 msec. After 300-500 msec, a subharmonic frequency is apparently imposed on this sinusoidal activity. The frequency and amplitude of the compound sinusoid decreases, until it merges imperceptibly into the intrinsic activity again. The envelope of the burst varies, and "flares" may appear. These may occur in a train of the usual spindle-shaped bursts, but were not seen regularly under the present conditions.

The apparent subharmonic frequency described here has been noted only rarely in other species. Biedenbach (1964, her p.9) described a shift of bulbar frequency from 34-54 Hz to a lower one of 20-30 Hz towards the end of the burst, in the awake cat.

Domino & Ueki (1960) and Ueki & Domino (1961) commented that the amygdalar induced wave had a frequency half that of the bulbar (bulb 40-44 Hz, amygdala 20-22 Hz).

Polson (1971) (his p.8-17, 9B-16) commented on the same phenomenon. He suggested that the 40 Hz activity was characteristic of the olfactory bulb, and acted as a "driving frequency" for the cortex. The 20 Hz activity was suggested as being characteristic of the cortical "analyser". A similar suggestion had previously been made by McLennan & Graystone (1965). They recorded 40 Hz activity in both bulb and amygdala of cats by telemetry. They thought that the initial activity was generated in the amygdala, and acted as a "gated scanning system".

3. *Induced waves: intact olfactory cortex*

The induced waves recorded in the olfactory cortex were also variable in amplitude and duration. They usually began 50-200 msec after the onset of the initial DC shift of the bulb with high-frequency, low amplitude sinusoidal activity. This became high-voltage sinusoidal activity, at about half the bulbar frequency. The latter frequency sometimes appeared to be added to it. Like the bulbar responses, they were variable in envelope and amplitude. The frequencies which they apparently contained remained approximately constant in the one animal, about half that of the bulb, i.e. 10-25 Hz.

In some cases this low-frequency activity was not evident in the cortical record, which simply reflected the bulbar frequencies. There was no apparent anatomical correlate; all recording electrodes were behind the nucleus of the lateral olfactory tract, in olfactory cortex lateral to the amygdaloid complex.

Elucidation of the origin and projection of the high-frequency bulbar sinusoidal activity, and the origin and course of the low-frequency cortical activity, will require additional research. This may include stereotaxic mapping, ablation and stimulation studies. Boudreau & Freeman (1963) were unable to show regional differences in the spectral analyses of prepyriform cortical induced activity in the cat.

4a. Induced waves: isolated olfactory bulb

Following transection of the olfactory peduncle just rostral to the anterior olfactory nucleus (see Fig. 3-2), induced activity could be recorded from the isolated bulb for as long as the animal was kept alive. Adrian (1950) showed this in rabbit, if the blood supply to the bulb was intact.

This implies that in these experiments there was no significant damage to the bulbar blood supply or intrinsic neuronal connexions. It has been shown that the bulb of opossum is supplied by end-arteries of a ring anastamosis of anterior, middle, and posterior cerebral arteries (Gillilan, 1972). There has been no such study of the phalanger bulb.

A permanent reduction in spontaneous activity followed transection of the peduncle in these experiments. The induced waves in such an isolated bulb were similar in duration to those of the intact side, and had a similar envelope. Immediately after the stimulus, the high-frequency component began to increase in amplitude and decrease in frequency. Maximum amplitude occurred at about 300 msec, during the burst of 0.8 sec duration illustrated in Fig. 3-9. This maximum amplitude was of the same order as that of the intact side in most cases, but on occasions was randomly higher or lower. The envelope was usually spindle-shaped, but "flares" were sometimes seen.

Initial frequency of the sinusoid was approximately 45-50 Hz, and this decreased to approximately 35 Hz at the end of the burst. A low-frequency component (less than 20 Hz) was never seen in the isolated bulb. Its absence was one of the criteria used at the time of the experiment to assess complete transection, which was universally confirmed by the post mortem anatomical observations.

Affanni, Morita & Samartino (1968) studied the effects of section of olfactory peduncle and for anterior commissure on the bulbar-induced waves of the opossum. They showed that section of the anterior commissure caused an increase in the amplitude and "synchronization" of the induced wave, and subsequent section of the peduncle caused a further increase in the amplitude. They attributed these changes to the influence of two efferent systems. The animals were studied with indwelling electrodes for a month postoperatively. No detailed analysis was made of the frequencies contained in the bursts. The frequency was said not to change from the value reported in a previous paper(40-50 Hz; Vaccarezza & Affanni, 1964). No comment was made of frequency changes within the burst.

Yamanto & Iwama (1961) made similar observations after sectioning peduncles of rabbits.

From the results presented in this thesis it would appear that the intrinsic mechanisms of the bulb are responsible not only for the generation of the induced wave, but also for its duration. Coding of some sort presumably is taking place, and in the normal case, the cortex demodulates this bulbar activity. The reasons for variation in amplitude of the induced wave after isolation of the bulb are not certain. There would probably have been minor interference to blood supply of the bulb; there may have been movement of the bulb relative to the recording electrode or tissue damage may have released CNS-active substances.

The "alerting response", discussed below, was not seen in the isolated bulb.

No certain explanation can be offered for the changes in appearance of the individual bursts. Despite the precautions, there might have been changes in the air flow of each puff; there might have been changes in odour (e.g. PVC) concentration in each puff, although none was added, or there might have been regional differences in air flow causing different patterns of receptor stimulation.

It can be seen in Fig.3-9 that after transection of an olfactory peduncle, the induced waves of the opposite side appear to be "purer"; more markedly sinusoidal. Kerr (1960) has shown that strong stimulation of one olfactory bulb depresses the induced activity of the contralateral bulb *via* the anterior commissure. If such depression were removed (by peduncular section), the contralateral bulb could be expected to show activity closer to that of an isolated bulb.

The results presented here show that the mechanisms responsible for the frequencies, duration, and amplitude of the bulbar induced waves are intrinsic to the bulb. Central influences are not necessary.

The concepts presented by Hernández-Peon et al.(1960) that their electrophysiological evidence indicated a central origin of the induced waves are refuted by the present studies. Since the phalanger has been shown to be so similar to other species in relation to these aspects of olfactory physiology, it is reasonable to conclude that induced activity of the kind recorded in these experiments is provoked only by olfactory stimulation.

4b. Electrical activity of deafferented cortex

Transection of the olfactory peduncle caused an immediate and permanent decrease in amplitude of electrical activity in the ipsilateral olfactory cortex. No induced waves were recorded from the cortex

afterwards in these experiments.

This is additional evidence to refute the claims of Hernández-Peon et al.(1960).

Biedenbach (1964, her Fig. 18) showed a decrease in prepyriform cortical activity after section of the lateral olfactory tract.

Becker & Freeman (1968) described similar effects in cat prepyriform cortex, and suggested that the low "ripple" of 14-20 Hz activity was generated locally. Although 40 Hz bursting cortical activity occasionally occurred on one side only in the non-alert state in their study, removal of the olfactory bulb or section of the lateral olfactory tract completely abolished bursting activity on that side.

Freeman (1968) showed by evoked potential experiments that the mitral cells are responsible for maintaining the cortical neurons in a state of activity. After removal of the bulb, section of the lateral olfactory tract, or local or general anaesthesia, the normal evoked potential (a damped sine wave) was reduced to a single negative peak. At the same time the cortical unit and EEG activity were suppressed. Artificial stimulation of the tract restored the evoked potential to normal. This report is consistent with the conclusions reached in the present study.

5. Alerting response

Olfactory stimulation causing arousal produced a different pattern of induced activity from that in the alert animal.

In the examples given in this chapter, it is not the high-frequency bulbar activity which persists, but the low-frequency activity shown here to arise centrally. In the first such response, low-frequency activity is seen in the contralateral (unstimulated) bulb after a delay of about 800 msec. The origin of this activity has not been investigated. It would not be unreasonable to assume that it has arisen, after numerous synapses, from the ipsilateral cortex. This phenomenon has not been described previously, and indicates a field for further investigation. This pseudo-induced wave might reflect intense cortical activity during the altering process, in which the dominant sensory modality at the time is being closely examined by central mechanisms.

Gault & Leaton (1963) described the onset of induced waves with arousal of cats by noise, but did not note alterations in the normal burst at the beginning of olfactory exploration.

Westecker (1970*a*, her p.95) noted that at the beginning of many experiments many areas of the bulb were silent (recording with tungsten microelectrodes). The first stimulation with air flow produced frequently continuous activity in many cells, which did not stop at the end of stimulatio

Walsh (1959) had previously shown that electrical stimulation of one bulb evoked a potential in the contralateral bulb. However, he assumed it was a result of the direct connexion of tufted cells with the opposite granule cells *via* the anterior commissure. The response was negative in the granule cell layer, and he thought that they were postsynaptic potentials of the internal granule cells. In the light of recent experiments (Polson, 1971), they were probably anterior olfactory nucleus impulses transmitted to the granule cells, and represent a current sink in the granule cell layer.

6. Transection of midbrain

Stimulation of the midbrain reticular formation may affect olfactory electrical activity. Intercollicular transection of the brainstem in the phalanger sometimes reduced the induced activity, but it is not certain whether this was due to swelling or haemorrhage into the brain, or as the primary effect of the transection.

Zernicki (1968) described the responses to olfactory stimuli of the pretrigeminal cat. They consisted of (1) EEG arousal; (2) pupillary dilatation; (3) small vertical eye movements and (4) change in the accommodation. He did not comment on the appearance of olfactory induced waves, but only showed the EEG of visual cortex, his Fig. 6. However, he predicted that it would have more precise olfactory responses than the intact animal because (1) the afferent olfactory input is separated from a possible trigeminal component (2) tracheostomy isolates the olfactory receptors from normal stimulation during nasal breathing, and (3) stimulation may be directly into the nostrils. The latter two situations apply to the present experiments.

7. Hypoxia - hyperoxia

Generalized hypoxia has been shown to interfere with normal induced waves, first increasing the amplitude (Schwartze, 1970b) and then eventually causing them to disappear. These studies confirmed this. It was not thought necessary to illustrate this finding.

Synaptic transmission is depressed in isolated slabs of guinea pig olfactory cortex in hypoxic conditions (Yamamoto & Kurokawa, 1970).

8. Carbon dioxide

Hyperventilation producing hypocapnia is used in clinical EEG studies to increase cortical excitability and unmask foci of epileptic activity. It has been suggested that carbon dioxide functions as an anti-convulsant-tranquillizer (Viukari, 1970-71). In the experiments reported here it has been shown that such minor increases in arterial carbon dioxide levels would not affect the induced waves.

Because of lack of adequate monitoring equipment, it was not possible to study the effects of hypocapnia on the olfactory electrical activity.

Ventilation with 5% carbon dioxide in oxygen made no significant difference to the appearance of the induced waves. High carbon dioxide concentrations have been shown progressively and reversibly to depress transmission in other sensory systems (Gellhorn, 1953; Morris, 1971; dorsal column - medial lamniscus).

9. Neuromuscular blockade

There were no gross changes in the induced waves able to be attributed to the neuromuscular blocking drugs. Evidence has been presented to the contrary by other workers.

Gallamine has been shown to elevate the seizure threshold for lignocaine in rhesus monkeys by some 42% (Munson & Wagman, 1973). This increase was not the result of altered oxygenation, acid/base status, blood-brain barrier, plasma binding of lignocaine, or gallamine-lignocaine chemical interation.

Depolarizing neuromuscular blockers (succinyl choline and decamethonium) were shown not to elevate the threshold in their study.

The study reported in this chapter of induced waves is unlikely to detect this minor anticonvulsant activity of gallamine. It would require a more sensitive parameter, for example, the average evoked response, to detect quantitative changes.

Von Baumgarten, Bloom, Oliver & Salmoiraghi (1963) applied gallamine micro-electrophoretically to cells in the olfactory bulb of rabbits. They found that it did not influence cell firing (their p.133)

10. Hypothermia

There were no apparent changes during cooling of the phalanger from 35°C to 28.5°C. The temperature was measured in the colon, and it was assumed that the brain was at a similar temperature.

Huggins et al. (1968) recorded electrical activity of the olfactory lobes of the caiman, and showed that the olfactory spindles decreased in frequency and amplitude during cooling from 24°C to 14°C, and increased during warming to 35°C. The range of frequencies of the induced waves was 5-21.5 Hz. However, frequencies were relatively constant at 22 Hz in the temperature range 30-36°C (their Fig. 7).

The caiman is a phylogenetically more primitive animal than the marsupial, and it is interesting to note that a species difference has

---

* For example see bibliography No.18 prepared by UCLA Brain Information Service, "Effects of Flaxedil on Electrical Brain Activity", 1963-August 1969

apparently been uncovered by the present experiments.

11. Accessory olfactory bulb

This part of the olfactory system has different receptors and central connexions from the main bulb. It was assumed that it did not contribute significantly to the induced wave recorded in the centre of the bulb, because of the small size of the former. It would require a separate set of experiments to determine its normal function, indeed, if it even produced induced waves. Accessory bulb responses have been examined by Tucker (1971). Transection of the peduncle was immediately rostral to the accessory bulb so the responses of the isolated bulb were from the main bulb only.

CONCLUSIONS

This study has shown that the phalanger is similar to other marsupials and mammals in its induced olfactory activity. In particular, the bulbar induced waves resulting from artificial olfactory stimulation are similar in frequency to those in other species, beginning at 50-60 Hz. The cortical induced waves appear to be subharmonics of the bulbar bursts, and are triggered by them.

Isolation of an olfactory bulb from the rest of the brain does not impair its ability to respond to olfactory stimulation. The resulting induced waves are similar in amplitude, contained frequency and duration to those in the intact bulb. This implies that mechanisms intrinsic to the bulb are responsible for these factors.

An 'alerting response' is described: the first induced wave after a quiescent period is different from subsequent ones. It is longer in duration, the prolongation being caused by an increase in the cortical activity. The significance of this response is not known.

The following manipulations had no significant effect on the olfactory induced waves: midbrain transection, 100% oxygen, 5% carbon dioxide, non-depolarizing neuromuscular blocking agents and mild hypothermia. Chloroform used for initial surgery prevented the production of induced waves.

CHAPTER FOUR

SUMMARY

1. Induced waves in the phalanger olfactory system were modified by nitrous oxide, chloroform, halothane, barbiturates, propanidid, and diazepam. Bulbar evoked potentials were modified by nitrous oxide, barbiturates, propanidid and diazepam.

2. Nitrous oxide produced minimal depression of induced waves or evoked potentials.

3. Chloroform had a local anaesthetic action on the olfactory mucosa in addition to its depressant action on the induced waves.

4. Halothane depressed induced waves in bulb and cortex.

5. Barbiturates caused a transient increase in induces waves of bulb and cortex, followed by depression of all brain function. Mitral cell inhibition following lateral olfactory tract stimulation was prolonged by barbiturates. The olfactory bulb was shown to be significantly depressed by small amounts of barbiturates (pentobarbitone, 2 mg/kg).

6. Propanidid had similar effects to barbiturates on the induced waves, but caused less depression of the bulbar evoked potentials.

7. Diazepam depressed all brain function, including induced olfactory activity.

8. No significant species differences in the response to these drugs were discovered.

9. There were no convincing common structure-action relationships discovered.

EFFECTS OF ANAESTHETIC AGENTS ON ELECTRICAL ACTIVITY IN THE OLFACTORY SYSTEM OF THE PHALANGER

INTRODUCTION

Agents which alter central nervous system function must do so by facilitating or inhibiting the generation, propagation or transmission of impulses. Such alterations may be by direct membrane (receptor) effects, or indirect (metabolic) effects. In simple organisms, such as aplysia, drugs can be applied directly to neural tissue. Resultant changes can be recorded easily, and confident hypotheses and predictions made.

In complex systems, however, there are increased difficulties. The standard routes of drug administration (topical application, parenteral or intraventricular injection) have problems associated with the presence of diffusional and enzymatic barriers, variable distribution throughout the organism, the possibility of indirect effects, and the complexity of the connexions of a particular system. These routes still are used widely for research purposes for several reasons: they are the usual clinical routes, no complicated, delicate and expensive equipment is necessary, and comparisons can be made between drugs given by the same route.

A major advance in neurophysiological methods was made by Curtis & Eccles (1958). They modified the then-common glass micro-electrode to enable them to apply small, known amounts of drugs by electrophoresis, recording from the electrode at the same time. This technique has been used extensively since, to study both central and peripheral nervous systems.

This method, and the standard ones, have all been used to study the effects of drugs on the olfactory system. The purposes of such studies are two-fold: to elucidate the "normal" physiology (and transmitters) of the olfactory system, and to elucidate the mechanisms of drug action and structure/action relationships.

Pharmacological studies on the olfactory system can be considered in six categories: (1) Induced activity; (2) Evoked activity; (3) Unit activity; (4) Behaviour (all on intact, *in vivo* systems); (5) "isolated slab" and (6) Histochemistry (*in vitro* studies). The following discussion will be carried out under these headings. Most emphasis will be given to the first, about which this chapter is primarily concerned.

Pharmacological studies can be divided also according to drug group, and such divisions will be made in this chapter, viz. volatile anaesthetic agents, barbiturates, benzodiazepines, other IV induction agents, other CNS depressants (e.g. phenothaizines) and other agents (e.g. radiation, hypoxia, neuromuscular blocking agents). Analeptics will be considered in the next chapter.

1. Induced activity: drug effects

This chapter is concerned with the effects on induced olfactory activity of anaesthetic agents and central nervous system depressants. Several agents were used in an attempt to uncover similar actions. The published results of other workers will now be discussed briefly under broad headings according to the nature of the drug being investigated.

(a) Volatile anaesthetic agents. This is a large group of substances, many of which are chemically unrelated. Not all in this review have been used in the study about to be reported, but have been included to enable comparisons to be made.

(i) *Nitrous oxide (N_2O).* Domino & Ueki (1959) reported comprehensive experiments on the effects of volatile anaesthetic agents on induced olfactory activity, nitrous oxide being one. They administered it to dogs (80% N_2O in O_2) and found that surgical anaesthesia could not be attained in three of five dogs. In these, there was no change in the neocortical or rhinencephalic electrical activity. In the other two, respiratory rate slowed and the corneal reflex was obtunded. They described high-voltage hypersynchronous waves of approximately 40 Hz, especially in the olfactory bulb. Their Fig. 6, panels B & C, shows this. It is interesting to notice that in their panel C these hypersynchronous waves are inhibited shortly after the onset of inspiration (downward deflexion). No comment was made by the authors of the significance of this observation.

de Jong & Wagman (1963) reported that nitrous oxide decreased the lignocaine seizure threshold from 12 mg/kg to 6 mg/kg in cats. They suggested that such seizures began in the anterolateral nuclei of the amygdala. Although this study was of rhinencephalic activity, no comment was made of the effects of nitrous oxide on induced waves.

de Jong, Heavner & de Oliveira (1972*a*) re-evaluated this finding. Using cats again, they were able to construct dose/response relationships of lignocaine infused at a standard rate (1 mg/kg/min). The

control CD_{50}* (lignocaine) was 7.6 mg/kg; after artificial ventilation with 70% nitrous oxide it rose to 11.4 mg/kg. Again, no comment was made on the effects on the induced waves.

In view of the ease of administration of nitrous oxide, and the certainty of obtaining constant blood levels, it is surprising that more studies have not been reported.

(ii) *Ethers.* Diethyl and divinyl ethers were used by Domino & Ueki (1959) in dogs. These agents were shown to suppress induced waves, reduce cortical activity, and eventually cause spiking and hypersynchronous 12-15 Hz activity (their Figs. 1, 2 & 3). Induced waves (difficult to see in their figures) were related to the spontaneous respiration, and disappeared during phases 1 & 2 of surgical anaesthesia. They did not return for an hour or more after cessation of general anaesthesia. Diethyl ether was also given through an artificial respirator, and hypersynchronous activity of 10-11 Hz was recorded from "the amygdala and related rhinencephalic structures". It is assumed that induced waves were not seen in this experiment. The mechanism of ether action was not clear after these experiments.

Hernandez-Peón, Lavin, Alcocer-Cuarón & Marcelin (1960) showed in cats that diethyl ether prevented the appearance of the "arousal discharges", which were probably induced waves.

Huggins, Parsons & Pena (1968) noted that ether caused the olfactory spindles of the caiman to disappear (for 24 hr or more). They did not comment on the general appearance of the EEG.

(iii) *Chloroform.* There have been few studies reported on the effects of chloroform on the olfactory system. Ottoson (1956) noted that chloroform abolished the electro-olfactogram of the frog.

Sasa, Nakai & Takaori (1967) noted high voltage slow waves and spindle bursts in the cortical EEG of cats after 0.3 - 1.5% chloroform. These spindle bursts were not induced waves.

The action of chloroform on the induced waves has not been adequately described previously.

(iv) *Halothane.* Domino & Ueki (1959) showed that halothane was similar in effect to the ethers on the rhinencephalic activity of dogs (their Fig. 4), both during induction and recovery.

(v) *Other agents.* Other agents which Domino & Ueki (1959)

---

* CD_{50}: Convulsive Dose 50: the dose of drug producing convulsions in 50% of subjects.

used included cyclopropane (28 Hz hypersynchronous amygdalar activity), ethylene 80% (similar to nitrous oxide), and trichloroethylene. This last agent caused 12-17 Hz spike-like activity in amygdala, olfactory bulb and hypothalamus during induction, and grand mal seizures during emergence (their Fig. 7). No mechanism of action was postulated for this phenomenon. In the post-ictal period, 40-80 Hz activity was seen in the amygdala and olfactory bulb.

(b) Barbiturates. The effects of the depressant barbiturates (usually pentobarbitone) have been studied since induced waves were first recognized. These barbiturates produce qualitatively similar changes, and they will be discussed together. The main differences are in duration of action.

Adrian (1942) recorded induced waves from the olfactory bulbs of hedgehogs anaesthetized with pentobarbitone. During light anaesthesia the frequencies within each burst were 25-45 Hz, apparently depending on stimulus intensity. Deep pentobarbitone anaesthesia reduced the frequency within the induced burst to 15 Hz. He subsequently (1950) described similar changes with thiopentone and diallylbarbitone.

Kerr & Hagbarth (1955) described the effects of pentobarbitone on the induced waves recorded in the olfactory bulbs of immobilized cats (their Figs. 4 & 7). They showed that a one-fifth anaesthetic dose of pentobarbitone suppressed the high amplitude asynchronous activity and allowed induced waves to appear. It was suggested that a centrifugal influence was eliminated at its source, because the induced waves could still be suppressed by appropriate anterior commissural stimulation. They also showed (in their Fig. 8) that transection of the anterior commissure also caused augmentation of the induced waves if these were obscured by the intrinsic activity. The implication of these observations is that there are two centrifugal inhibitory systems: one barbiturate-sensitive (basal rhinencephalon), and the other barbiturate-resistant (anterior commissure). At higher (anaesthetic) levels of pentobarbitone, induced waves were eventually suppressed (their Fig. 7F).

Domino & Ueki (1959) included pentobarbitone in their study using dogs with chronically-implanted electrodes. At sub-anaesthetic doses (10 mg/kg) the olfactory bulbar induced waves occurred more often, and were slightly slower in frequency (their Fig. 8B, 30 Hz). After an increment in dosage (20 mg/kg, 30 min after the first dose) the amygdalar and bulbar bursts increased in amplitude, but decreased

further in frequency to 15 Hz and 28 Hz, respectively. A further 20 mg/kg caused surgical anaesthesia, with depression of all electrical activity, except for neocortical *delta* waves (their Fig. 8D). They concluded that the increased burst activity in the amygdala was due to increased afferent input, after noting a reduction after occlusion of the ipsilateral nostril.

Hernandez-Peón *et al.* (1960) showed that pentobarbitone and thiopentone, like ether, prevented the appearance of the "arousal discharges".

Hughes & Mazurowski (1962*b*) reported a decrease in the olfactory bulbar burst frequency (43 - 23 Hz) in the monkey during pentobarbitone anaesthesia (their Fig. 4). They later reported similar changes in the rabbit (Hughes & Mazurowski, 1964; their Fig. 9).

Yamamoto & Yamamoto (1962) reported the changes caused by pentobarbitone 2.5 mg/kg in the rabbit. The control bursts (onset 45 Hz, termination 30 Hz) were slowed to 15 - 20 Hz (by measurement of their Fig. 2B).

Biedenbach (1964) illustrated the effects of deep pentobarbitone anaesthesia in her Fig. 22. The dose was probably 40 mg/kg, given in increments over one hour (her p. 50). The usual activity was replaced by isolated spikes (0.5 - 1/sec), with an isoelectric record between. She suggested that pentobarbitone may have had a similar mode of action to that in the spinal cord (Domino, 1962) of blocking inhibition at low doses, and depressing all neurones at high doses.

Ueki, Tanaka & Sugano (1964) described the effects of thiopentone on artificial sniffing imposed on immobilized cats, an experiment similar to the ones about to be reported in this thesis. Their Fig. 6 shows the changes following IV thiopentone, 2.5 mg/kg. Within 15 sec the induced waves had disappeared without transient amplitude increase. Spike activity began to occur in the bulb and cortex, and the bulbar slow wave was markedly reduced. Return to control appearances took 25 - 30 min.

Ueki & Fukuda (1966) used a similar experimental method with rabbits, and showed again that pentobarbitone depressed the amplitude and frequency of the induced bursts. In the example illustrated in their Fig. 5, the isolated bulb (L-OB) appears to be more severely depressed than the intact one. No comments were made about this, or whether there was a transient increase in amplitude (noted by Domino & Ueki, 1959).

Fukuda (1966) illustrated similar changes with thiopentone 3 mg/kg in intact and isolated bulbs of rabbits (his Fig. 1E & F). Electrical stimulation of the anterior commissure reduced the amplitude of the bulbar burst in the intact bulb (his Fig. 2A). This depression was not affected by thiopentone 3 - 5 mg/kg. On the other hand, the depressant effect of amygdalar stimulation was eliminated (his Fig. 3A). These findings are remarkably similar to those of Kerr & Hagbarth (1955). It may be noted that Fukuda's Fig. 10A shows depression of the burst in both intact and isolated (L-OB) olfactory bulbs following anterior commissural stimulation. This casts some doubt on the completeness of bulbar isolation.

Khazan, Kandaluft & Sulman (1967) made similar observations in rabbits, as did Huggins, Parsons & Pena (1968) in the caiman. Timiras, Woolley, Silva & Williams (1967) showed similar changes in X-irradiated rats (their Fig. 1).

(c) Benzodiazepines. There is only one previous report on the effects of benzodiazepines on induced activity in the olfactory system. Fukuda (1966) used diazepam (3 mg/kg) and chlordiazepoxide (1 - 10 mg/kg) in rabbits, and showed that both reduced the amplitude and frequency (38 - 42 Hz to 15 - 25 Hz) of the bursts in the intact (his Fig. 1A & B) or isolated bulbs. They had minimal, if any, effect on the depressive influence of anterior commissural stimulation (his Fig. 2). The effects of amygdaloid stimulation were blocked by both agents (his Figs. 3 & 4). These were similar effects to those of thiopentone (reviewed in his Table 1), suggesting a dual efferent system with differential drug sensitivity.

de Jong & Heavner (1971, 1972) implied that systemic diazepam acted in the limbic system to prevent or modify lignocaine-induced seizures in cats. Wale & Jenkins (1973) used intravenous and discrete intracerebral micro-injections of diazepam in cats to modify similar seizures. They found that micro-application of diazepam to brain stem reticular formation prevented the lignocaine convulsions "in every instance" (their p. 148). Diazepam at other sites (hippocampus, amygdala, hypothalamus) incompletely blocked the lignocaine convulsions.

(d) Other anaesthetic agents. Various other central nervous system depressants have been used to influence olfactory electrical activity.

(i) *Chloralose.* Adrian (1942) noted that chloralose had a depressant effect on bulbar induced waves. This was also noted by

Domino & Ueki (1960) who commented on concurrent high amplitude, low frequency (3 - 6 Hz) cortical activity, as did Biedenbach (1964, her Fig. 23).

(ii) *Urethane.* Adrian (1950) was the first to comment on the effects of urethane, and showed that it caused less depression of the induced waves than the barbiturates. Ottoson (1959*a*) showed that the induced wave was eventually abolished by urethane (his Fig. 5), a finding which was confirmed by Schwartze (1970*a*, his Fig. 1a & b).

(iii) *Phencyclidine.* This hallucinogenic agent (Sernyl, Parke-Davis), now proscribed in this country, was reported by Adey & Dunlop (1960) to cause spiking activity in amygdala and pyriform cortex of cats. Domino (1964) reported depression and eventual obliteration of induced bursts recorded from the olfactory bulb and amygdala of dogs (his Fig. 2). Similar changes were seen in the monkey (his Fig. 3). Ueki, Tanaka & Sugano (1964) reported a transient increase in the bulbar bursts of cats, to be followed by their abolition (their Figs. 7 & 8).

(iv) *Phenothiazines.* Domino & Ueki (1960) reported the effects of chlorpromazine, 1 mg/kg, on the induced waves of monkeys and dogs. There appeared to be a reduction in tidal volume and respiratory rate. The olfactory bulbar bursts decreased with respiration. Frequencies were not stated, neither could they be counted in the figures. Arousal of the animal improved the burst for a short time.

Chlorpromazine was shown to enhance burst activity in cats at low doses (1 - 2 mg/kg) without change in frequency (Ueki, Tanaka & Sugano, 1964). Higher doses (to 10 mg/kg) caused further increase in amplitude and a reduction in frequency. Large doses (45 - 50 mg/kg) caused seizure activity (their Fig. 10). Bulbar bursts during the phase of post-ictal depression were prominent, and shown to have a frequency of 36 Hz (their Fig. 109, last 3 responses). In their Fig. 9, however, they show that 5 mg chlorpromazine/kg abolished the bulbar induced waves.

Fukuda (1966) reported a reduction in amplitude and frequency of the bulbar bursts in rabbits (his Fig. 1C). There was also antagonism of the depressant effects of stimulation of hypothalamic and mid-brain reticular formation (his Figs. 6 & 9), similar to that of thiopentone (his Table 1). Comparable findings were made by Khazan, Kandaluft & Sulman (1967).

(v) *Opiates.* Morphine was given to monkeys and dogs by Domino & Ueki (1960). At 1 mg/kg there was respiratory depression with parallel olfactory bulbar depression.

Hughes & Mazurowski (1962*a*) illustrated the depth of anaesthesia which could be attained with pethidine (their Fig. 5). Induced waves were reduced in duration and frequency. Fukuda (1966) showed that morphine (5 - 10 mg/kg) had minimal effects on the induced waves of rabbits.

2. Evoked activity: drug effects

Evoked potentials in the nervous system have been the subject of much research since Caton first described visually evoked responses nearly 100 years ago (1875). Added impetus was given by the development of computer hardware to allow on-line averaging of sensory- or electrically-evoked potentials.

Potentials may be electrically evoked in the olfactory system by orthograde or retrograde spread of the stimulus. For example, stimulation of the lateral olfactory tract will cause electrical activity at least in the bulb and olfactory cortex. Interpretation of the resulting potentials has been facilitated by the work of Freeman (numerous papers 1959-1974), Rall, Shepherd, Reese & Brightman (1966), and Rall & Shepherd (1968), discussed earlier.

Pharmacological modification of the various potentials evoked in the olfactory system has been undertaken by numerous workers. The aims of these experiments usually have been to determine drug action, or olfactory system function. Numerous agents have been used in this field, and include studies on interacted evoked potentials. A brief review of some of the published work of R. A. Nicoll will be followed by a review of others' work.

R. A. Nicoll

Evoked potentials in rabbit olfactory bulb following lateral olfactory tract stimulation were described fully (1969). The inhibition of a test stimulus by a preceding conditioning stimulus supported the hypothesis of a dendrodendritic inhibitory pathway. The inhibition was related to the conditioning-test interval of the paired lateral olfactory tract stimuli (his Fig. 7). Maximum inhibition occurred at about 15 msec, with no inhibition beyond 150 msec.

There are some technical points which may complicate interpretation of his results. Initial surgery was performed with ether anaesthesia, but maintenance was with pentobarbitone, which has been shown to be

very active in altering olfactory system function (e.g. Kerr & Hagbarth, 1955; this thesis). The bulb under study was isolated from the rest of the brain by transection of the "peduncle", illustrated in his (1969) Fig. 1. Such a transection was admitted to leave some pre-pyriform cortex and olfactory tubercle (his p. 159), and also would have left most of the anterior olfactory nucleus. The claim that this would be "chronically decentralized" is inaccurate. His experiments in which the anterior commissure were stimulated (his Fig. 10) may need re-evaluation in the light of the presence of ipsilateral anterior olfactory nucleus.

Effects of olfactory nerve stimulation were reported in a subsequent paper (Nicoll, 1972*a*), again in rabbits under pentobarbitone anaesthesia. Comparisons were made between potentials evoked in the bulb by surface and lateral olfactory tract stimulation. Interaction between the two were also studied, and a proposed sequence of events was explained (his p. 196).

These papers formed the background for a study of the effects of anaesthetics on excitation and inhibition in the olfactory bulb (Nicoll, 1972*b*; which appeared after my experiments were completed). The evoked potential (recorded in the external plexiform layer after lateral olfactory tract stimulus) had an initial negative component (N_1) said to be due to the antidromic invasion of mitral cells and their dendrites, and a second negative component (N_2, often with an inflexion) said to be due to depolarization of the mitral-to-granule component of the dendrodendritic synapse (his Fig. 1A). All anaesthetics tested (halothane, ethanol, hexobarbitone and chloralose) depressed both negative components, the second more than the first. (The depression was expressed as the ratio of the two negative amplitudes, not with reference to the pre- and post-drug amplitudes.) All were shown to depress both synaptic excitation of granule cells and antidromic invasion of mitral cells.

Interactions between a conditioning tract stimulus and a following test stimulus were used to assess drug effects on synaptic inhibition. Pentobarbitone was said to prolong this inhibition in the dose range 3 - 60 mg/kg. His (1972*b*) Fig. 4 illustrates the effects of 30, 50 and 70 mg/kg. The control (drug-free) conditioning stimulus caused inhibition of the test response for some 140 msec (the 11th test stimulus by direct measurement of his Fig. 4A). Pentobarbitone 30 mg/kg caused some inhibition for 400 msec. The data presented in

his Fig. 4 for pentobarbitone 70 mg/kg are difficult to interpret. His dose-response curve (his Fig. 7B) shows that the test response reaches 80 - 85% of the control value in 170 msec. However, the conditioning response in this case is 1 mV, compared with the control value of 3 mV (his Fig. 4A). No explanation is given. Chloralose (50 and 150 mg/kg), halothane (2.0 and 2.5%) and chloral hydrate (80 and 200 mg/kg) were all shown to prolong inhibition of the test response by the conditioning response (his Fig. 5A, B & C). The same drugs, and hexobarbitone, were also shown to prolong the P-wave (which is probably the late negative response recorded in the granule cell layer, representing a source of current during inhibition by granule cells). The P-wave duration was shown to correspond in one example (his p. 80a) to the duration of inhibition of a test tract stimulus (his Fig. 6).

There now follows a brief review of some drug effects on evoked potentials in the olfactory system.

(a) Barbiturates. Biedenbach (1964) studied the effects of pentobarbitone (*inter alii*) on the damped sinusoidal potential evoked in the prepyriform cortex by stimulation of the lateral olfactory tract in cats. A reduction in the oscillations was shown (her Figs. 19 & 22), but no firm conclusions were drawn regarding the mode of action of pentobarbitone. The relationship between loop gain and feedback delay (assuming a single loop - probably not a valid assumption bearing in mind Kerr & Hagbarth's 1955 findings) was altered, but the site of pentobarbitone action was not determined by this method.

Freeman (1968*a*) reported that pentobarbitone suppressed background unit activity and tract-cortex evoked responses in cats. He also (1968) depicted the changes (his Fig. 1) of an oscillatory to a non-oscillatory response. Willey & Freeman (1968) confirmed these findings in the cortex. They also showed that the bulbar response, although converted to the open-loop (non-oscillatory) state, was still present at low (1.5 threshold) stimulus levels.

Jalfre, Monachon & Haefely (1971) showed that hexobarbitone reduced the amplitude of the amygdalo-hippocampal evoked potential in cats, without changing its latency (their Fig. 4).

(b) Propanidid. Fujita, Ishikura & Kitana (1972) examined the effects of propanidid on olfactory evoked potentials in the immobilized rabbit (electrical stimulation of olfactory mucosa, recording in bulb and amygdala, "terminal nucleus". Propanidid (5%) was given at a rate

of 40 - 50 mg/kg/min, an order higher than that used in clinical anaesthesia. Latency of the bulbar response was unchanged (35 msec), but amplitude was reduced by some 30% in 1 min, and after-potentials disappeared. In the same time the amygdaloid potential disappeared completely. These results were said to demonstrate incomplete inhibition of the monosynaptic pathway, and complete inhibition of the polysynaptic pathway.

(c) Benzodiazepines. The limbic system, including the olfactory system, has been shown to be sensitive to the benzodiazepines (for example, de Jong & Heavner, 1972). Jalfre, Monachon & Haefely (1971) reported the effects of four of this group of drugs on the amygdalo-hippocampal evoked potential in the cat. Nitrazepam, diazepam, chlordiazepoxide and medazepam depressed the amplitude of this evoked response and increased its latency (their Figs. 3, 4 & 5). They concluded that the benzodiazepines acted on the amygdala itself, or on the multisynaptic efferent pathway. (Hexobarbitone, however, depressed the amplitude without affecting latency, implying that it affected the "target organ", the hippocampus.)

There have been many other studies to determine the central nervous system sites of action of diazepam. Stratten & Barnes (1971) refer to some of these in their report which showed that diazepam enhanced presynaptic inhibition in the spinal cord of decerebrate immobilized cats.

A similar implication can be drawn from the results of Sharer & Kutt (1971). They showed that peripheral motor activity following penicillin-induced focal seizures was abolished, although cortical activity was still present (their Figs. 2, 3 & 5).

3. Unit activity: drug effects

The study of unit activity in the olfactory system has been useful because of the certainty of identification of the mitral cell by antidromic activation by lateral olfactory tract stimulation. It must be remembered that the dendrodendritic synapse, the key to olfactory bulbar function, was not described until 1966 (Rall, Shepherd, Reese & Brightman). This did not discredit the obviously well-designed and executed experiments of workers such as Green, Mancia & von Baumgarten (1962); von Baumgarten, Green & Mancia (1962*a*); Yamamoto, Yamamoto & Iwama (1963); Ochi (1963); or Bloom, Costa & Salmoiraghi (1964) to name only a few.

Following the description of the gemmule and dendrodendritic

synapse, Nicoll (1970, 1971*b*) showed that γ-aminobutyric acid (GABA) was probably the inhibitory transmitter to the mitral cell secondary dendrite. Felix & McLennan (1971), working in Professor Curtis's laboratory, reached the same conclusion independently, also using the GABA antagonist, bicuculline.

The pharmacology of neurones in the olfactory cortex has been reported by various groups. Randić & Straughan (1965), and Legge, Randić & Straughan (1966) reported the effects of various amino acids and putative transmitters on cat prepyriform cortical cells after micro-electrophoretic application. L-glutamate was an invariable excitant, and GABA inhibited spontaneous or glutamate-induced firing. No firm conclusions about drug actions or transmitters could be made.

4. Olfaction and behaviour: drug effects

It is not within the scope of this thesis to review this aspect of olfaction, which has been intensively studied since MacLean & Delgado (1953) applied acetylcholine to the olfactory cortex of cats, noting behavioural changes similar to those caused by electrical stimulation of the same area. For example, Penaloza-Rojas & Zeidenweber (1965) showed that injection of adrenaline into the olfactory bulbs of cats caused sleep, while acetylcholine caused alertness.

5. Cortical slabs: drug effects

(a) In vitro. Yamamoto & McIlwain (1966) described the preparation and maintenance of thin slices of guinea pig brain consisting of lateral olfactory tract and an attached thin sheet of olfactory cortex. Electrical stimulation of the tract enabled an orthodromic volley to be transmitted to the cortex, and the resulting surface fields to be measured.

This experimental method subsequently has been defined more fully. Richards & Sercombe (1968) described more fully the cortical surface and depth electrical changes. Changes in the artificial extracellular fluid altered the evoked response (Richards & Sercombe, 1970). Their interpretation was that calcium ions increased transmitter output, and magnesium ions decreased it.

Functional distribution of the transmitter was described by Richards (1972*a*). Using a conditioning stimulus or train, he measured the test response at varying intervals afterwards. At conditioning intervals up to 10 msec, and again from 300 msec to 5 sec, the test response (extracellular e.p.s.p.) was smaller than the conditioning

one (his Fig. 1A). The test response was potentiated with conditioning intervals between 10 and 200 msec. Post-tetanic depression (for 30 sec) was followed by potentiation for at least 2 min (his Fig. 6). He postulated the existence of a three-compartment transmitter system: (1) an immediately available store with slow replenishment; (2) conditionally available transmitter, "primed" by a lateral olfactory tract volley above 5/sec; and (3) main depot transmitter.

Having defined his model, Richards investigated the mechanism of pentobarbitone anaesthesia (1972*b*). He considered four cellular mechanisms which could have been affected: (1) conduction of impulses along incoming or outgoing axons; (2) chemical transmission; (3) propagation of excitatory impulses from synapse to axon hillock; and (4) the threshold at which impulses are initiated. The most likely mechanism, on the basis of his results, was that pentobarbitone reduced the output of the unknown transmitter from the presynaptic nerve terminals of the olfactory cortex.

He used the same model to study the mechanism of halothane anaesthesia (Richards, 1973), considering the same four possible mechanisms. He concluded that halothane reduced excitatory synaptic transmission by reducing transmitter output or sensitivity of the post-synaptic membrane to the released transmitter.

(b) In vivo. Goldring, O'Leary, Holmes & Jerva (1961) described the application of drugs (GABA, pentobarbitone and procaine) to acute and chronically undercut cortical slabs in the cat. The drugs were applied topically, and the pentobarbitone and procaine also given parenterally, modifying the responses to direct cortical stimulation. Freeman (1968*b*) and Becker & Freeman (1968) undercut, deafferented or isolated areas of the olfactory cortex of cats, and defined the characteristics of each preparation. This method has not yet been fully utilized in pharmacological studies.

Despite the variety of pharmacological investigations into the olfactory system, no excitatory, and only one inhibitory, transmitter has been proven. In this setting, it was considered reasonable to investigate drug effects on this system, with two major objectives: elucidation of transmitters, and determination of structure-action relationships of the drugs themselves.

A major problem in the olfactory system is that the nature and function of the inhibitory systems are not known, particularly with respect to physiological activity (intrinsic and induced).

Pharmacological alterations of inhibition of the mitral cell via the dendrodendritic synapses (the only inhibitory inputs to the mitral cell) would alter mitral cell excitability. If inhibition were to be removed for a prolonged period, there could be three possible results: (1) an increased number of mitral cells would discharge in response to a standard stimulus; (2) the rate of firing of the mitral cells would increase after a standard stimulus or (3) instability would be caused in the system by removal of negative feedback. It is premature to speculate that the results of these actions would be respectively: (1) an increase in the amplitude of the bulbar induced waves; (2) an increase in the frequencies within the induced wave, or (3) oscillations or convulsions within the bulb.

Such changes could be studied best in the isolated bulb, assuming that no significant damage was done to its components. Any changes seen in an intact bulb reflect not only local (bulbar) changes, but also alterations in efferent activity.

The responses of the olfactory system of the phalanger to standardized stimuli (olfactory and electrical) have been described in Chapters 2 and 3. It was decided to attempt to modify these responses with drugs generally classified as anaesthetic agents, in the hope that this might provide insight into both the working of the olfactory system and the mode of action of these anaesthetic agents.

The hypotheses to be tested are: (1) that anaesthetic agents alter olfactory activity in the phalanger in the same ways as in other species; (2) that there is a well-defined structure-action relationship between these drugs acting on the olfactory system; (3) that all parts of the olfactory system are equally susceptible to the action of these drugs.

These will be examined within the constraints imposed by the experimental methods, recognizing that the olfactory stimulus to the immobilized, artificially ventilated animal does not replicate normal sniffing in the unrestrained state.

METHODS

Surgical preparation for recording was carried out as described in Chapter 3. Briefly, nitrous oxide/oxygen/halothane anaesthesia was used during all surgery and until recording began. Recording electrodes were placed in the dorsal granule cell layer of each bulb, and in the olfactory cortex. The usual stereotaxic co-ordinate was

A15 L7. Electrode positions were adjusted until electrical stimulation of the cortical electrode evoked a maximal positive response in the ipsilateral bulb.

At the completion of surgery and electrode placement, local anaesthetic solution was infiltrated into pressure points and tympanic membranes, surgical wounds were coated with benzocaine ointment, and each cornea anaesthetized with amethocaine solution. The animal was then paralyzed with gallamine and artificially ventilated. Transection of the olfactory bulbar peduncle, when performed, was achieved with a fine hand-held spatula under direct vision. Intercollicular brain stem section was carried out with a stereotaxically-positioned spatula.

Olfactory stimulation was by "puffs" of untreated compressed "medical air" (C.I.G.), duration 100 - 300 msec, peak flow rate 3 l/min, repeated every 5 - 10 sec to one or both nostrils.

After suitable preamplification, the electrical signals were recorded on analogue magnetic tape, and monitored by oscilloscope and polygraph. All illustrations have been retrieved from tape, replayed either at the recording speed, or, more commonly, at one-tenth recording speed.

Recovery from the general anaesthetic was rapid, often within five minutes, judged by the onset of induced waves. Despite this, at least one and a half hours were allowed to elapse before the first drug was given. If the first agent tested was volatile, at least an hour was allowed after it had been withdrawn before another was given. When a non-volatile agent was used, at least two hours were allowed before it was repeated, judged by the return of the induced waves to their control appearance. Gallamine was given when indicated clinically, and no records were used within 15 min of such administration.

The effects of drugs on evoked potentials were tested in some experiments. One of the bipolar cortical electrodes was able to be switched to allow an electrical stimulus to be applied to it. The resulting evoked potential was displayed on the Tektronix 502A CRO and photographed. One evoked potential was recorded each minute; for the rest of the time (55 sec) this electrode was used to record the cortical activity.

The resulting photographs were projected onto graph paper and traced (usual magnification x 20). Graphic subtraction was able to be carried out.

Each drug used will be described and discussed separately.

Nitrous oxide

Introduction

Joseph Priestly discovered the respirability and extraordinary effects of nitrous oxide in 1799. Humphry Davy (1800) was induced to carry on an investigation into the composition, properties, combinations and mode of operation on living beings. He noted that it had similar effects to those of alcohol, thereby beginning research into the anaesthetic state.

Nitrous oxide is administered during the majority of all anaesthetics given in this country, but its mode of action is still unknown. It does not produce "surgical" anaesthesia in man in concentrations of 75% - 80%, but obtunds consciousness, producing amnesia and analgesia (Parbrook, 1967). It has been shown to have minimal effects on various brain systems in animals (trigeminal system, cats: Haugen & Melzack, 1957; Kitahata, McAllister & Taub, 1973; thalamic relay nuclei of cats: Mori *et al*., 1972 ; auditory system of cats: Sasa, Nakai & Takaori, 1967; mesencephalic reticular neurones of cats: Shimoji & Bickford, 1971; dorsal horn of cats: de Jong, Robles & Heavner, 1970), and recently reviewed by Yamamura & Kato (1970), Clark & Rosner (1973) and Rosner & Clark (1973). Its general pharmacology has been reviewed recently (Smith, 1971).

Methods

Nitrous oxide, 75% in oxygen, was administered *via* the respiration pump to seven animals from which induced waves were able to be recorded. These gases were metered with the anaesthetic machine and led to a reservoir at the inlet of the respiration pump (Palmer). The flow rate to this reservoir exceeded the minute volume; excess gas escaped to the room. This mixture was chosen to reduce the likelihood of hypoxia resulting from inadvertent minor hypoventilation. Respiratory or blood gases were not monitored. Recording of electrical activity continued during administration and withdrawal of the nitrous oxide. Olfactory stimulation was at the constant rate, and in two cases, evoked potentials were recorded also.

Results

It was discovered early and painfully that the phalanger could still respond vigorously and purposefully to minor sensory stimuli despite apparent sedation with 75% nitrous oxide.

Induced waves. Induced waves were recorded from olfactory bulbs

and cortex throughout the administration of nitrous oxide to the immobilized phalanger. Pre-drug records are illustrated in Figs. 4-1A (upper, real-time) and 4-1B (lower, 0.1 real-time) from both intact bulbs and cortices of the immobilized phalanger. The usual induced waves are seen, with initial high frequency onset, and low frequency cortical component apparently added later in the burst. After 10 min ventilation with 75% nitrous oxide, there was an increase in the amplitude of the intrinsic activity of bulbs and cortex. This activity became more sinusoidal in character. The induced waves became less prominent with the increase in intrinsic activity (Fig. 4-1C, upper). When written out slowly (Fig. 4-1D, lower), the bulbar high frequency activity was still present, but there was a reduction in the low frequency cortical component. There was no apparent change in the frequencies within the bulbar burst.

There was usually a change in the intrinsic activity of bulb and cortex in the other experiments, but it varied from experiment to another without apparent reason. The change was usually an increase in the amplitude of the background activity, with an increase in "synchronization" of this, with a dominant frequency of 40 - 50 Hz. This was seen within 5 min of beginning the nitrous oxide. On other occasions, there was high frequency activity (40 - 100 Hz) at increased amplitudes, with occasional apparently random spikes.

Maximal peak-to-peak amplitude of the induced waves was measured in one experiment, and the mean of the six bursts in each minute calculated. In the cortical record, the amplitude was reduced by 25% after the second minute of nitrous oxide administration. It returned to control levels after a further six minutes. During the early phase of amplitude depression, there was a decrease in duration of the bursts. (This parameter is difficult to measure, however, because of the indistinct end-point of the burst, as it merges into the intrinsic activity.) The bulbar induced wave was relatively unaffected in this experiment. There was no significant change in the duration or frequencies within each burst, but a slight (15%) increase in mean maximum amplitude was noted between the third and sixth minutes.

Results from another experiment are illustrated in Fig. 4-2. The induced waves from an intact bulb and its cortex are shown at times zero, 2, 5 and 15 min of ventilation with 75% nitrous oxide. In this case, there is little change in the induced waves of the bulb. The intrinsic activity of bulb and cortex becomes more regular in frequency

and sinusoidal in character. The low frequency induced waves of the cortex are never prominent in this example, and are less obvious after nitrous oxide.

Evoked potentials. In two experiments, a study was made of the effects of nitrous oxide on responses evoked in the bulb by paired electrical stimuli to the cortical electrode. The stimuli were supra-maximal, 45 msec apart. Tracings of the responses after 15 min nitrous oxide are shown in Fig. 4-3. The solid line in A and C is the control response; the broken line is the response after nitrous oxide. Fig. 4-3A shows the responses with preamplifier band pass set at 0.15 Hz - 1 KHz; Fig. 4-3C is the same signal with band pass 7 - 100 Hz (which differentiates below 7 Hz). There is a decrease in the initial positive response of the conditioning (first) stimulus, and a slight increase in positivity of the test (second) response. The late negative is slightly augmented. Graphic subtraction of the nitrous oxide from the control response produces the result illustrated in Fig. 4-3B & D. This shows that nitrous oxide has removed part of the initial positive of the conditioning response, and augmented the late negative of that response. There is also some subtraction from the late negative component of the test response.

Discussion

This study on the effects of nitrous oxide has shown that the agent produces only minor electrophysiological or behavioural changes in the phalanger. It was not expected to induce surgical anaesthesia, and it did not cause major changes in the induced waves or evoked potentials.

The significance of the minor increase in bulbar burst amplitude is not known; it might represent the removal of an inhibitory influence. The bulbar bursts were affected least when the cortical component was minimal. When the spontaneous cortical activity was increased, however, all induced waves were shorter in duration. This implies that the bulbar mechanisms themselves are relatively resistant to the effects of nitrous oxide, but reflect cortical (efferent) activity. These conjectures will require further studies.

Interpretation of the observations on the evoked potentials in the bulb is also difficult. It is suggested that nitrous oxide produces non-specific inhibition of both excitatory (positive) and inhibitory (negative) mechanisms. This aspect will also require the full conditioning-test evoked potential experiment (tract to bulb). Stimulus

parameters and conditioning-test stimulus intervals would have to be varied to allow estimates to be made of conduction times, delays, and duration of inhibition of mitral cells. Nitrous oxide administered by controlled ventilation has the advantage that a constant blood (and presumably brain) level can be maintained during data acquisition.

My experiments do not correspond precisely with those of Domino & Ueki (1959) in several respects, the most important being in their reliance on spontaneous inspiration to supply the olfactory stimulus. The findings, however, are compatible.

Conclusions

In the phalanger, 75% nitrous oxide produces minimal changes in the induced waves recorded in the olfactory bulb. When cortical induced waves are present, they are reduced by the agent. If an increase in cortical activity is produced, there is a reduction in the duration of the induced wave, as if bulbar inhibition were increased. These effects will require further assessment.

Nitrous oxide produces depression of both components of the bulbar evoked potential, suggesting non-specific depression. Again, further investigation will be required to clarify this point.

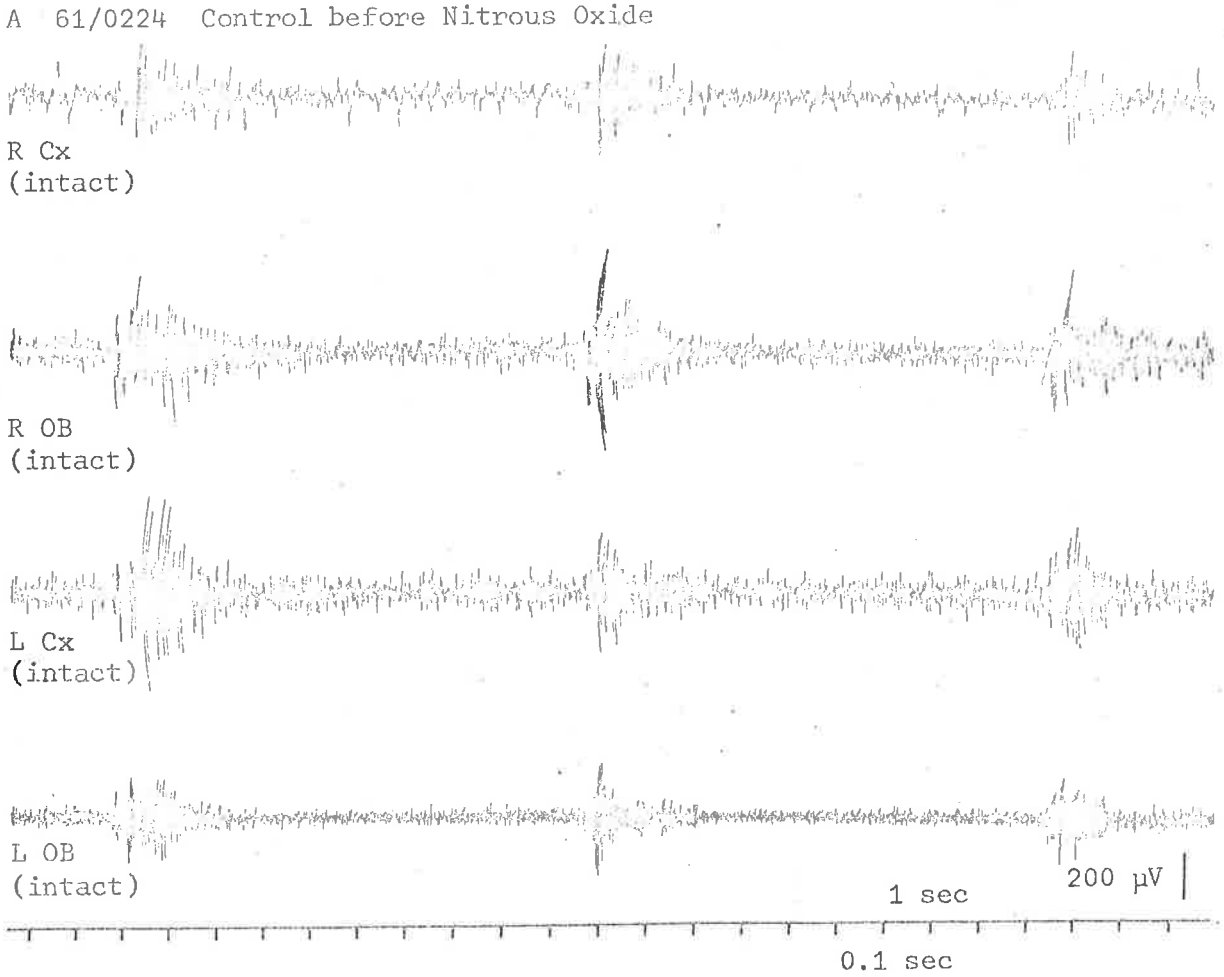


Fig. 4-1A (Upper) Induced waves recorded from both intact bulbs and olfactory cortices of the phalanger. The usual spindle-shaped burst is produced with each stimulus. Real time.

B (Lower) The first induced wave written out at 0.1 recording speed. No drugs.

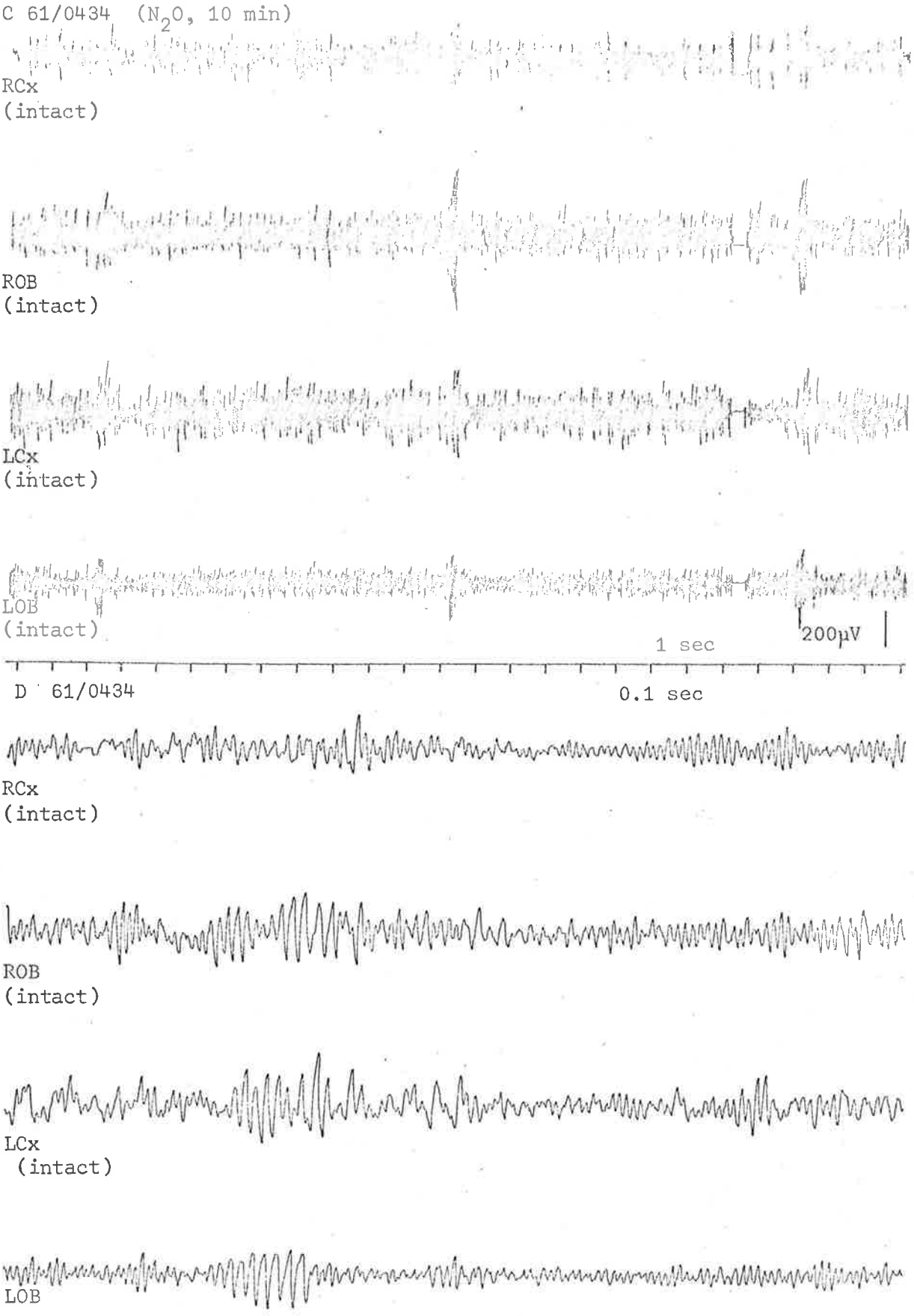


Fig. 4-1C (Upper) Olfactory bulbar and cortical records after 10 min administration of nitrous oxide 75% to the phalanger. The induced waves are masked by the high frequency activity.

D (Lower) The first induced wave written out at 0.1 recording speed.

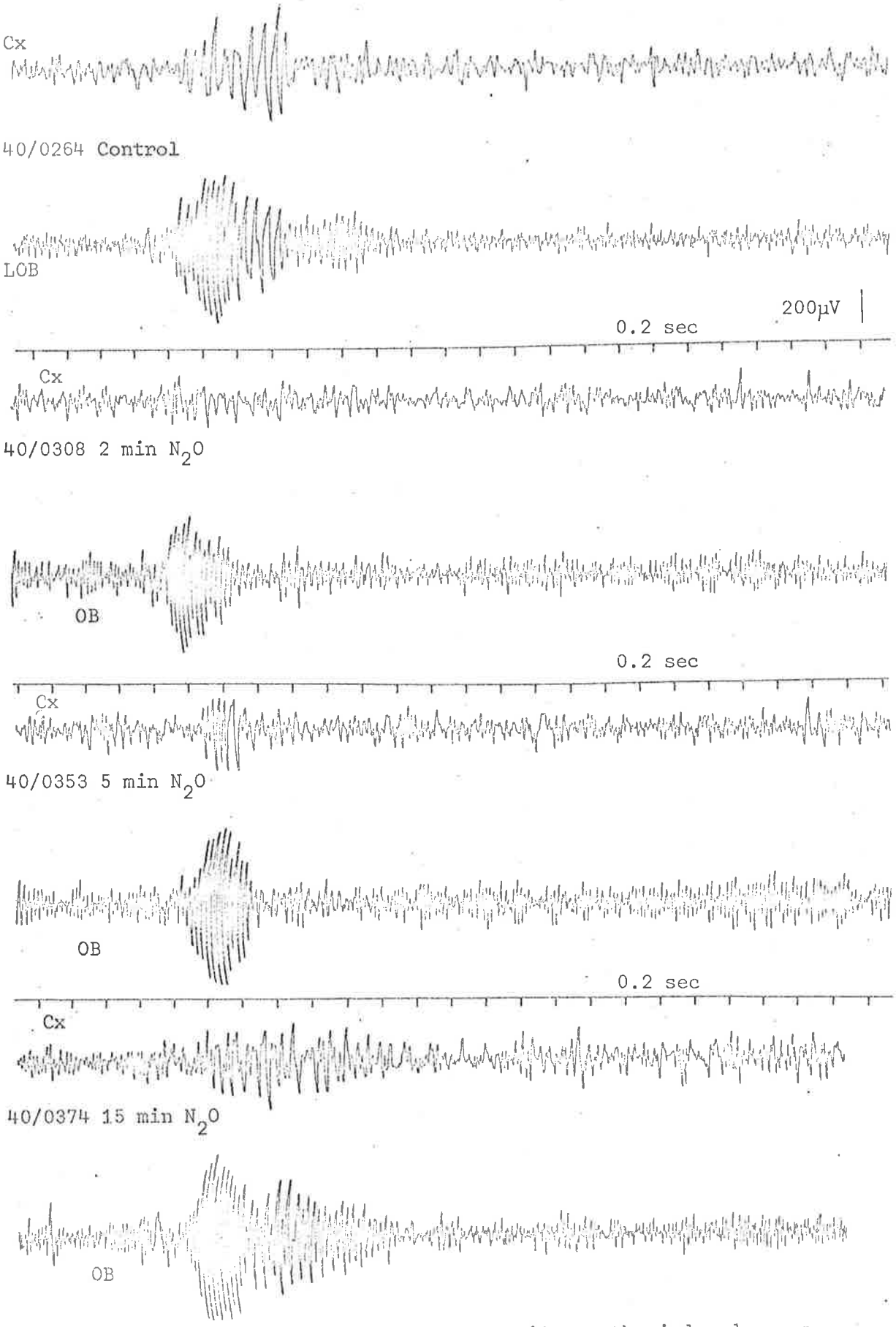


Fig. 4-2. Progressive effect of 75% nitrous oxide on the induced waves recorded from the intact olfactory bulb and cortex of the phalanger at times zero, 2, 5 and 15 minutes.

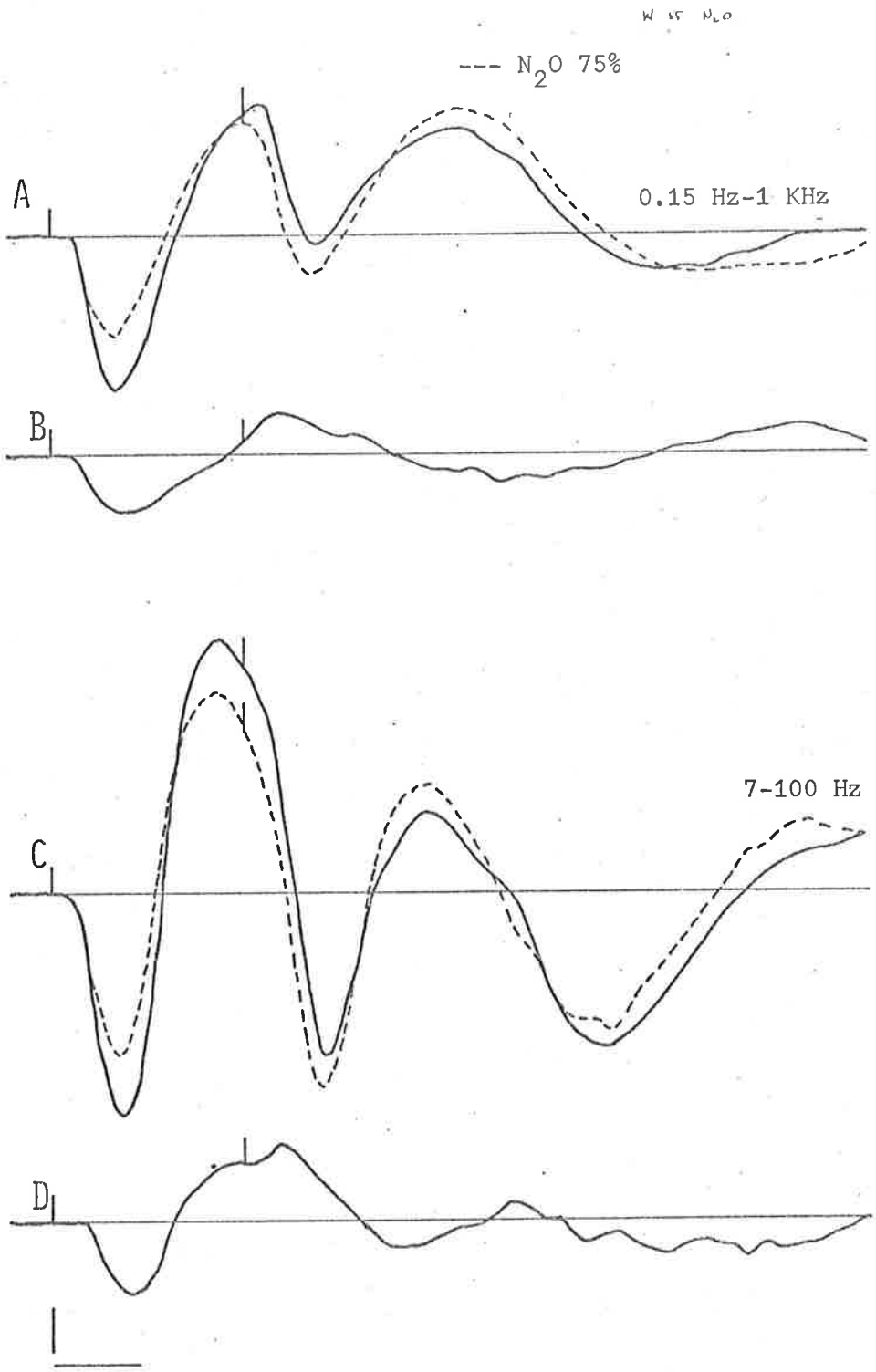


Fig. 4-3 A. Responses evoked in the olfactory bulb of the phalanger by stimulation of olfactory cortex with two electrical pulses, 45 msec apart. Drug-free response solid line, nitrous oxide 75% dotted line. Band-pass 0.1 Hz - 1 KHz.

B. Graphic subtraction of the nitrous oxide response in A from the control response.

C. The same signals as A, but band-pass set at 7-100 Hz.

D. Graphic subtraction of the two responses in C.

(Scale 20 msec, 1 mV)

Chloroform

Introduction

Chloroform, trichloromethane, was first prepared in 1831 by Liebig. It was first employed for anaesthesia by James Young Simpson in 1847 in Scotland. Physical and chemical data are available (Secher, 1971). It is not used widely in contemporary anaesthetic practice because of its hepatotoxicity.

Chloroform was used as the general anaesthetic in the initial experiments, until it was decided to try halothane. This single change in anaesthetic technique enabled induced waves to be recorded in all subsequent experiments. The effects of chloroform were studied in two animals prepared with halothane, in an attempt to determine the reasons why induced waves could not be recorded after chloroform.

Methods

The experimental system was devised to allow chloroform vapour to be applied to the olfactory mucosa or lungs independently, to attempt to differentiate between local and systemic effects.

The first part consisted of blowing chloroform vapour into the nasal cavity *via* the catheter used for olfactory stimulation. The concentration of chloroform was the same as that used for induction of anaesthesia (the same arbitrary vapourizer setting and gas flow; a portion only being used). It was blown continuously for a varying period, and the usual olfactory stimuli were resumed. The effects of intermittent olfactory stimulation with the chloroform vapour were not investigated.

In the second part, the animals were ventilated with the anaesthetic mixture *via* the tracheal cannula. The usual olfactory stimuli with air continued throughout the administration, and during recovery.

Results

Nasal chloroform. After chloroform vapour had been blown over the nasal mucosa for 10 min, no induced waves could be recorded for a similar period. When they resumed, they were shorter in duration and lower in amplitude than before. They contained the same frequencies. It required at least an hour for the bursts to return to their former appearance.

Tracheal chloroform. Ventilation with chloroform vapour caused the induced waves to become less apparent.

Figure 4-4 (A, B, C, D) shows the progressive effect of chloroform absorption on the induced waves recorded from an intact olfactory bulb and its cortex. Fig. 4-4A is a record of drug-free induced waves, and the first wave written at one-tenth real time. Fig. 4-4B shows the change after two minutes' exposure to chloroform. Intrinsic activity is unchanged, but the cortical induced wave is reduced in amplitude. There is a consequent reduction in its contribution to the bulbar induced wave. Fig. 4-4C is the record after five minutes, and shows the same changes. Fig. 4-4D is after ten minutes' ventilation with chloroform. Olfactory stimuli were given where indicated by the arrows. Induced activity has been abolished both in cortex and bulb. Cortical activity has slowed, but bulbar intrinsic activity is unchanged.

There was a progressive decrease in frequency within the burst during chloroform administration. Control values were 50 - 55 Hz at onset and 35 - 40 Hz at termination of the burst. After 8 min chloroform, these values were 35 - 40 Hz and 25 - 30 Hz, respectively.

Recovery from the effects of the chloroform is shown in Fig. 4-5 (A, B, C, D). Three minutes after cessation of chloroform the induced waves had the appearance recorded in Fig. 4-5A. Cortical slow wave activity is still present, but the induced wave is brief (400 msec), high in amplitude and contains low frequencies (approximately 20 Hz). It dominates the bulbar record, where minimal high-frequency activity is seen at the beginning of the burst. Five minutes afterwards (Fig. 4-5B), the cortex has high-voltage, slow, spiking activity (20 - 30 Hz). This becomes less prominent with time. Figs. 4-5C & D show the activity after ten and thirty minutes, respectively. The bulbar high-frequency activity becomes more prominent, and the bursts begin to resemble their pre-drug condition.

Discussion

There has been no previous study of the effects of chloroform on olfactory induced waves. It may be that other workers also have found that induced waves are difficult to record after chloroform anaesthesia.

This preliminary study has shown that chloroform has a peripheral action on the olfactory mucosa, as well as a central one. In the initial experiments of the series, chloroform was in use for up to 2 hr during preparative surgery. During the first 15 - 30 min of this, the animals breathed through the nose until the trachea was cannulated.

For the rest of the surgery, ventilation was *via* this route.

Chloroform has similar uptake/excretion properties to halothane, and it would be expected to produce anaesthesia of similar duration. The effects of nasal halothane were not investigated, but it would be desirable to see whether this agent also has local effects on the olfactory mucosa.

In some of the experiments, high voltage spiking was seen with chloroform anaesthesia. Under these conditions, induced waves could not be obtained. These changes were not the same as those described by Domino & Ueki (1959) after trichloroethylene in the dog. The chloroform spikes were apparently random, not synchronized, as the trichloroethylene ones.

It is notable that the cortical response is depressed before the bulbar response during induction of chloroform anaesthesia. During this phase, the bulbar induced waves lose the low-frequency cortical component. In view of this, it is curious that a cortical response returns before a significant bulbar one. There must be a bulbar signal which reaches the cortex to initiate the burst recorded there. Such a signal is not clearly evident under the conditions of this experiment. One implication is that inhibition of bulbar mechanisms is high during the recovery phase. The increased cortical intrinsic activity, if generalized, would imply an increase in centrifugal inhibition of the bulb. (An extension of this inhibition during seizure activity will be discussed in the next chapter.)

Further experiments again will be required to clarify the observations recorded here.

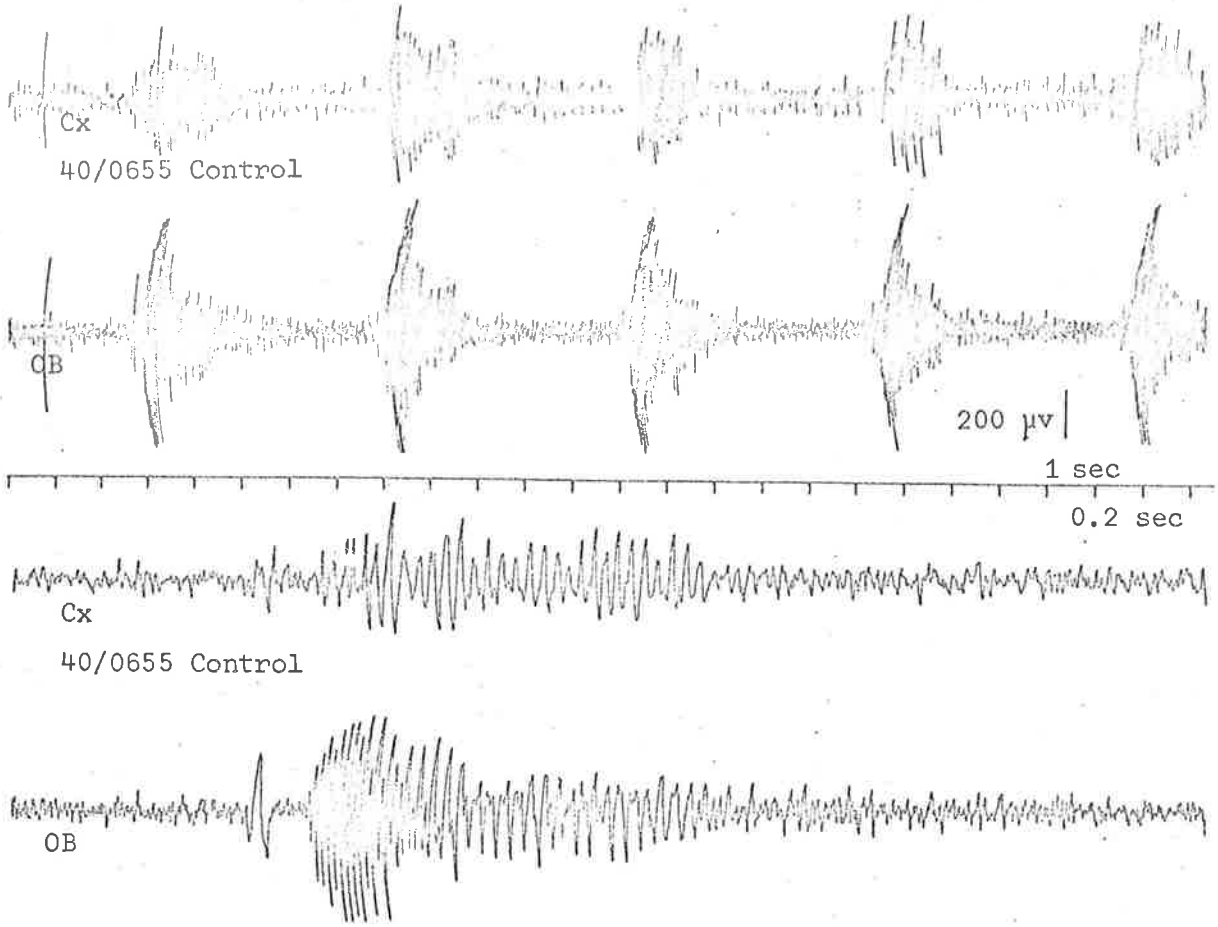


Fig. 4-4 A. Induced waves of intact olfactory bulb and cortex. No drug.

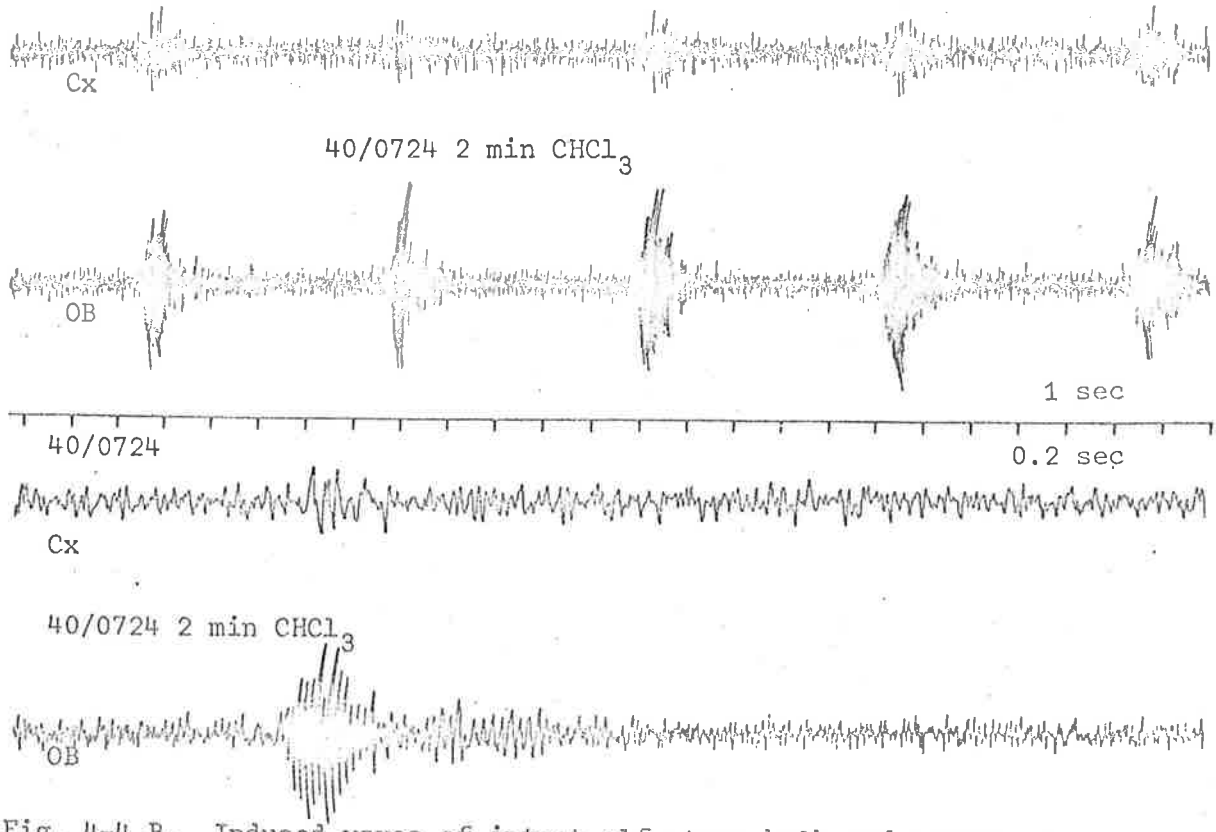


Fig. 4-4 B. Induced waves of intact olfactory bulb and cortex after 2 min. ventilation with chloroform.

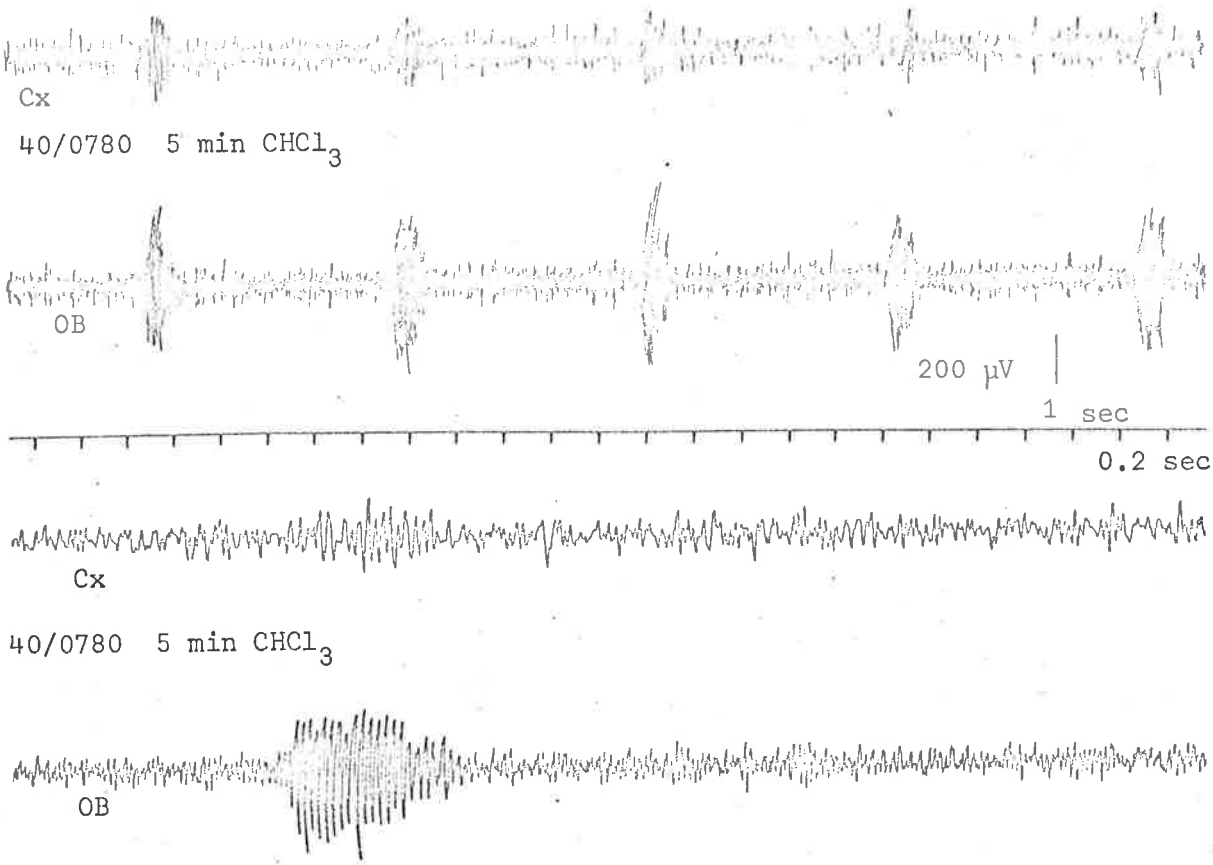


Fig. 4-4 C. Induced waves after 5 min. chloroform

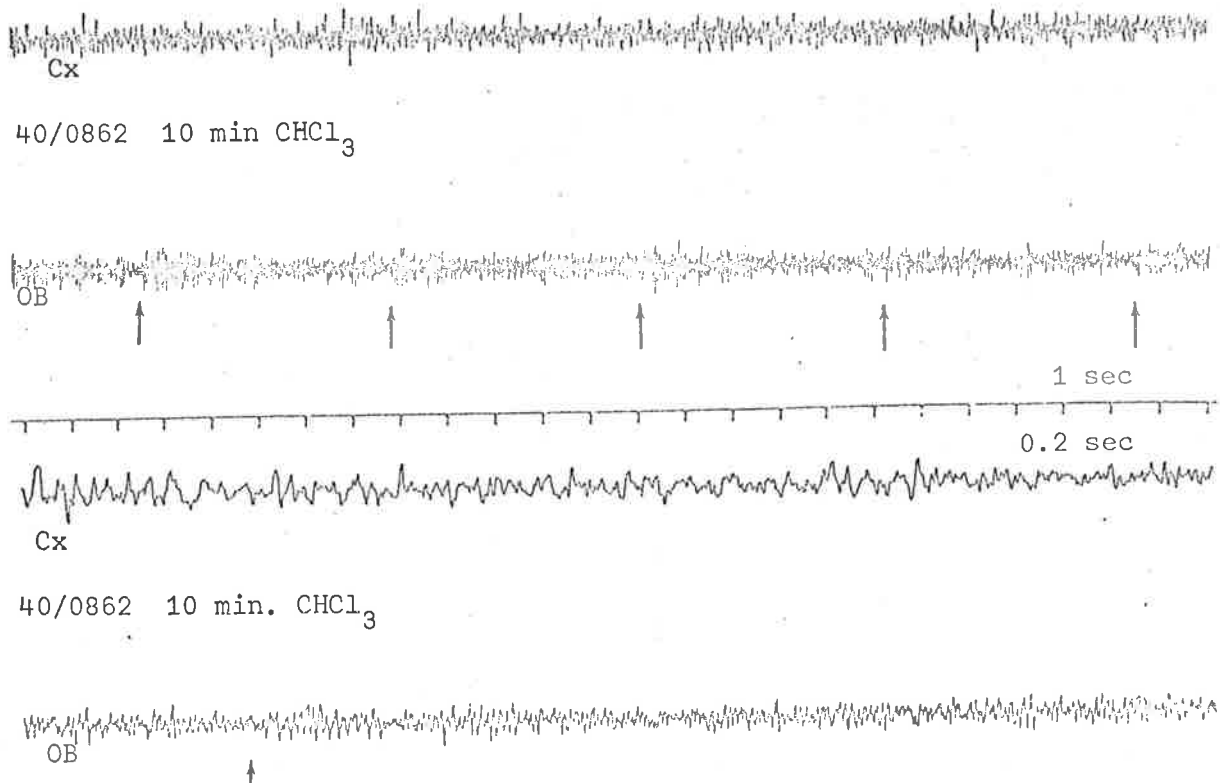


Fig. 4-4 D. Olfactory bulbar and cortical activity after 10 min. ventilatio with chloroform. The standard olfactory stimulus was applied where indicated by the arrows.

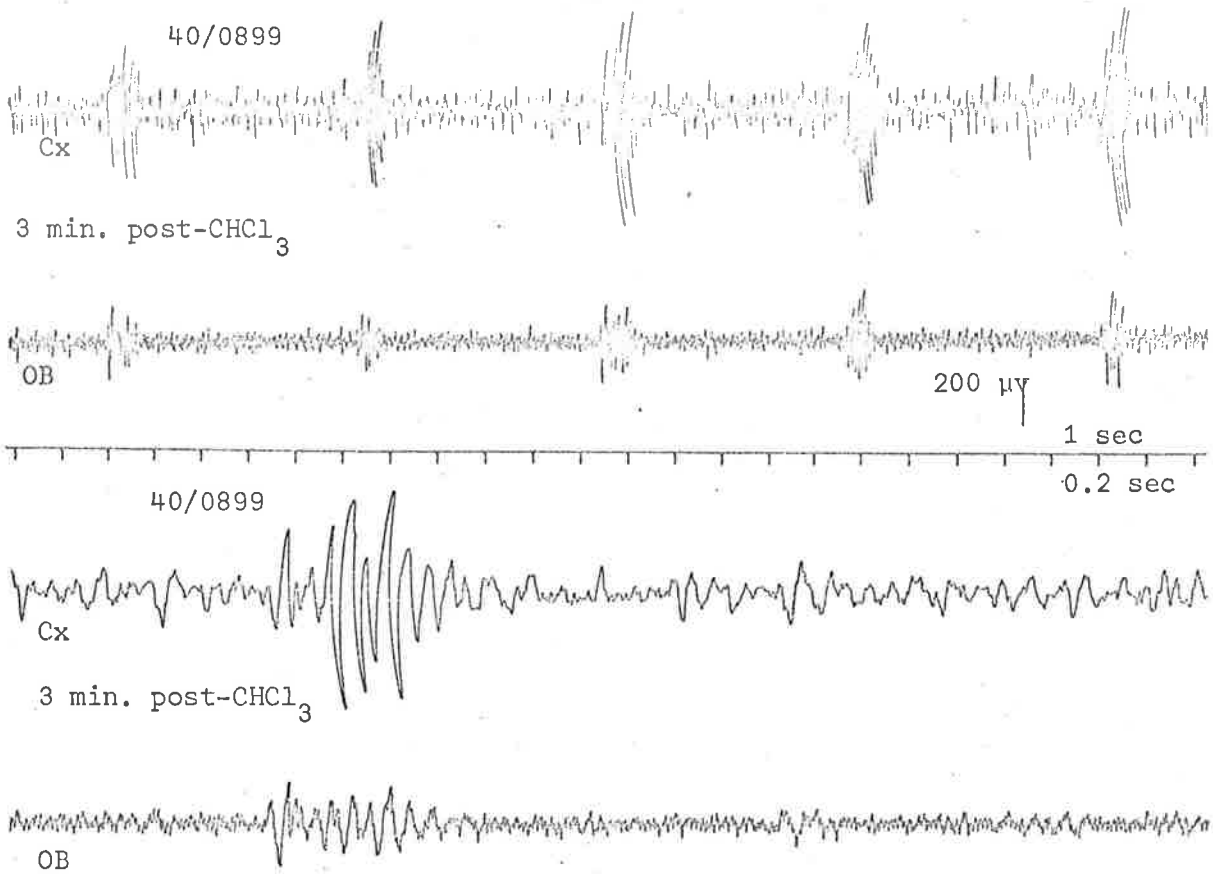


Fig. 4-5A. Induced waves during recovery from chloroform.

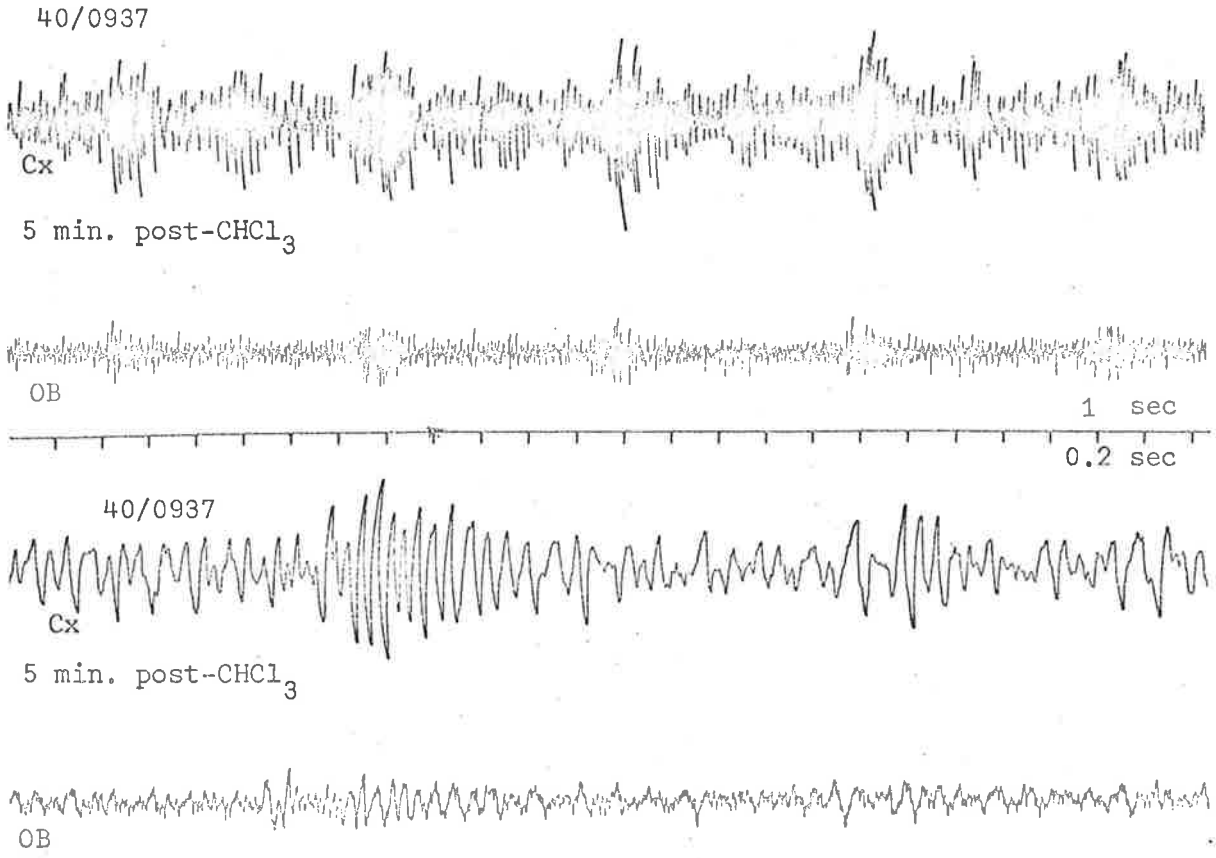


Fig. 4-5 B. Induced waves recorded from intact olfactory bulb and cortex of phalanger 5 min. after cessation of chloroform administration

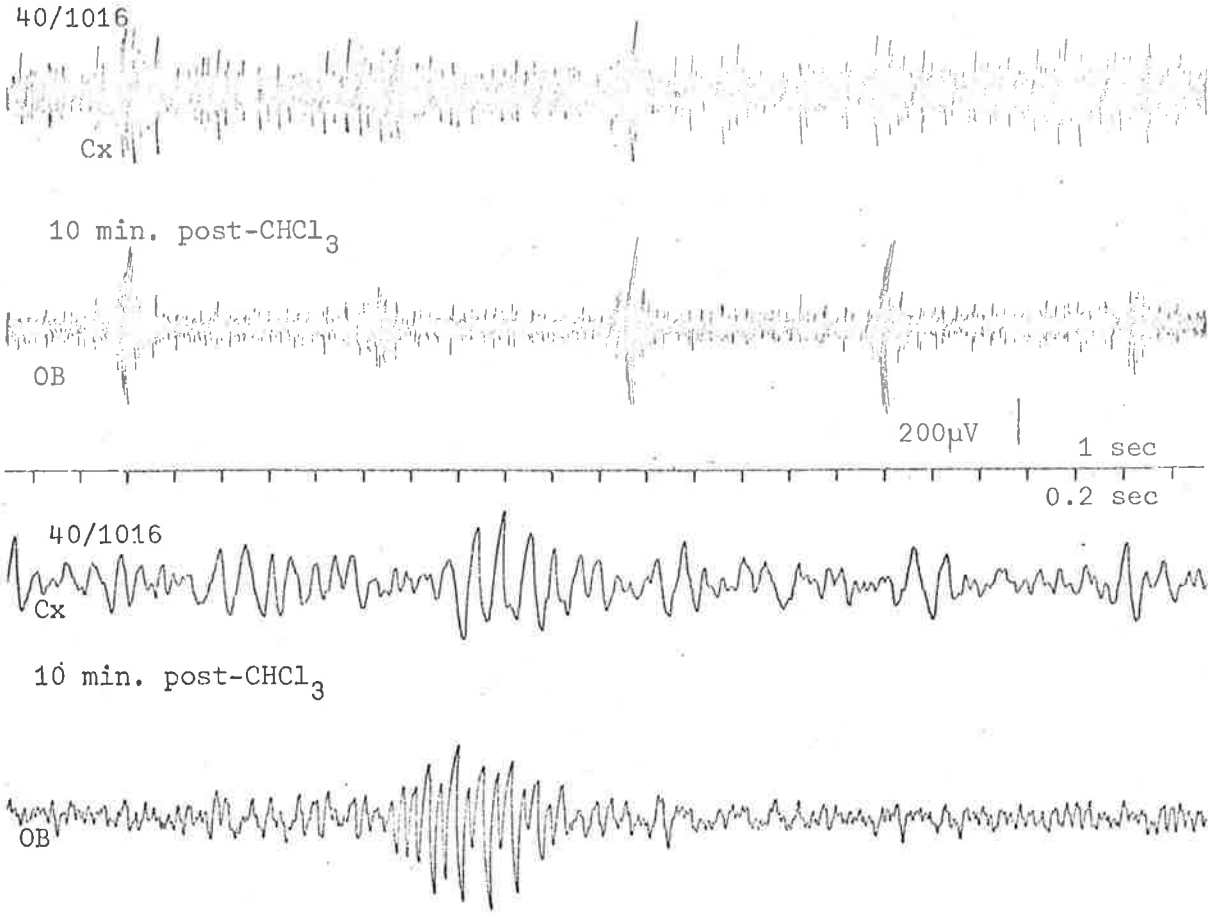


Fig. 4-5 C. Induced waves 10 min. after cessation of chloroform.

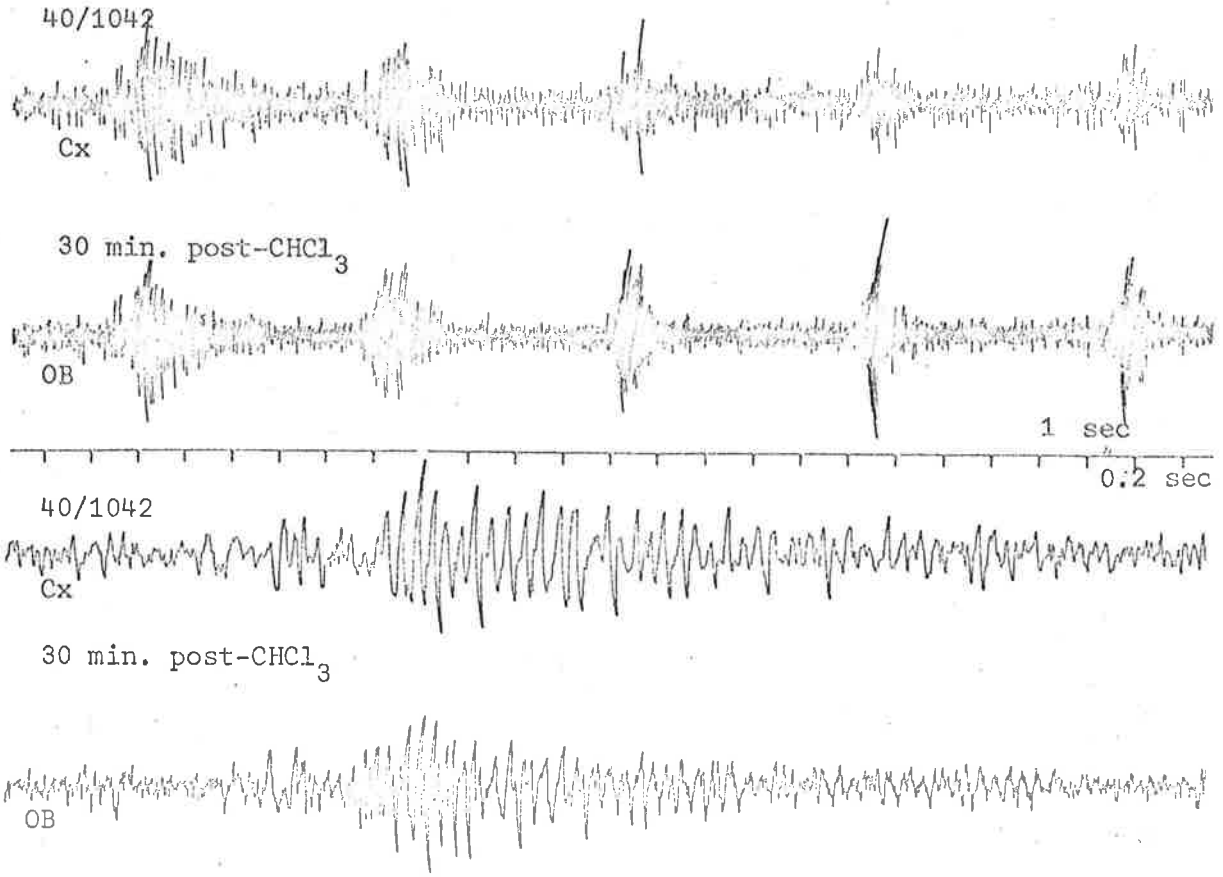


Fig. 4-5 D. Induced waves from phalanger intact olfactory bulb and cortex, 30 min. after cessation of chloroform administration. They are similar to the pre-drug control (Fig. 4-4A).

Halothane

Introduction

Halothane is the most widely used volatile anaesthetic agent in clinical practice (Chenoweth, 1972). Its history and clinical use have been reviewed recently (Ngai, 1972). Some of its neuropharmacological properties have been enumerated by Bimar & Naquet (1972).

Methods

Halothane was found to be the most effective induction and maintenance agent in these experiments. An accurate vapourizer (e.g. C.I.G. Fluotec or Dräger Vapor) was not available however, and a Rowbotham's bottle (Burton, 1958) had to be used. This is uncalibrated, but able to deliver adequate concentrations for surgical anaesthesia. Oxygen was used as the carrier gas in the present series of experiments.

The gas mixture was led, as before, to the reservoir proximal to the respiration pump. The animals were ventilated with the halothane vapour, recording continuing during administration and recovery.

Five animals were used for the formal study of this agent, which was added and withdrawn several times in each case. In all other experiments, records were made of the electrical activity during recovery from the surgical anaesthesia.

Results

The changes caused by halothane were similar on all occasions on which it was tested, and typical changes are illustrated in Fig. 4-6 (A-F).

Fig. 4-6A shows induced waves recorded in the absence of any general anaesthetic from intact olfactory cortex and bulb (RCx and ROB), deafferented cortex (LCx) and isolated bulb (LOB). The upper record is in real-time; the lower is written out at one-tenth recording speed. The pens had been aligned vertically with a marker pulse previously. The induced waves of intact bulb and cortex and isolated bulb have the now-familiar appearance. A spike-and-wave was recorded from the deafferented cortex about 100 msec after the induced waves began.

Fig. 4-6B shows the induced waves from the same sites after halothane (approximately 2% in oxygen) had been administered for one minute. The most remarkable change is the appearance of random spike activity in the deafferented cortex. The induced waves are somewhat

shorter in duration, but contain the same frequencies and have amplitudes of the same order. Intrinsic activity is unchanged.

Fig. 4-6C depicts the activity after three minutes' halothane. There is still spike activity in the deafferented cortex. The induced wave in the isolated bulb has become lower in amplitude, and begins at a slightly lower frequency (40 - 50 Hz approximately instead of 45 - 50 Hz). The induced wave in the intact bulb shows a reduction in its initial high-frequency component; its amplitude is unchanged.

Fig. 4-6D is after five minutes' halothane, and the preceding changes continue, and spiking begins to appear in the intact cortex and bulb.

Fig. 4-6E is five minutes after the previous record (now ten minutes' ventilation with halothane). The induced wave in the isolated bulb is greatly attenuated in amplitude and duration, and begins at 35 Hz. The induced waves of the intact bulb are apparently increased in duration and amplitude, but the frequency they contain is similar to the cortical frequency of 15 - 25 Hz. Spiking is more marked.

Fig. 4-6F is the record after fifteen minutes' halothane, and shows a similar pattern. The induced wave of the isolated bulb appears to begin at 40 - 45 Hz, while that in the intact bulb begins at half this frequency. The isolated induced wave has a duration of about one second, whereas the sinusoidal activity in the intact side lasts about twice as long as this. Intrinsic activity is reduced in amplitude in the isolated bulb.

Recovery from the halothane anaesthesia is shown in Fig. 4-7 (A & B). Five minutes after the halothane was removed from the circuit, and oxygen substituted, the changes had begun to reverse (Fig. 4-7A). Spike activity is seen to be greatly reduced. The burst in the isolated bulb is increasing in duration, amplitude and frequency (initially 50 Hz). The induced wave in the intact bulb still contains predominantly low frequencies. By fifteen minutes (Fig. 4-7B), appearances are similar to the pre-drug state. The induced wave of the isolated bulb has an initial frequency of 50 - 60 Hz, but the intact bulb has not regained its prominent initial high frequency. Recovery to control appearances took another 30 - 45 min.

Discussion

This study has shown that halothane depresses the mechanisms responsible for generating the induced wave in the isolated bulb. With increasing halothane dosage, the burst becomes smaller, slower and

shorter. This implies that excitatory mechanisms are depressed and/or inhibitory ones are augmented. This study cannot distinguish the two possibilities, but further studies, including evoked potential interaction, might clarify the point.

Despite the reduction in isolated bulbar activity, there was an increase in the intact cortical activity, manifested by spikes and induced wave activity which was of greater duration and amplitude than the controls. Indeed, there seemed to be an almost inverse relationship between isolated bulbar activity and cortical induced activity. This low-frequency activity was reflected in the intact bulbar activity. In the anaesthetized animal (15 min halothane) there seemed to be a minimal stimulus necessary to initiate the cortical activity. In the control situation (Fig. 4-6A), the cortical burst was delayed 200 - 250 msec during the intense bulbar activity, but at 15 min halothane (Fig. 4-6F) the cortical burst began at about 100 msec. This observation suggests that the bulbar activity initially inhibits the cortical activity. Again, more work (including unit studies) will be required to clarify this.

This part of the study has shown that there are differences in the action of chloroform and halothane. If the intact bulb and cortex records are compared, it is seen that the high-frequency bulbar component is more resistant to chloroform than halothane, and the cortical component is apparently depressed. Because no quantitative measures of intrinsic activity have been made, it is not possible to say whether chloroform or halothane is more potent as a central depressant. Again, studies of mitral cell inhibition using evoked potential methods might help.

Domino & Ueki (1959) showed the effects of halothane anaesthesia in the dog (their Fig. 4). The respiratory bursts in the bulb were said to be markedly reduced. There were probably no bulbar induced waves illustrated after their Fig. 4A. Some spike activity began to occur. They also described an increase in frequency and voltage (of the spontaneous activity), especially in the amygdala and posterior hypothalamus. This 20 - 33 Hz activity was present in the olfactory bulb. The 15 Hz spiking occurred first in the amygdala and olfactory bulb. Rapid behavioural recovery from the anaesthetic was described and EEG changes were illustrated in their Fig. 4G. The induced bursts were absent from the olfactory bulbar and amygdalar records. No comment was made on the time taken for the bursts to recover. These

changes caused by halothane were similar to the changes after other volatile anaesthetics. An exception was trichloroethylene, after which undoubted *grand mal* seizures occurred. The causes of the hypersynchrony were suggested to be (a) direct rhinencephalic stimulation; (b) 'release' of rhinencephalic structures associated with the cortical depression or (c) production of rhinencephalic "circus" movements allowing reverberation within the system. They were unable to corroborate any of these suggestions.

The results described in this thesis agree with some of these findings of Domino & Ueki (1959). It was noted that the bulbar induced wave was reduced by the unknown concentration of halothane. However, the nature of the reduction has been more precisely described. The slowing and shortening of the burst suggests that an inhibitory system may be augmented or that the excitatory system may be inhibited.

No studies were undertaken in this thesis on the effects of halothane on evoked responses. It would be useful to attempt to correlate such effects with changes in the induced waves. This study has shown a decrease in initial burst frequency from 50 Hz to 35 Hz. If a frequency of 50 Hz implies a refractory period of 20 msec, then a frequency of 35 Hz implies a refractory period of 28.6 msec. This aspect of olfactory bulb function, attempting to relate duration of inhibition to induced wave frequency, has not been studied previously.

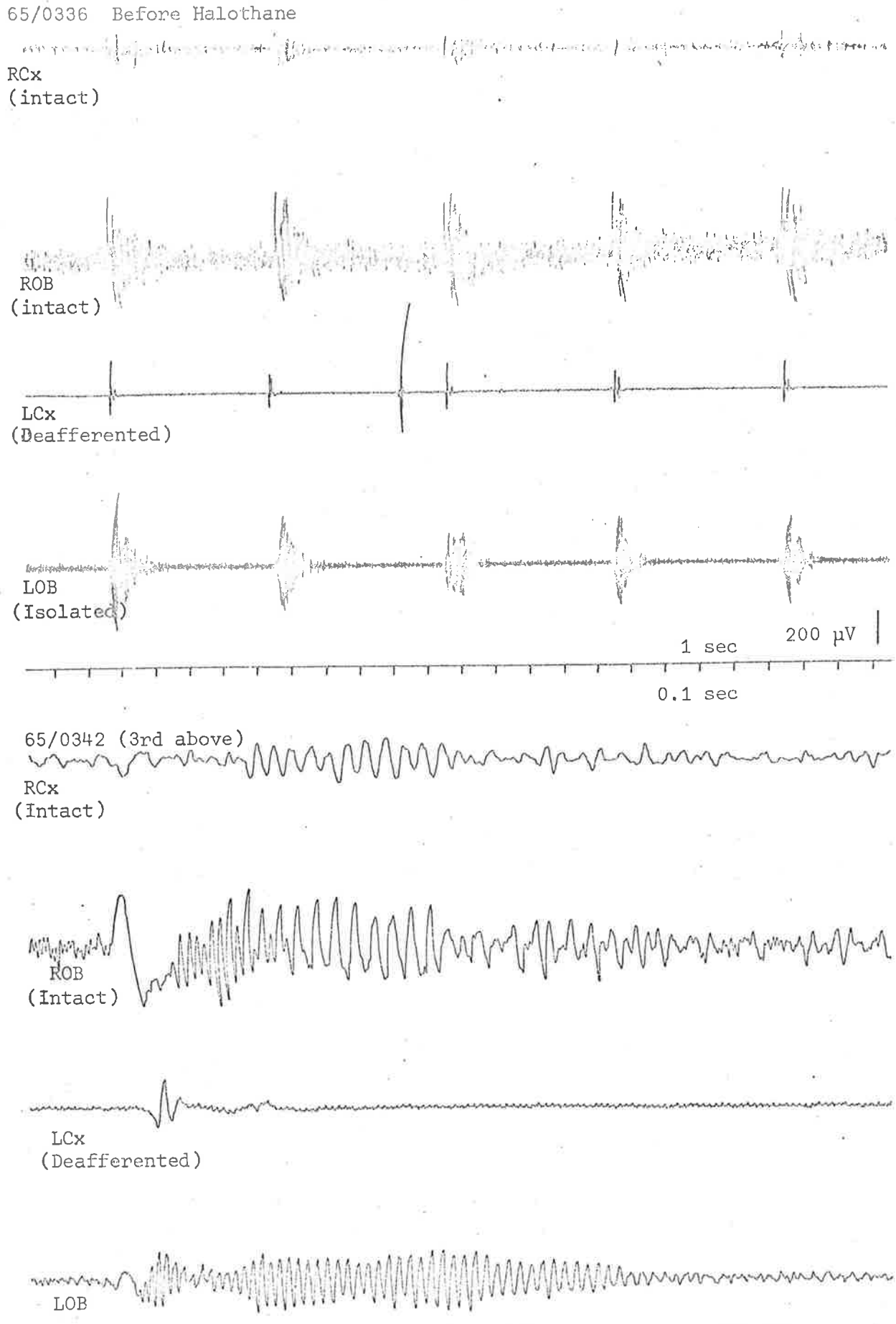


Fig. 4-6 A. Induced waves recorded from intact olfactory bulb and cortex, isolated bulb and deafferented cortex of phalanger in the absence of drugs, before administration of halothane. Upper part real time, lower 1/10 real time.

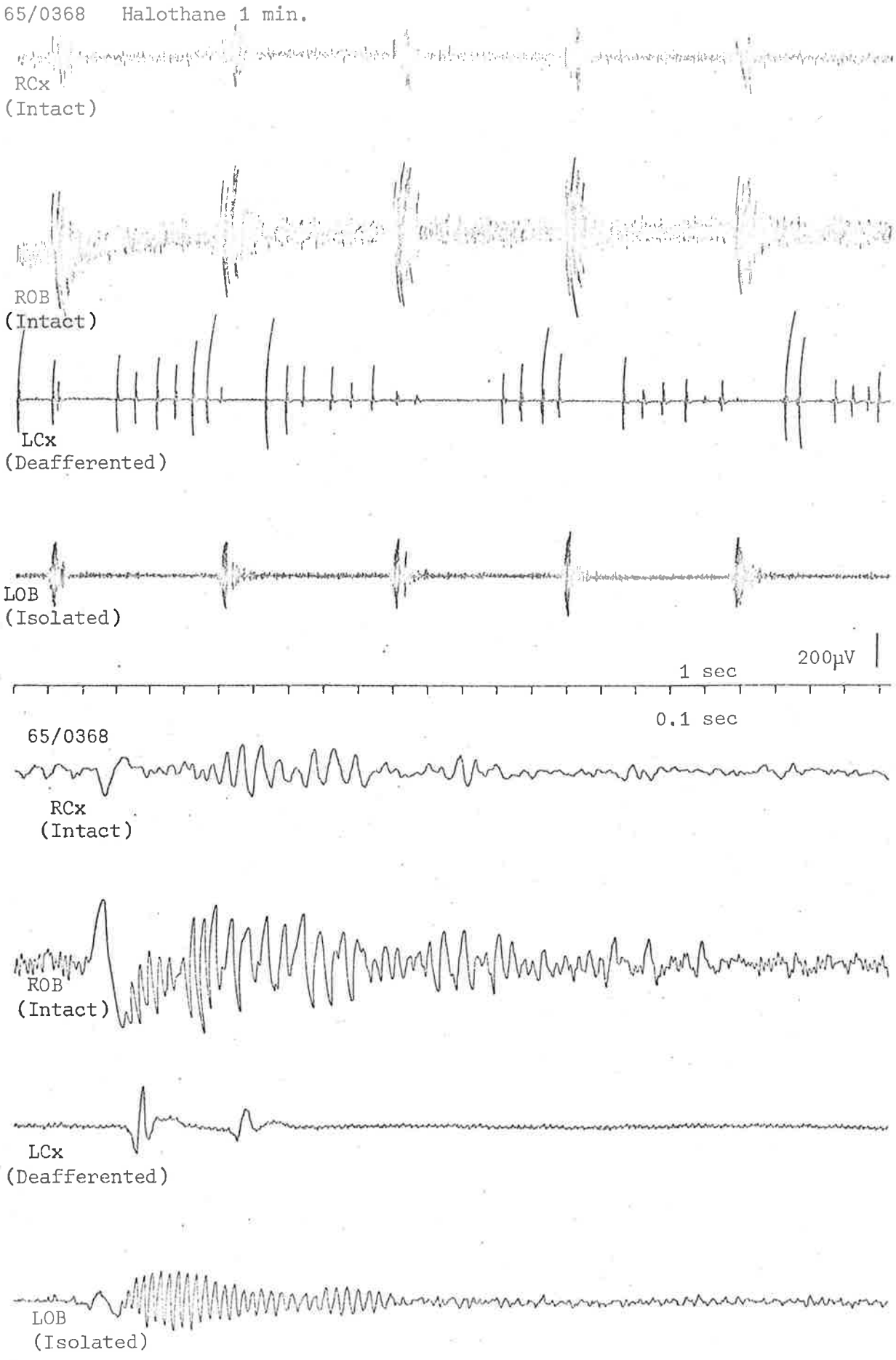


Fig. 4-6 B Induced waves recorded from the same sites as in the preceding figure, after ventilation of the phalanger with halothane (2%) for 1 min. Note the spiking in the deafferented cortex.

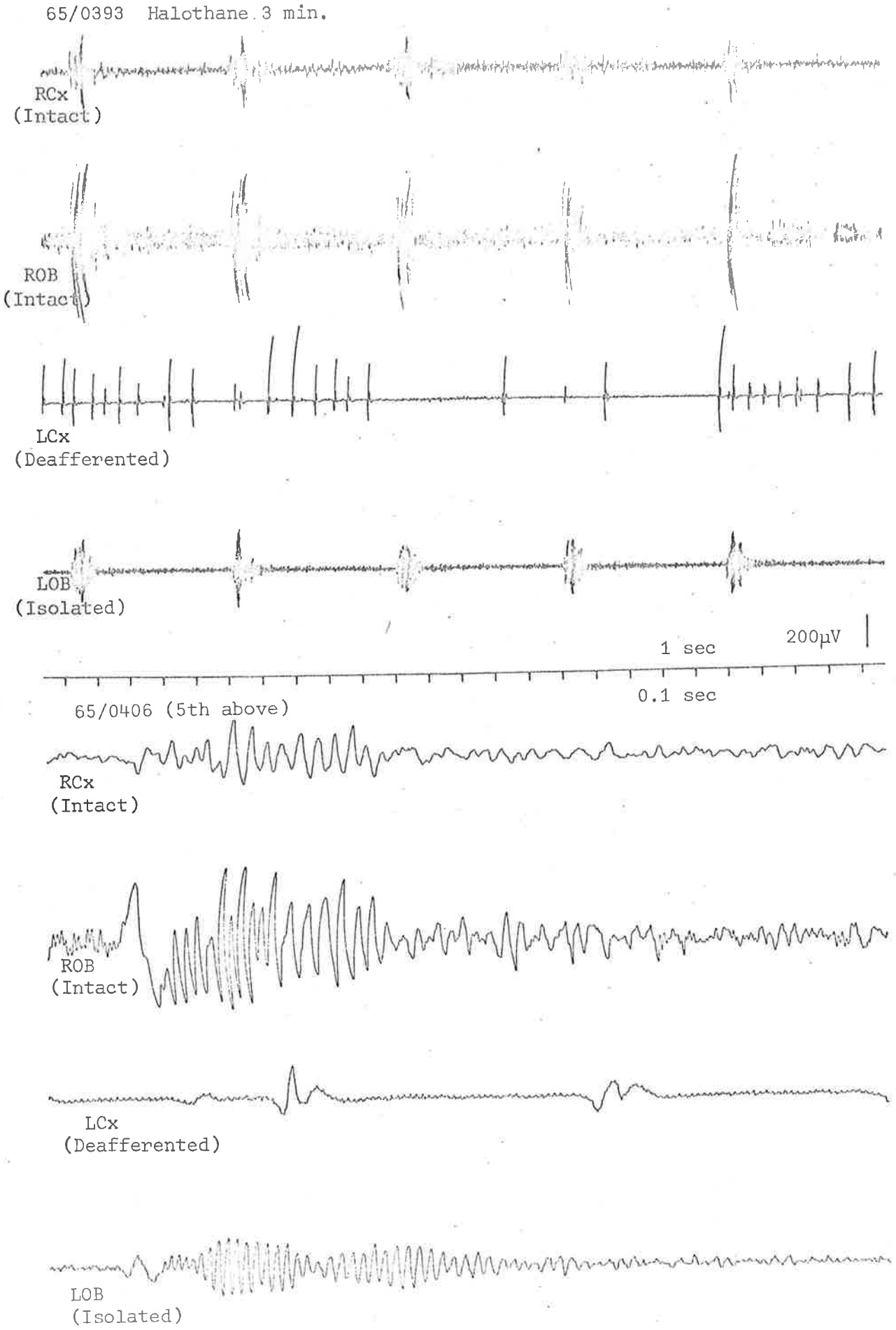


Fig. 4-6 c Induced waves from the same sites as before, after 3 min ventilation with halothane.

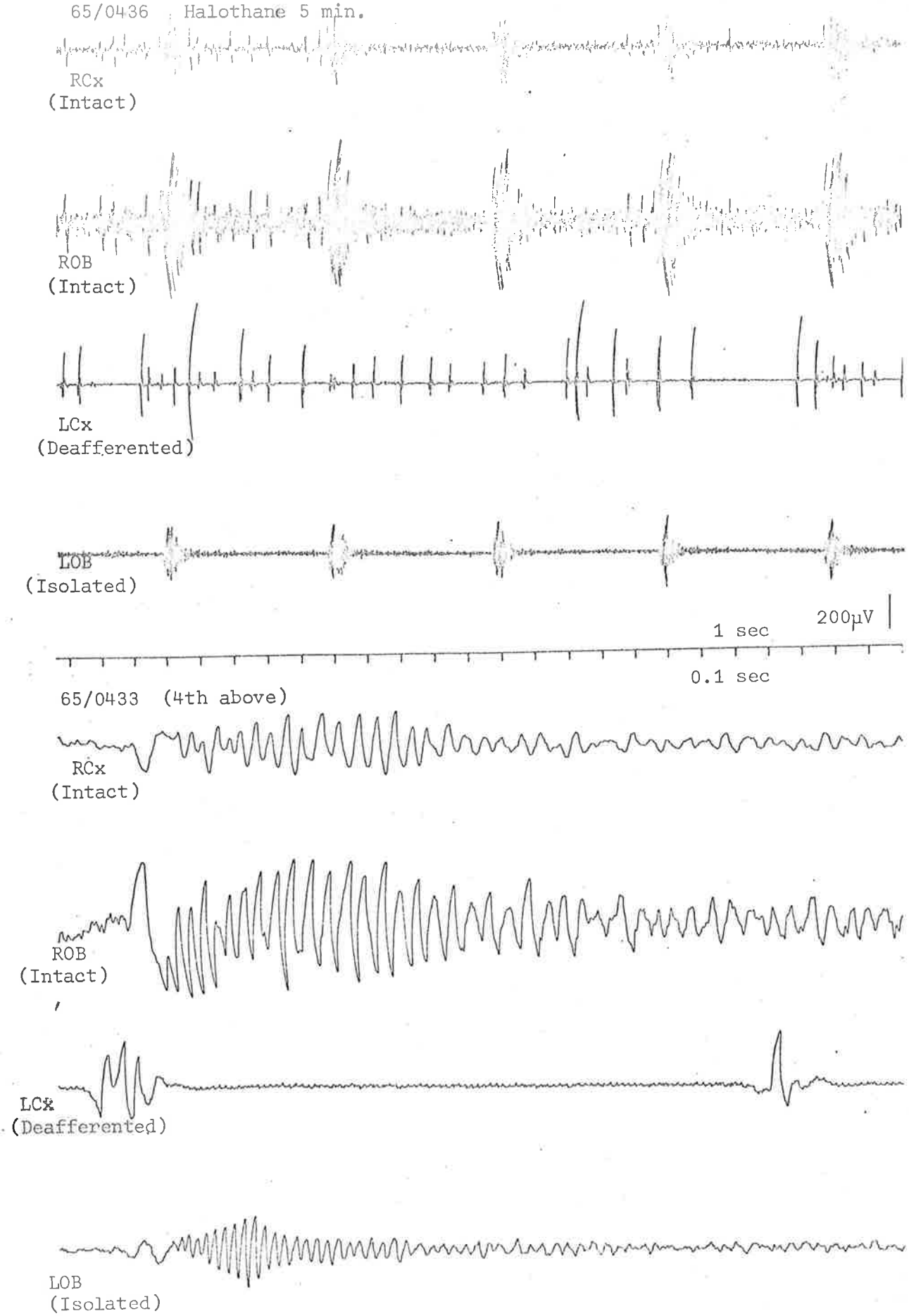


Fig.4-6 D Induced waves from the same sites as before, after 5 min ventilation with halothane.

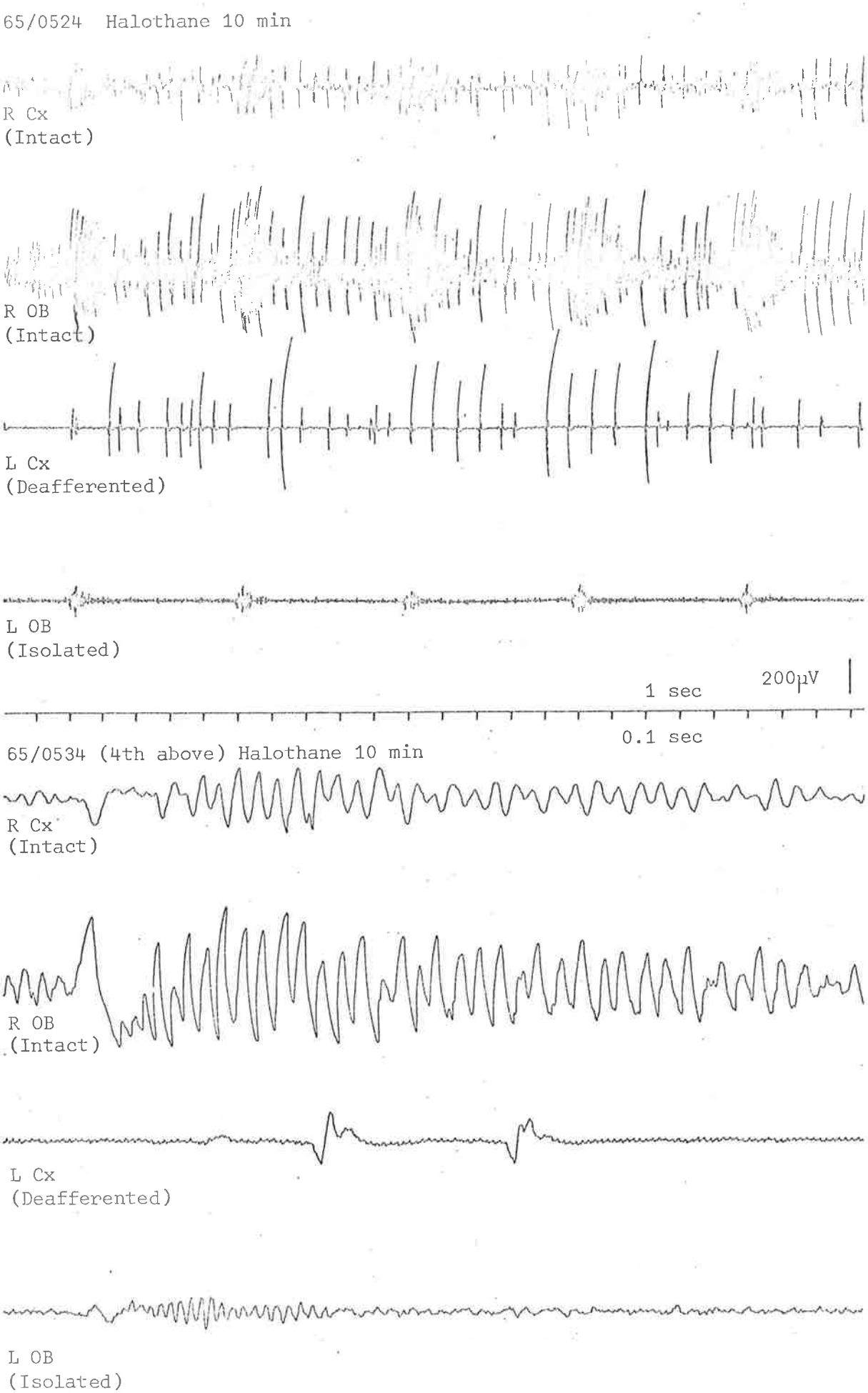


Fig. 4-6 E Induced waves from the same sites as before, after 10 min ventilation with halothane. The induced wave of the isolated bulb is greatly attenuated, and there is an increase in the intact cortical wave.

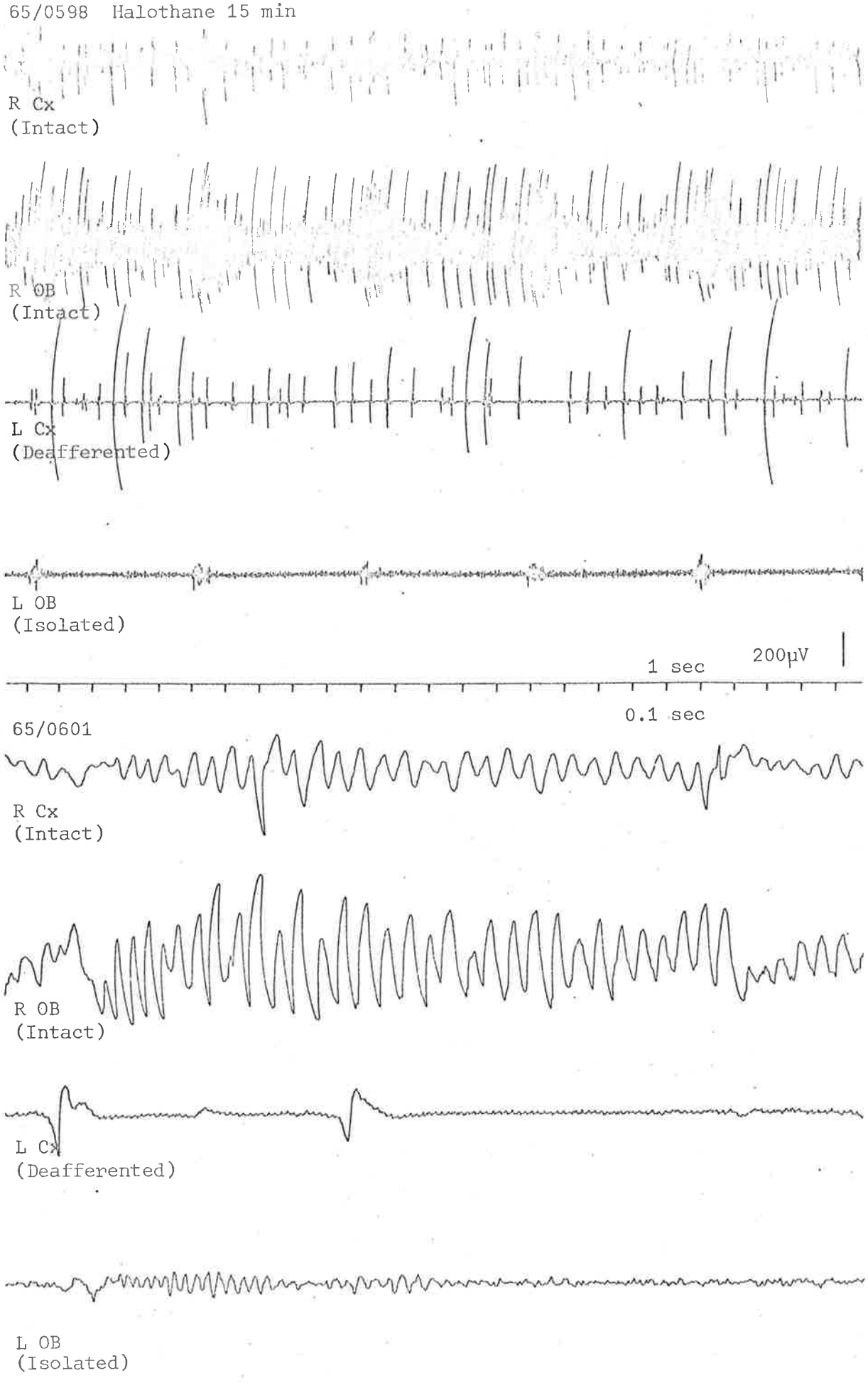


Fig. 4-6 F Induced waves recorded from the same sites as before, after 15 min ventilation with halothane. The induced wave of the isolated bulb is further attenuated. Spiking continues in the deafferented cortex and intact sites.

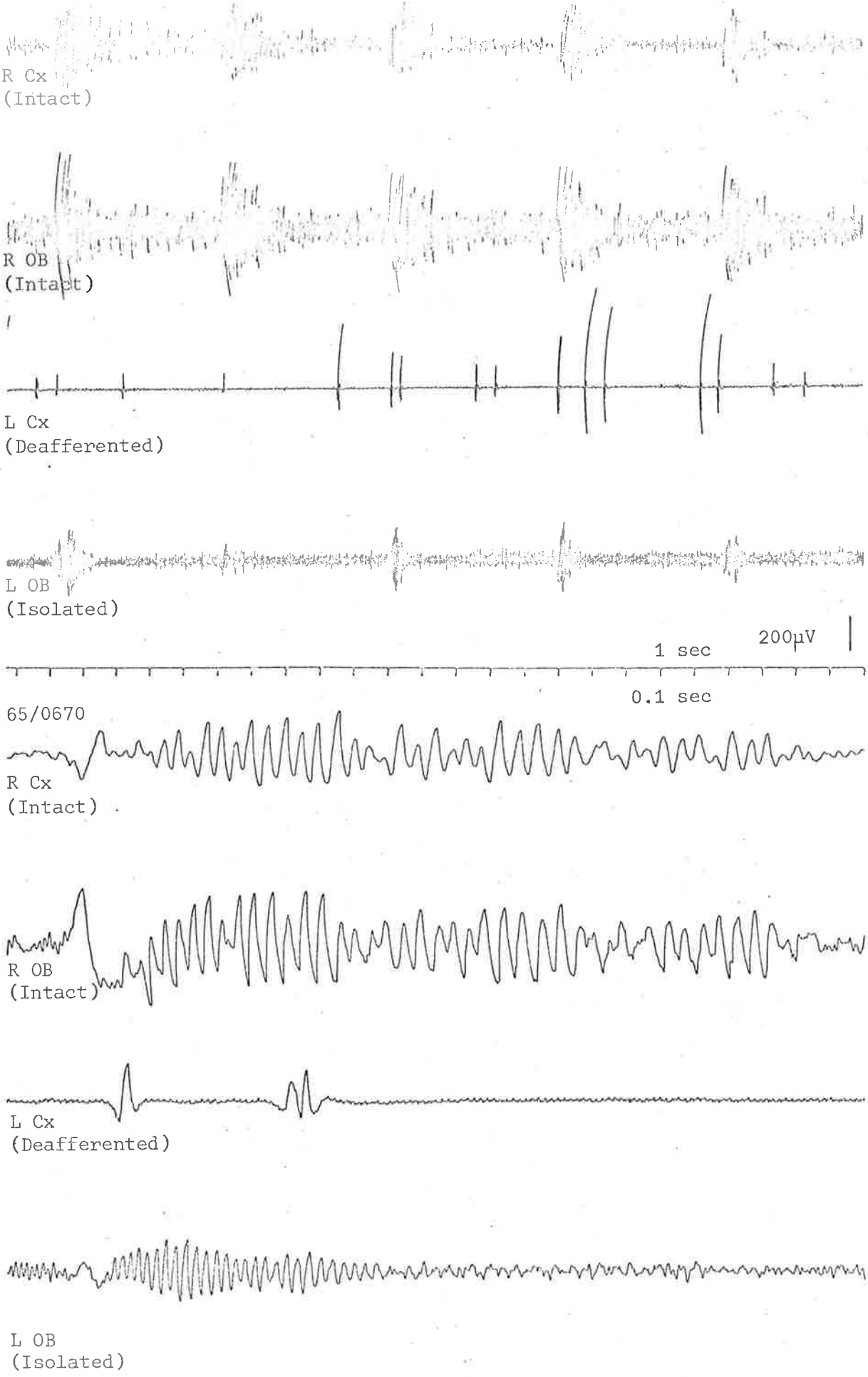


Fig. 4-7 A Records from the same sites as Fig. 4-6, 5 min after cessation of halothane administration and resumption of ventilation with oxygen

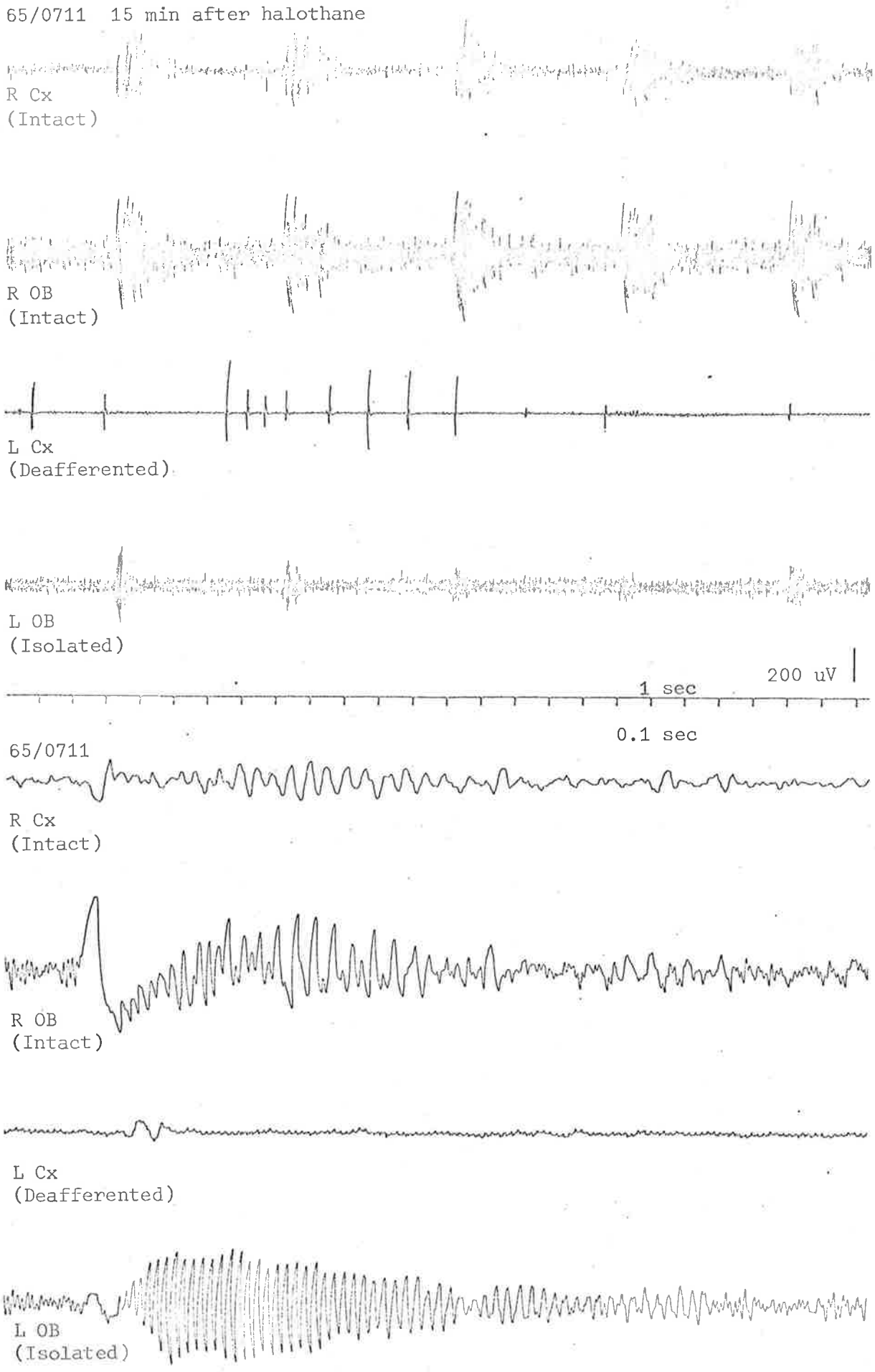


Fig. 4-7 B Induced waves from the same sites as before, 15 min after cessation of halothane.

Barbiturates

Introduction

Barbiturates are the main group of drugs used to produce alterations of consciousness (Dundee, 1971). Since the introduction of the first hypnotic barbiturate in 1903 (diethylbarbituric acid), more than 2500 others have been synthesized (Sharpless, 1970). Among these are barbiturates with a convulsant activity (for example, see Swanson & Chen, 1939).

Reference has already been made to several papers concerning the action of barbiturates on the olfactory system. Richards (1972*b*) had suggested that barbiturates reduced transmitter output at presynaptic terminals in the isolated slab of olfactory cortex. Other evidence referred to (Richards, 1972*b*; his p. 765) suggested that barbiturates might potentiate recurrent inhibition of spinal motoneurones, and potentiate presynaptic inhibition by unknown mechanisms. These three suspected effects of barbiturates would be expected to reduce total neuronal activity and form the basis of barbiturate anaesthesia. They do not explain the occurrence of barbiturate spindles or spikes, which may be the result of net loss of inhibition.

It was decided to investigate the effects of barbiturates on induced and evoked activity in the olfactory system of the phalanger.

Pentobarbitone had been used as a general anaesthetic for phalangers in another study. It was found to have an unexpectedly brief duration of action. Thiopentone has a duration of action similar to that of pentobarbitone in the phalanger.

Methods

Pentobarbitone (MW = 248.3; 1 mg = 4.03×10^{-3} mM) was given to 8 animals. Four received incremental IV injections (1 - 2.5 mg/kg/bolus), and the other four received IV infusions (1 mg/kg/min). Thiopentone (MW = 264.3; 1 mg = 3.78×10^{-3} mM) was given to two animals by intermittent IV injection (2 mg/kg/bolus).

In two experiments in this series, bulbar evoked potentials were recorded at the same time as the induced waves, one potential per minute. The sequence of events was controlled by a pair of digitimers.

Olfactory stimuli	Time 0, 10, 20, 30, 40, 50 sec
Switch electrode	Time 53 & 58 sec
Stimulate	Time 55 sec

The evoked potentials were photographed and plotted by hand, as before.

Results

Induced waves. Small doses of pentobarbitone (2 - 2.5 mg/kg) consistently increased the amplitude and duration of the induced waves recorded in the intact bulb and corresponding cortex. Accompanying this increase was an unexpected and significant decrease in the amplitude and duration of the induced wave of the isolated bulb. Fig. 4-8A is a real-time continuous record of induced waves of intact bulb and cortex, isolated bulb and deafferented cortex. Between the third and fourth responses (approximately 1060') a bolus IV injection of pentobarbitone 2 mg/kg was given. The changes in the next 90 sec are shown in the bottom half of Fig. 4-8A and Fig. 4-8B, which are continuous. In the pre-drug state (upper part of Fig. 4-8A), induced waves are clearly seen in the isolated bulb, less well seen in the intact bulb, and not discernible in the intact cortex. Within 20 sec of the pentobarbitone (last burst of Fig. 4-8A) a large induced wave has emerged from the intact cortical record, and the induced wave of the intact bulb is correspondingly increased. The induced wave of the isolated bulb is decreased in amplitude and duration. Fig. 4-8B shows the next six induced waves resulting from identical olfactory stimuli. The intact cortical response wanes slightly, and the isolated bulbar response is depressed further.

Examination of these induced waves written out at 1/10 tape speed shows their characteristics. Fig. 4-9 shows the third and sixth responses illustrated in Fig. 4-8A, before and after pentobarbitone, 2 mg/kg. Before pentobarbitone (61/1059), there is a barely perceptible induced wave in the intact cortex, a low-frequency induced wave in the intact bulb, and the usual burst in the isolated bulb. Twenty-five seconds after 2 mg pentobarbitone/kg (61/1068) there is a dramatic increase in the intact cortical induced wave, which is reflected by a corresponding increase in the induced wave of the intact bulb. The frequency is 25 - 15 Hz, and duration approximately 1.4 sec. There is no change in the appearance of the intrinsic activity of the deafferented cortex. The amplitude and duration of the burst in the isolated bulb are reduced, but the contained frequencies remain the same.

This pentobarbitone-induced increase in induced waves was most marked when there was a high level of intrinsic activity, as just illustrated. When background activity was low, or induced waves were

prominent, the increase was not as marked.

Higher doses of pentobarbitone eventually depress the induced waves at all sites. The effects of incremental IV dosage are shown in Figs. 4-10 A-D. Recording sites were intact bulb and cortex, deafferented cortex and isolated bulb. (In this case, isolation was shown subsequently to be incomplete at the lateral part of the peduncle, a small area of tissue approximately 1 mm^2 maintaining connexions). Fig. 4-10A (upper) shows an induced wave in the drug-free state. The lower part of that figure shows minimal changes after pentobarbitone, 2 mg/kg. Fig. 4-10B shows induced waves after 4 (upper) and 6 mg/kg (lower). The frequencies at all sites are reduced, and the amplitude and duration of the induced wave of the isolated bulb are also reduced. Notice that spiking has begun to occur at both cortical sites and intact bulb. Fig. 4-10C shows the progressive effects of pentobarbitone after 8 (upper) and 10 mg/kg (lower). Frequencies are lower still, and spiking is more marked. The induced wave has almost disappeared from the isolated bulb, but is still readily seen at the intact sites. Fig. 4-10D is the electrical activity (tape speed normal) before and after pentobarbitone, 10 mg/kg, showing spiking, and reduction in isolated bulbar activity. Spike activity was never seen in the isolated bulb. Higher doses of pentobarbitone (to 30 mg/kg) suppressed spike activity and induced waves, and the EEG eventually became iso-electric.

Recovery from the effects of pentobarbitone 20 mg/kg took about 30 min, judged by the return of the induced waves to their pre-drug appearances.

Thiopentone (0.5 - 10 mg/kg) produced similar changes to pento-barbitone, and these will not be illustrated.

Evoked potentials. The effects of pentobarbitone 24 mg/kg were studied on the bulbar potentials evoked by paired electrical stimulation of the olfactory cortex. Supramaximal stimuli were applied to the cortical electrode (A15, L7), a test response 45 msec after a conditioning one. The responses were amplified with two preamplifier settings, photographed and projected onto graph paper. Fig. 4-11 shows the results from experiment W51, and Fig. 4-12 is from experiment W55. Results were similar in each case. After pentobarbitone 24 mg/kg (dotted line), there was no change in latency of the conditioning response, but the initial positivity (band pass 0.15 Hz - 1 KHz, upper trace A in each figure) was increased. This

change was not seen when the band pass of the preamplifiers was 7 - 100 Hz (trace C in each figure). The late negative of the conditioning response was augmented. The major effect was on the test response, which was almost obliterated. Graphic subtraction of the pre-drug and drug responses (traces B and D in each figure) show that the test response was depressed.

Discussion

This study has described the effects of depressant barbiturates on the induced and evoked activity of the olfactory system of the phalanger. The most striking result has been the demonstration of high sensitivity of the isolated bulb to barbiturates. Such sensitivity has not been described previously. A parallel reduction in the high frequency activity of the intact bulb has also been described.

Despite the reduction in amplitude and duration of the burst in the isolated bulb by the low pentobarbitone dosage (2 mg/kg), the frequencies contained in it remained essentially the same (measured by zero-crossing analysis). This suggests the possibility of two intrinsic bulbar systems. The first controls the rate of discharge of mitral cells, and is barbiturate-resistant at low doses. The second controls the number of mitral cells which discharge in response to a standard stimulus, and is barbiturate-sensitive. This speculation would assign the frequency-controlling role to the dendrodendritic synapse between the mitral and granule cell. Control of the amplitude and duration of the burst by lateral and intraglomerular inhibition would reside in the short-axon and periglomerular cells.

A remote and unlikely possibility is that mitral and tufted cells are differentially sensitive to barbiturates. Tufted cells have been suggested as having a role in signal amplification (Nicoll, 1970*b*). If their action were to be specifically inhibited, a smaller output (amplitude and duration) would result. There has been no such suggestion by any other worker.

The changes seen in the intact bulb and cortex are also difficult to explain. Such changes have been described previously (for example, Kerr & Hagbarth, 1955; their Fig. 7). The high intrinsic activity in the cortex could imply that olfactory-inhibitory activity is also high, causing suppression of induced waves. The small dose of pentobarbitone (2 mg/kg) appears to be enough to remove this inhibition and allow the bulbar signal to reach the cortex, initiating the low-frequency burst in the cortex which is fed back to the bulb.

The occurrence of cortical spiking was not unexpected, as barbiturates may have convulsant activities (Leonard & Harrison, 1953).

Spiking was never seen in the isolated bulb, a point to be discussed in the next chapter.

Interpretation of the evoked potential studies is equally difficult. The experimental design was similar to that of Nicoll (1972*b*), reported after mine were completed. He recorded his results in a more accessible format, however. In my series, each conditioning-test pair was recorded on a separate photographic frame.

Graphic subtraction of a single response may not give valid results. Subtraction of responses could be carried out with the Computer of Average Transients (SUB mode) to yield a result more likely to be statistically significant. Such a method would rely on a constant anaesthetic level during acquisition of data.

Results of the graphic subtraction suggest that pentobarbitone may have two effects: (1) inhibition is removed from the conditioning response, and potentiation of the initial positive potential occurs, and (2) the inhibition following the conditioning response is sufficiently powerful to block the test response at 45 msec.

Nicoll (1972*b*) has shown that pentobarbitone increases the duration of inhibition following a conditioning lateral olfactory tract stimulus. The present study has confirmed his findings in two animals. Quantitation of the inhibition is not possible with the experiments described above.

It is interesting to notice that even when the intact bulb is apparently severely depressed (with little visible high frequency activity), there is still adequate stimulus transmitted to the cortex/amygdala to initiate the induced activity at the low frequency, which feeds back to the bulb. Freeman (1960*b*) showed that it was possible to evoke burst-like activity in the olfactory cortex of cats with appropriate electrical stimuli to amygdala (his Fig. 4).

The study reported here has pointed out that the olfactory bulb of the phalanger is very sensitive to the action of barbiturates. It suggests that barbiturates are unsuitable anaesthetic agents for olfactory physiology. Any results so obtained must be interpreted in the light of the evidence presented here, that as little as 2 mg pentobarbitone/kg can have profound effects on olfactory function.

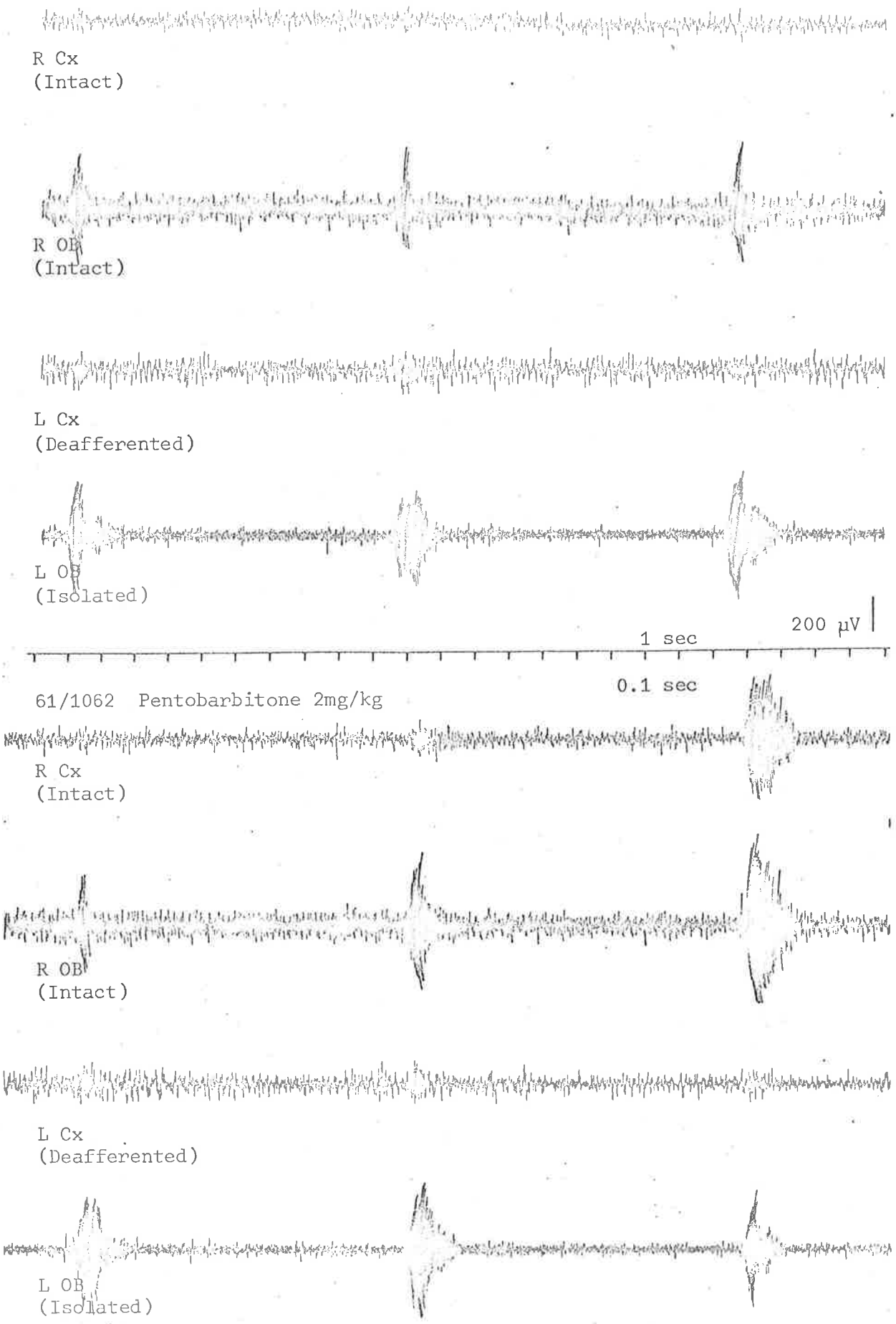


Fig. 4-8 A Induced waves recorded before (upper) and after (lower) pentobarbitone 2mg/kg IV, given between the 3rd and 4th of the six consecutive responses shown here.

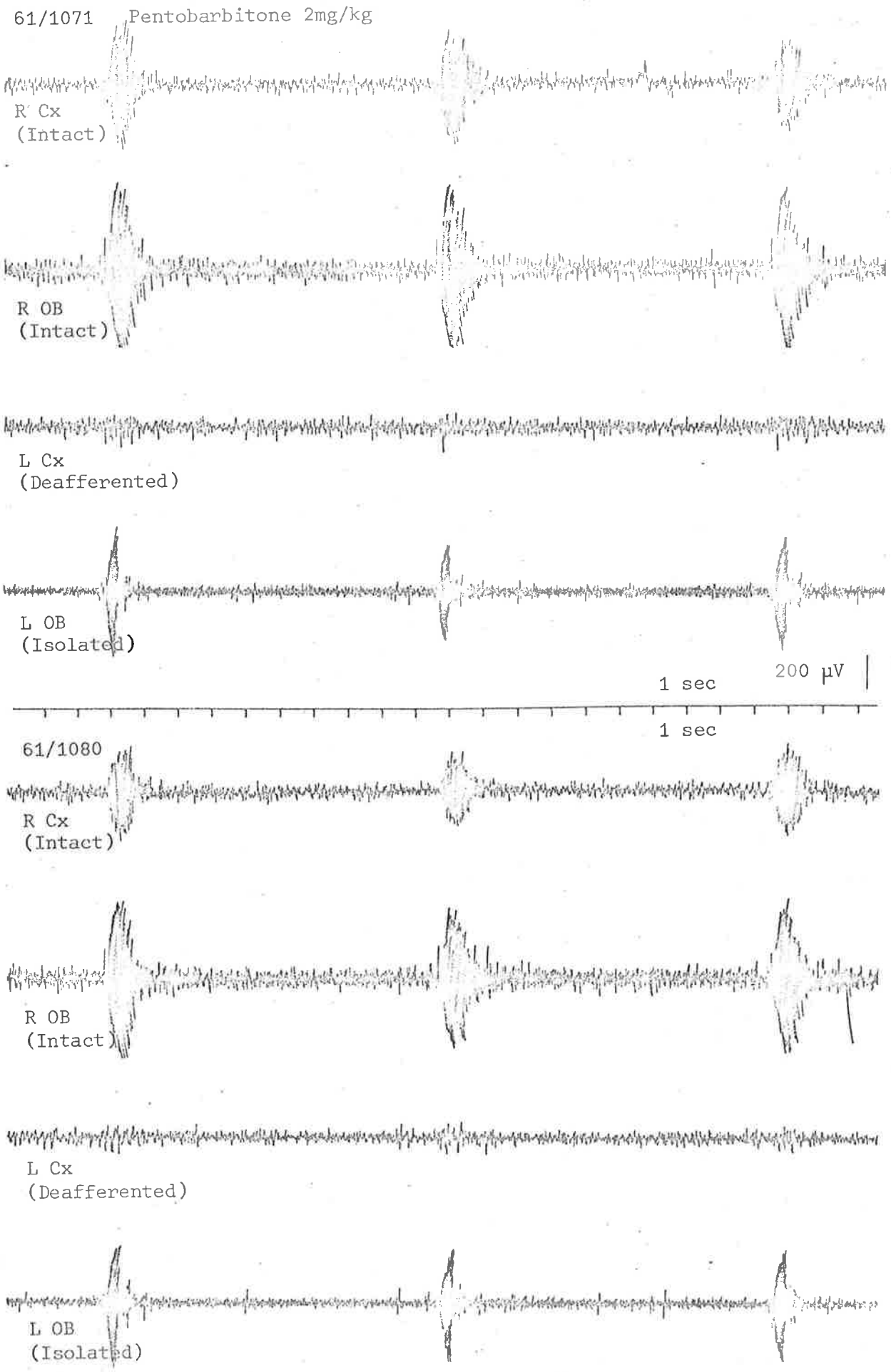


Fig. 4-8 B The six induced waves which followed the last on Fig. 5-8 A, after pentobarbitone 2mg/kg.

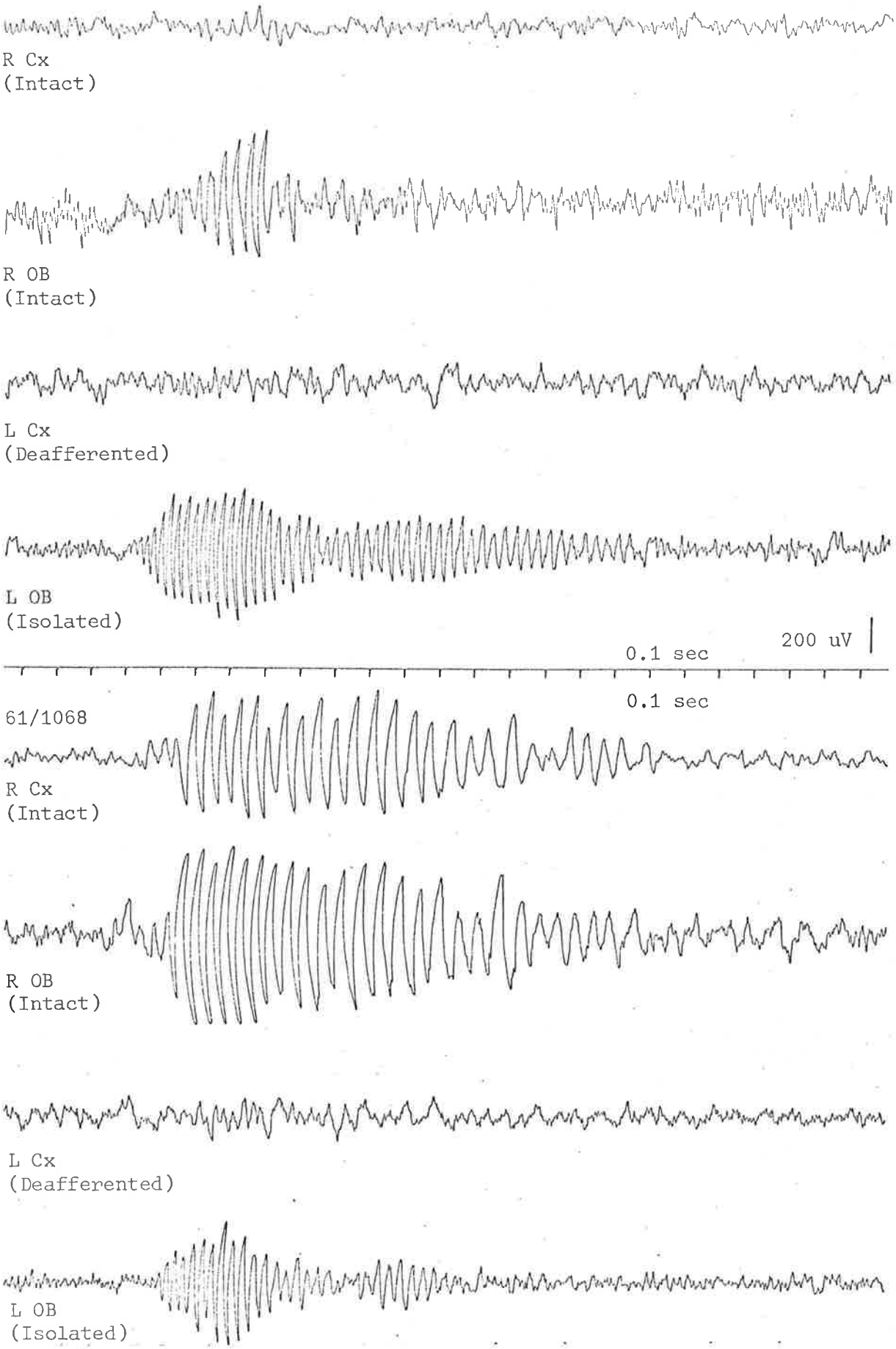


Fig. 4-9 Induced waves recorded before (upper) and after (lower) pentobarbitone, 2mg/kg.

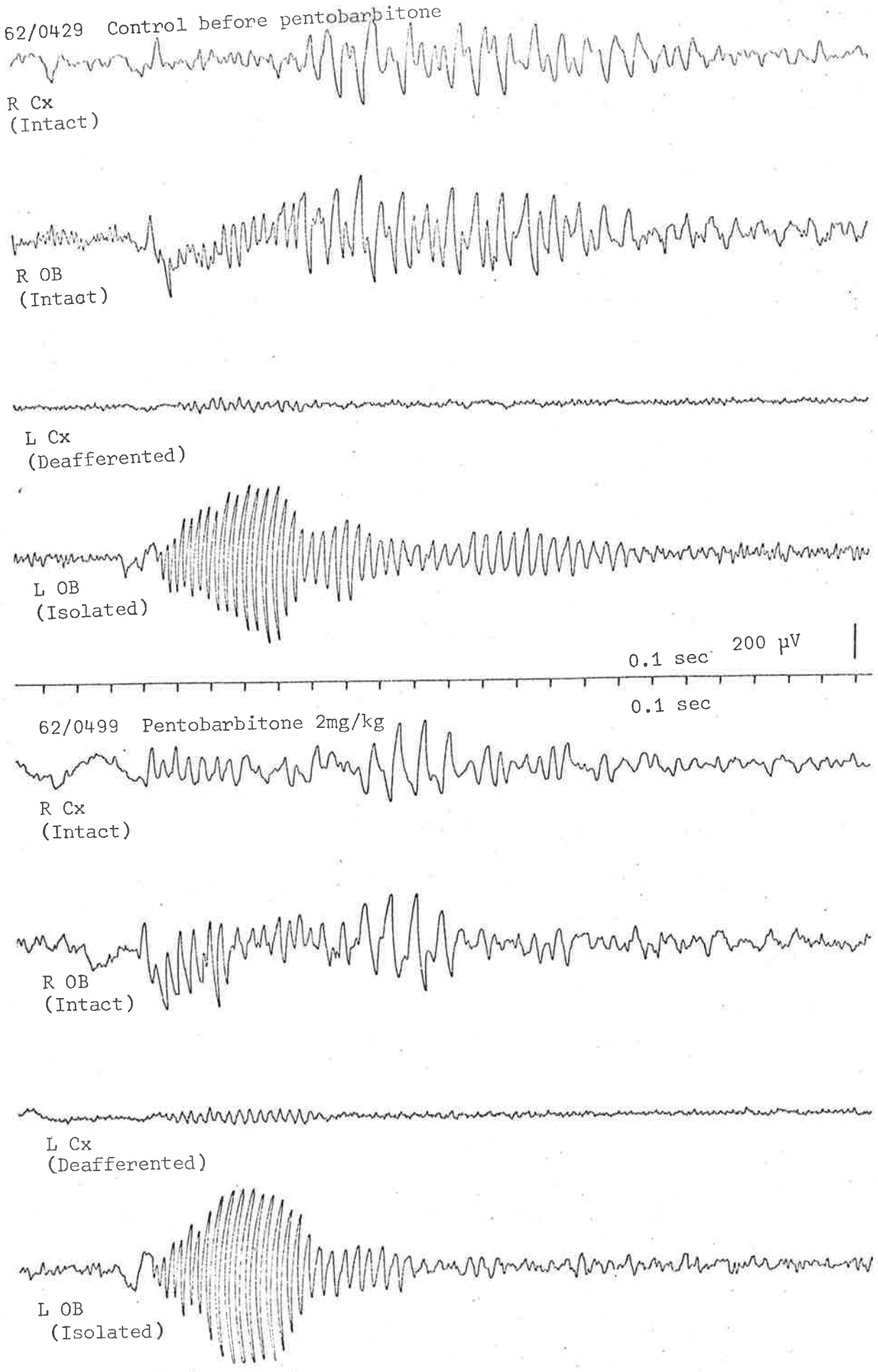


Fig. 4-10 A Induced waves recorded before pentobarbitone (upper) and after 2mg/kg (lower).

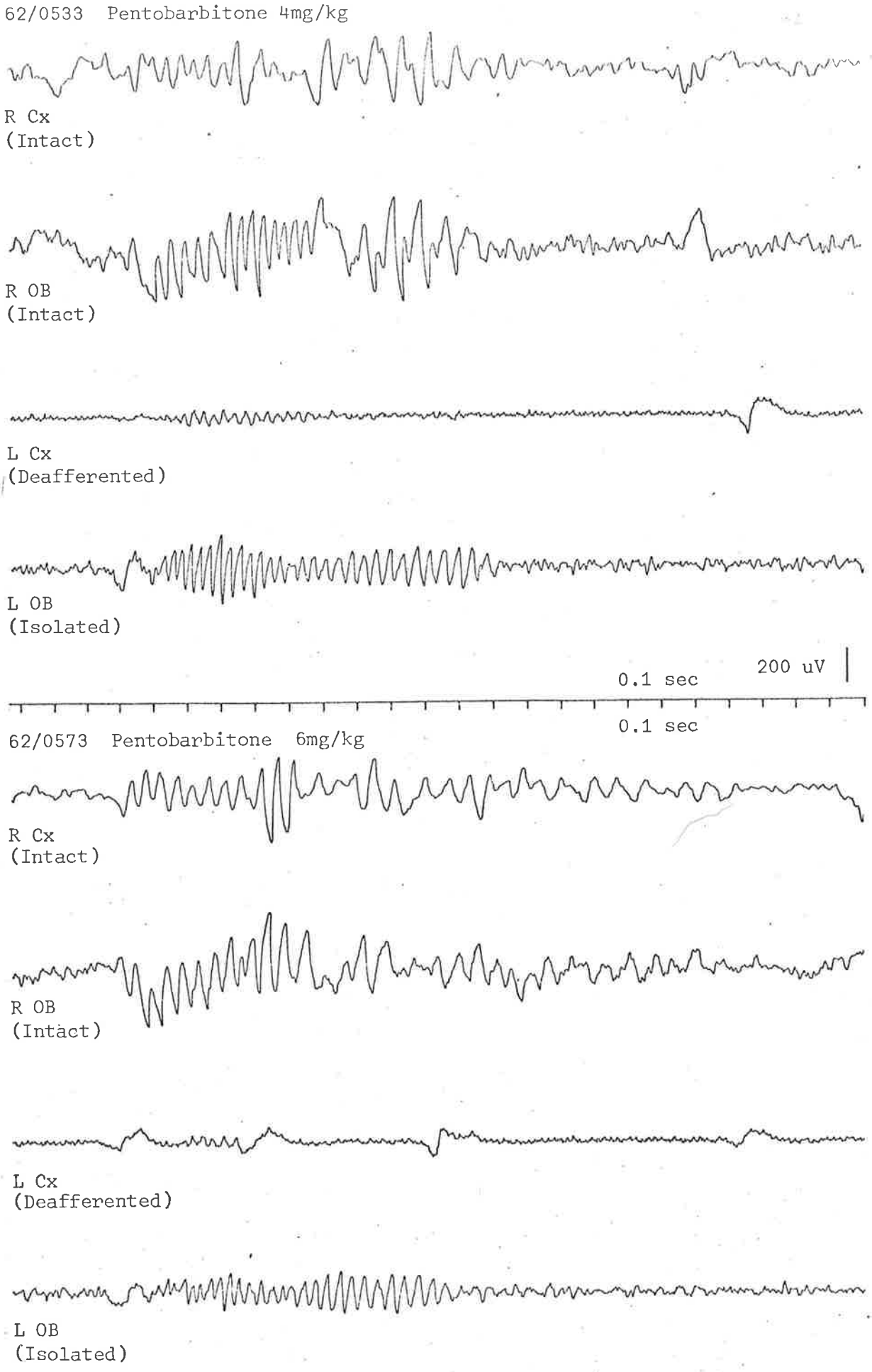


Fig. 4-10 B Progressive effect of pentobarbitone, 4mg/kg (upper) and 6mg/kg (lower) on induced waves of the phalanger.

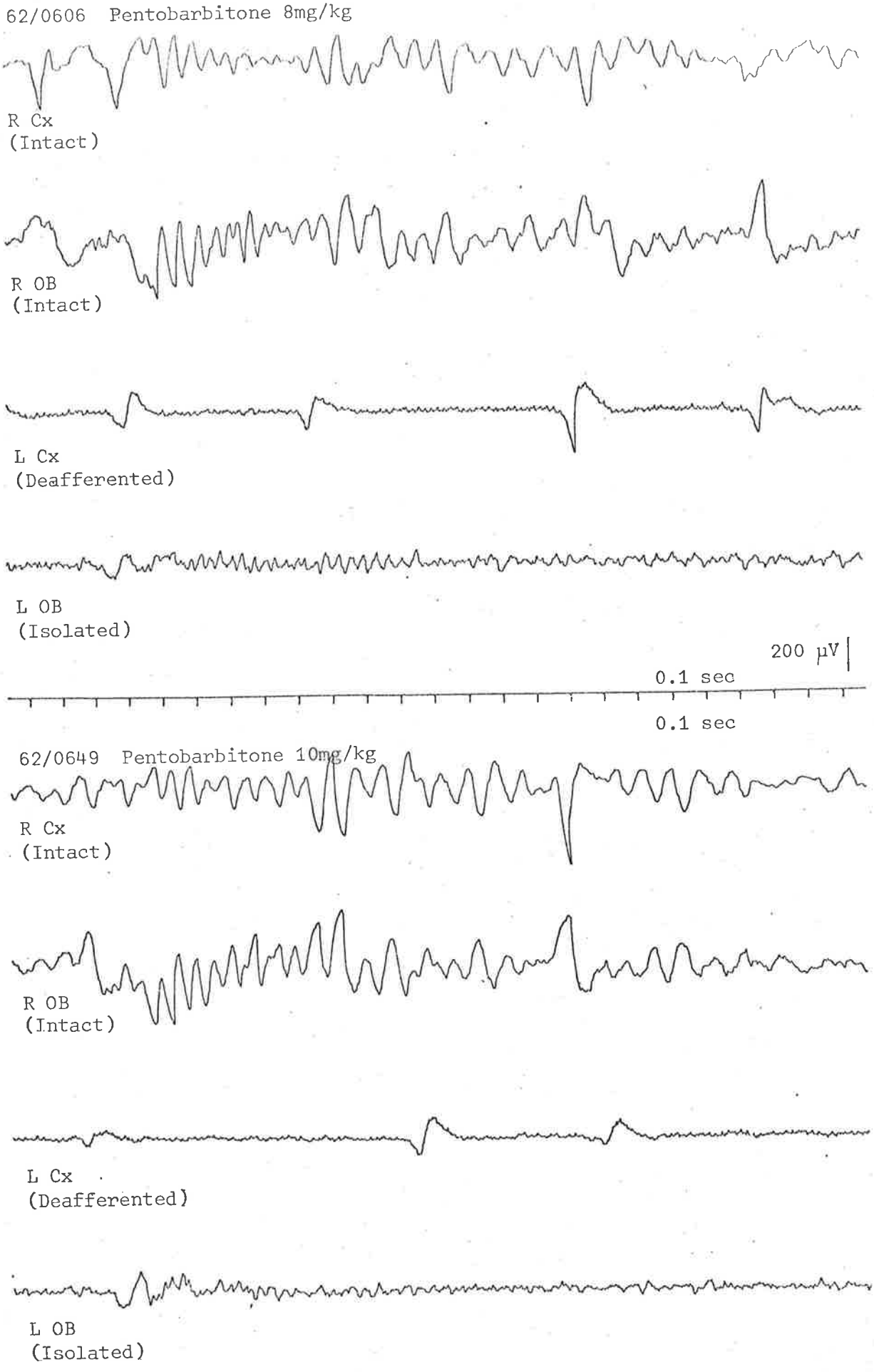


Fig. 4-10 C Continuation of series showing effects of pentobarbitone 8mg/kg (upper) and 10mg/kg (lower).

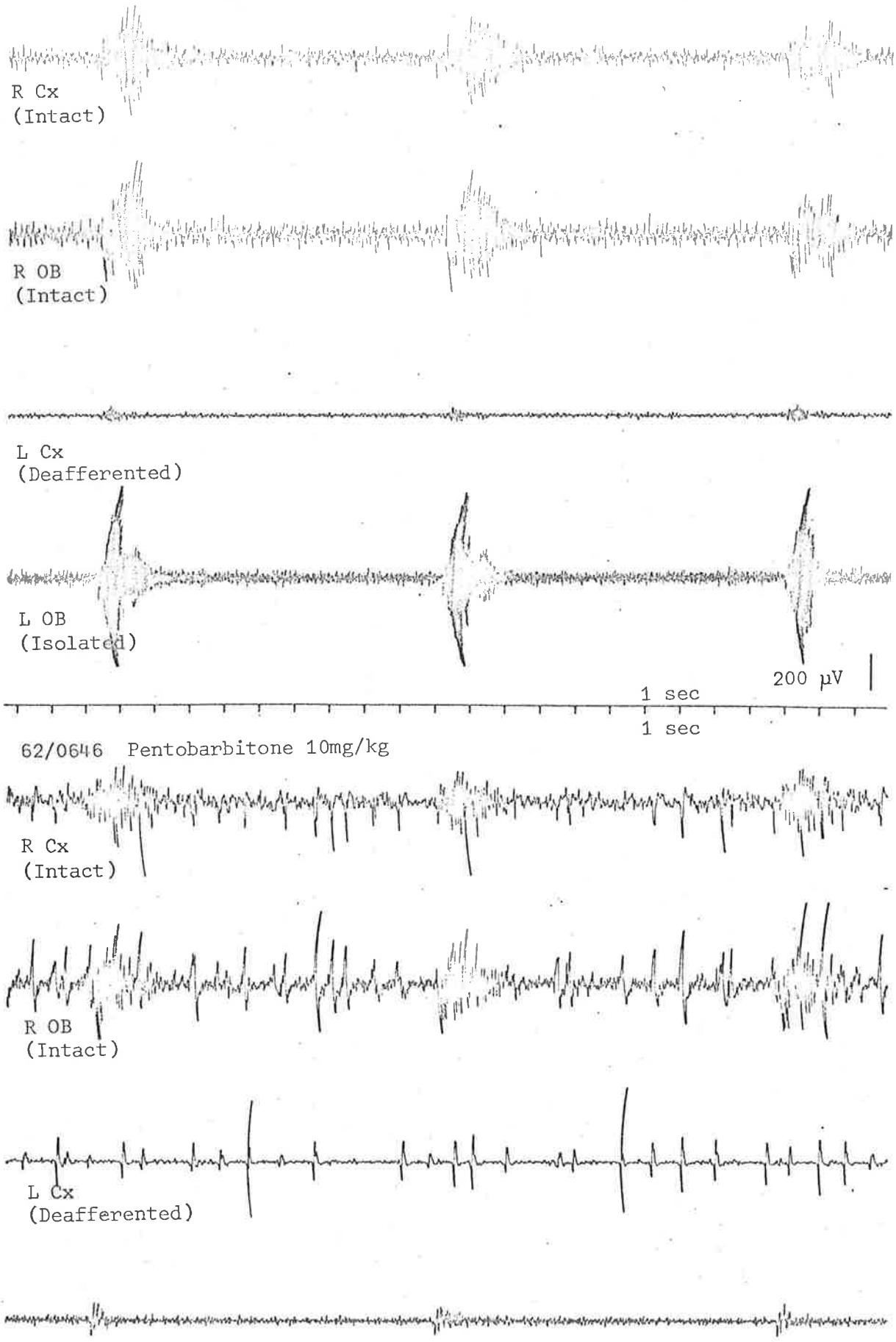


Fig. 4-10 D Real-time records of induced waves from the stated sites before (upper) and after (lower) pentobarbitone, from the same experiment as the preceding examples (Fig. 4-10 A-C).

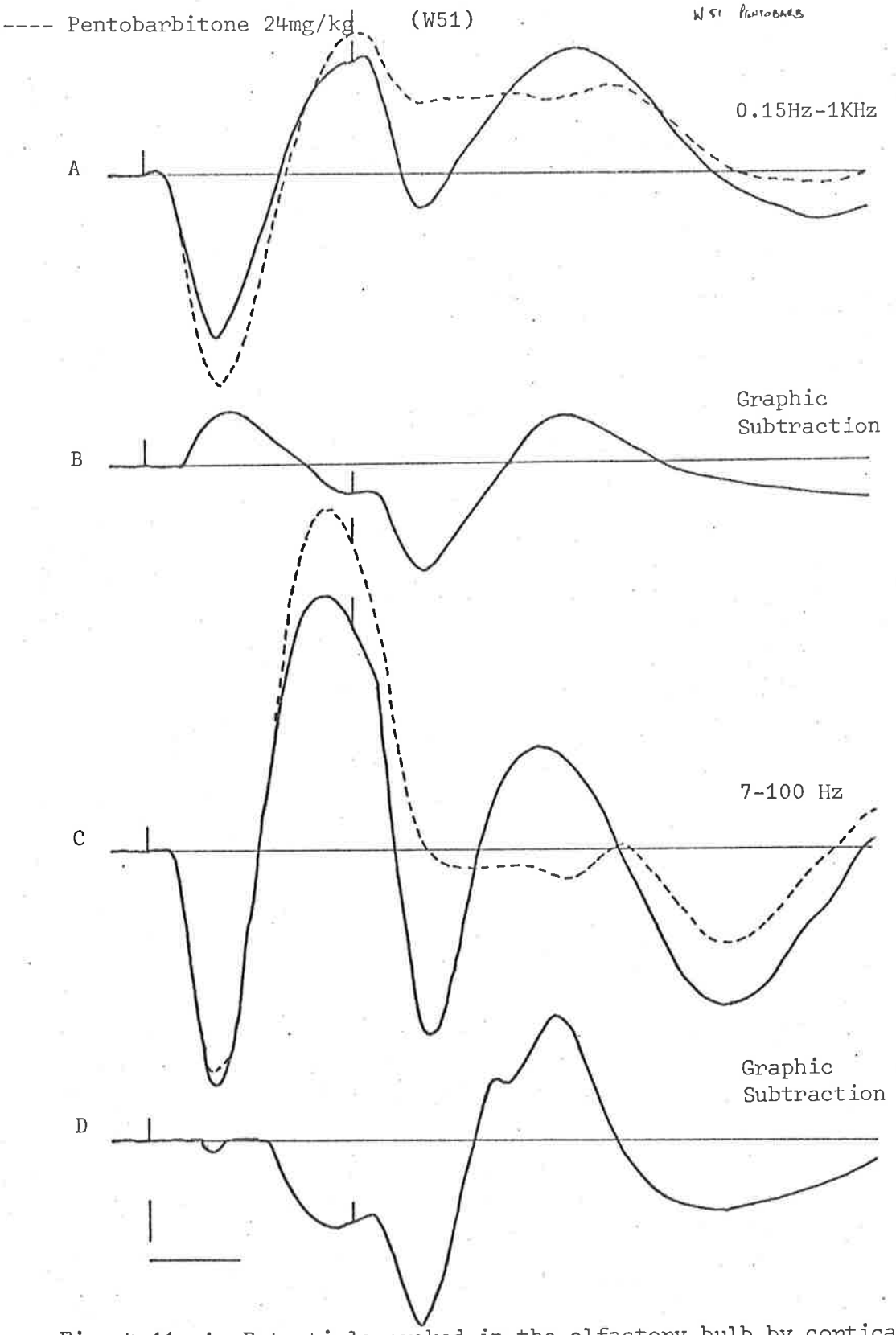


Fig. 4-11 A. Potentials evoked in the olfactory bulb by cortical stimulation before (continuous line) and after (broken line) pentobarbitone 24 mg/kg. Test response 45 msec after conditioning response. Band-pass 0.15 Hz-1 KHz.

B. Graphic subtraction of the two responses.

C. The same evoked potentials, but pre-amplifier band-pass 7-100 Hz.

D. Graphic subtraction of responses in C. (Calibration 20 msec, 1 mV)

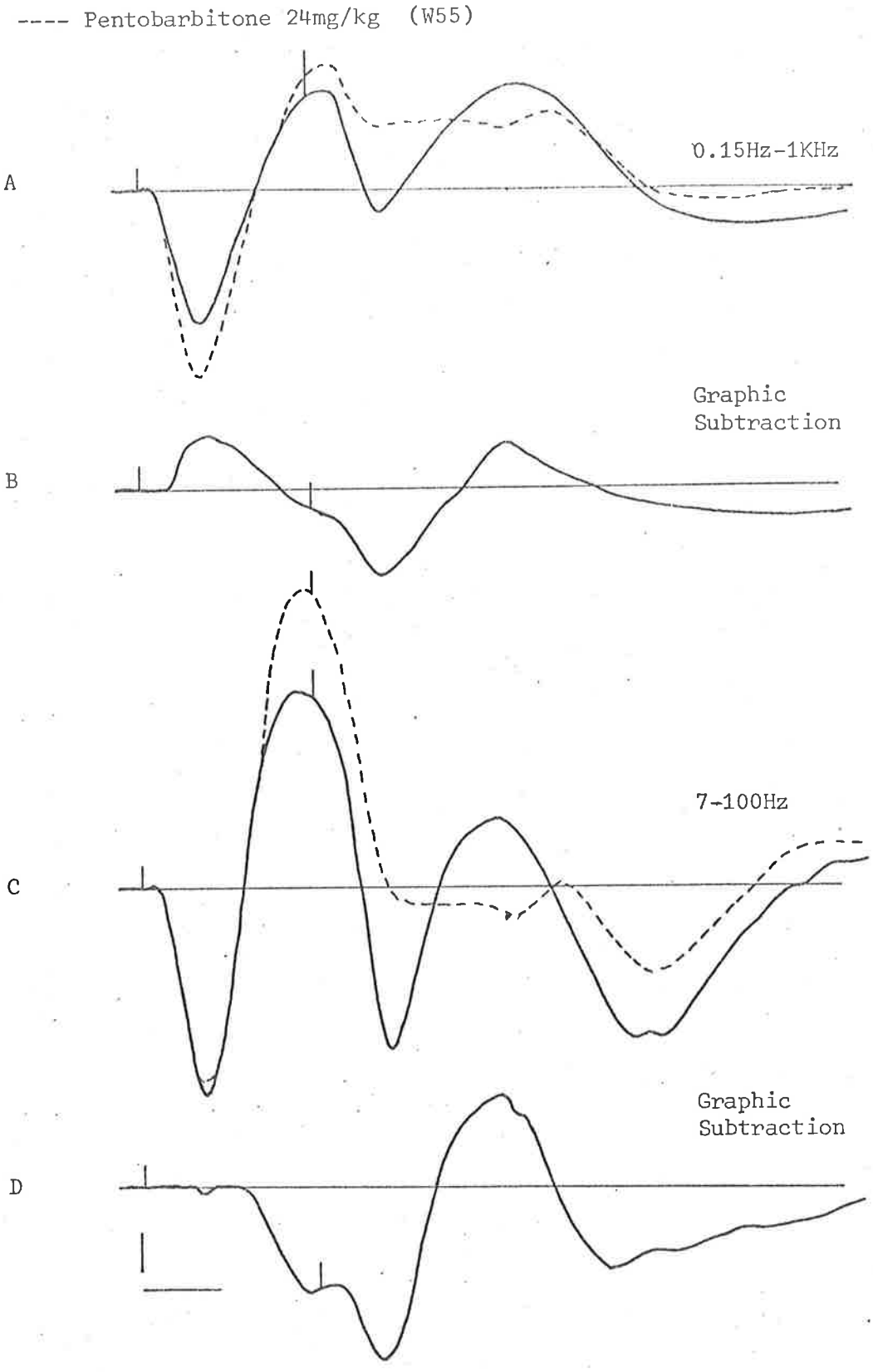


Fig. 4-12 A Potentials evoked in the olfactory bulb by cortical stimulation before (continuous line) and after (broken line) pentobarbitone 24mg/kg. Test response 45 msec after conditioning response. Band pass 0.15Hz-1KHz.

B Graphic subtraction of the two responses.

C The same evoked potentials, but with preamplifier band pass set at 7-100Hz.

D Graphic subtraction of responses in C.

(Calibration 20 msec, 1 mV)

Propanidid

Introduction

Propanidid (Epontol, Bayer) in a non-barbiturate anaesthetic induction agent (MW = 337.4; 1 mg = 2.96×10^{-3} mM), first prepared in 1963 and reported as an anaesthetic agent for humans in the following year (Dundee & Clark, 1964). Its pharmacology and clinical use have been reviewed recently (Conway & Ellis, 1970; Dundee & Clark, 1971). Electroencephalographic changes in man during and after propanidid anaesthesia have been described (Doenicke, Kugler, Schellenberger & Gurtner, 1966; Torda & O'Brien, 1971).

Darbinjan, Golovchinsky & Plehotkina (1971) studied the effects of propanidid, thialbarbitone (a short-acting barbiturate) and diethyl ether on brain function in the cat. They assessed drug effects on spontaneous activity of cerebral cortex (EEG) and mid-brain reticular formation. This was supplemented by examining the effects on non-specific sensory-evoked responses in the reticular formation, an electrically-evoked trans-callosal response, and EEG changes associated with electrical stimulation of the mid-brain reticular formation. Propanidid and thialbarbitone increased callosal potential amplitudes. Both depressed the evoked potentials in the reticular formation, propanidid less than thialbarbitone. It was concluded that the three drugs had different mechanisms of anaesthetic action, depending on whether cortex, reticular formation spontaneous activity, or transmission of information through the reticular formation was most affected.

Kavan, Besson & Aleonard (1971) studied the effects of propanidid on evoked potentials in various neuronal systems of the cat (primary sensory cortex, nuclei ventralis postero-lateralis and centremedian, and the mid-brain reticular formation). They noted EEG changes with doses to 30 mg/kg, including spiking, similar to those reported in man. The amplitudes of the potentials evoked in the primary sensory cortex by stimulation of the contralateral forelimb of the animal were increased in both their positive and negative phases. Maximum increase occurred within 5 min, and the potentials had returned to approximately control values in 20 min. A long-latency negative potential appeared in half the records from the ventral postero-lateral nucleus of the thalamus. Their conclusion was that propanidid was similar in action to the short-acting barbiturates. Its cellular or synaptic action was not determined.

In view of the sparse and conflicting information about propanidid, a study was made of its effects on olfactory activity of the phalanger.

Methods

Propanidid (5%) was given to six animals as the first non-volatile drug, and repeated once to three times each. Four were given incremental IV injections (1 - 5 mg/kg/bolus), and two had IV infusions at a rate of 2 mg/kg/min. Control injections of equivalent volumes of the propanidid vehicle* were given before the propanidid itself.

Induced waves were recorded from intact bulb and olfactory cortex of all animals. No records were made from isolated bulbs. Cardiovascular parameters were not measured.

Evoked potentials were recorded from the bulbs of the two animals receiving the infusions. Switching from record to stimulate mode of the cortical electrode was carried out each minute as before. Band pass filters of the preamplifier were set at 7 - 100 Hz and 0.15 Hz - 1 KHz. In one animal, intercollicular transection of the mid-brain was performed and the infusions repeated.

Results

Induced waves. Propanidid in small doses (1 - 2 mg/kg) caused an increase in the amplitude of the induced waves. On slow replay of the magnetic tape, it was seen to be a similar effect to that of the barbiturates. Fig. 4-13 illustrates the changes. The upper part shows the dramatic change resulting from the injection of a bolus of 2 mg propanidid/kg. Within 15 sec, the bulbar and cortical induced wave had become greater in amplitude and duration. The middle record is the induced wave before the injection, and shows the usual appearance. The lower record is that of the third induced wave of the series, 15 sec after the injection. There is reduction in the high frequency bulbar component, and a marked increase in the low frequency central component.

At higher doses (5 - 10 mg/kg), spiking occurred in the cortical and bulbar records. Induced waves of both cortex and bulb were suppressed. Duration and amplitude were both reduced, and frequency slowed until it was in the 15 - 20 Hz range.

Above 20 mg propanidid/kg, the induced waves were not able to be recorded. The intrinsic activity of the bulb was reduced in amplitude, but appeared normal otherwise. The olfactory cortical record appeared

---

*Samples of propanidid vehicle (Epontol OW, Bayer) were supplied generously by the Medical Director of Bayer Pharmaceutical Company (Australia).

to contain more low frequency (5 - 10 Hz) activity than without the drug.

After propanidid 20 mg/kg was given within 10 min, induced waves returned to their control appearance within 20 min.

Evoked potentials. Propanidid infusion (2 mg/kg/min for 12 min) caused an increase in the initial positive potential of the conditioning (first) response, and a minimal increase in the late negative response. There was minimal alteration in the test response 45 msec later (Fig. 4-14).

Because of the ephemeral action of propanidid, it was not possible to carry out the full sequence of conditioning-test responses at a constant blood level. There was, however, absence of induced waves by the end of the infusion.

Propanidid vehicle had no effect either on induced waves or evoked potentials.

Discussion

Propanidid has a brief duration action because of its rapid metabolism by esterases in several species. That the phalanger has usual levels of esterases may be implied from the observation that succinyl choline does not have a prolonged action.

Propanidid has an effect on the induced waves which is similar to that of the barbiturates. There is an increase in the low frequency component of the burst at the expense of the high frequency bulbar component (compare Figs. 4-9 and 4-13) in the intact bulb. It would have been a more convincing study if the effects on an isolated bulb had been recorded.

It has been demonstrated that there needs to be a minimal bulbar signal to cause the cortical burst. In the examples illustrated (Figs. 4-9 and 4-13) it is difficult to determine the precise onset of the induced wave in either bulb or cortex. It is not surprising, therefore, that Boudreau (1963) concluded that the prepyriform cortex led the bulb (his Fig. 21 and pp. 104 & 108).

Mechanisms of action of propanidid and pentobarbitone are not clear. The design of this experiment obviously does not allow definition of the precise sites of action, let alone effects on membranes or quanta of transmitters. However, the induced wave-generating mechanism of the bulb seems to be extremely sensitive to both these drugs.

If it is assumed that the mitral cells generate the induced waves, these results would be explicable by (a) inhibition of olfactory

nerve-mitral cell transmission; (b) increase in inhibition within the glomerulus; (c) increase of inhibition of mitral cells at the dendro-dendritic synapse between mitral secondary dendrites and somata and granule cell gemmules; or (d) direct metabolic depressant effect on mitral cells. Within the isolated bulb, it is assumed that the only feedback inhibitory loops are the dendrodendritic synapses (in glomeruli and external plexiform layers) and *via* the mitral recurrent collaterals. It will require, for example, unit studies of mitral cell inhibition to clarify sites of action of barbiturates and propanidid.

The two components of the intact bulbar record could have two generators: mitral cells for the high-frequency component, and granule cell dendrites or bodies for the low-frequency component. Again, unit studies of mitral cells would be required to determine this. The positive and negative components of the evoked potentials have different generators (Polson, 1971). The short-axon cells have been ignored in this discussion: their role is unknown.

No definite cause can be given for the increase in the induced wave recorded in the cortex. The implication of the increase is that there was (a) increased excitation or (b) reduced inhibition of the generators of the cortical burst, presumably the pyramidal cells.

It would be helpful if the experiments were repeated with the following design: (1) one bulb isolated, with electrodes in it, and its cortex; (2) electrodes recording from intact bulb (external plexiform and granule cell layers), lateral olfactory tract, and olfactory cortex. This would indicate the activity in the lateral olfactory tract (predominantly mitral cell axons). It might show, in addition, if efferent fibres in the tract are the conductor to the bulb of the low-frequency activity which arises centrally, or if it travels in one of the other efferent systems.

Unit studies of mitral cells, induced and evoked, would also be necessary, with particular reference to nature and duration of inhibition.

In view of the low numbers of experiments and defects in experimental design, the results presented in this thesis must be regarded as being preliminary only.

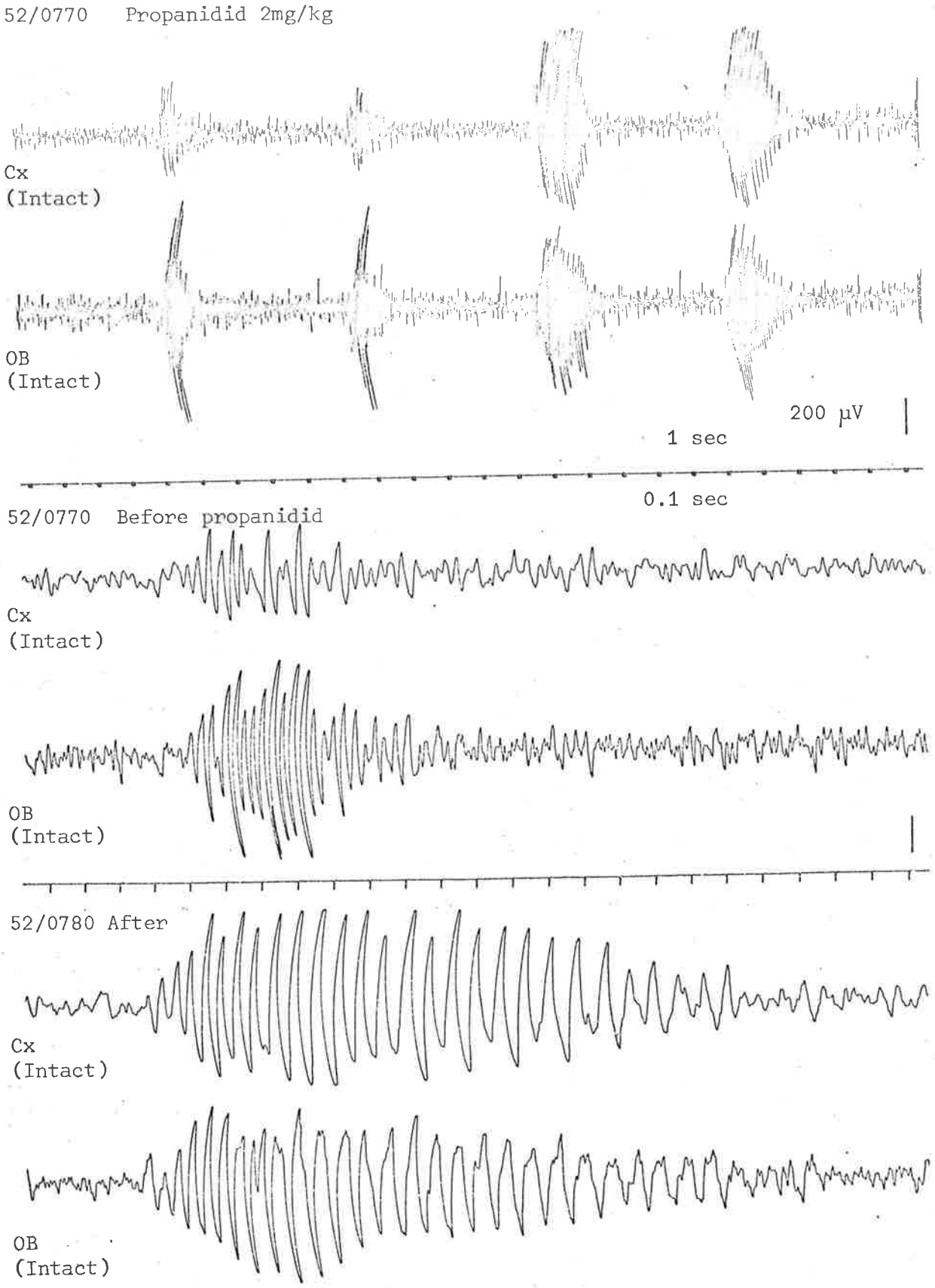


Fig. 4-13 (Upper) The effect of propanidid 2mg/kg on the induced waves recorded from intact bulb and cortex of the phalanger. Injection was made between the first and second responses.
(Middle) The induced wave immediately before the injection, written out at 0.1 recording speed.
(Lower) The third induced wave of the series.

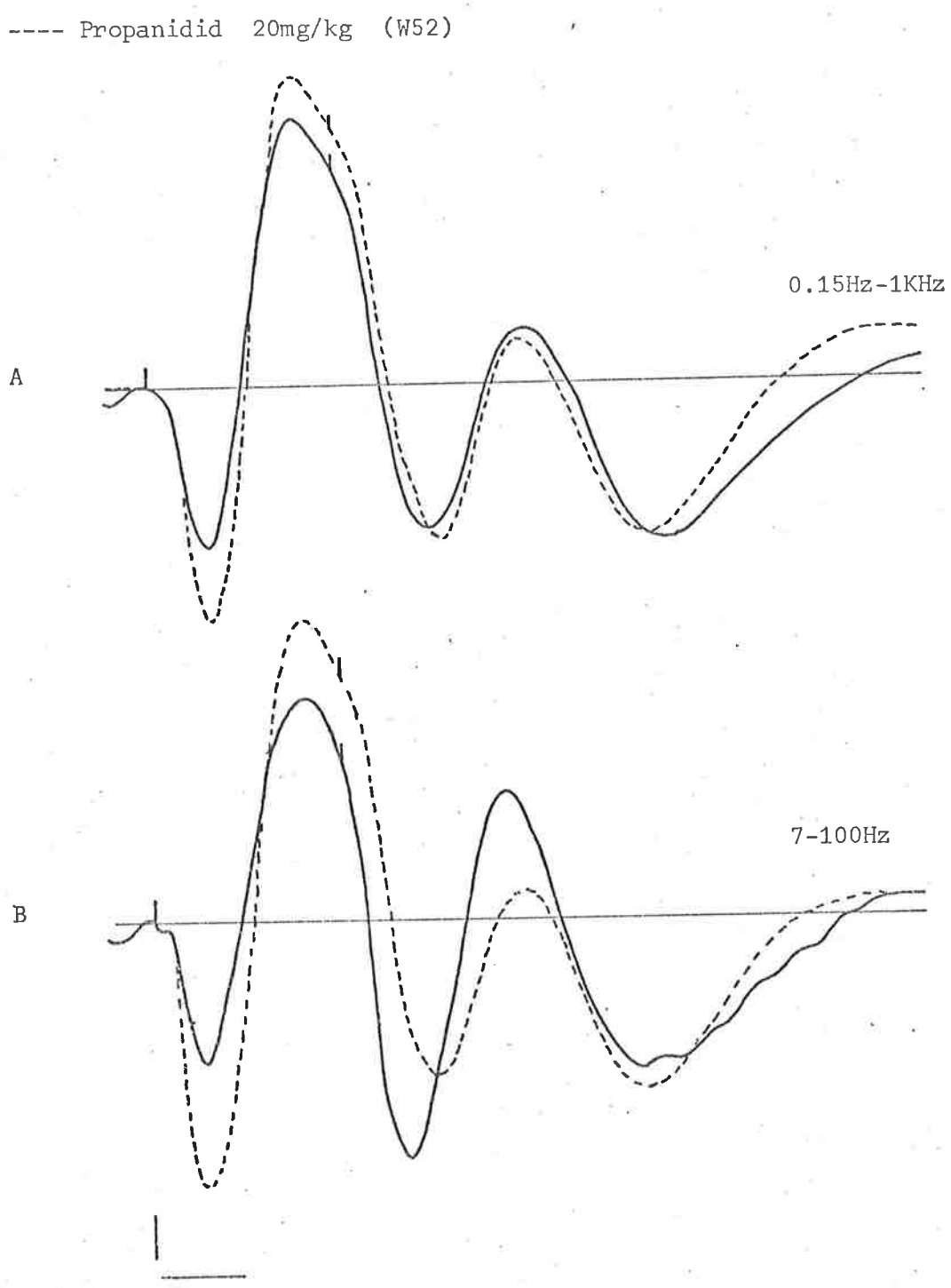


Fig. 4-14 A Potentials evoked in the bulb by stimulation of the olfactory cortex before (continuous line) and after (broken line) propanidid 20mg/kg. Band pass 0.15hz-1KHz. Test stimulus 45msec after the conditioning stimulus.

B The same potentials, with band pass set to 7-100Hz.

(Calibration 20msec, 1 mV

Diazepam

Introduction

The 1,4-benzodiazepines comprise a relatively new group of compounds whose pharmacology and clinical use is still being assessed. This group of drugs is being intensively investigated as (a) anti-convulsants, (b) minor "tranquillizers", (c) anaesthetic induction agents and (d) "centrally-acting muscle relaxants".

Structure-activity relationships of this group have been discussed by Sternbach, Randall, Banziger & Lehr (1968). They used standard tests for motor co-ordination, sedation, timing, and seizure protection. Results from some 27 compounds were described, and similarities and differences discussed.

Clinical use of the benzodiazepines was reviewed by Dundee & Haslett (1970), with particular reference to their use in anaesthetic practice. At that time, four were in clinical use: diazepam, chlordiazepoxide, oxazepam and nitrazepam. Of these, diazepam enjoys the distinction, in Australia at least, of being the most extensively used in clinical practice.

Diazepam (MW = 284.76; 1 mg = 3.51×10^{-3} mM) has been widely studied, and it was decided to investigate its actions on the olfactory system of the phalanger.

Methods

Three phalangers received diazepam* (0.5%) as the first drug after recovery from general anaesthesia, two by incremental bolus IV injection, and one by IV infusion. Comparable volumes of the vehicle were also tested for central actions. Effects on the induced waves of the intact olfactory system were studied in the three animals. In the one of these receiving the infusion, bulbar potentials were evoked by olfactory cortical stimulation each minute. Band pass of the pre-amplifier was set at 7 - 100 Hz, and stimuli and switching were controlled by the Digitimers as before. The conditioning-test interval was constant at 45 msec.

The phalanger receiving the infusions was subjected to

---

* General supplies of diazepam were received from the Associate Medical Director, Roche Products Pty Ltd. The commercial formulation of the vehicle was indicated as: Propylene glycol, 40% v/v; Ethyl alcohol, 10% v/v; Sodium benzoate, 97.6 mg; Benzoic acid, 2.4 mg; Benzyl alcohol, 1.5% v/v; NaOH at pH 6.4 - 6.6; Water to 2 ml; containing 10 mg diazepam as Valium for injection.

intercollicular transection of its brain stem. The induced and evoked potentials were recorded during the two hours following the procedure. Diazepam was infused during this time also.

Results

Induced waves. Diazepam in doses of 1 mg/kg caused an increase in amplitude and duration of the induced waves, superficially similar to those produced by pentobarbitone and propanidid. Real-time records of induced waves in intact bulb and cortex before and after diazepam, 1 mg/kg, are seen in the upper parts of Figs. 4-15 A & B. Records produced at 0.1 recording speed are shown in the lower part of these two figures. It can be seen that there is an increase in the cortical low frequency component, but there is still a significant amount of the bulbar high frequency activity.

Higher doses of diazepam caused a progressive decrease in cortical and bulbar bursts, with bulbar activity persisting after the cortical induced wave had been suppressed. Figure 4-16A is a control record of activity in the intact bulb and its cortex, and the contralateral, unstimulated bulb. With increasing dosage (Figs. 4-16B, 1 mg/kg; 4-16C, 2 mg/kg; 4-16D, 3 mg/kg) there is a progressive decrease in the amplitude and duration of the bulbar and cortical induced waves. These induced waves appear to contain the control frequencies.

Recovery from diazepam was slow, and bursts did not resume their control appearance for 2 - 3 hr.

Diazepam vehicle was without effect.

Evoked potentials. There were only minor changes in the evoked potentials at 3 mg diazepam/kg, despite the profound depression of the induced waves. Latency was unchanged (measured to peak positivity, 16 msec). The initial positive potential was increased, and the positive potential of the test stimulus was also increased (Band pass 7 - 100 Hz). Changes in the negative components were minimal (Fig. 4-17 A & B).

Intercollicular transection had no effect on the induced or evoked potentials, or the effect of diazepam on these.

Discussion

There has been only one previous report of the actions of diazepam on the olfactory system (Fukuda, 1966). His study is said to have used aqueous solutions of diazepam, which is insoluble in water. The solvent used in my study was that recommended by the suppliers of the drug. Crankshaw & Raper (1971) showed that the propylene glycol-based solvent

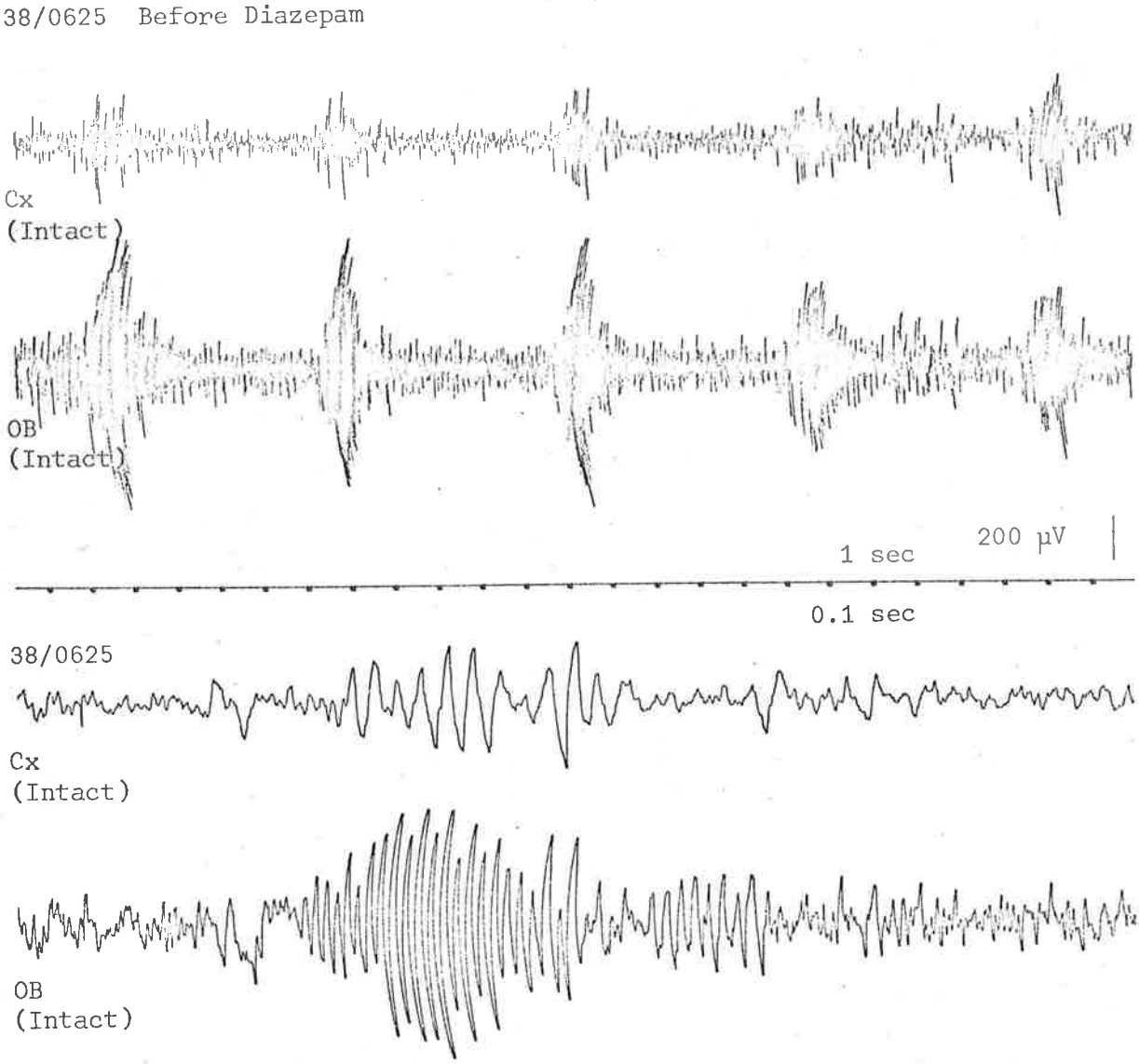


Fig. 4-15 A Induced waves recorded from intact bulb and cortex of phalanger before diazepam. The high frequency bulbar component is prominent, with little evidence of the low frequency cortical component.

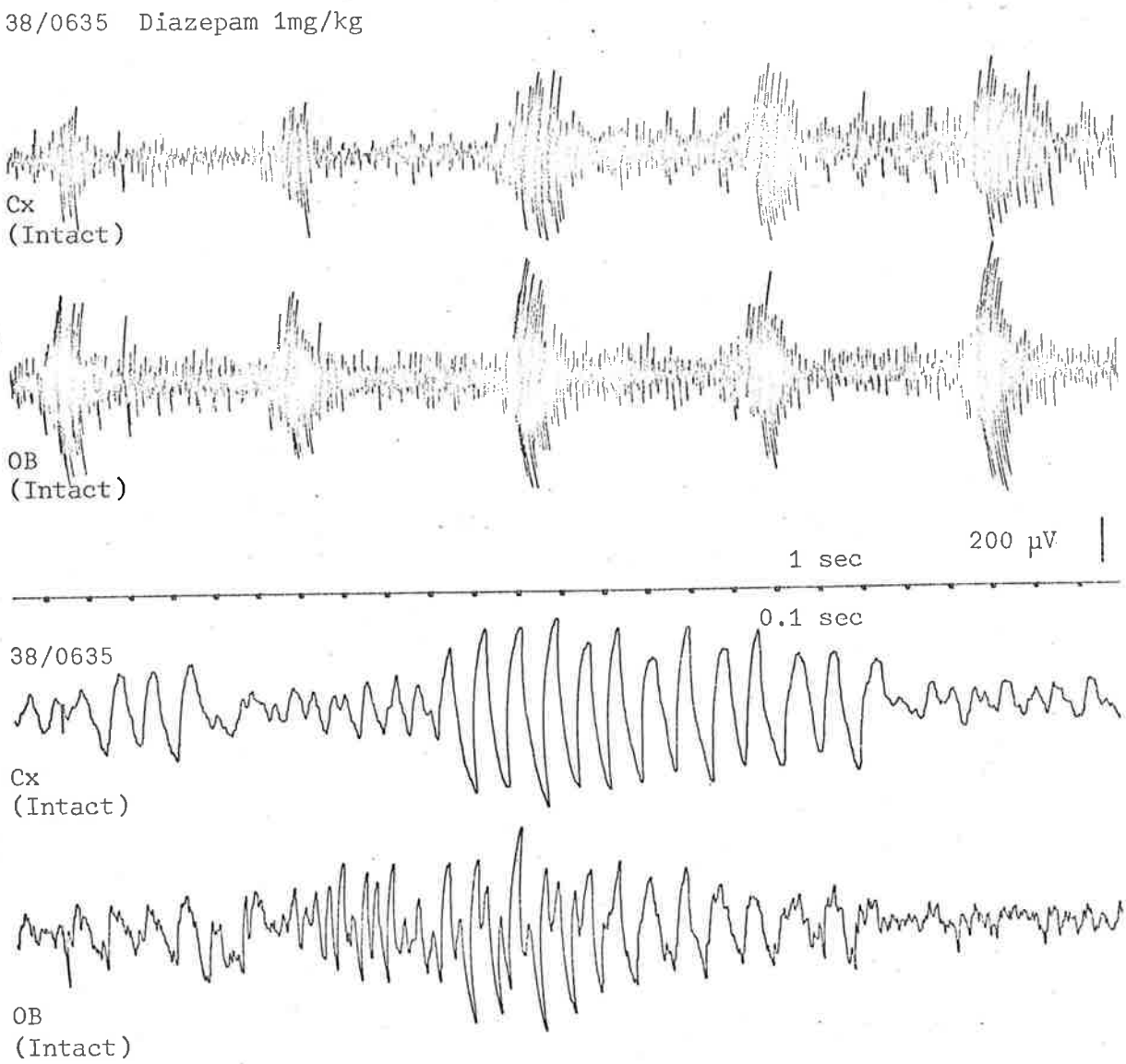


Fig. 4-15 B The effects of diazepam, 1mg/kg, on induced waves from the same sites as the preceding figure. Notice that the high frequency bulbar component is reduced, and the low frequency cortical component is prominent at both sites.

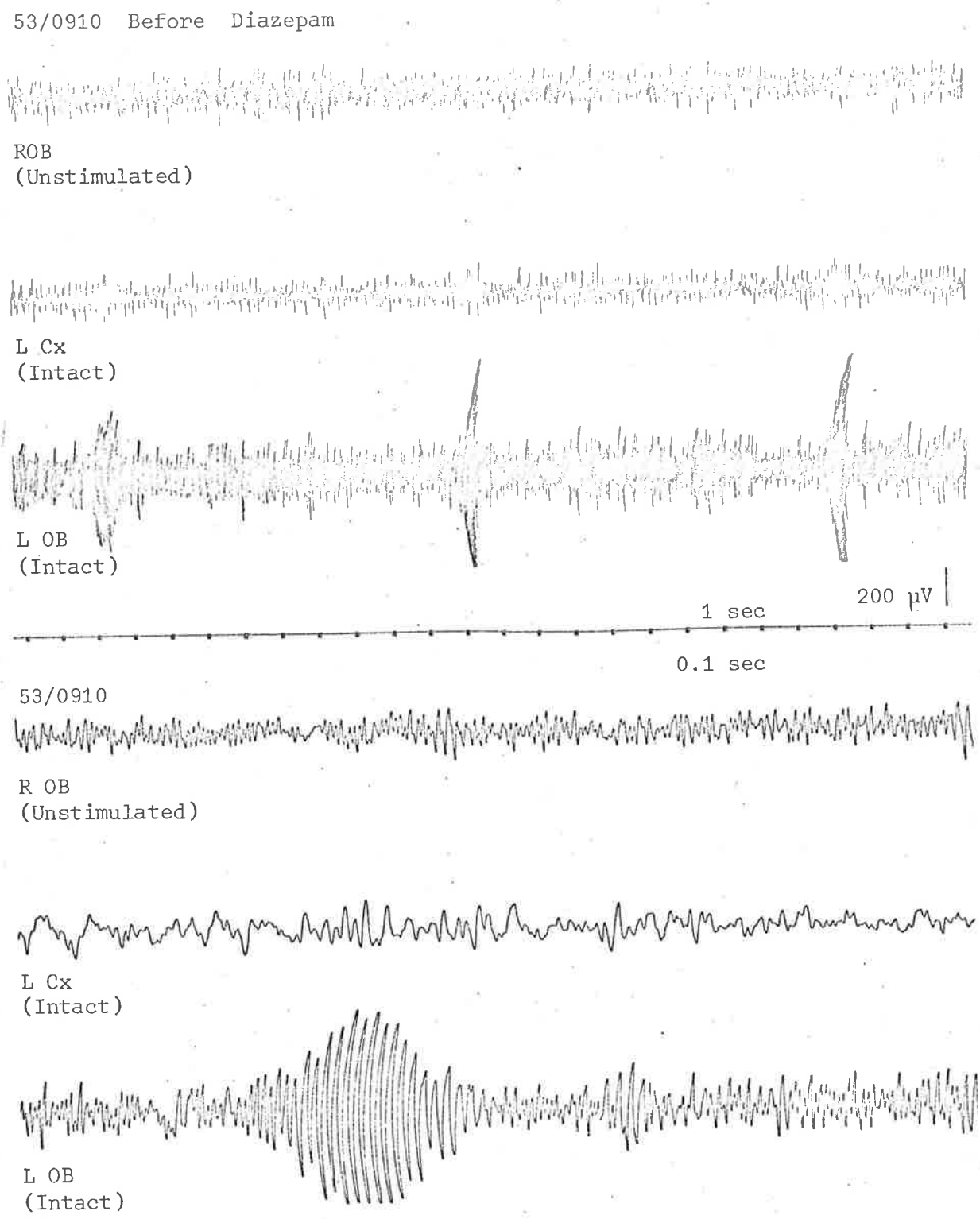


Fig. 4-16A. Induced waves from intact bulb and cortex before diazepam. There is minimal induced activity in the cortex, and no low-frequency activity in the bulb.

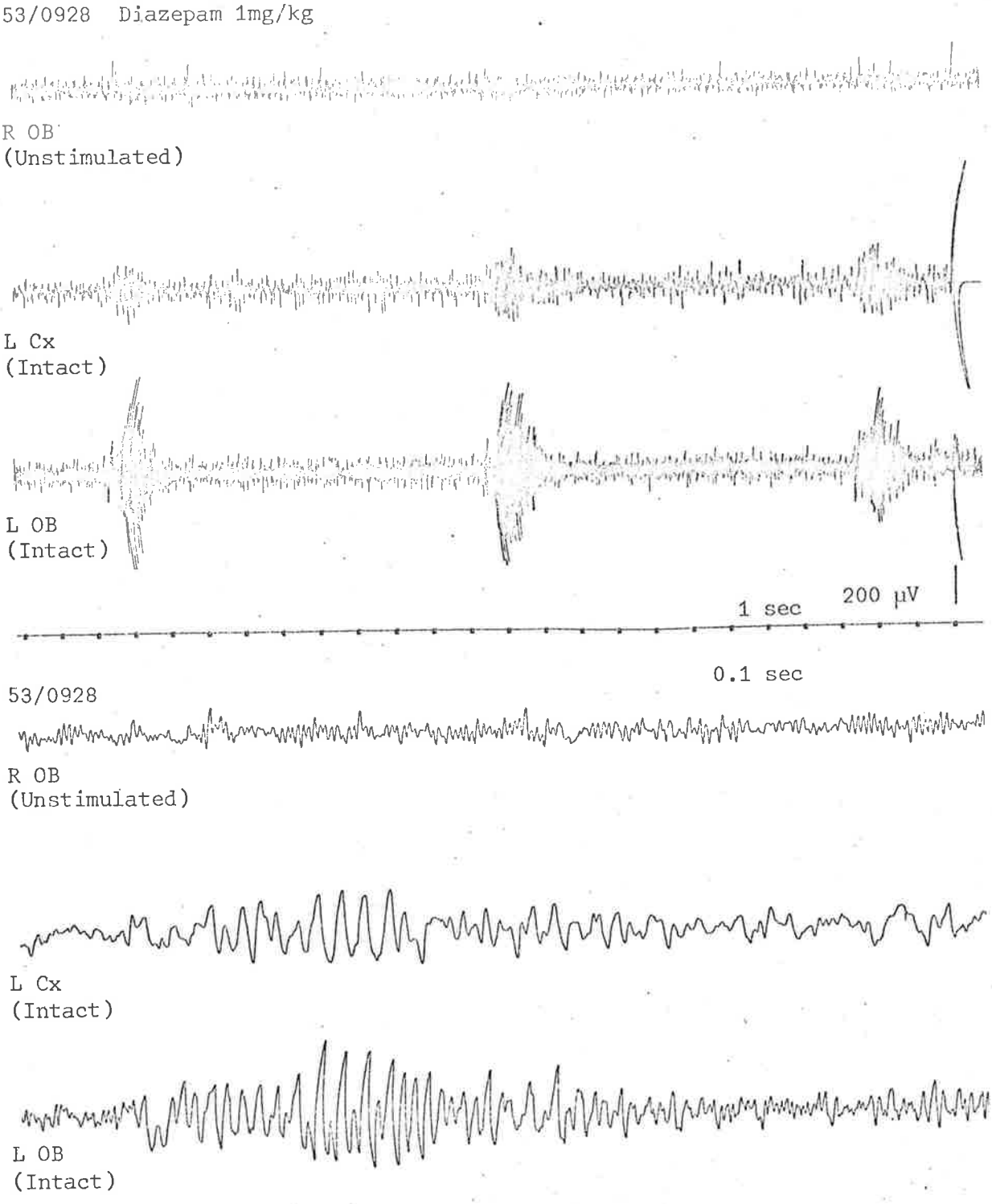


Fig. 4-16B. Induced waves recorded from the same sites as the preceding figure after diazepam 1 mg/kg.

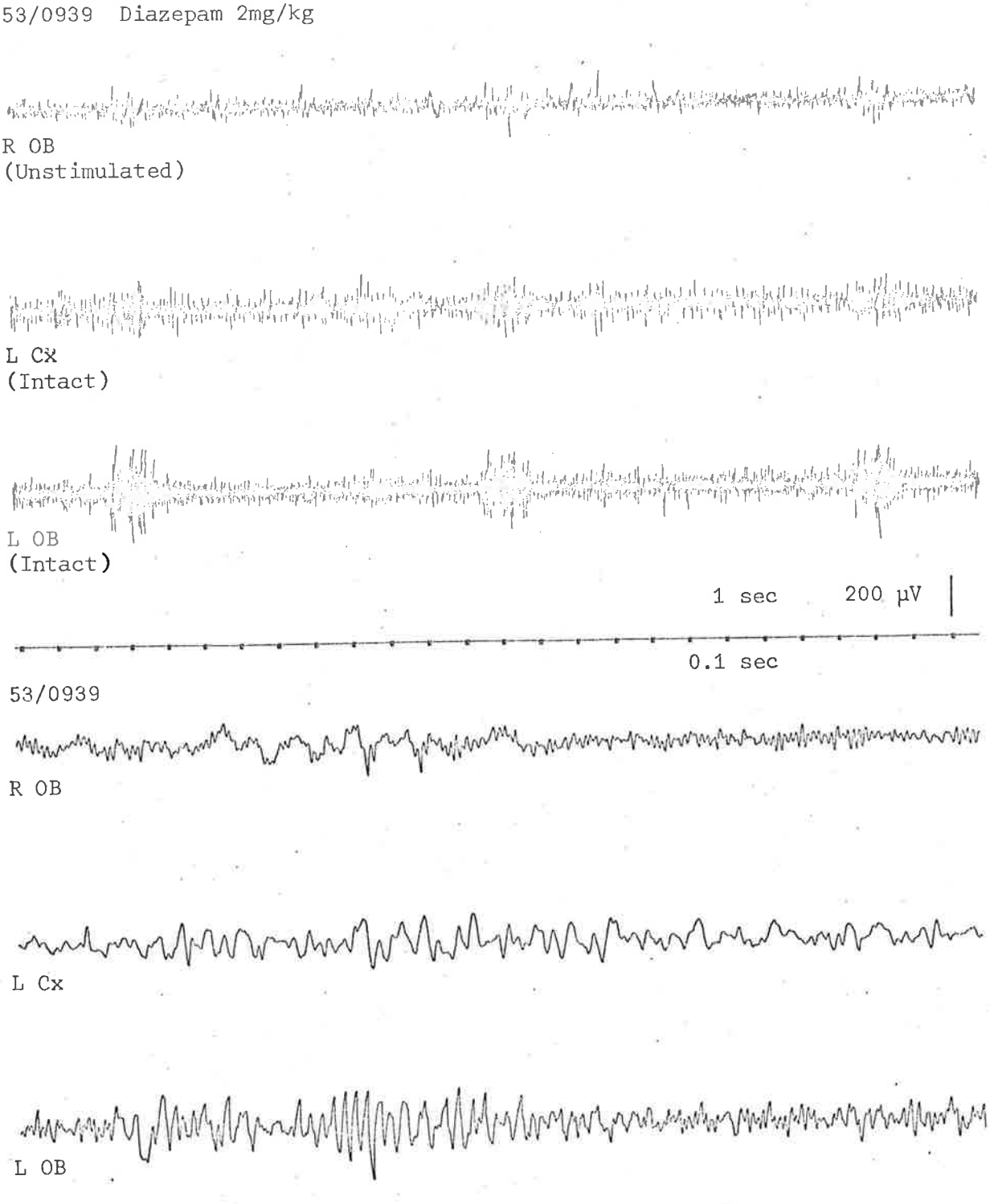


Fig. 4-16C. Induced waves recorded from the same sites as the preceding figure after diazepam 2 mg/kg.

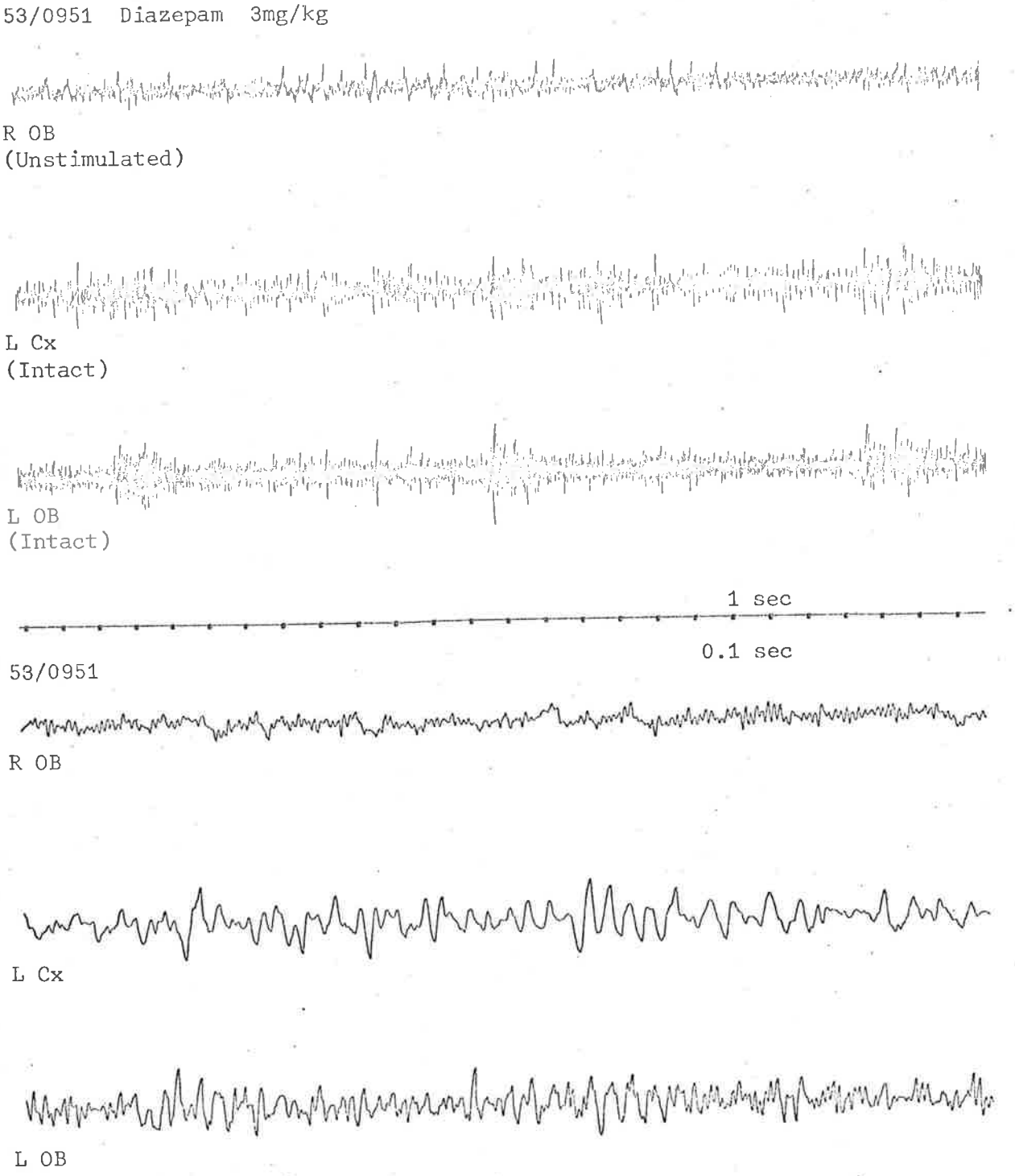


Fig. 4-16D. Induced waves recorded from the same sites as the preceding figure after diazepam 3 mg/kg.

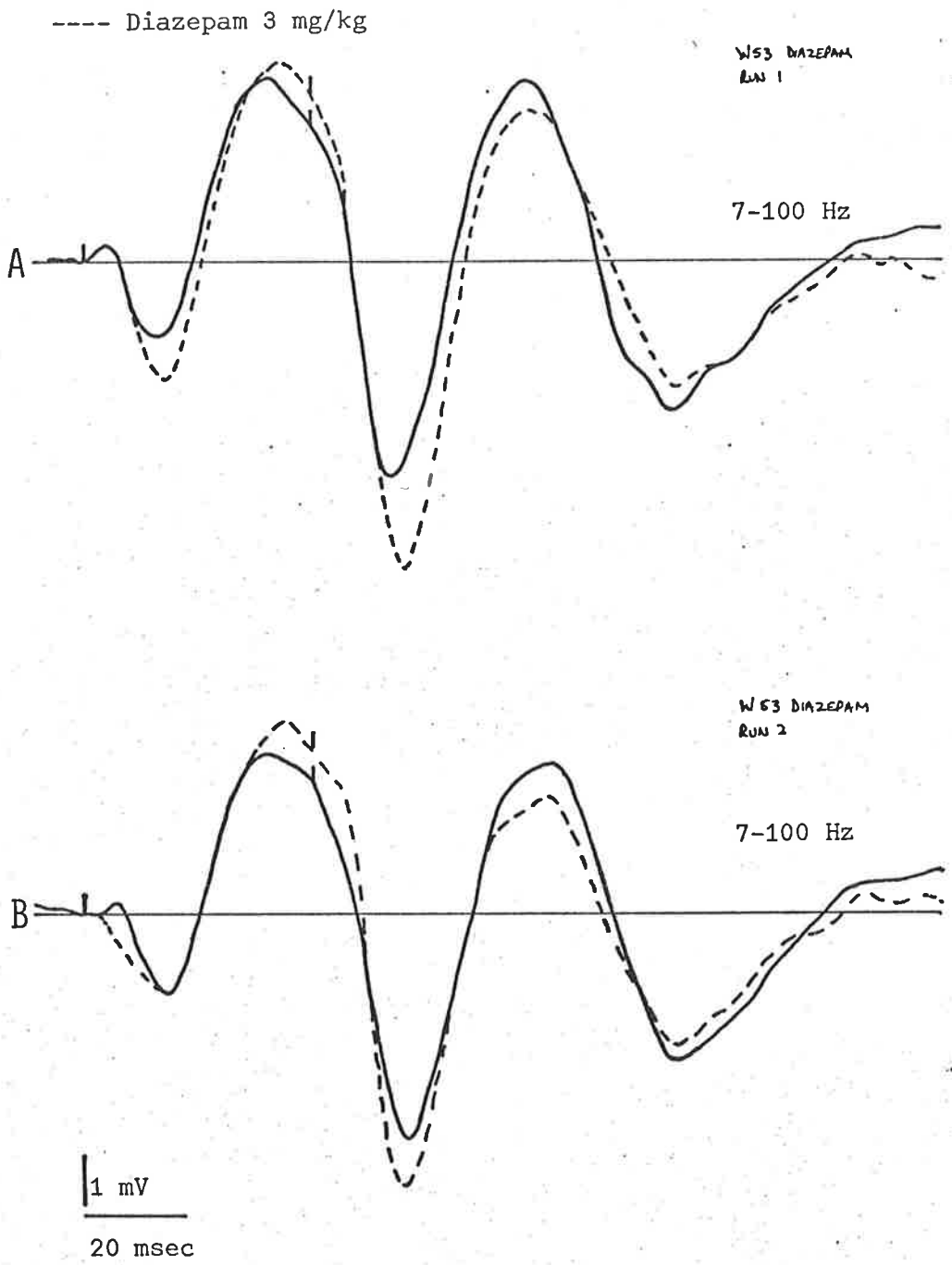


Fig. 4-17 A. Responses evoked in the olfactory bulb of the phalanger by stimulation of olfactory cortex with two electrical pulses, 45 msec apart. Drug-free response solid line, response after diazepam 3 mg/kg broken line.

B. Evoked responses in the phalanger four hours later after an additional dose of diazepam, 3 mg/kg.

gave the lowest ED_{50} in mice.

Sharer & Kutt (1971), however, have shown that 40% propylene glycol injected intravenously caused respiratory arrest, hypotension, and cardiac arrhythmias. The magnitude of these cardiorespiratory effects was approximately two-thirds of those caused by the diazepam in solution. These parameters were not measured in this thesis. The diazepam vehicle had no apparent effect on olfactory activity.

Changes in the induced waves superficially resembled those of propanidid and pentobarbitone. There was an initial increase in the cortical contribution, but not the same degree of bulbar depression which would have been expected with the other agents. At the doses used here, there was some suppression of the intrinsic activity, which may have removed inhibition early, to be followed by non-specific depression of all activity later.

Alterations to the evoked potential were markedly different from those caused by pentobarbitone. At the doses used (pentobarbitone, 24 mg/kg; diazepam, 3 mg/kg), induced waves had been abolished. Pentobarbitone, however, caused inhibition of the test response by the conditioning one, where diazepam had no such effect in three infusions in the one experiment.

These results, which also must be regarded as preliminary, indicate that there may be a major difference in basic action of pentobarbitone and diazepam in the olfactory bulb. In order to confirm the observation and elicit the differences, a formal study of the evoked potentials, alone and interacted (tract-cortex, tract-tract, tract-commissure, etc.), will need to be carried out. Such an experiment could be carried out on a deafferented animal. Data could be acquired according to the equipment available: oscilloscope traces photographed, as here, or in the manner of Nicoll (1973), or responses digitized and stored on magnetic tape for subsequent numerical analysis. This may be a fruitful field.

Care will have to be taken in the experimental design, because of the prolonged action of diazepam. Although the induced waves and evoked potentials appeared normal to the eye (after slow writeout) after 2 - 2½ hours, evidence has been presented that suggests that EEG changes may persist for significantly longer. Joy, Hance & Killam (1971) used diazepam 2 mg/kg in primates and showed that the spectral transform of the EEG was altered for more than 24 hours, with recovery appearing 'essentially complete' within 48 hours after

administration. This suggests that diazepam may be slowly eliminated or metabolized, or may have a prolonged effect on enzyme or transmitter systems, as well as its acute effects.

CONCLUSIONS

Studies were carried out to make comparisons between the effects on olfactory activity in the phalanger of a group of diverse chemical agents having the common action of anaesthesia. The influence of these agents on different parts of the olfactory system of the phalanger was investigated. Comparisons were also made between their effects in the phalanger and those reported in other species.

Species differences

No major differences in olfactory responses to drugs were uncovered between the phalanger and those reported in other species.

In particular, nitrous oxide caused a reduction in induced waves, an increase in background activity, and generalized but minor depression of both components of the evoked potential.

Induced waves were not able to be recorded if chloroform had been used as the anaesthetic for preparation. Chloroform depressed induced waves and, on occasions, caused cortical spiking. This had not been reported previously in other species. It was shown to have a local anaesthetic action on the olfactory mucosa in addition.

Halothane was shown to depress the generation of induced waves in the isolated bulb. It increased the activity of the cortex initially, including that of the induced wave, with eventual depression. The action was not the same as that of chloroform, but the mechanism was able to be determined.

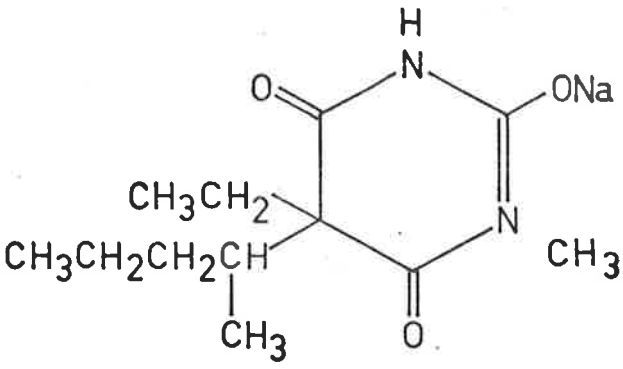
Barbiturates (pentobarbitone and thiopentone) in small doses produced apparent increases in bulbar and cortical induced waves, as in other species. Larger doses produced the expected depression. Mitral cell inhibition following electrical stimulation of the lateral olfactory tract was increased.

Propanidid was shown to have a similar effect on the induced waves as the barbiturates. This had not been reported previously.

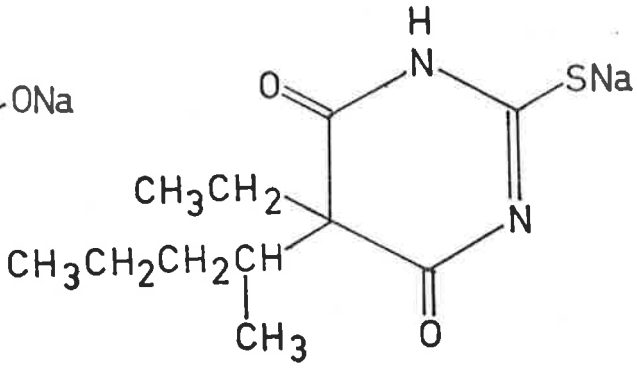
Diazepam in small doses was shown to cause a previously-unreported increase in the induced waves. Larger doses of diazepam depressed the induced waves.

Structure-action relationships

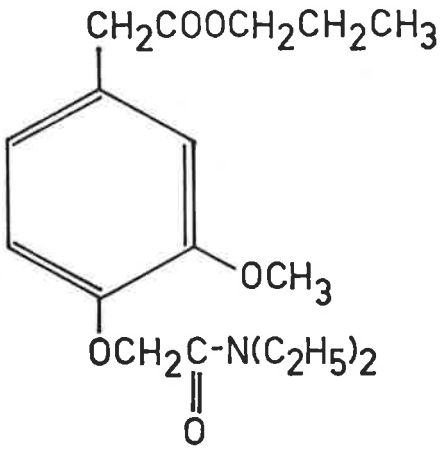
Conventional chemical structures of the drugs used in the study



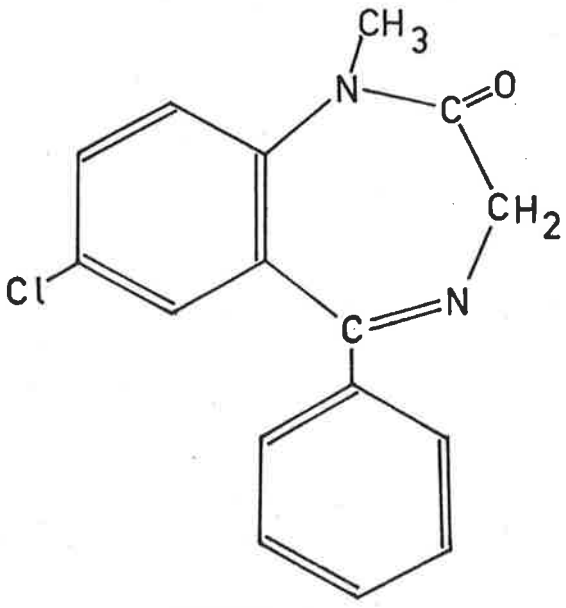
PENTOBARBITONE



THIOPENTONE



PROPANIDID



DIAZEPAM

Fig. 4-18. Chemical structures of the drugs used in this study.

are shown in Fig. 4-18.

Chloroform and halothane could have been expected to have similar actions, but did not, chloroform interfering with the induced activity to a greater extent.

Barbiturates and propanidid had similar effects on the induced waves, but propanidid produced less depression of the evoked potential. The structural similarities are not clear.

Diazepam also produced eventual depression of induced waves, but relatively less depression of the evoked potential. Again, common structural similarities are not apparent.

Differential sensitivity

The olfactory bulb has been shown to be sensitive to the action of barbiturates, being significantly depressed by a one-fifth anaesthetic dose. The bulb was more readily depressed than the cortex by propanidid and chloroform.

CHAPTER FIVE

SUMMARY

1. Induced waves were recorded from intact and isolated olfactory bulbs, and from corresponding cortices of immobilized, locally-anaesthetized phalangers.

2. Strychnine, picrotoxin, bicuculline and tetramine were shown to be analeptic agents in the phalanger.

3. These agents had minimal effects on the induced waves recorded from isolated olfactory bulbs, despite generalized seizure activity.

4. Induced waves were not recorded from intact olfactory bulb or cortex during the seizure activity.

5. During periods of post-ictal cortical depression, induced waves were recorded in the intact bulb, and were similar to those in the isolated bulb.

6. Lignocaine was shown to be a depressant of induced waves, despite its analeptic activity at high doses.

7. Sinusoidal activity has been recorded in some cases from intact and isolated bulbs. Its significance is not known.

8. No structure-action relationship between these analeptics emerged.

EFFECTS OF ANALEPTIC AGENTS ON INDUCED OLFACTORY ACTIVITY IN THE PHALANGER

INTRODUCTION

'No disorder of brain function has revealed more about the organization of the human brain and behaviour than that subsumed under the term epilepsy. And none has been the subject of more intense experimental inquiry. Epileptic manifestations are unique among the various disturbances of brain function in providing the neurobiologist with a wealth of opportunity to examine the intimate neuronal mechanisms underlying a major health problem. At the same time, studies of convulsant activities provide fruitful approaches to the analyses of neuronal structure and function and of biochemical, genetic, and environmental factors contributing to normal and abnormal neuronal synchronization processes. It is little wonder that the search for knowledge concerning the basic mechanisms of the epilepsies has been so closely related to the historical development of neurobiological research in general' (Purpura, Penry, Tower, Woodbury & Walter, 1972).

Numerous substances are capable of producing convulsions in man and experimental animals. There are only two general mechanisms by which such substances can act:by block ofinhibition or by enhancement of excitation (Esplin & Zablocka-Esplin, 1969). Studies of these actions has helped in elucidating the nature of transmitter substances, mechanisms of transmission and actions of inhibitory systems. Because of the large number of publications concerning the chemical convulsants, a comprehensive review is neither possible nor relevant. A survey by Hahn, 1962 (completed in 1959), reviewed 724 papers. It must be remembered that this was before the wide acceptance of microelectrophoresis: Curtis had just published his paper on pharmacological inhibition in spinal motoneurones (1959). Mention will be made in this chapter of significant new concepts, and relevant experiments reporting the effects of analeptics on the olfactory system.

Analeptics have been used in the study of the olfactory system in several fields: (1) neuronography, (2) induced waves, (3) evoked potentials and (4) unit studies. There are fewer published reports of the effects of analeptics on isolated (*in vitro*) cerebral tissue, histochemistry and behaviour, and these will be discussed briefly.

1. Neuronography: analeptic effects

Various agents are capable of causing local electrical spikes when applied topically to the brain, a subject recently reviewed by Ajmone-Marsan (1969). Strychnine is such an agent, and had been used topically in many systems to evoke discrete electrical spiking so that neuronal projections could be mapped. Frankenhaeuser (1951) showed that this method could not be used for mapping olfactory bulbar projections. Strychnine applied to the olfactory bulbs of cats did not cause spike discharges in those structures. Morrison & Kerr* found that it was not possible to create a penicillin focus in the olfactory bulb of the guinea pig. No such bulbar focus is mentioned in the reviews of Prince (1972) or Ward (1972) concerning topical epileptogenic agents.

It can be implied from these findings that the olfactory bulb does not possess the neuronal components or pharmacological susceptibility to those agents necessary for spike-and-wave generation.

2. Induced waves: analeptic effects

Analeptic agents have not been used commonly to modify induced olfactory activity. These drugs will be discussed separately below.

Strychnine. Strychnine (0.05 - 0.2 mg/kg) was used by Ueki & Fukuda (1966) to modify induced waves in immobilized rabbits. They reported that it increased the amplitude, but not frequency, of the bursts both in intact and isolated bulbs. No difference was noted between these two. Strychnine did not antagonize pentobarbitone (15 mg/kg) depression of the bulbar response.

Pentylenetetrazole. The same authors (Ueki & Fukuda, 1966) showed that pentylenetetrazole (2 - 20 mg/kg) augmented the amplitude of the bursts, without altering their frequency. The same effect was noted in isolated as well as intact bulbs. No comment was made of the duration of the bursts, but examination of their Fig. 2 suggests that it was increased. In three of their cases, spike discharges and seizures occurred. They reported that the seizure could occur in the isolated bulb. They neither illustrated nor described this phenomenon. Pentylenetetrazole antagonized the depression of amplitude and frequency of the bursts caused by pentobarbitone.

Fukuda (1966) reported the same changes caused by this agent, and that it did not invariably alter the efferent effects of electrical

---

* Unpublished observations, Department of Human Physiology, University of Adelaide, 1969

stimulation of central structures.

Tuttle & Elliott (1969) reported similar experiments in cats implanted with electrodes and cannulae. They showed that systemic administration of pentylenetetrazole, 20 - 25 mg/kg, caused generalized synchronous 3-5/sec waves. These later became 8-10/sec spikes. Infusion of this drug into amygdala, hippocampus or mesencephalic reticular formation caused generalized spike activity. The amygdaloid discharges were synchronous with respiration, suggesting that the threshold of the amygdala was lowered to olfactory input.

Lignocaine. Local anaesthetics are known to be anticonvulsants in low doses (lignocaine, 1 - 3 mg/kg), but analeptic at high doses (Bernhard & Bohm, 1955; Bernhard, Bohm & Wiesel, 1956; Eidelberg, Lesse & Gault, 1961; Julien, 1973).

Lignocaine has been the subject of extensive research, particularly with reference to the limbic system. de Jong & Wagman (1963) studied the effects of intravenous lignocaine on the brain of the cat. They implicated the antero-lateral amygdala as the focus for the consequent convulsions. They noted that nitrous oxide halved the convulsive dose of lignocaine from about 12 mg/kg. Ether also facilitated its convulsive effects, whereas halothane had no such effect.

Wagman, de Jong & Prince (1964) recorded amygdaloid and pre-pyriform cortical bursts in cats and rabbits, such bursts being dependent on nasal air flow. They thought that lignocaine had excitatory effects on the amygdala, independent of respiratory effects. This excitation was thought to be by block of inhibition.

Wagman & de Jong (1964) demonstrated in cats that increasing inspired carbon dioxide tension caused increased sensitivity to lignocaine. The mechanism was thought to have been caused by increases in brain permeability to lignocaine. Alterations in the amount of available lignocaine base were not thought to be significant.

Prince & Wagman (1966) reported that cryogenic lesions in the amygdalae of cats and rabbits were more sensitive than normally to activation by lignocaine. Metrazole did not activate these damaged areas to become foci of spike activity, an unexpected property of this known analeptic agent. They postulated an effect of lignocaine on transmitter amines within the amygdala, or blockade of small-fibre inhibition of that structure.

Wagman, de Jong & Prince (1967) reviewed the central effects of

lignocaine, and described experiments showing behavioural and electrical changes in cats and rabbits. Convulsive threshold for intravenous lignocaine was 10 - 12.5 mg/kg in cats, and half that dose for rabbits. Olfactory bulb activity of a cat was shown in their Fig. 4A. The control showed a large DC shift and high-frequency activity. Minimal simultaneous amygdaloid activity was present. Lignocaine (4.5 mg/kg) caused marked slowing of the bulbar record and the appearance of spindles of equivalent frequency in the amygdala 65 sec after the injection. The olfactory bulbar induced wave had recovered to a high frequency by 150 sec, and the amygdaloid spindle had almost disappeared. Their Fig. 4B showed essentially the same changes after a higher dose of lignocaine (6.2 mg/kg). Synchronous spindle activity was seen in the contralateral (and to a lesser extent ipsilateral) fronto-orbital cortex as well as amygdalae of both sides. The effects of a convulsant dose (10 mg/kg) were shown in their Fig. 5. Spiking was shown to occur first in the right basal amygdaloid nucleus, spreading to the other areas, including the olfactory bulb. It is worth noting that bulbar activity at this site continued between the spikes early in the convulsion (the interspike saw-tooth pattern). By 120 sec, the slow burst had appeared. Arterial carbon dioxide tension was varied between 15 and 95 mm Hg, and the convulsant dose of lignocaine was shown to vary inversely with this tension. It was also noted that pentylenetetrazole, although producing hyperpnoea, did not produce a similar pattern. This implied that the lignocaine effects were not solely by ventilatory changes. Possible mechanisms of action on the amygdala considered were: (1) removal of a normally afferent inhibition, and (2) effects on serotonin or noradrenaline. Membrane stabilization was not considered to be important. Factors modifying the effects of lignocaine were discussed. Species differences were apparent, and arterial carbon dioxide tension altered responses. Nitrous oxide lowered, and pentobarbitone raised seizure threshold. However, the basic question of why lignocaine has anticonvulsant properties at low dosage, and is convulsant at high dosage, remained unanswered.

This study was extended (Wagman, de Jong & Prince, 1968), and they described the dose-related sequence of exaggerated spindling, spike-spindle complexes, rhythmic spiking progressing to frank electrical seizure. The mechanisms of action of lignocaine were undetermined. (The same figures were used in three places: Figs. 4B,

5 & 6, and 3, 5 & 7, respectively.)

Tuttle & Elliott (1969) showed that systemic infusion of lignocaine caused changes first in the basal amygdaloid nucleus of the cat and later in the hippocampus. Hypersynchronous 25 - 35 Hz activity was recorded, related to respiration and blocked by nasal occlusion (their Fig. 2). Infusion of lignocaine (and butacaine) into the basal nucleus caused similar EEG changes, but which were not self-sustaining.

Riblet & Tuttle (1970) continued this study using *cerveau isolé* cats (deafferented except for cranial nerves I and II by intra-collicular transection), and deafferented cats with intact reticular cores. These were severe surgical procedures with a reported survival rate of 30%. Intraperitoneal lignocaine (60 mg/kg) was given to these survivors. The *cerveau isolé* cats showed no change in the EEG of the amygdala, hippocampus, olfactory tract or fronto-occipital cortex. The other group, however, with intact reticular core and periaqueductal gray, showed the previously-described 25 - 35 Hz activity in the same sites as before. Such bursting depended on inspiratory nasal air flow, and began in the lateral olfactory tract (their Fig. 3a). Their modified hypothesis was that lignocaine lowered the threshold of the olfactory apparatus, and subsequently that of the amygdala. An activated cerebrum or intact reticular core was necessary for the genesis of these EEG changes.

Having previously defined their experimental model of epilepsy, de Jong & Heavner (1971) reported the activity of diazepam in preventing the lignocaine seizures. Intramuscular pretreatment with diazepam, 0.25 mg/kg, doubled the median convulsive dose of lignocaine in cats. They subsequently (1972) compared the anti-convulsant activities of diazepam and pentobarbitone (0.2 mg/kg versus 10 mg/kg, respectively) against these lignocaine convulsions. These doses were approximately equipotent. Diazepam, however, caused fewer unwanted effects than pentobarbitone (their Tables 3, 4, 5 & 6).

In a subsequent study, de Jong, Heavner & de Oliveira (1972*a*) made an accurate assessment of the nitrous oxide effect on lignocaine convulsions in cats. They found that 70% nitrous oxide (with artificial ventilation maintaining end-tidal carbon dioxide at 4.5%) raised the CD_{50}* of the intravenous lignocaine infusion from 7.6 mg/kg to

---

* CD_{50}: Convulsive Dose 50: dosage required to produce convulsions in 50% of subjects.

11.4 mg/kg, contrary to their previous findings. They also reported (1972*b*) that 50% nitrous oxide had a similar effect. The mechanism of action was not determined.

Munson, Pugno & Wagman (1972) were unable to show that oxygen possessed an anticonvulsant action in mice, rabbits or monkeys. If the increased oxygen tension was combined with a decreased carbon dioxide tension in rabbits, the dose-response curve of lignocaine was shifted to the left (their Fig. 2 and Table 2). Munson, Martucci & Wagman (1973) measured the seizure threshold in monkeys of lignocaine (26.1 ± 1.8 µg/ml arterial blood) and bupivicaine (5.5 ± 1.78 µg/ml). They were unable to detect seizure foci. Munson & Wagman (1973) were able to show that gallamine raised lignocaine seizure threshold in the monkeys by about 40% (25.5 ± 3.0 to 36.2 ± 4.0) in eleven experiments involving five animals. They did not make any firm conclusions about the mechanisms of central action of gallamine. It might be expected, however, that with all motor end-plates blocked, total neuronal traffic would be reduced. This would possibly reduce the likelihood of generalized seizures.

Wale & Jenkins (1973) have produced evidence which conflicts with some that is reported above. They showed that diazepam was capable of completely blocking the analeptic effect of lignocaine only when applied to the brain stem reticular formation. The effect was incomplete if diazepam was applied to the hippocampus. There was no effect when applied to the amygdala.

These reported studies have shown that prominent sites of action both of lignocaine and diazepam are in the limbic system. Mechanisms of action have not been determined.

Other analeptic agents. Various other central nervous system stimulants have been used to modify olfactory induced activity. Ueki & Fukuda (1966) also used nikethamide, d-amphetamine and caffeine. These agents increased the amplitude but not frequencies within each burst, in a manner similar to strychnine and pentylenetetrazole. They commented that these agents caused seizure activity both in the isolated and intact bulbs. (These seizures were neither described nor illustrated.) The induced waves were not depressed during the generalized post-ictal depression. This phenomenon had been reported previously for chlorpromazine (Ueki, Tanaka & Sugana, 1964; their p.91). These interictal bursts have also been reported by Ueki & Fukuda (1966; their p.913) and Timiras, Woolley, Silva & Williams

(1967; their p.399). Khazan, Kandalaft & Sulman (1967) reported the potentiation of the "respiratory waves" of the rabbit by LSD-25 and amphetamine sulphate.

No unifying mechanism of action of these analeptic agents has emerged from these studies.

3. Evoked potentials: analeptic effects

Convulsant drugs have also been used to modify evoked olfactory activity.

Strychnine. MacLean, Rosner & Robinson (1957) showed that strychnine applied to the pyriform cortex (of rabbits, opossums, and one monkey) caused spike activity (their Fig. 7). These spikes were unaffected by stimulation of the lateral olfactory tract at rates from 12/sec to 40/sec. This suggested that the generators of the evoked spike and strychnine spike were different structures. Repetitive stimulation of the bulb or tract was also shown to cause recruitment (at rates of 3 - 40/sec), alternation (6 - 110/sec), "decrementation" (fatigue, 20 - 40/sec), and post-tetanic potentiation.

Evoked potentials within the bulb were examined by Iwase, Uruha & Ochi (1961). Strychnine solution (2%) was applied to the surface of the bulb. This abolished the spike component of the potential, while augmenting the slow component. No strychnine spikes were described. No explanations were given for these observations.

Yamamoto & Yamamoto (1962) applied strychnine to the surface of olfactory bulbs of immobilized rabbits. When a single electric shock was applied to the olfactory epithelium, the "N-potential" (negative deflexion) was followed by the "R-wave" of 30 - 50 Hz lasting for 200 - 400 msec, similar in appearance to an ordinary induced wave. Pentobarbitone slowed both the R-waves and the induced waves (45 Hz slowing to 30 Hz). Strychnine was thought to block inhibition in the bulb, while picrotoxin was said to facilitate excitation. No comment was made about the presence of spikes, nor changes in the spontaneous activity of the bulb.

Green, Mancia & von Baumgarten (1962) used intravenous strychnine in their investigations of recurrent inhibition in the olfactory bulb of the rabbit. They stimulated the lateral olfactory tract to the isolated olfactory bulb. Their olfactory peduncular section, however, probably left some anterior olfactory nucleus, olfactory tubercle and olfactory cortex, as did Nicoll's (1969). The diagrams in their Figs. 2, 3, 8A, 10A & 11 show the levels of transection, which would

have been caudal to the above-named structures. This is demonstrated in their Fig. 10. The effects of a second more rostral transection (dotted line, the approximate site of my transections) are shown. The removal of the "reflected discharge" probably represents removal of anterior olfactory nuclear inhibition via recurrent collaterals. Their Fig. 11 shows that strychnine 0.5 mg/kg had no effect on the inhibition of spontaneous mitral cell firing caused by 100/sec stimulation of the lateral olfactory tract.

Post-inhibitory rebound was present before and after the strychnine. These results imply that strychnine does not interfere with inhibitory mechanisms within the bulb.

In their subsequent paper (von Baumgarten, Green & Mancia, 1962*a*) they showed that a similar dose of strychnine did not modify the inhibition of spontaneous mitral cell firing caused by stimulation of commissural fibres. This confirmed their previous findings. It would have been interesting if they had used strychnine when they studied the interaction between lateral olfactory tract and anterior commissural stimulation. This may have shown if these systems had different inhibitory transmitters, or were differentially sensitive. If not, the possibility of a final common transmitter could have been contemplated.

Biedenbach (1964, 1966) found no change in the evoked prepyriform cortical potential with subcutaneous, subdural or intracortical strychnine.

4. Unit activity: analeptic effects

Salmoiraghi & Nicoll (1968) reviewed the published effects of drugs on the olfactory bulb, and reported further studies on bulbar unit activity, including the effects of strychnine and LSD-25. They concluded that mitral cell inhibition was mediated via an adrenergic system. Clarification of the mechanism of mitral cell inhibition came with independent reports by Felix & McLennan (1971) and Nicoll (1971*b*).

Felix & McLennan (1971) used barbiturate-anaesthetized, immobilized cats. They noted that microelectrophoretic application of DL-homocysteate (DLH) commonly produced inhibition of spontaneous mitral cell firing, comparable to γ-aminobutyric acid (GABA), glycine or β-alanine (their Fig. 1A). Bicuculline, a putative GABA antagonist, reversed this unexpected depressant effect of DLH when both were applied simultaneously. It also lengthened the pause in mitral cell firing consequent upon lateral olfactory tract stimulation (their Fig. 1E).

This was suggested as evidence that granule cells were under a tonic inhibitory influence, able to be removed by bicuculline. Intravenous bicuculline (0.1 - 0.6 mg/kg) converted DLH depression to excitation, but did not alter the period of inhibition following tract stimulation. The implication of these observations was that DLH excited granule cells (or their gemmules) which secondarily inhibited mitral cells, the inhibitory transmitter being GABA. Strychnine, on the other hand, blocked the depressant effect of glycine, but did not affect GABA- or DLH-depression. It also did not affect the inhibitory pause following tract stimulation. An explanation for observed noradrenaline-induced depression was that this transmitter excited the granule cells, causing secondary mitral inhibition.

McLennan (1971) extended this study, using the same microelectrophoretic model with pentobarbitone 35 mg/kg as the anaesthetic agent. Mitral cells were identified and their firing rate was measured. The depressant effects of microelectrophoretic GABA and noradrenaline were reversibly blocked by simultaneous ejection of bicuculline; depressant effects of glycine were unaffected. In this series of experiments, the firing pause of some mitral cells after tract stimulation was found to be increased after intravenous bicuculline (0.1 - 0.38 mg/kg; his Fig. 4A, B, C). Bicuculline was also shown to decrease the inhibition of a conditioning lateral olfactory tract stimulus. The conclusion drawn from this paper was that the site of action of bicuculline could be only at the granule-to-mitral synapse. Another hypothesis was that a tonic inhibitory system (possibly from the nucleus of the horizontal limb of the diagonal band; Price & Powell, 1970) ending on the gemmule had GABA as an inhibitory transmitter.

It must be remembered that these preceding experiments had been carried out on cats anaesthetized with pentobarbitone 35 mg/kg. Maintenance dosage was not stated. The doses of bicuculline used (0.1 - 0.6 mg/kg) would produce generalized convulsions in the absence of central depressant drugs. Such convulsions could alter the appearance of the post-stimulus histograms (McLennan's 1971 Figs. 4A, B, C). Mitral cell firing before the stimulus might be influenced by the convulsive activity within the efferent systems (which are probably all inhibitory). If the lateral olfactory tract stimulus was enough to trigger a seizure, the efferent systems would be activated also. In such a case, inhibition could possibly be prolonged. No comment is made about the presence or absence of generalized convulsions. If

present, this argument prevails. If absent, the possibility of drug interaction between bicuculline and the barbiturate must be contemplated. Clarification of the question could be achieved by altering the methods slightly: use of midbrain deafferentation, stimulus of the bulbar end of the lateral tract of an isolated bulb, and monitoring of EEG, preferably olfactory cortical or amygdaloid records. However, these criticisms do not invalidate the microelectrophoretic studies.

Nicoll (1971*b*) reached similar conclusions using pentobarbitone-anaesthetized rabbits immobilized with gallamine. Multibarrel microelectrodes were used to record units and to apply drugs. Mitral cells were identified by antidromic invasion following lateral olfactory tract stimulation, or by their anatomical position. Dose-response curves were constructed for GABA and glycine. GABA was shown to be a more potent depressant of mitral cells than glycine. Picrotoxin and bicuculline both moved the dose-response curves to the right. Strychnine selectively blocked the depressant action of glycine, but not of GABA. These studies were generally limited to those cells (an unstated proportion) on which pharmacological antagonism could be demonstrated. The usually excitant amino acid derivatives D-L homocysteate (DLH), aspartate and glutamate also depressed the mitral cells, sometimes after initial excitation.

The question of whether this was a direct effect, or mediated via another transmitter, was tested in the presence of magnesium ion. This ion prevents transmitter release from terminals, and did not interfere with the direct action of GABA. The effects of DLH, aspartate and glutamate were blocked, implying that they had an indirect effect. Bicuculline and picrotoxin also blocked the DLH-induced depression.

These convulsants were also given intravenously, but little effect was seen on GABA- or DLH-depression of the mitral cells until "convulsant doses" were reached. Seizure activity was reported in the olfactory bulb after the peduncle had been transected. The type of seizure activity was not stated nor illustrated. It must be remembered that Nicoll's transection probably left some more central structures in continuity with the bulb.

At convulsant levels of the drugs, there was a reduction of the duration of inhibition following lateral olfactory tract antidromic stimulation. This was interpreted as antagonism of dendrodendritic inhibition. Unit activity was also shown to increase and "the cell fired in bursts". In the unit studies, however, it was not possible

to show reliable antagonism between the convulsants and granule-to-mitral inhibition. The possibility that the convulsants were directly exciting mitral cells was not excluded.

Banerjee, Feldberg & Georgiev (1970) made microinjections of tubocurarine, leptazole, strychnine and picrotoxin into various areas of cat brain. All agents caused spike discharges in the pyriform cortex.

5. Isolated olfactory tissue: analeptic effects

The isolated lateral olfactory tract-olfactory cortex preparation has also been used to examine analeptic effects. Katsuda & Hori (1972) reported the effects of lignocaine (*inter alii*) on such a preparation. It reduced the 20 msec negative (N) wave, and modified post-tetanic potentiation in the same way as a calcium ion deficit in the bathing solution. (This abstract was unillustrated.) They concluded that lignocaine affected presynaptic structures, but did not speculate on whether these were excitatory or inhibitory axons or terminals. They also suggested (Hori & Katsuda, 1972) that picrotoxin and tubocurarine blocked an inhibitory pathway in their experiments. Strychnine did not have the same effect, but suppressed both the slow surface-negative wave and its positive notches.

6. Histochemistry of olfactory system

Histochemical studies of the olfactory system have contributed to the understanding of neural mechanisms. Feldberg & Vogt (1948) first showed choline acetylase in olfactory cortex. Since then, various authors have studied other transmitter systems. For example, Graham (1973) has described the distribution of glutamic acid decarboxylase (GAD) activity and GABA content in the olfactory bulbs of rats. The highest levels of each were in the external plexiform layer. There were also high levels of GAD in the glomerular and granule cell layers, and of GABA in mitral and granule cell layers.

Beart & Johnston (1973) examined the uptake of GABA into rat brain slices and reported that bicuculline and tetramine (*inter alii*) had no effect.

Brownson, McDougal & Suter (1972) reported that parathion reduced the cholinesterase content of the olfactory bulb of immature rats.

Uptake of various labelled amino acids into the olfactory bulbs of mice was described by Neidle, Kandera & Lajtha (1973). Intact bulbs took up these substances most rapidly, and glycine was the most readily taken up.

Histochemical studies are not relevant to this thesis, except that they provide confirmatory evidence that the putative transmitters do exist in the olfactory system.

7. Behaviour: analeptics

Modification of olfactory behaviour by analeptics has been reported. Tuttle & Elliott (1969) reported behavioural changes in cats which had been given lignocaine. Ellinwood & Escalante (1970) reported that methyl amphetamine caused stereotyped and obsessive sniffing in cats. They also reported histochemical studies of catecholamine fluorescence.

Olfactory bulbar ablation has been shown to alter sensitivity to convulsion-producing agents and the nature of the convulsions in mice (Araki & Ueki, 1972). Electroshock threshold was increased, and duration of its clonic phase and coma were reduced. The tonic phase of pentylenetetrazole, picrotoxin and strychnine seizures was reduced, but the clonic phase of strychnine was increased. These results indicate that alterations in olfactory system input alter the course of subsequent seizures. It will be shown in this chapter that olfactory input can be sufficient to initiate spiking activity after convulsants have been used.

Convulsants have been used to examine the function of many neuronal systems by their two previously-mentioned mechanisms.

The mechanisms of action of strychnine (anti-glycine), picrotoxin and bicuculline (anti-GABA) on various neural systems have been fully documented (for example, see Curtis & Crawford, 1969; Curtis, 1970; and Curtis, 1971). Their actions on the intact olfactory system are less clear. The actions of lignocaine on the limbic system have been comprehensively described, but there has been nothing reported on the effects of tetramine.

It was decided to assess the actions of these agents on the olfactory system of the phalanger to determine if different effects would be elicited.

METHODS

Adult phalangers were prepared for the recording of their induced olfactory activity as before. Nitrous oxide/oxygen/halothane anaesthesia was used during preparative surgery and placement of electrodes. After appropriate and adequate local anaesthesia and neuromuscular paralysis, ventilation with air was commenced. Analeptic drugs were given after

recovery from the initial volatile anaesthesia, by the intravenous route in all cases.

Lignocaine (Xylocaine, Astra; M.W. = 234.33, 1 mg = 4.27×10^{-3}mM) was given to eight animals. Five received intermittent bolus injections (0.5 - 2.0 mg/kg/bolus) and the other three received infusions (1 mg/kg/min). It must be remembered that lignocaine (2%, up to 0.5 ml, i.e. 10 mg) was infiltrated around pressure points at two-hourly intervals. The test doses, however, were not begun until an hour after such injections.

Strychnine (M.W. = 334.4; 1 mg = 2.99×10^{-3}mM) was given to two animals, by incremental bolus injections of 1 mg/kg, 5 min apart.

Picrotoxin (M.W. = 602.6; 1 mg = 1.66×10^{-3}mM; pictrotoxinin M.W. = 292.28, 1 mg = 3.42×10^{-3}mM) was given to five animals, in increments of 1 mg/kg, 5 min apart. A saturated aqueous solution (0.3%) was used.

Bicuculline (K. & K. Laboratories; M.W. = 367.34; 1 mg = 2.72×10^{-3}mM) was dissolved in 0.1 M HCl, pH then adjusted to approximately 6.0 with 0.1 M NaOH. It was given to three animals. Intermittent injections of 0.1 mg/kg were repeated up to three times at 5 min intervals until the desired effects were obtained.

Tetramine* (tetramethylenedisulphotetramine; M.W. = 240.27; 1 mg = 4.16×10^{-3}mM) was given as the first non-volatile drug to two animals in a dose of 0.7 mg/kg, repeated once after 3 min. It was given in increments to another animal after diphenylhydantoin 80 mg/kg had been given over the preceding two hours.

---

* Tetramine was synthesized in this department by Mr R. M. King, who used the basic method of Wood & Battye (1933) of condensing sulphamide and paraformaldehyde in 60% sulphuric acid. The structure of Mr King's product was confirmed at the Australian National University by Mr P. Beart (see also Beart & Johnston, 1973). Tetramine is soluble in dimethyl sulphoxide, the vehicle used in the experiments to be reported here.

RESULTS

Lignocaine

Isolated bulb: induced waves

At all doses to 15 mg/kg (the highest used in these studies), lignocaine had minimal effects on the induced waves recorded from the isolated bulb. The burst duration and amplitude remained of the same order as the control. There was, however, a reduction of the frequencies within the bursts. The bursts also appeared to slow more rapidly to lower frequencies than before. Control bursts began after the initial slow deflexions at 50 - 55 Hz, and slowed to 24 - 28 Hz after 1.2 sec or so. During the infusion, the initial high frequency activity became less prominent, and the burst as a whole slowed, final frequencies after 1.2 sec being of the order of 18 - 24 Hz. There was a reduction in the amplitude of the background intrinsic activity. No assessment was made of its contained frequencies.

Fig. 5-1A is a record of induced waves recorded from the isolated and intact bulbs, deafferented and intact olfactory cortices of the phalanger. The upper part is the real-time record, while the lower has been written out at 0.1 recording speed.

The succeeding figures are from the same sites during and after a continuous lignocaine infusion of 1 mg/kg/min. Fig. 5-1B, 1 min; C, 2 min; D, 4 min; E, 6 min; F, 8 min; G, 10 min; H, 2 min after cessation of infusion, and I, 1 hr afterwards.

Intact bulb: induced waves

The most noticeable alteration in the induced waves of the intact bulb was the reduction in the high frequency component. Superficially, the bursts appeared to be unchanged, but slow write-out showed the increase in low frequency content at the expense of the high frequency component. Intrinsic activity in the intact bulb apparently increased and contained spikes synchronous with those of the ipsilateral cortex. After 8 mg lignocaine/kg, the interspike intrinsic activity was reduced in amplitude. Burst amplitude remained roughly constant. There was, however, the qualitative impression that burst amplitude increased as intrinsic activity decreased. Burst duration was probably increased to 1.5 - 2 sec, but this last parameter is difficult to measure. Half an hour after the infusion, the bursts had returned to their pre-drug appearance.

Intact cortex: induced waves

Induced waves recorded in the intact olfactory cortex during lignocaine infusion showed an increase in low frequency activity. There was a reduction in the bulbar high frequency contribution to the burst. The low frequency sinusoidal activity slowed during the infusion, from approximately 20 Hz to 10 Hz. During the infusions spike activity occurred, sometimes within a minute of beginning. It had always occurred after 8 mg lignocaine/kg. There was an eventual reduction in the amplitude of the interspike intrinsic activity. Spike activity increased with increasing dosage, and electrical convulsions were caused by lignocaine 15 mg/kg in most animals. During the periods of post-ictal depression, no induced waves were recorded from the olfactory cortex, although they were present in the intact bulb of that side.

Recovery occurred rapidly. Intrinsic activity and induced waves appeared grossly normal 30 min after a lignocaine infusion of 10 mg/kg.

Small doses of lignocaine (1 - 4 mg/kg) sometimes caused enhancement of the induced waves of intact bulb and cortex. This was most likely to occur if there was high frequency, high amplitude background cortical activity. (This is not illustrated in this thesis, but the changes were similar to those of small doses of pentobarbitone or propanidid.)

Deafferented cortex: electrical activity

No induced waves or "arousal spindles" were recorded from the cortex on the side of olfactory peduncular transection. During lignocaine infusion, intrinsic activity was reduced in amplitude, and spike activity began to occur. In the example illustrated (Fig. 5-1B), a few spikes had begun to appear at a dosage of 1 mg/kg. Such spikes increased in frequency of occurrence, to reach a maximum in the example illustrated at a dosage of 6 mg/kg. Seizure activity eventually occurred.

Discussion

This study has shown that lignocaine reduces the frequencies within the induced waves of the isolated olfactory bulb, but does not significantly alter the duration or amplitude of such bursts. Intrinsic activity was depressed. Even the large doses of lignocaine which caused generalized convulsions had minimal effects on the isolated bulbs. Lignocaine was shown to be analeptic at high doses, but convulsions were never recorded from the isolated bulb. During the

lignocaine infusion, there was a reduction in the high frequency component of the induced waves recorded from intact sites (cortex and bulb).

Small doses of lignocaine (1 - 4 mg/kg) often caused enhancement of the induced waves at intact sites. This was a similar action to that of small doses of pentobarbitone. The implication of this observation is that lignocaine in small doses either (1) suppresses efferent inhibitory mechanisms, or (2) facilitates mitral cells directly. The former is more likely, because the isolated bulb was relatively unaffected at the dose levels reported there. This may be an example of the anticonvulsant action of lignocaine, but the experiments were not designed to show this.

No attempts were made to locate the site of origin of the convulsions, nor estimate dose-response relationships of lignocaine.

The similarity between low doses of pentobarbitone and lignocaine in augmenting the cortical induced waves has not been reported before. These two agents also share the ability to reduce the frequencies within the burst in the isolated bulb. Large doses of lignocaine, however, caused convulsions. On some occasions, pentobarbitone caused isolated spiking, but it never caused seizures. These observations indicate that at low levels, both drugs probably suppress efferent activity to the bulb, and at higher doses inhibit the bulb itself.

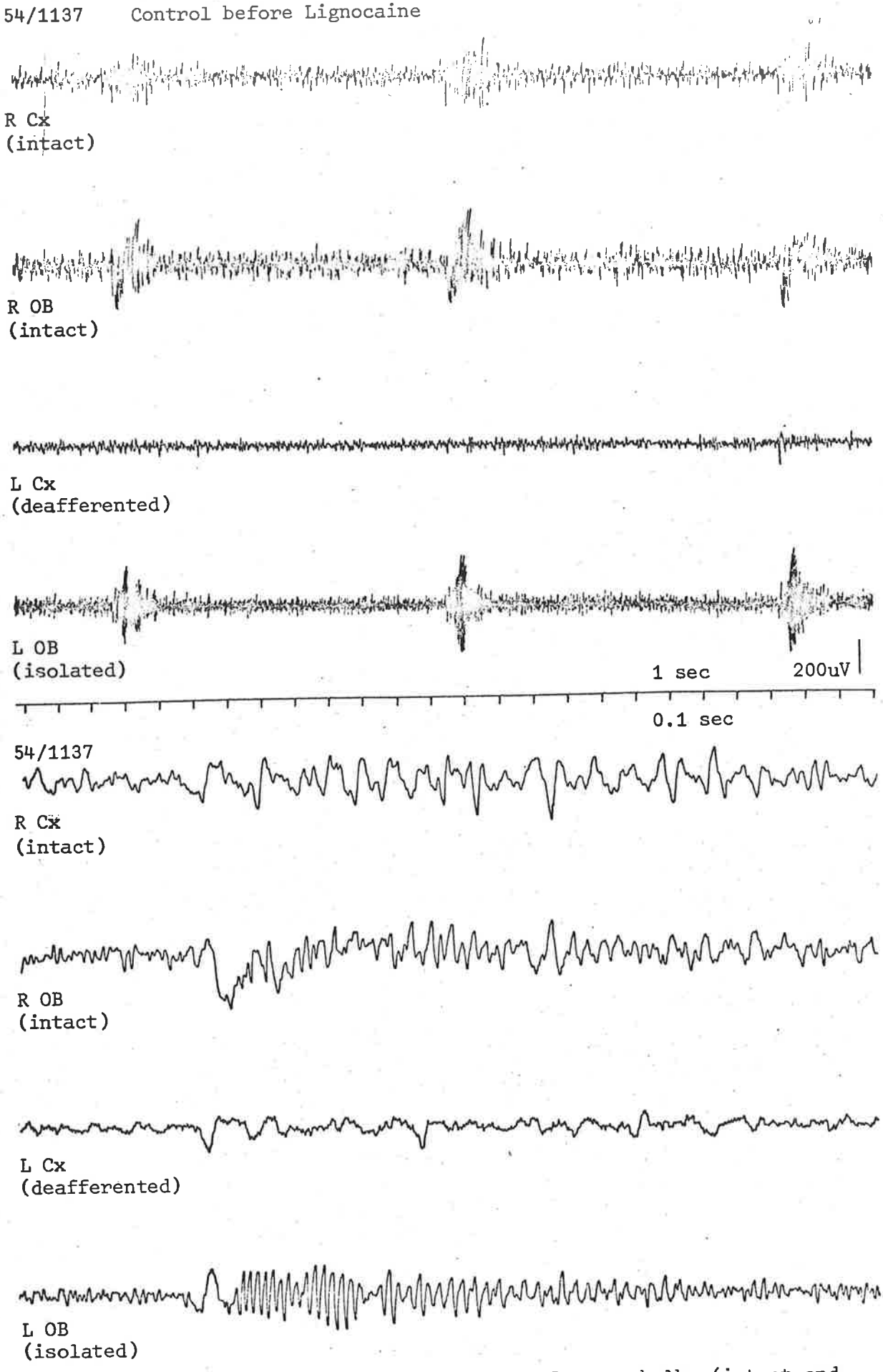


Fig. 5-1A Induced waves recorded from the olfactory bulbs (intact and isolated) and corresponding cortices of an immobilized phalanger before a lignocaine infusion was begun.

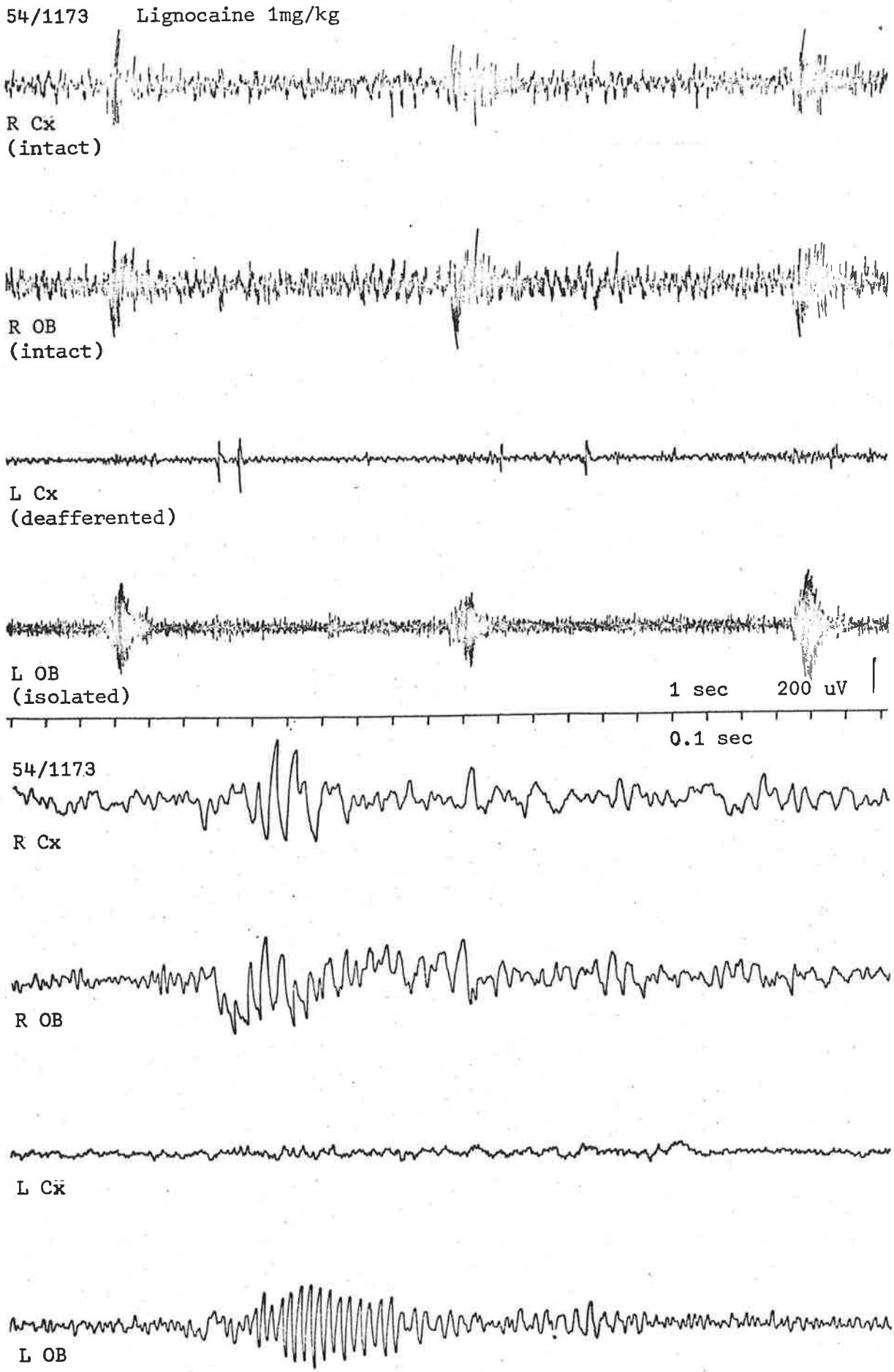


Fig. 5-1B Induced waves from the same sites after lignocaine infusion of 1mg/kg.

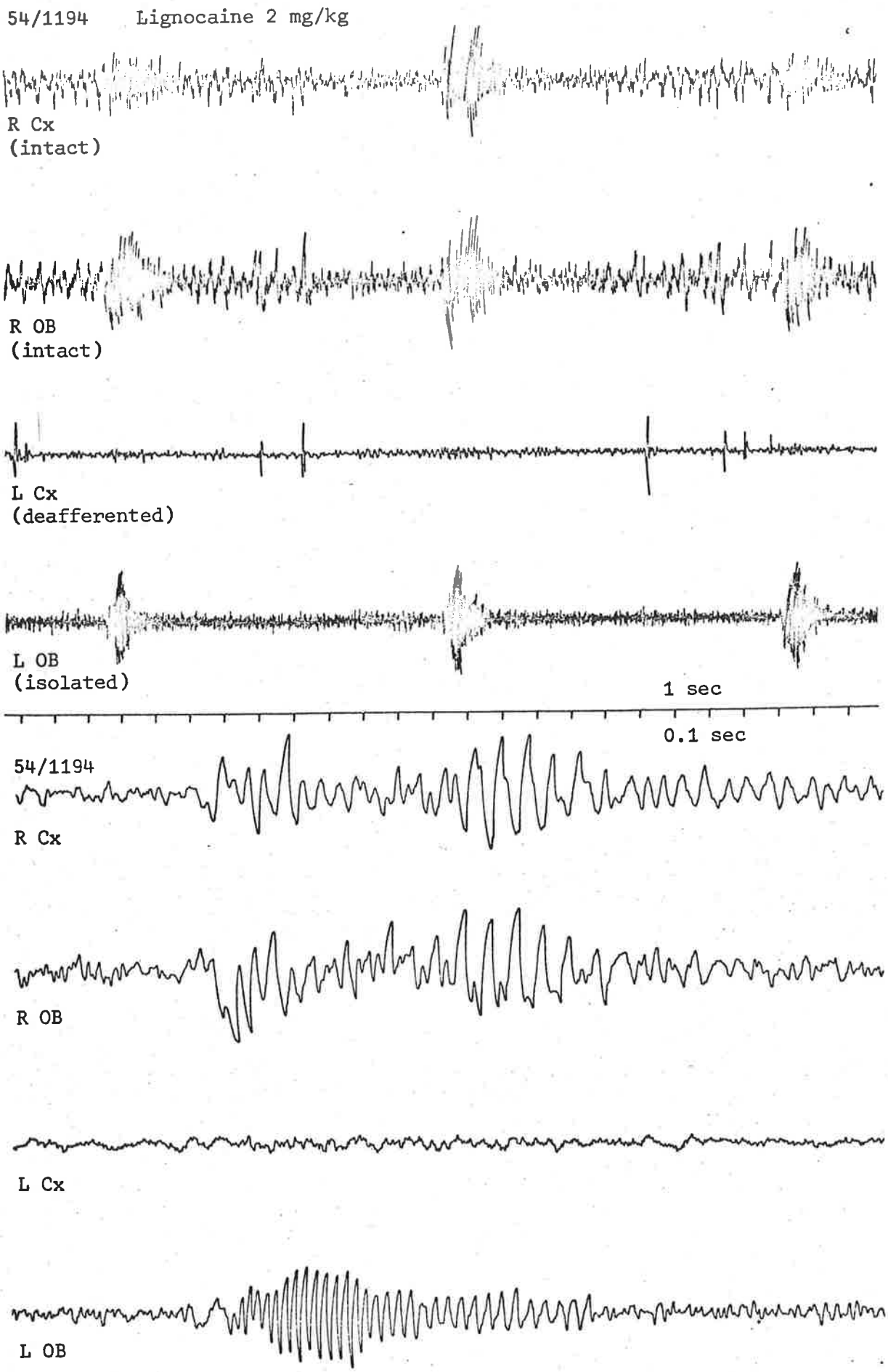


Fig. 5-1C Induced waves after lignocaine 2 mg/kg, recorded from the same sites.

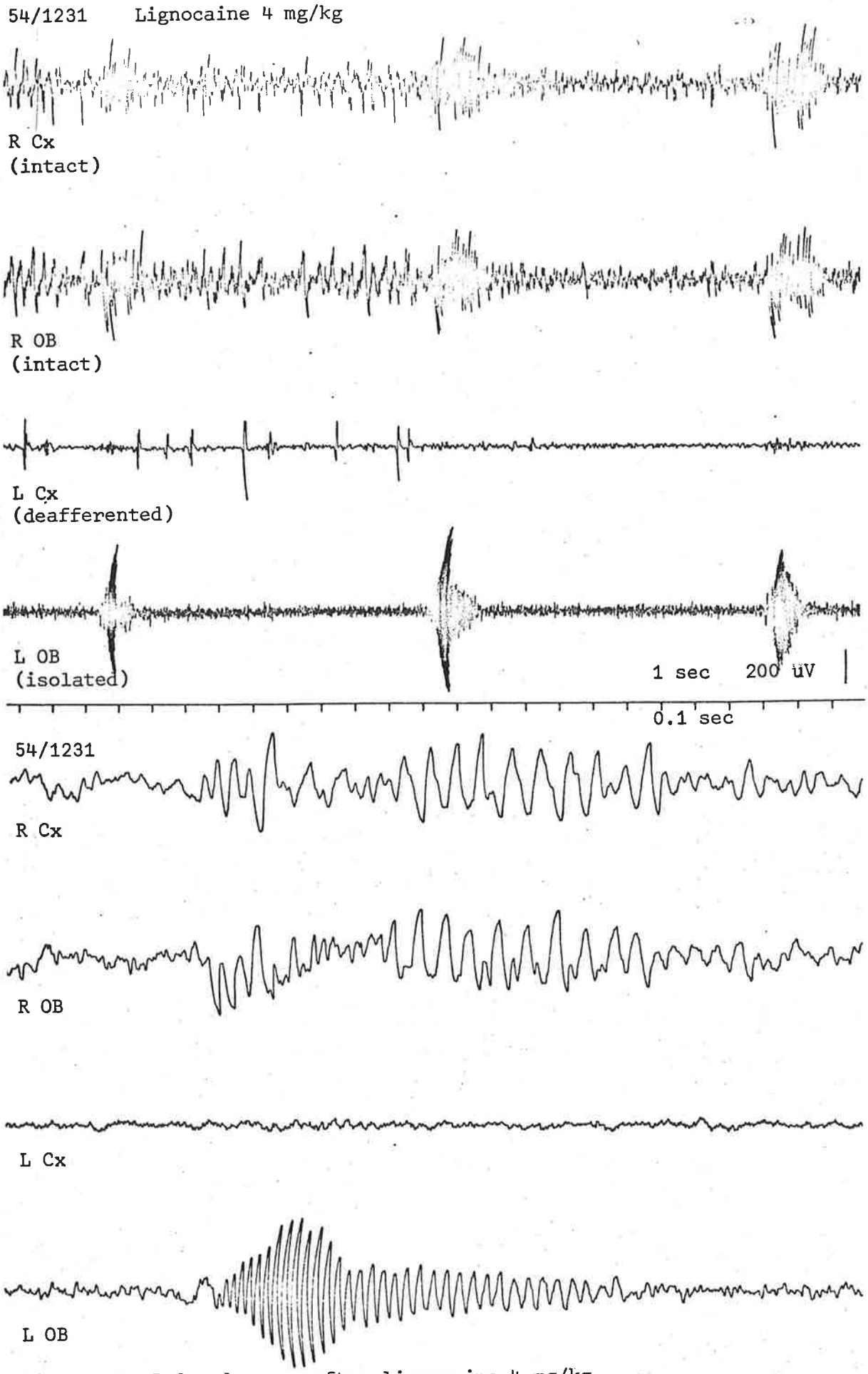


Fig. 5-1D Induced waves after lignocaine 4 mg/kg.

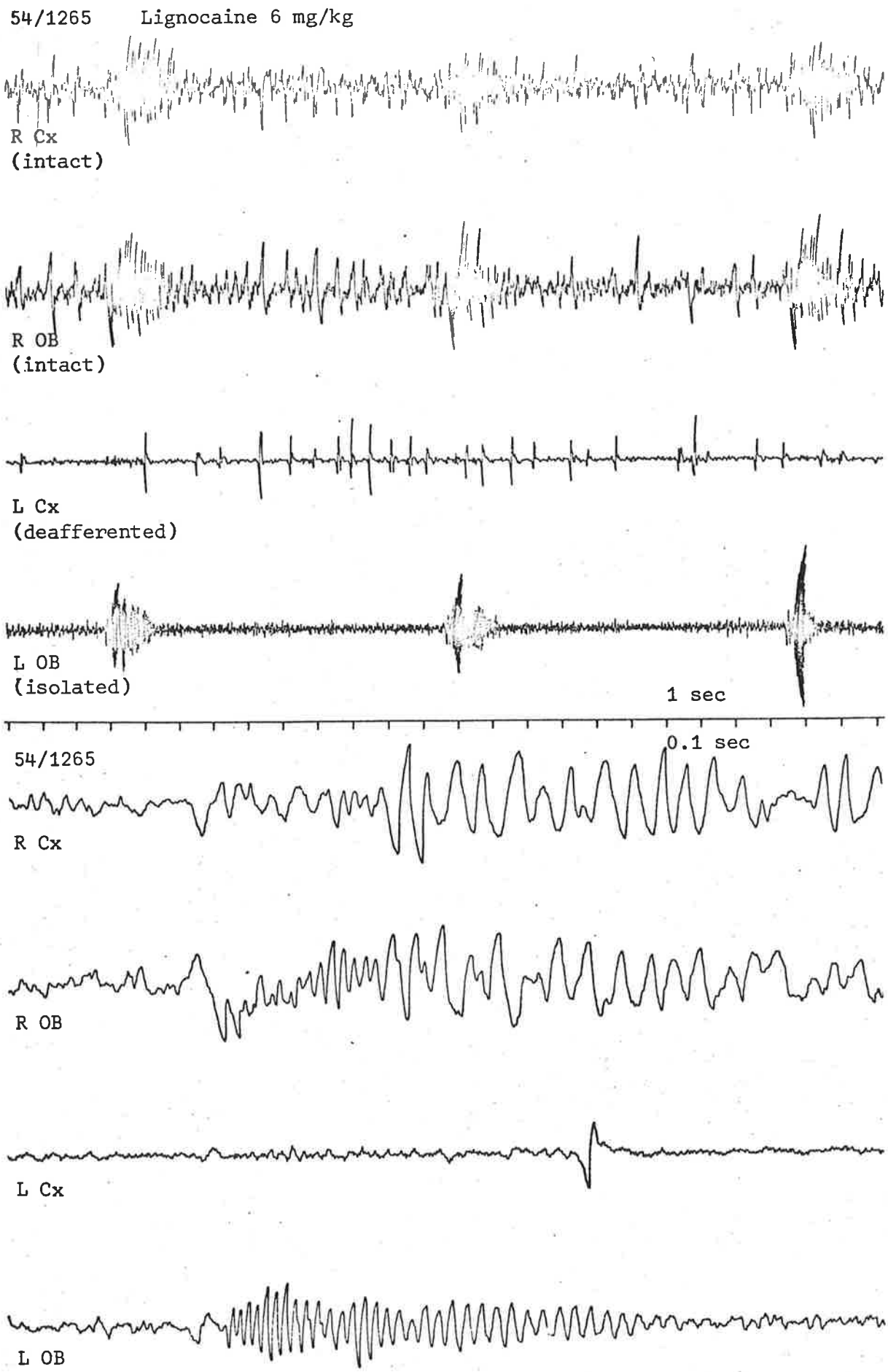


Fig. 5-1E Induced waves after lignocaine infusion, 6 mg/kg.

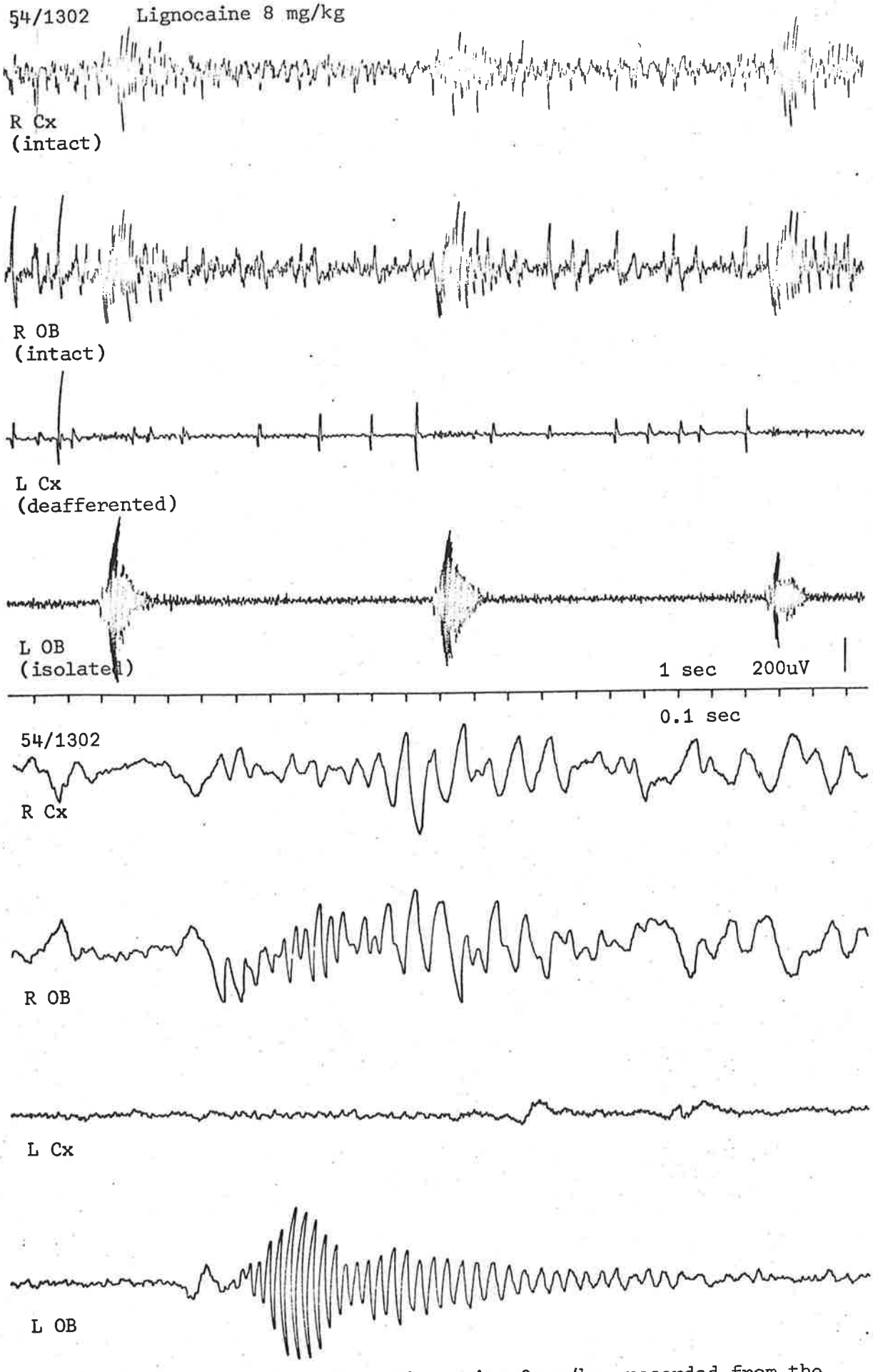


Fig. 5-1F Induced waves after lignocaine 8 mg/kg, recorded from the same sites as before.

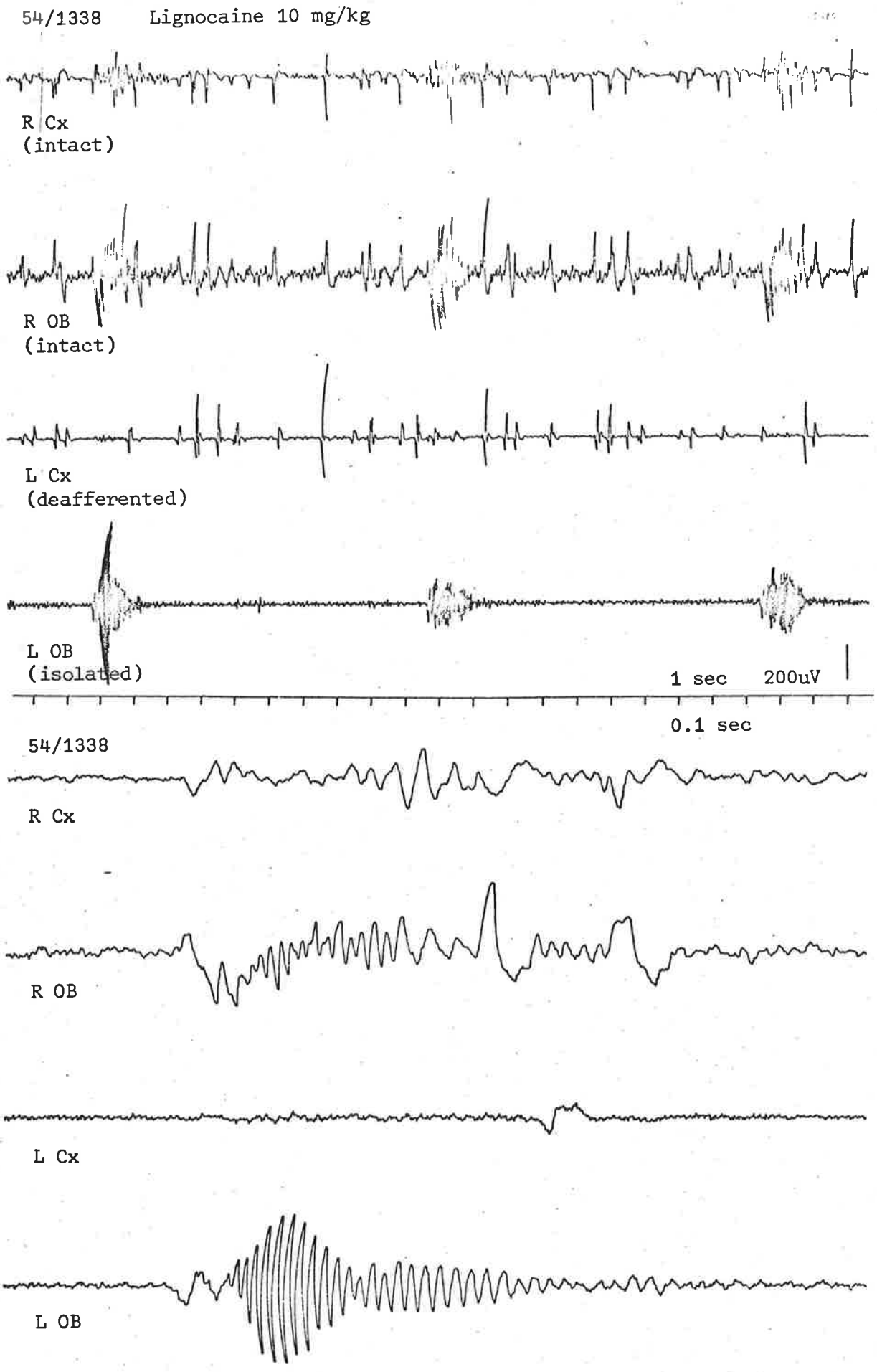


Fig. 5-1G Induced waves after lignocaine, 10 mg/kg.

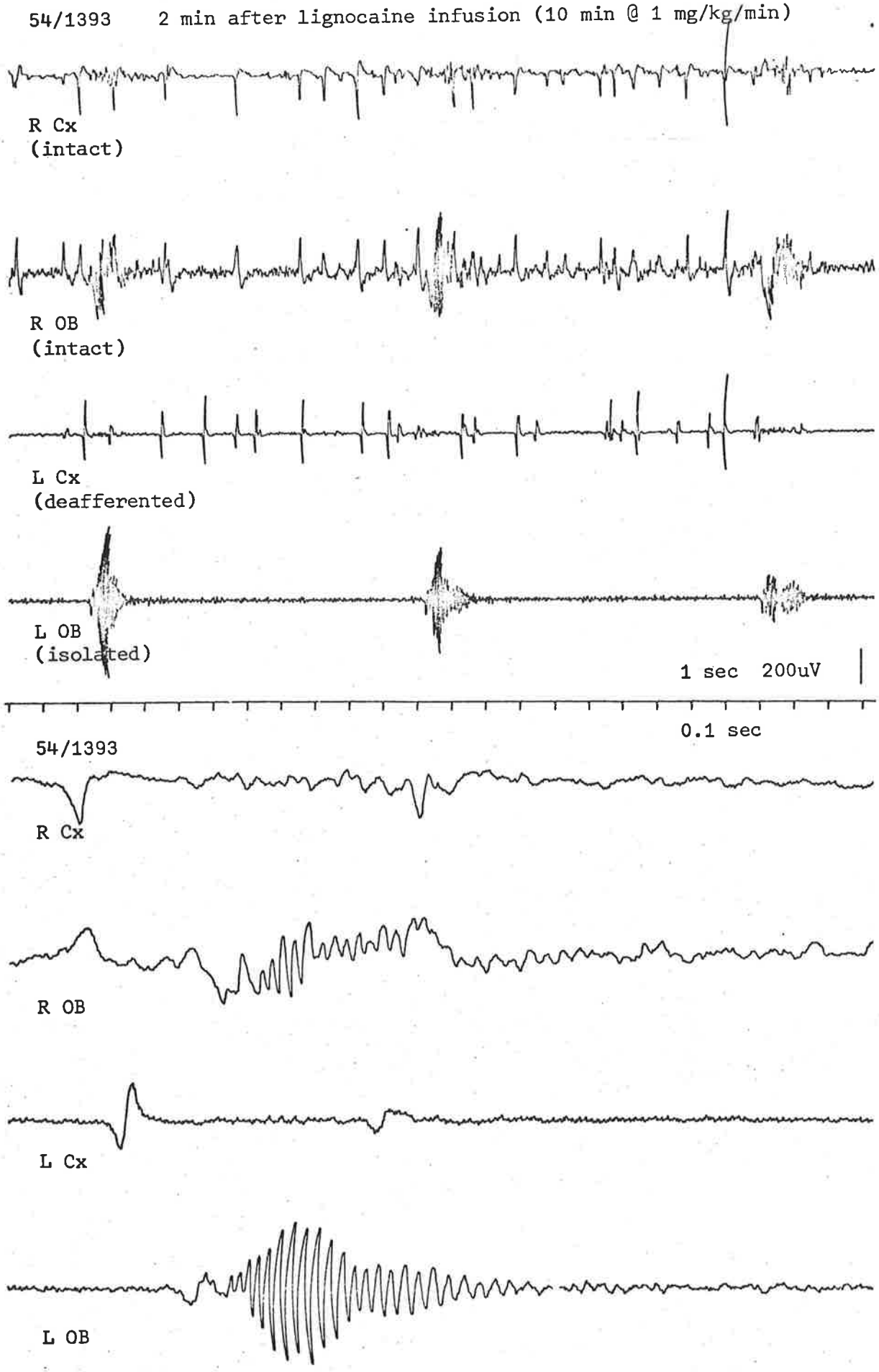


Fig. 5-1H Induced waves recorded 2 min after cessation of lignocaine infusion (10 min at 1 mg/kg/min).

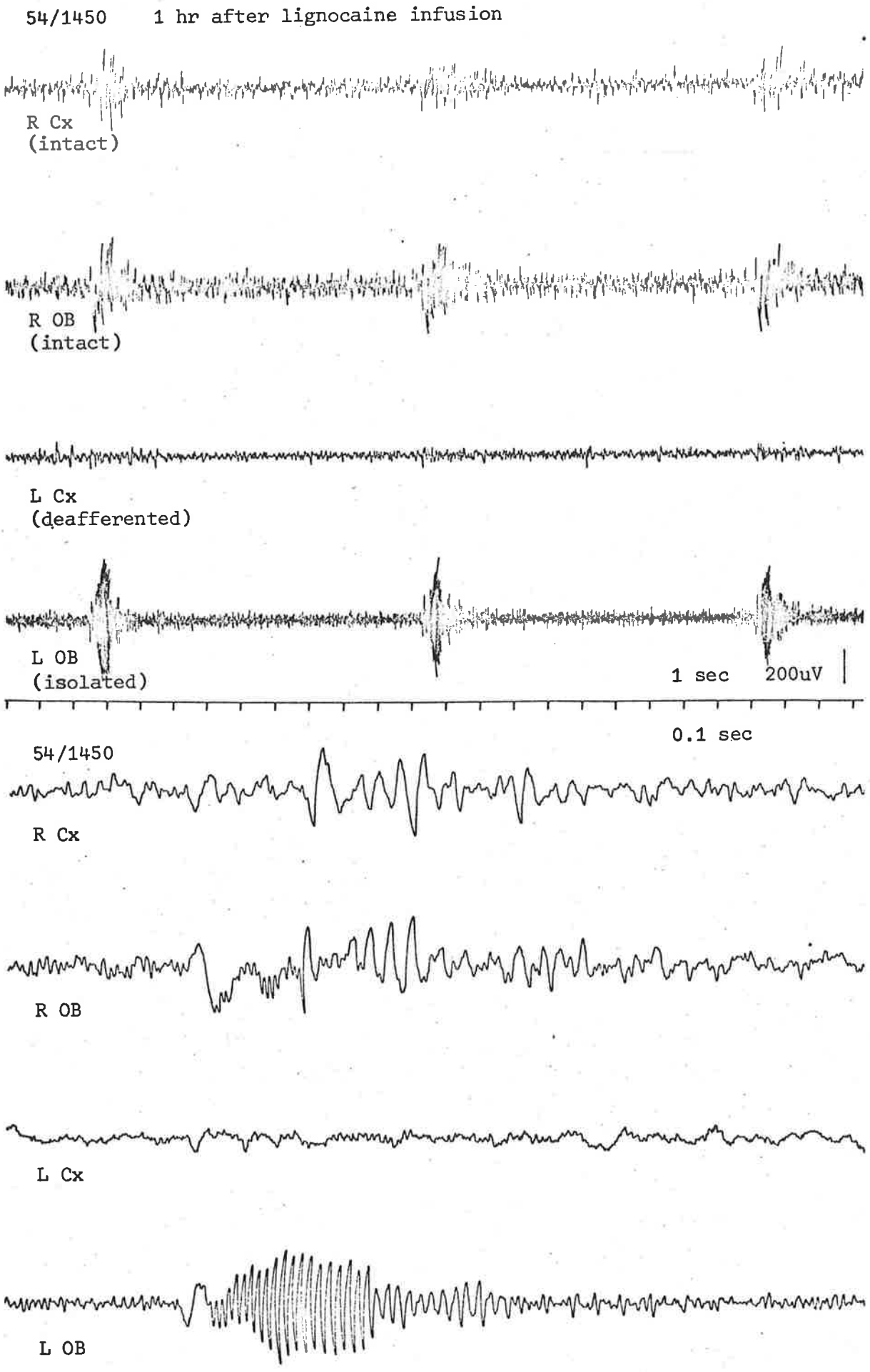


Fig. 5-1I Induced waves recorded 1 hour after cessation of the lignocaine infusion.

Strychnine

Strychnine produced generalized electrical seizure activity in both phalangers (one with 2 mg/kg, the other with 3 mg/kg). Such seizures began after about five minutes.

Fig. 5-2 shows the changes in one of these experiments after two injections of 1 mg strychnine/kg. Fig. 5-2A records from intact bulb and cortex, isolated bulb and deafferented cortex in the absence of any drug. B, 1 min after strychnine, 1 mg/kg; C, 1 min after B; D, 1 min after another 1 mg strychnine/kg, total 4 min after first injection; E, 30 sec after D; F, G, H & I show the electrical activity from the four sites, at times from the first injection approximately 5, 7, 8.5 and 12 min, respectively.

Isolated bulb: induced waves

Strychnine, in the doses used here (0.2 and 0.3 mg/kg), had no effect on the induced waves of the isolated bulb. There were no significant changes in duration, amplitude or contained frequencies within the bursts. The intrinsic activity also appeared unchanged, despite the generalized seizure activity in the other areas.

Intact bulb: induced waves

Induced waves were barely able to be distinguished in the control record (Fig. 5-2A). One minute after the initial strychnine 1 mg/kg, the bursts had become more prominent. With increasing strychnine effect they increased in amplitude and duration. The contained frequencies, however, were lower than in the drug-free state. When generalized convulsions began to occur (Fig. 5-2E), induced waves were masked by the spike-and-wave activity in the bulb. In the interspike interval, however, there appeared to be sinusoidal activity (Figs. 5-2F & G). This was at 25 - 30 Hz, and appeared to be inhibited for 200 msec by the positive (downward) deflexion of the spike. The spike activity was random at first, but became regular at 5-8/sec. A small section of this is seen in Fig. 5-2F.

During the phase of post-ictal depression (Fig. 5-2I), the intact bulb produced induced waves which were similar in all respects to those of the isolated bulb, before or after the strychnine. It is worth noting that the onset of each induced wave was marked by a spike both in intact and deafferented cortices.

Olfactory cortex: electrical activity

Strychnine produced the expected generalized spike-and-wave seizure activity in olfactory cortex. Before this occurred, however,

induced waves were enhanced in the intact cortex (Figs. 5-2C & D). There was an increase in amplitude and duration of the low frequency burst, which was reflected in the bulbar record of that side.

Electrical activity in the deafferented cortex was essentially unchanged until spiking began. It was not possible to tell in which cortex such spiking began. Other agents (lignocaine, halothane) initiated this activity in the deafferented cortex first.

Post-ictal depression was seen in both cortices, and during these periods induced waves were not recorded from the intact cortex (Fig. 5-2G & I).

Discussion

These results indicate that strychnine has minimal effect, if any, on the function of the olfactory bulb isolated from its central connexions. When the central connexions were intact, the bulb reflected the electrical activity in its own cortex, and induced waves were not seen in the midst of the seizure activity. The bulb, however, maintained its ability to produce sinusoidal activity. It did so in the inter-spike intervals (Fig. 5-2F & H). Spikes were not seen in the isolated bulb, confirming Frankenhaeuser's (1951) observations.

Yamamoto & Yamamoto (1962), however, showed that strychnine applied to the surface of an olfactory bulb caused a change in the bulbar response evoked by electrical stimulation of the olfactory mucosa. The resulting oscillating wave was accentuated until it resembled an induced wave, lasting approximately 1 sec, beginning at 45 Hz and ending at 30 Hz (their Fig. 1). They also reported, but did not illustrate, spontaneous paroxysmal discharges resulting from prolonged contact of the bulb with strychnine. All other bulbar activity was said to be suppressed during this phase.

Ueki & Fukuda (1966) reported that strychnine (0.05 - 0.2 mg/kg) increased the amplitude of the induced bursts. They held that there was no difference between the responses of intact or isolated bulbs. They did not comment on the presence of seizures, but they had used strychnine in a dose an order smaller than that used in this study.

The mechanism of action of strychnine has not been determined in this thesis. It does have its predicted convulsant action on the cortex, but does not affect the mechanisms responsible for intrinsic activity and induced waves generated in the olfactory bulb. An implication of this is that glycine is probably not the inhibitory transmitter of the bulb. It also implies that strychnine does not

block inhibitory mechanisms, or augment excitatory ones in the bulb at the doses used here. Nicoll (1971*b*) showed that strychnine antagonized glycine but not GABA in the olfactory bulb of rabbits (microelectrophoretic methods were used). In that paper he stated that his studies were limited to mitral cells on which pharmacological antagonism of GABA could be demonstrated. This could be taken to imply that there were mitral cells depressed by GABA, which was not antagonised by the convulsants used. He does not state the relative number of cells of each type.

The two experiments reported in this chapter bring into question the observations of other workers that "convulsions" were seen in the isolated bulb. It will be necessary to repeat these experiments on other species, and using higher doses, including injections of strychnine into the bulb itself. The meaning of the interspike sinusoidal activity in the intact bulb is not clear. It may imply again that the bulb does not have the neuronal elements to produce 'spike-and-wave' activity.

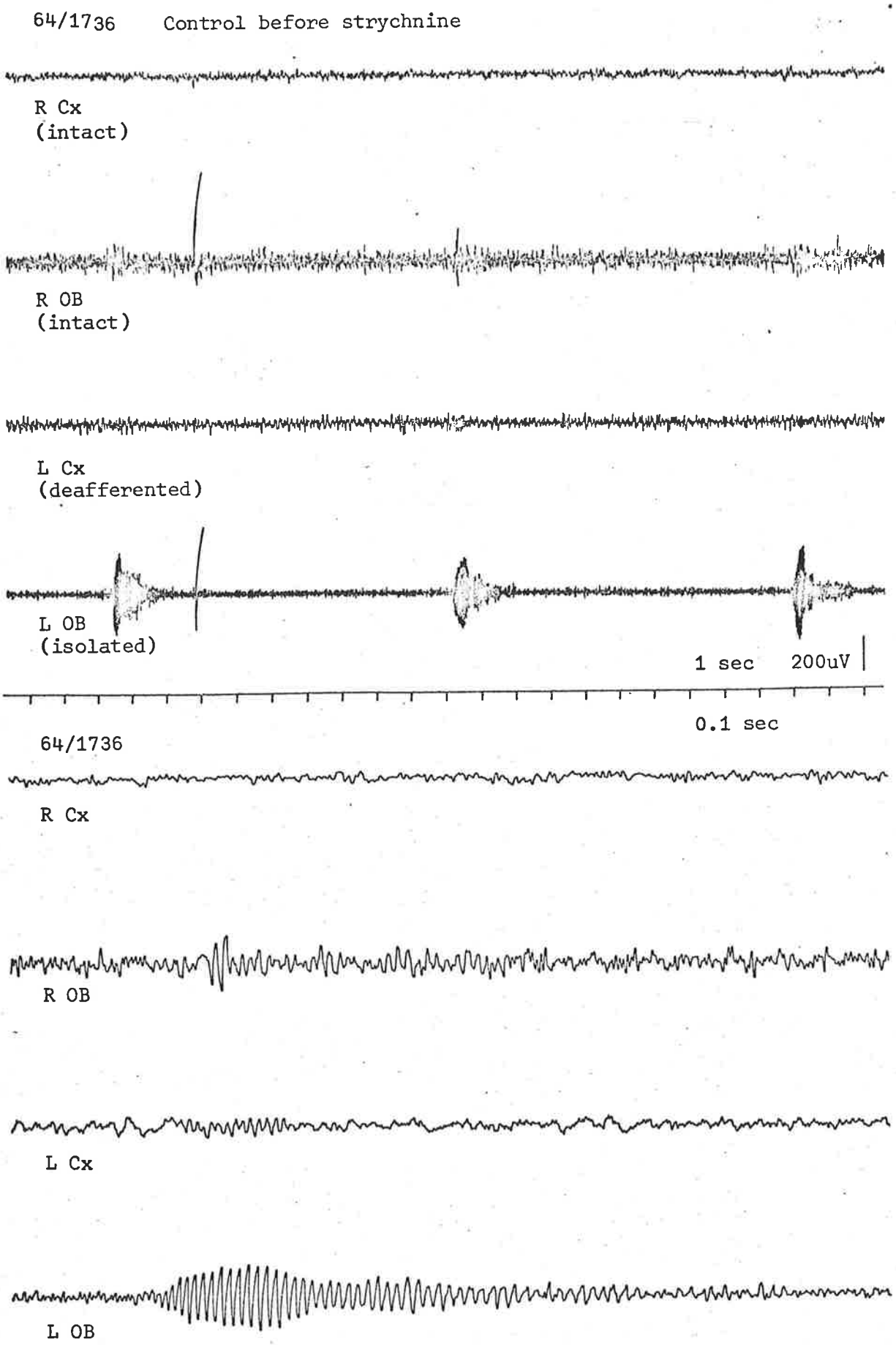


Fig. 5-2A Induced waves recorded from the olfactory bulbs (intact and isolated) and corresponding cortices before strychnine.

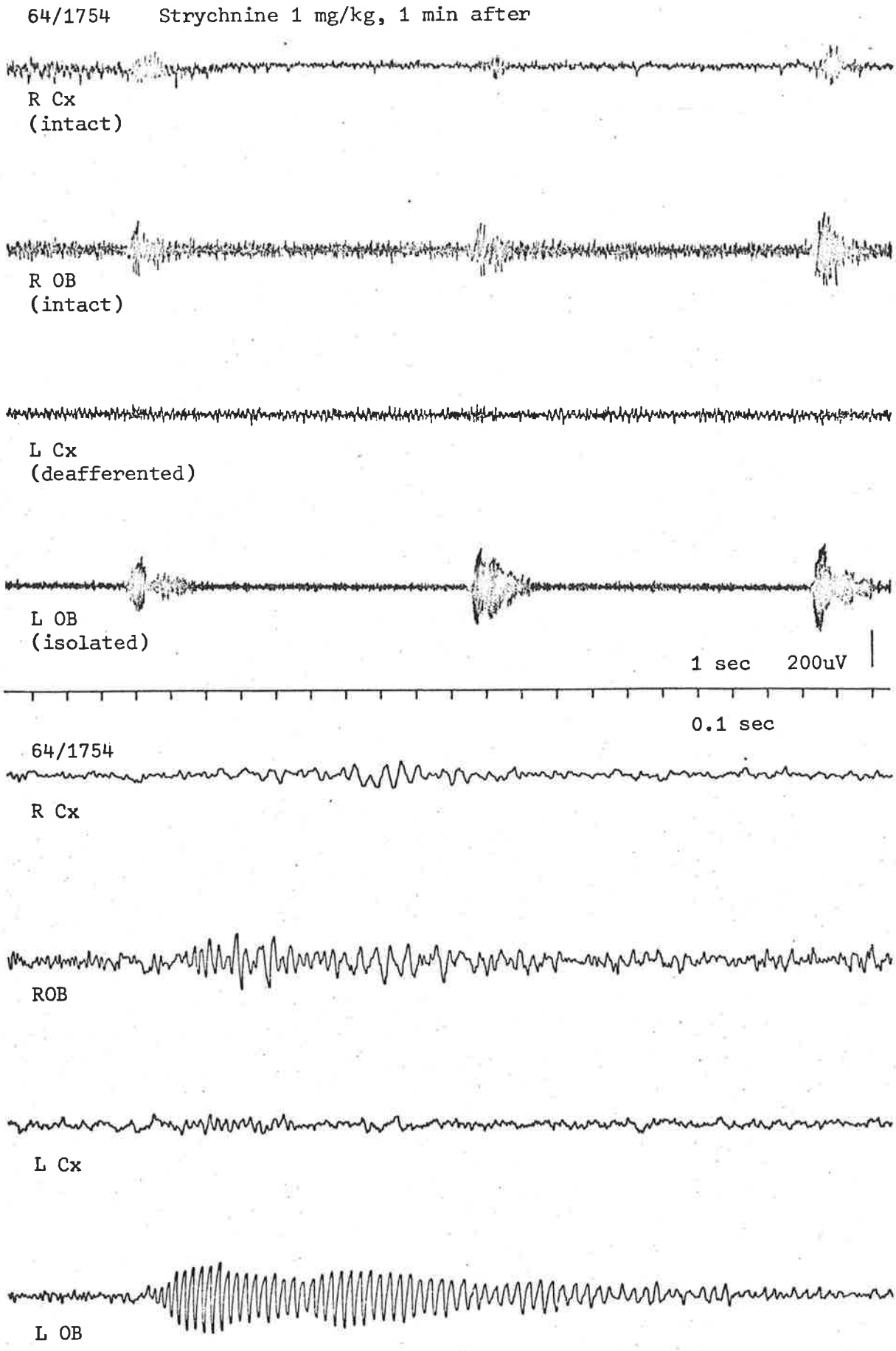


Fig 5-2B Induced waves recorded from the same sites as before, 1 min after strychnine 1 mg/kg had been given. Note that the intact cortical burst is becoming clearer.

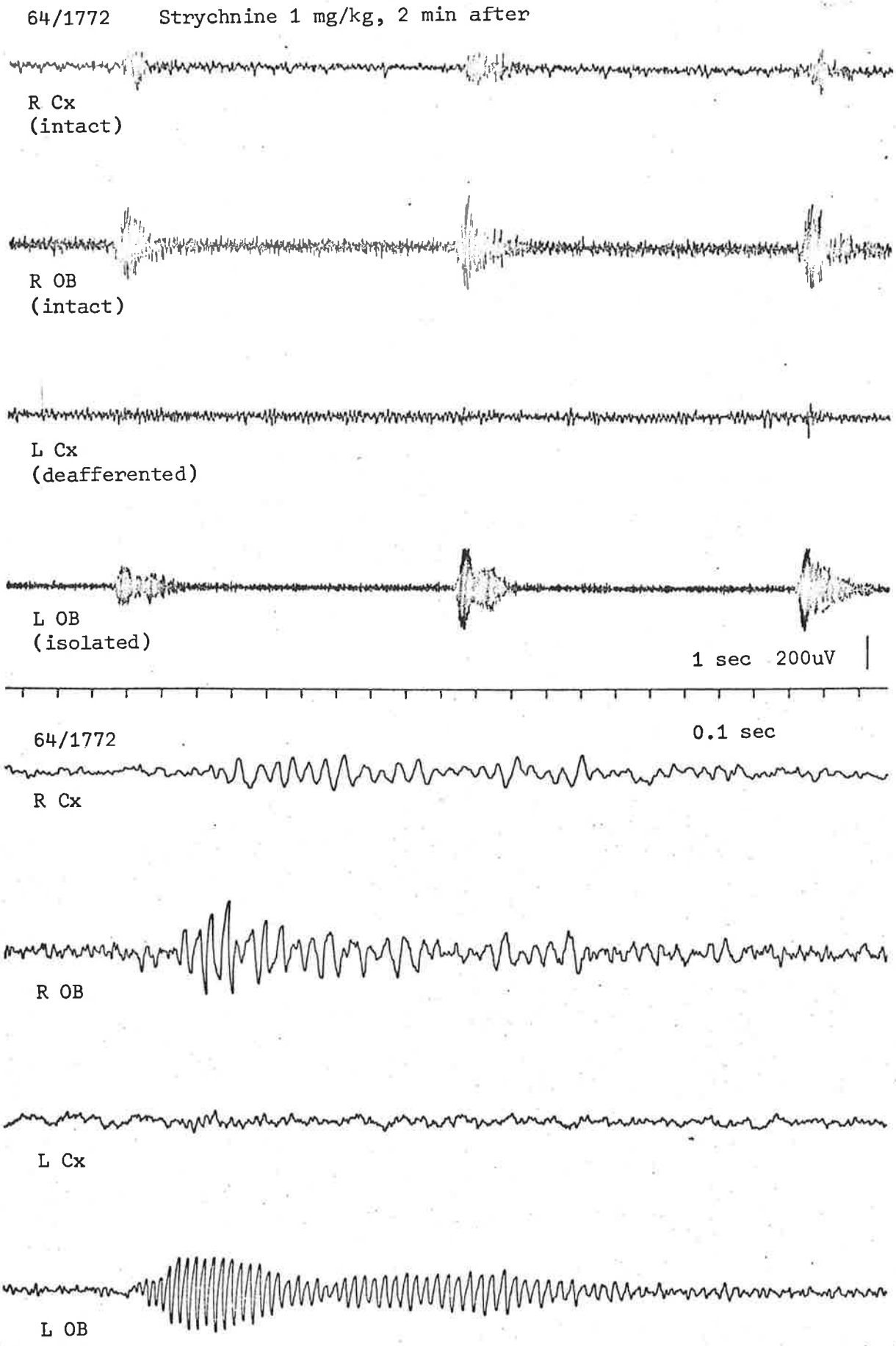


Fig. 5-2C Induced waves 2 min after strychnine 1 mg/kg.

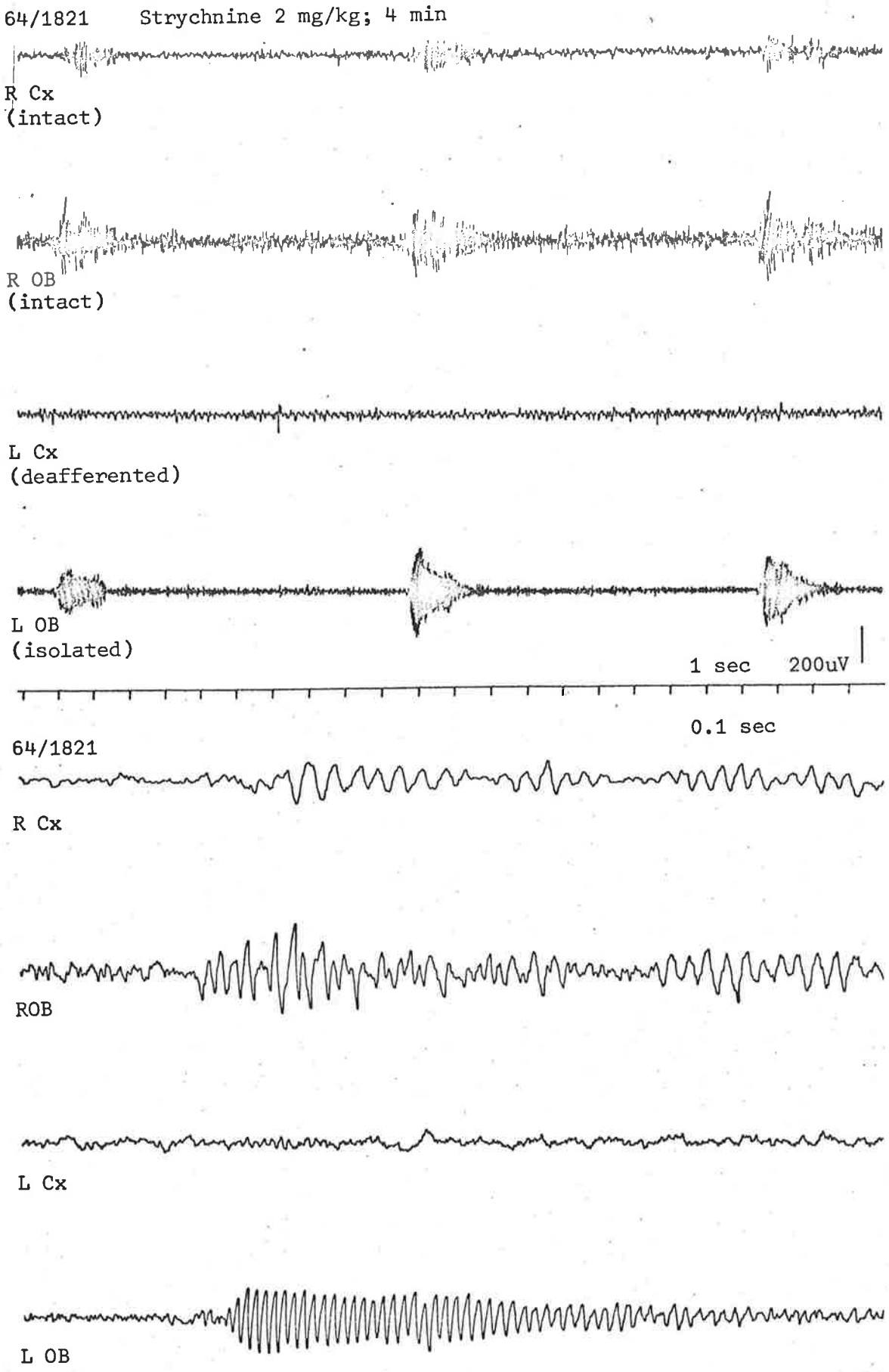


Fig. 5-2D Induced waves 1 min after a second increment of strychnine had been given (now 2 mg/kg); total time 4 min after 1 mg/kg.

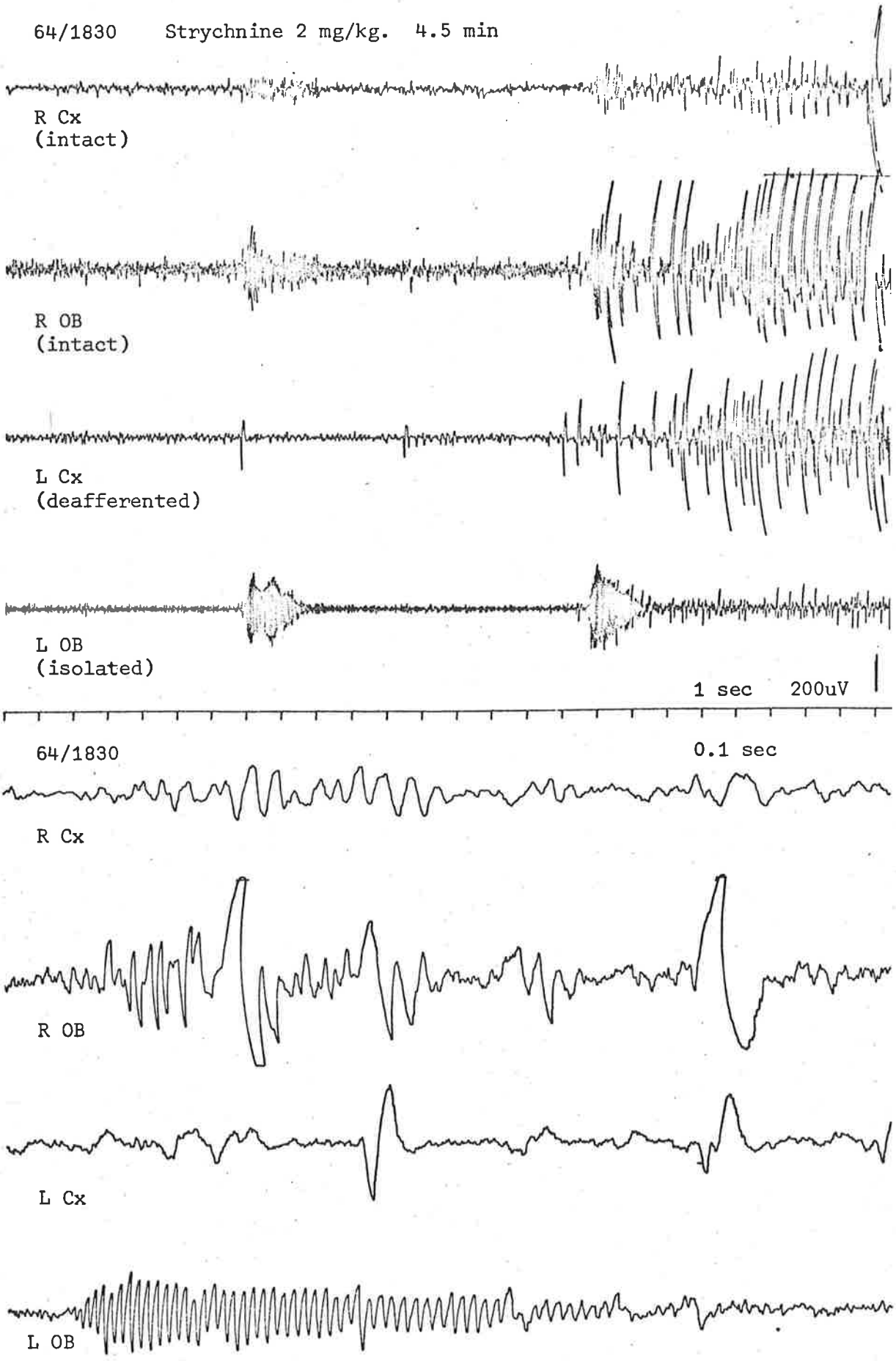


Fig. 5-2E Induced waves 30 sec after the preceding record, 4.5 min after the first injection of strychnine. Total dose 2 mg/kg.

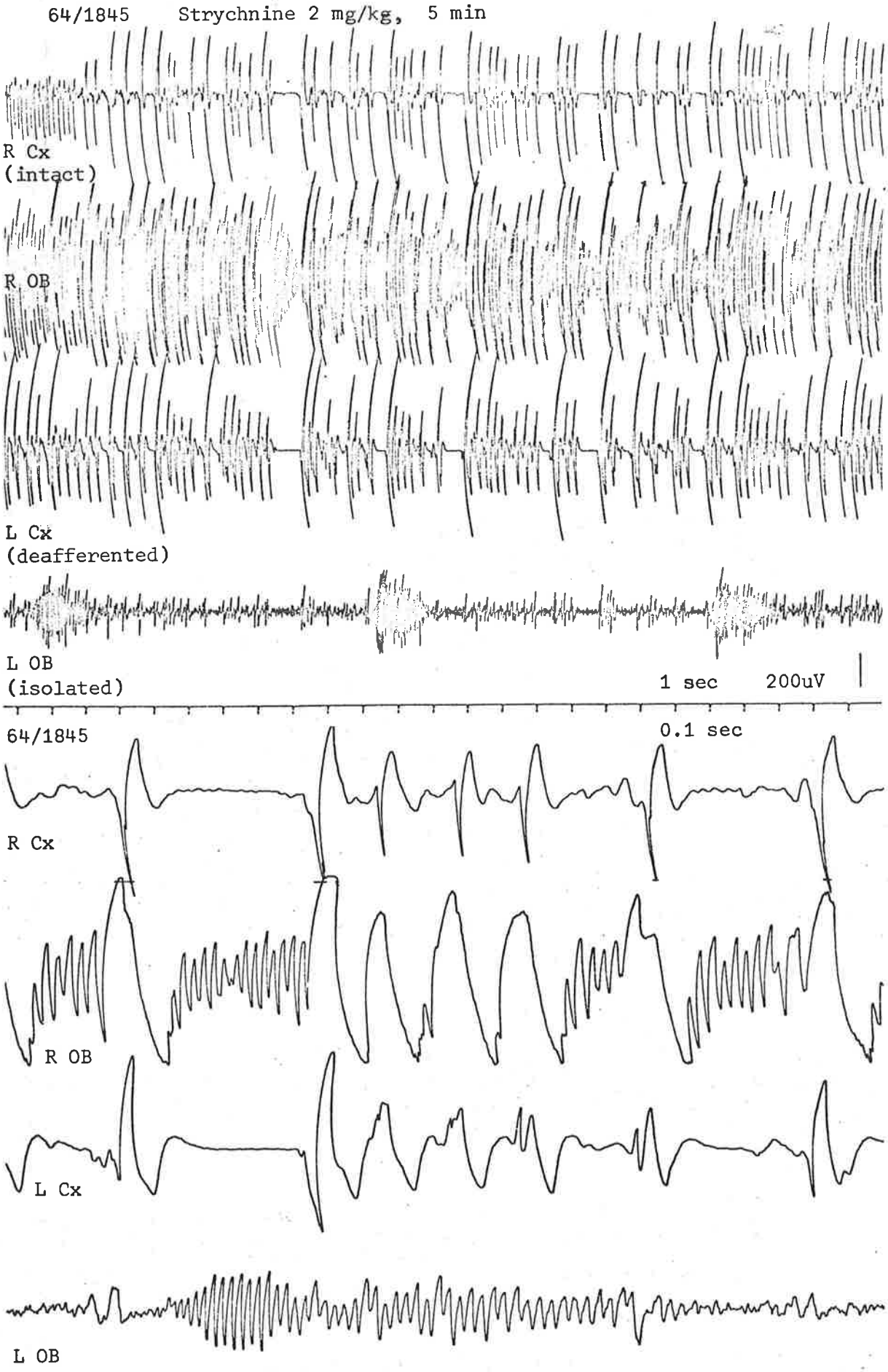


Fig. 5-2F Electrical activity recorded from the same sites as before, 5 min after the initila injection of strychnine.

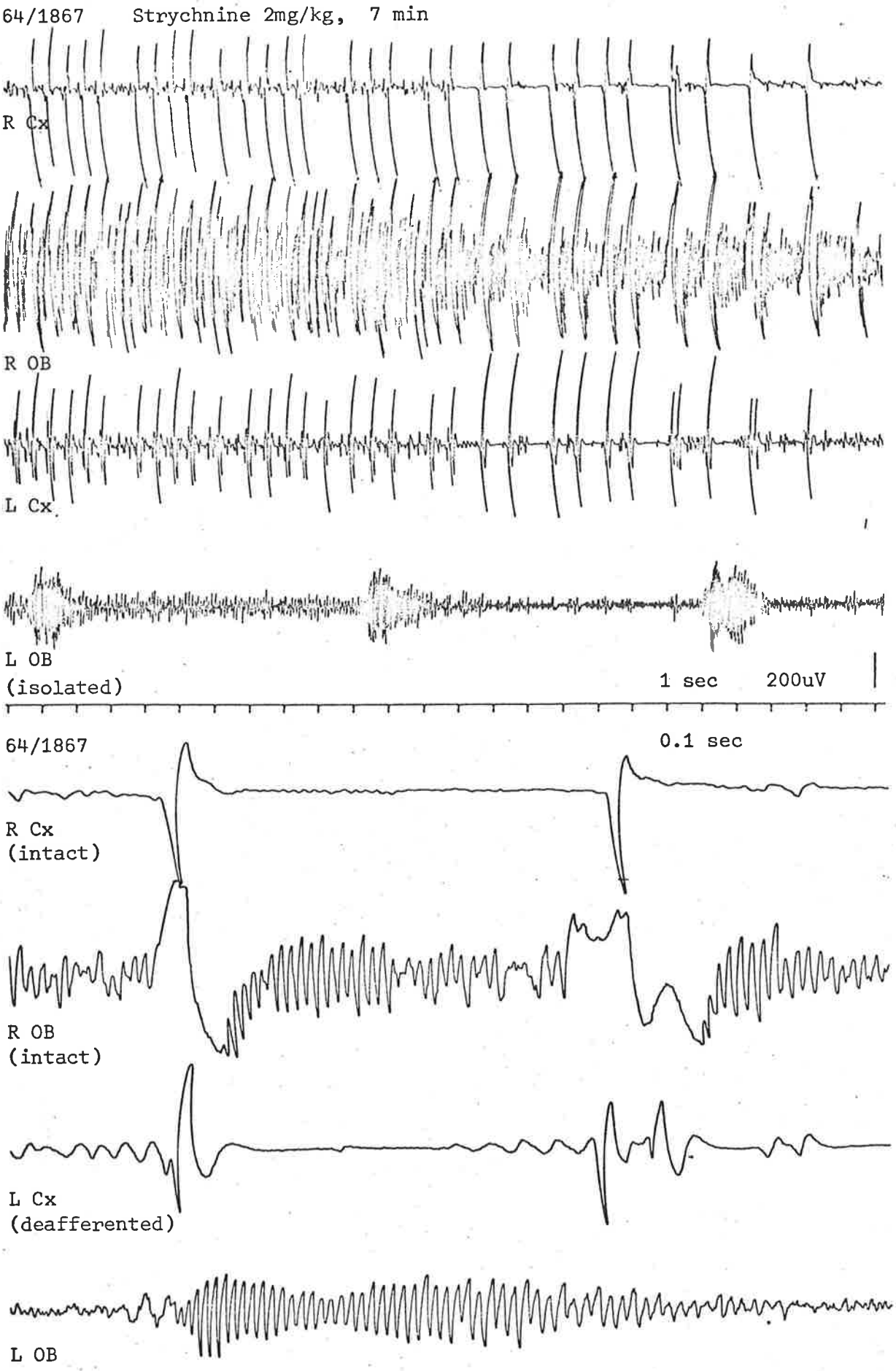


Fig. 5-2G Electrical activity recorded from the same sites, 7 min after the initial injection of strychnine, total now 2 mg/kg.

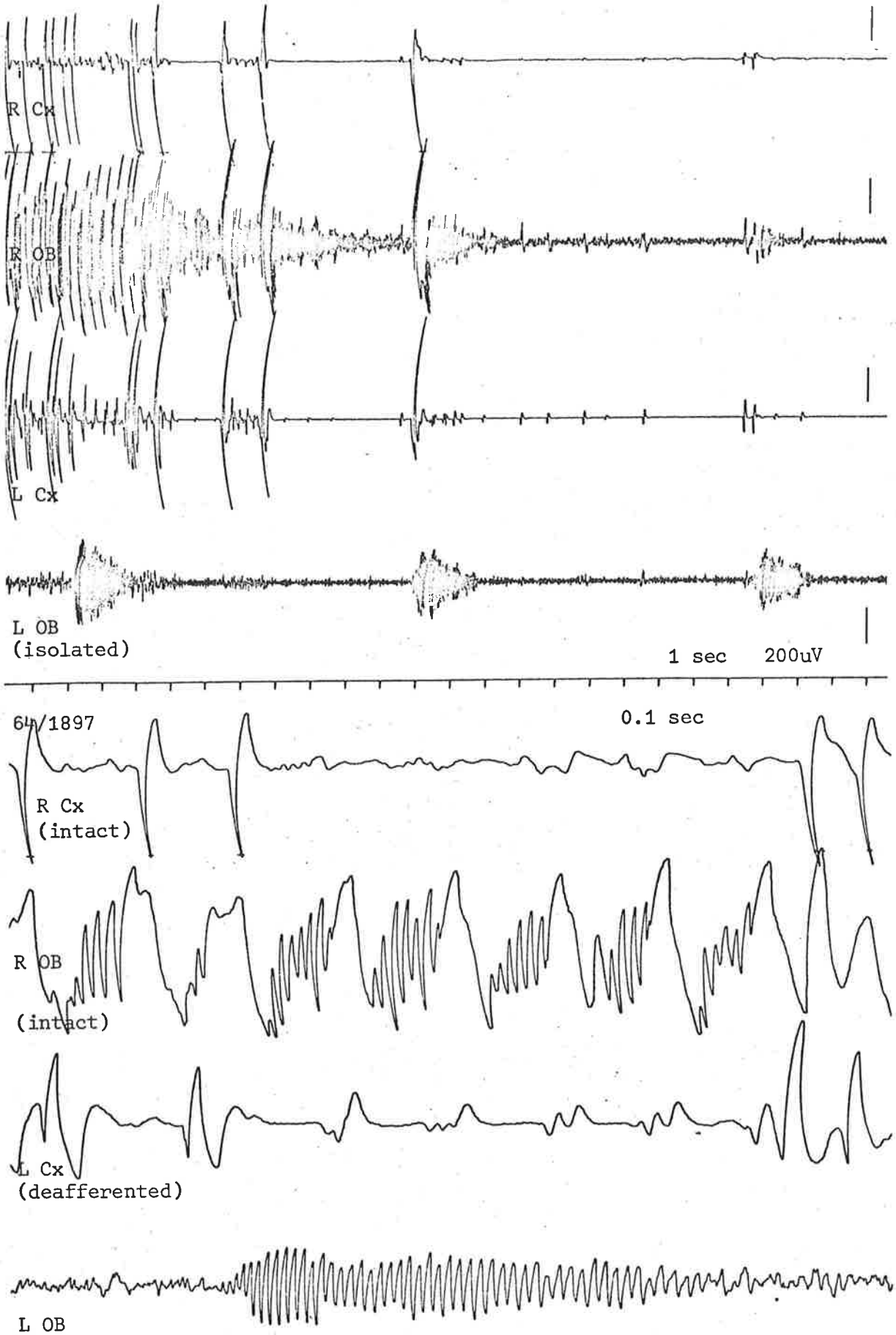


Fig. 5-2H Electrical activity from the same sites, 8.5 min after the the initial injection of strychnine. This record is continued as the upper part of the next figure.

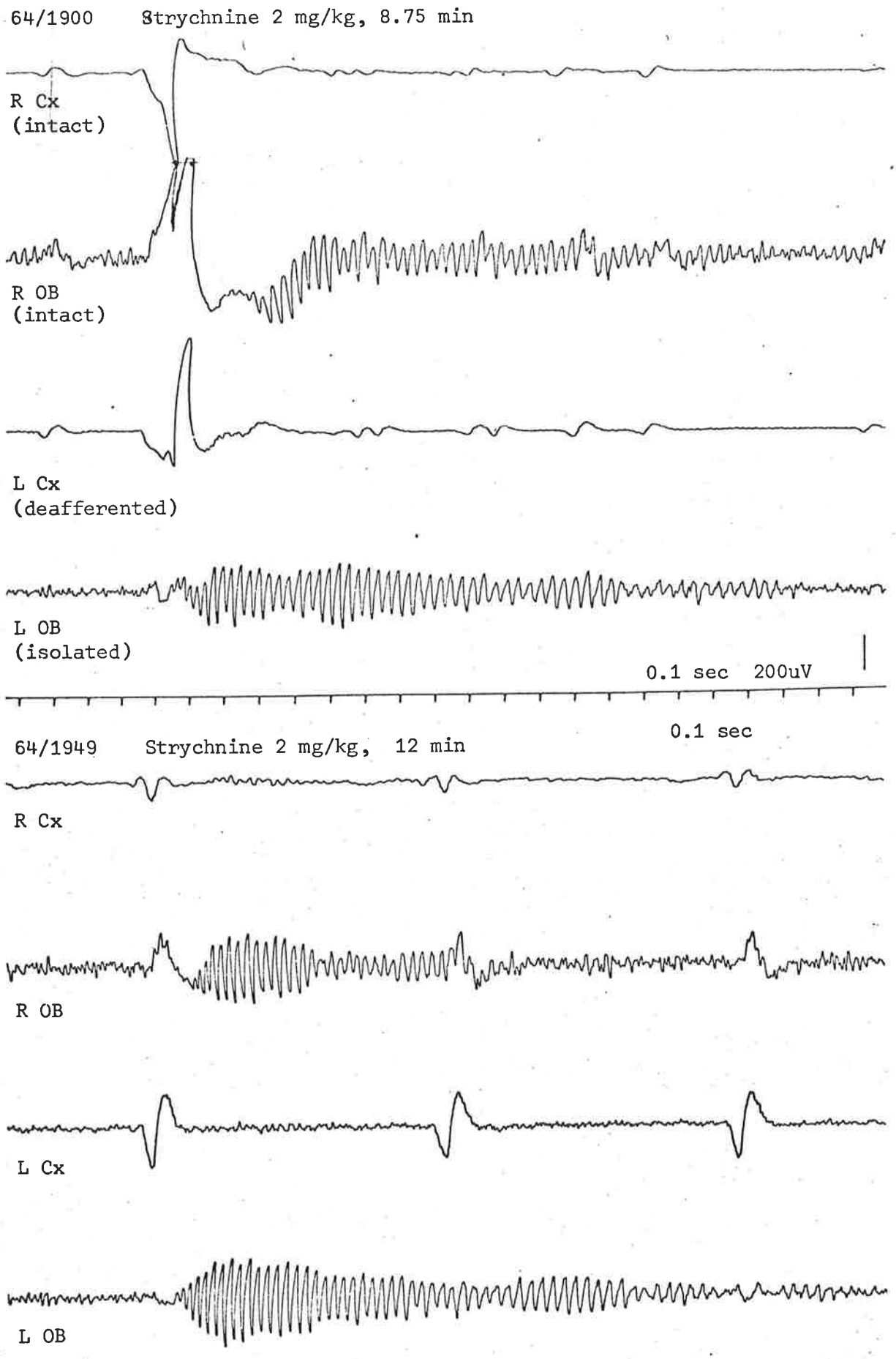


Fig 5-2I (Upper) Continuation of the preceding figure.
(Lower) Activity 12 min after initial injection of strychnine.

Picrotoxin

Picrotoxin caused generalized electrical seizures after the initial injection of 1 mg/kg in three cases, and after 3 mg/kg in the other two. Fig. 5-3 illustrates typical changes in records from intact and isolated olfactory bulbs, and the corresponding cortices of an immobilized, artificially-ventilated phalanger. Fig. 5-3A is the control recording of the induced waves before picrotoxin was given; 5-3B is 2 min after the second 1 mg picrotoxin/kg was given; C shows the second and third induced waves in the upper record of B; D is the record 2 min after B (4 min after the second, and 9 min after the first injection); E shows the second and third induced waves of D written out at 0.1 recording speed; and F shows the activity one hour after picrotoxin had been given.

Isolated bulb: induced waves

Picrotoxin had no effect on the induced waves of the isolated bulb. Amplitude, duration and contained frequencies remained constant. Intrinsic activity appeared to be unchanged also.

Intact bulb: induced waves

The intact bulb reflected the generalized seizure activity of its cortex, and spike activity was recorded from it. Despite this, there was still evidence of induced activity in the intact bulb. Fig. 5-3B shows the induced wave immediately before the seizures began, and the induced wave of the intact bulb (ROB) has the expected appearance. Ten seconds later (Fig. 5-3C, upper part), the induced wave in the intact bulb has a spike superimposed on it 600 msec from its onset. The next induced wave (Fig. 5-3C, lower) occurred when spike activity was fully developed. There is sinusoidal activity in the interspike intervals. This activity occurs during the period of the induced activity of the isolated bulb. It is inhibited for about 100 msec by each spike. The same phenomenon is shown in Fig. 5-3D (lower), but the duration of inhibition following the spike is longer.

Another example of this sinusoidal activity was seen in another animal and is illustrated in Figs. 5-4A, B & C. The upper part of Fig. 5-4A shows a control induced wave in the absence of drugs recorded from the usual intact bulb and cortex, isolated bulb and deafferented cortex. The expected features are seen. The lower part of that figure is the record 30 sec before generalized seizures began, 8 min after 1 mg picrotoxin/kg and 3 min after a second 1 mg/kg. It is essentially the same as before picrotoxin.

Fig. 5-4B (upper) shows an induced wave during the picrotoxin seizure. The isolated bulb is unaffected. Spike activity is recorded in the other three leads. An induced wave is present in the intact bulb, but it is interrupted by this spike activity. The lower part of this figure shows a period of post-ictal cortical depression, during which time 20 Hz sinusoidal activity was recorded from the intact bulb. Such activity was only rarely seen. It apparently occurred spontaneously, with no preceding olfactory stimulus.

Another form of sinusoidal activity was recorded from the intact bulb some 30 min later. Fig. 5-4C (upper) shows an induced wave of the isolated bulb (perhaps greater in duration than the control). The induced wave of the intact bulb is interrupted by the spikes, as before. In this case, however, the intact bulb apparently produces 35 Hz sinusoidal activity during the period of post-ictal cortical depression. The lower part of the figure is continuous with the upper, and the 35 Hz activity continues. It was terminated by the recurrence of the generalized seizure.

Intact cortex: induced waves

No induced waves were recorded from the intact cortex, either during the seizure activity or during the periods of post-ictal cortical depression.

Deafferented cortex: electrical activity

The deafferented cortex showed seizure activity synchronous with the intact cortex. There were also identical periods of post-ictal depression when electrical activity was minimal.

Discussion

Picrotoxin has been used as a central nervous system stimulant and barbiturate antagonist (for example, see Marshall, Walzl & Le Messurier, 1937; Werner & Tatum, 1939). Its recent use has been reviewed by Prince (1972). It has been shown to be a GABA antagonist at various sites; frog retina (Burkhardt, 1972), feline spinal cord (Levy & Anderson, 1972, 1973), feline cerebral cortex (Hill, Simmonds & Straughan, 1972), and feline nodose ganglion (de Groat, 1972).

At the dosage used in the experiments reported in this thesis, picrotoxin had the expected effect on cerebral cortical electrical activity; spike-and-wave seizure activity. There was little effect, if any, on either spontaneous or induced electrical activity of the isolated olfactory bulb. This was unexpected, for if GABA were the only inhibitory transmitter in the bulb, an effective GABA antagonist could

be expected to cause significant functional changes. Several possibilities could be considered to explain the lack of effect:

(1) Picrotoxin did not reach the receptor sites in adequate concentrations because (a) systemic dosage was too low, (b) the bulbar blood-brain barrier was more efficient than elsewhere, or (c) there had been significant interference with the bulbar blood supply.

(2) Picrotoxin did not compete for the GABA receptor in the bulb, the receptor being different from the GABA receptor elsewhere (for example, receiving "folded" not "straight" GABA).

(3) Another inhibitory system was present, with a transmitter other than GABA.

(4) Inhibitory mechanisms are not required for normal bulbar functioning.

Of these possibilities, (2) is the most likely. There are examples in the peripheral nervous system of different receptors for the same transmitter (muscarinic and nicotinic receptors for acetylcholine, α and β receptors for noradrenaline).

Nicoll (1970c, 1971b) showed that the microelectrophoretic application of picrotoxin blocked the action of GABA applied in the same way to the mitral cells of olfactory bulbs of rabbits. He also reported that intravenous picrotoxin, to 4 mg/kg, caused severe seizures in the bulb, even when isolated. These were neither described nor illustrated. These reports by Nicoll were not able to be substantiated by my results, but he used higher picrotoxin doses, a different method of bulbar isolation, and a different species.

The experiments reported in this chapter have shown that picrotoxin causes the expected seizures in the phalanger. At such doses, picrotoxin has minimal effect, if any, on the electrical activity of the isolated bulb. It may have some effect on the intact bulb, as continuous spontaneous sinusoidal activity (35 Hz) was seen. It is clear that the effects of picrotoxin will have to be investigated further, both on intact and isolated bulbs.

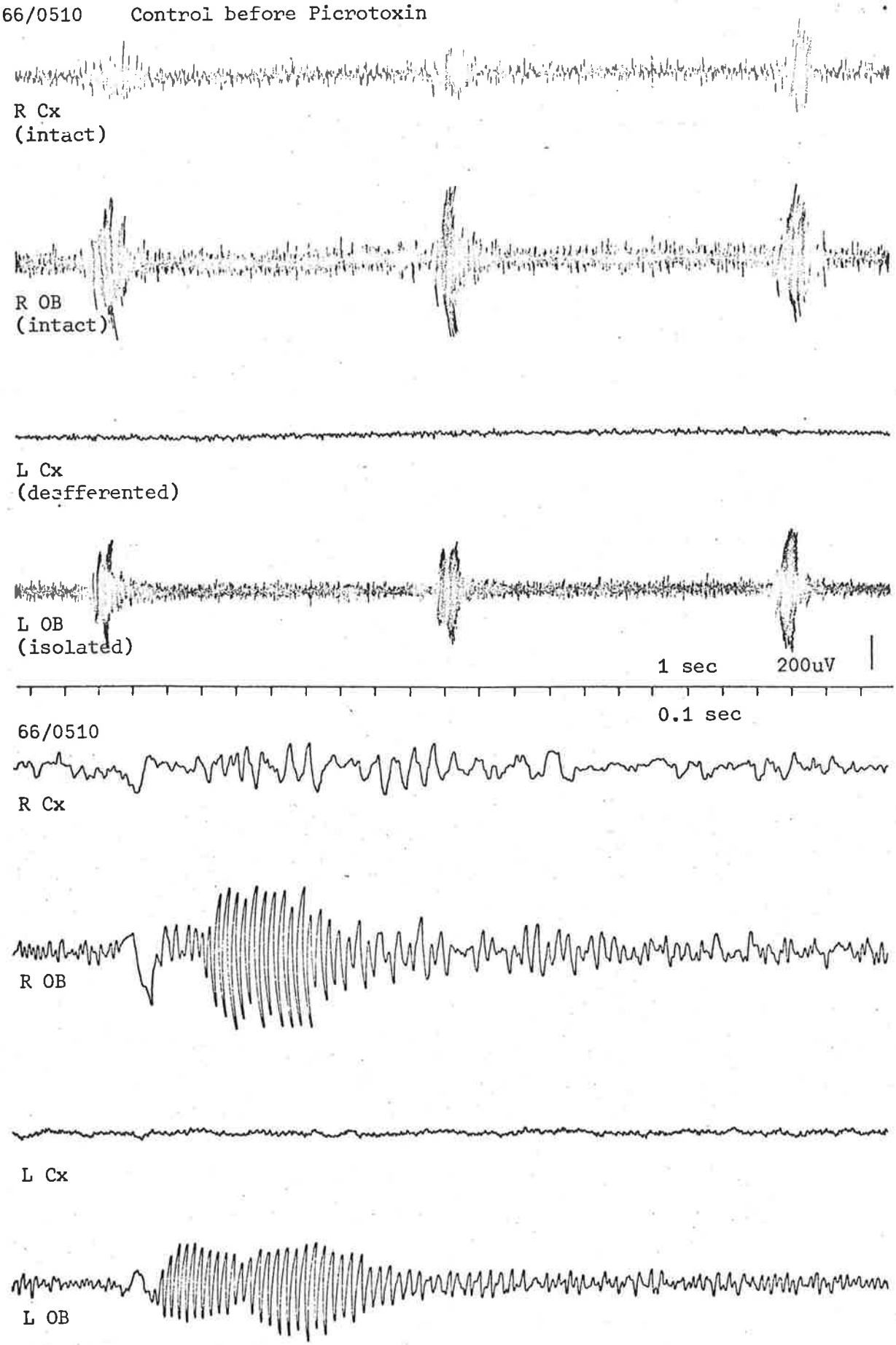


Fig. 5-3A Induced waves recorded from intact and isolated olfactory bulbs, and the corresponding olfactory cortices of the phalanger, before picrotoxin.

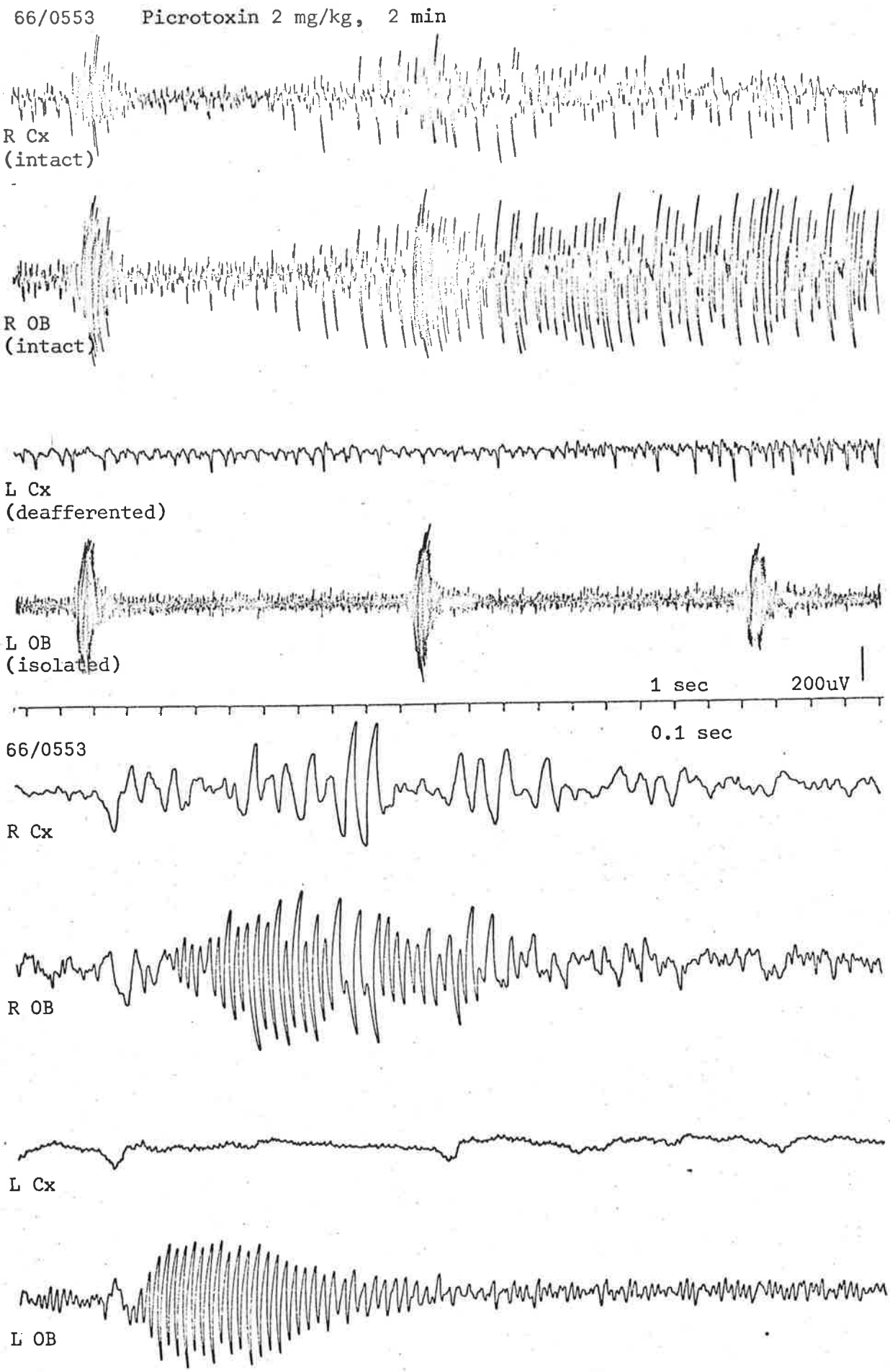


Fig. 5-3B Records from the same sites as before, 2 min after picrotoxin 2 mg/kg. The lower record is the first response written out at 0.1 recording speed.

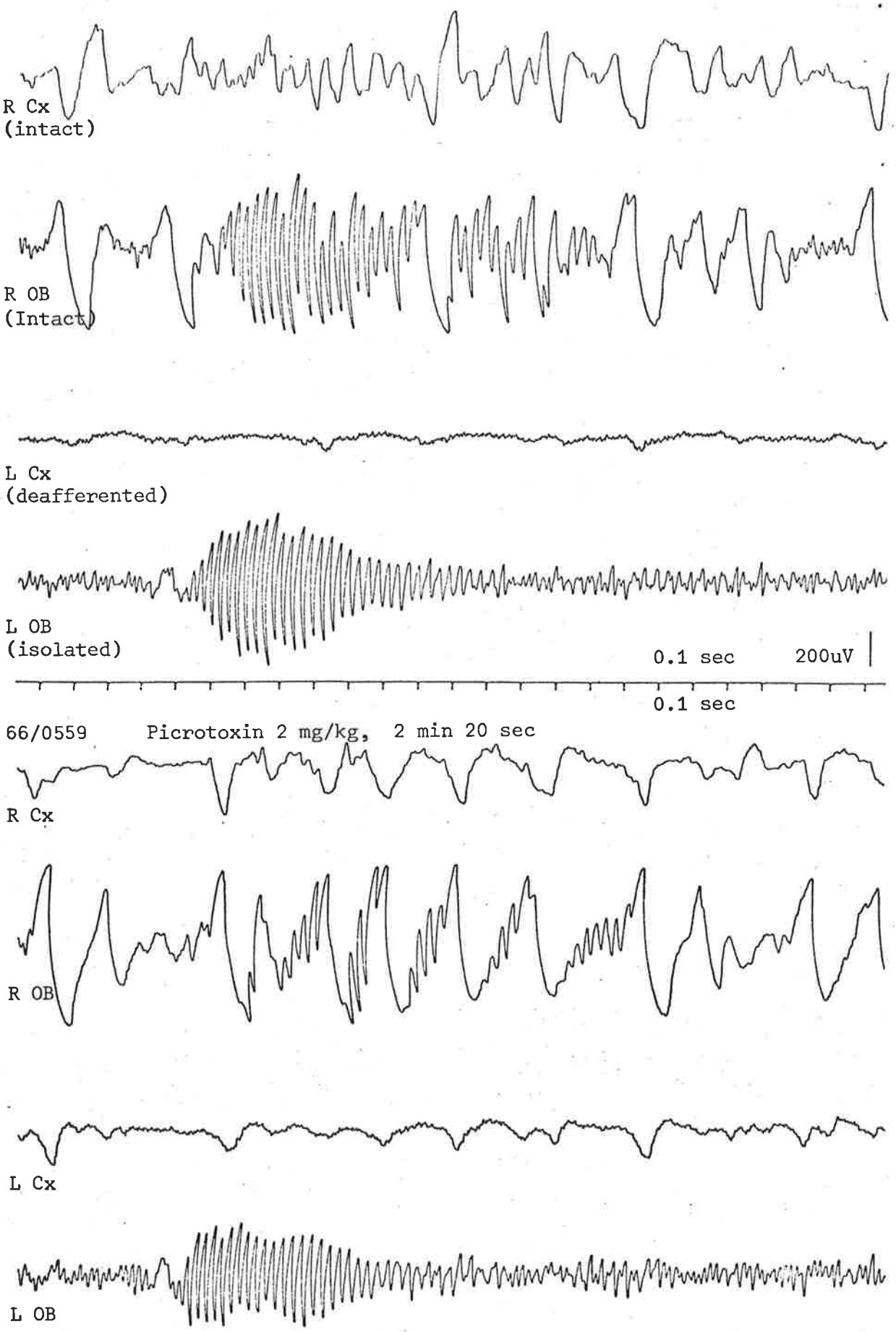


Fig. 5-3C The two induced waves which followed that illustrated on the lower part of the preceding figure at the beginning of the picrotoxin seizure.

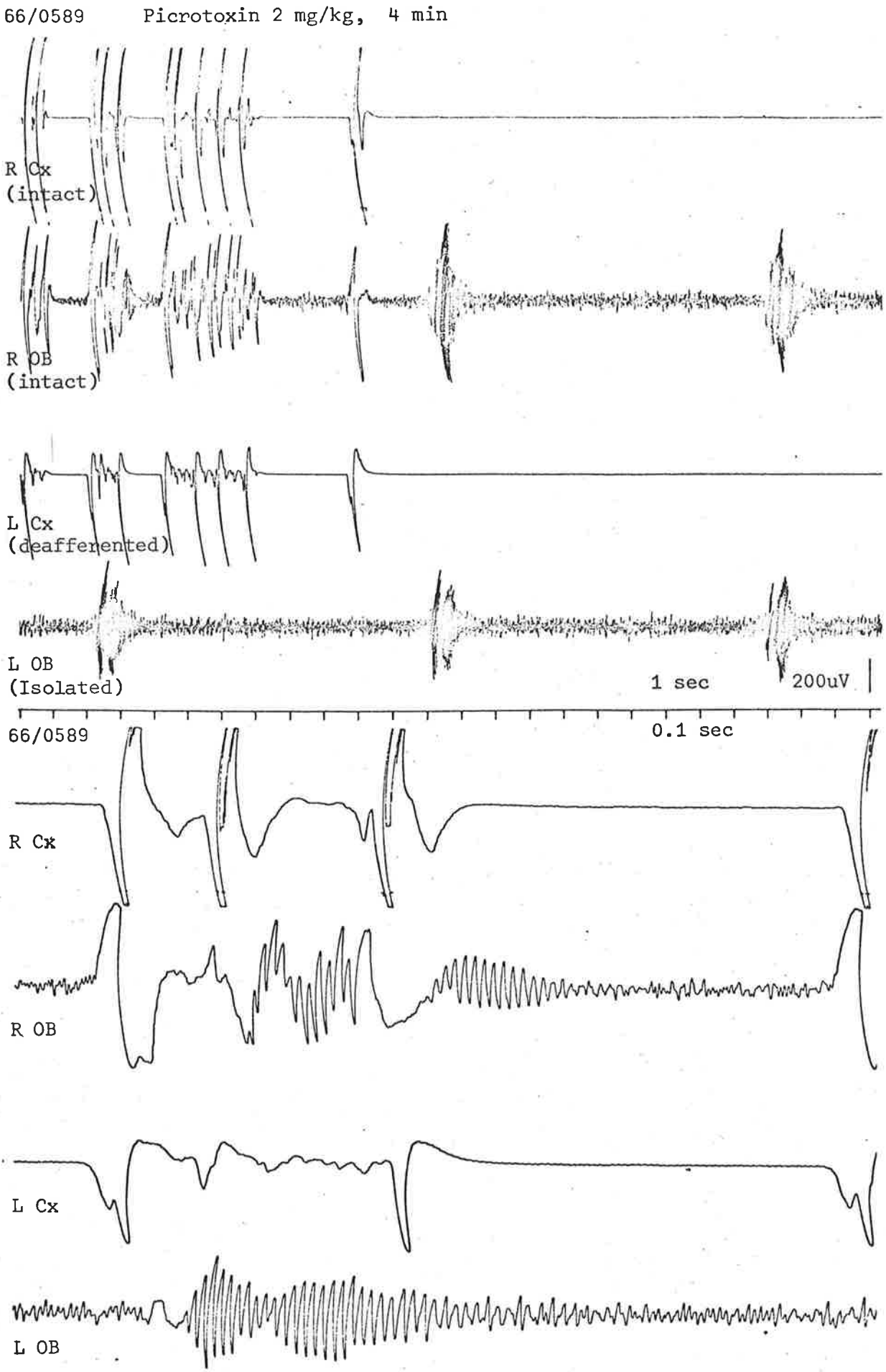


Fig. 5-3D Electrical activity from the same sites 4 min after picrotoxin 2 mg/kg. The lower part shows the first induced wave of the upper trace written out at 0.1 recording speed.

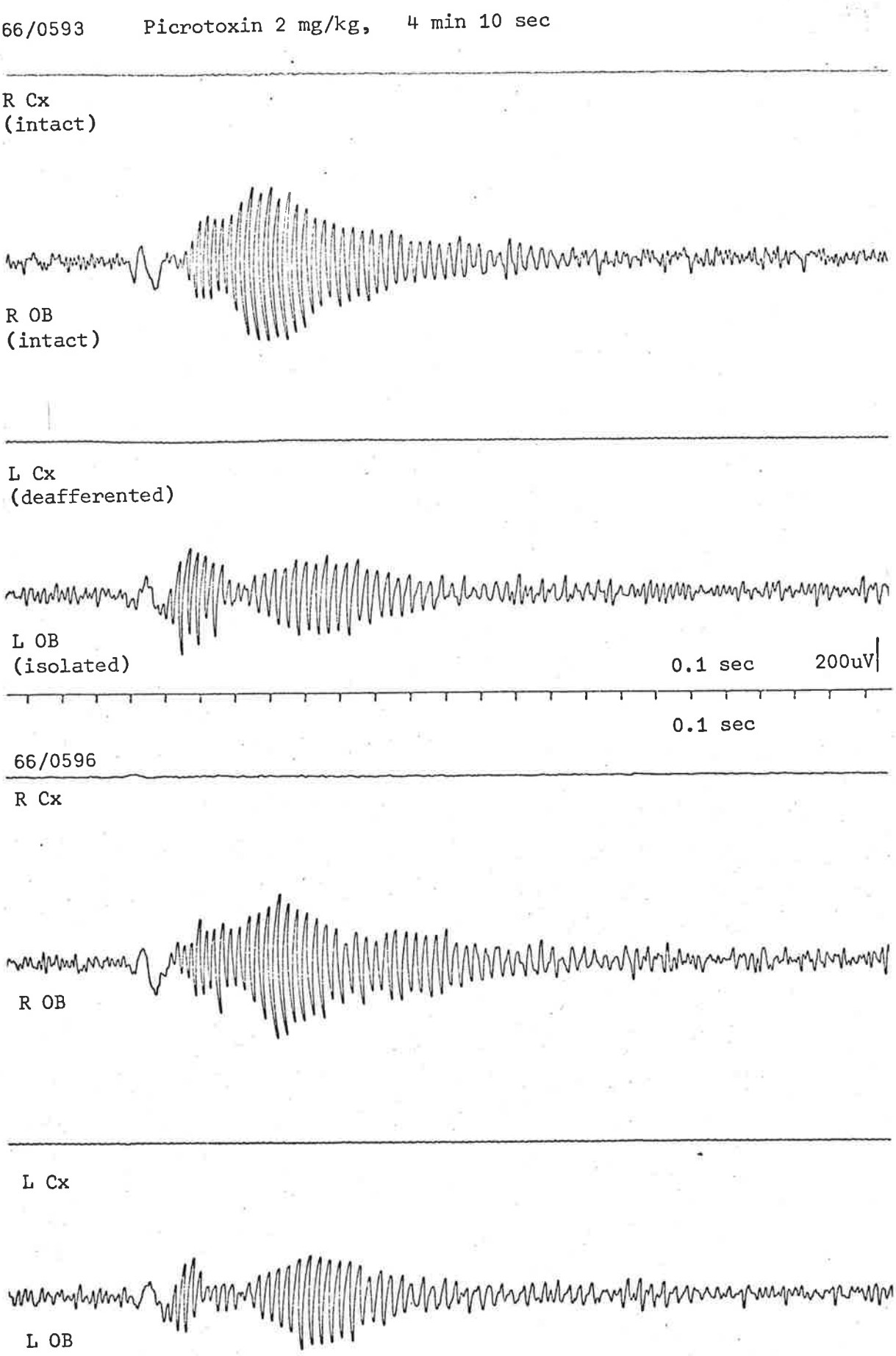


Fig. 5-3E The two induced waves which followed the one illustrated at the bottom of the preceding figure, during the period of post-ictal depression of cortical function.

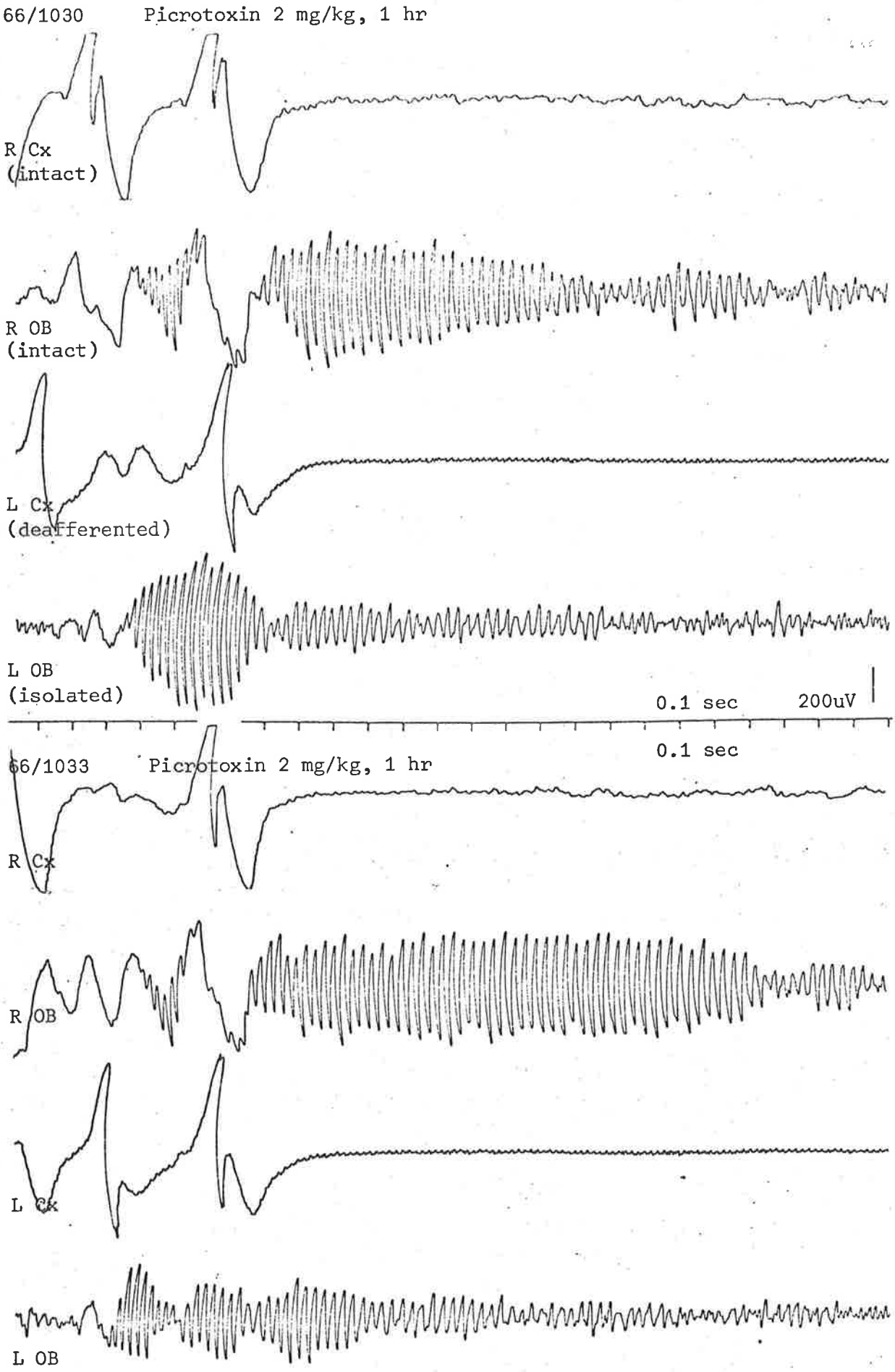


Fig. 5-3F Electrical activity recorded from the same sites 1 hr after picrotoxin 2 mg/kg had been given.

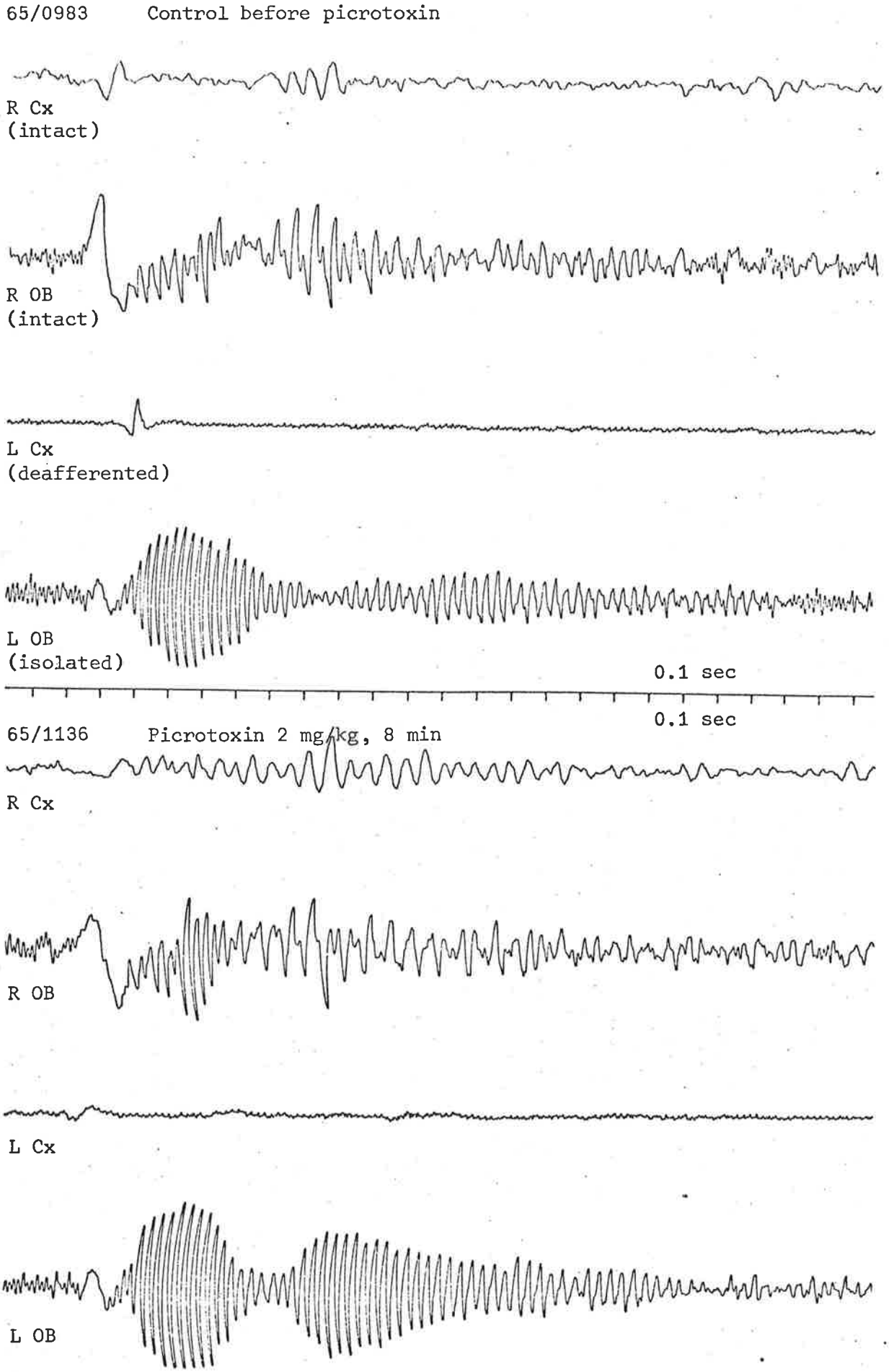


Fig. 5-4A Upper. Induced waves recorded from the four sites before picrotoxin was given to an immobilized phalanger.
Lower. Induced waves 8 min after picrotoxin 2 mg/kg, and 30 sec before generalized seizures began.

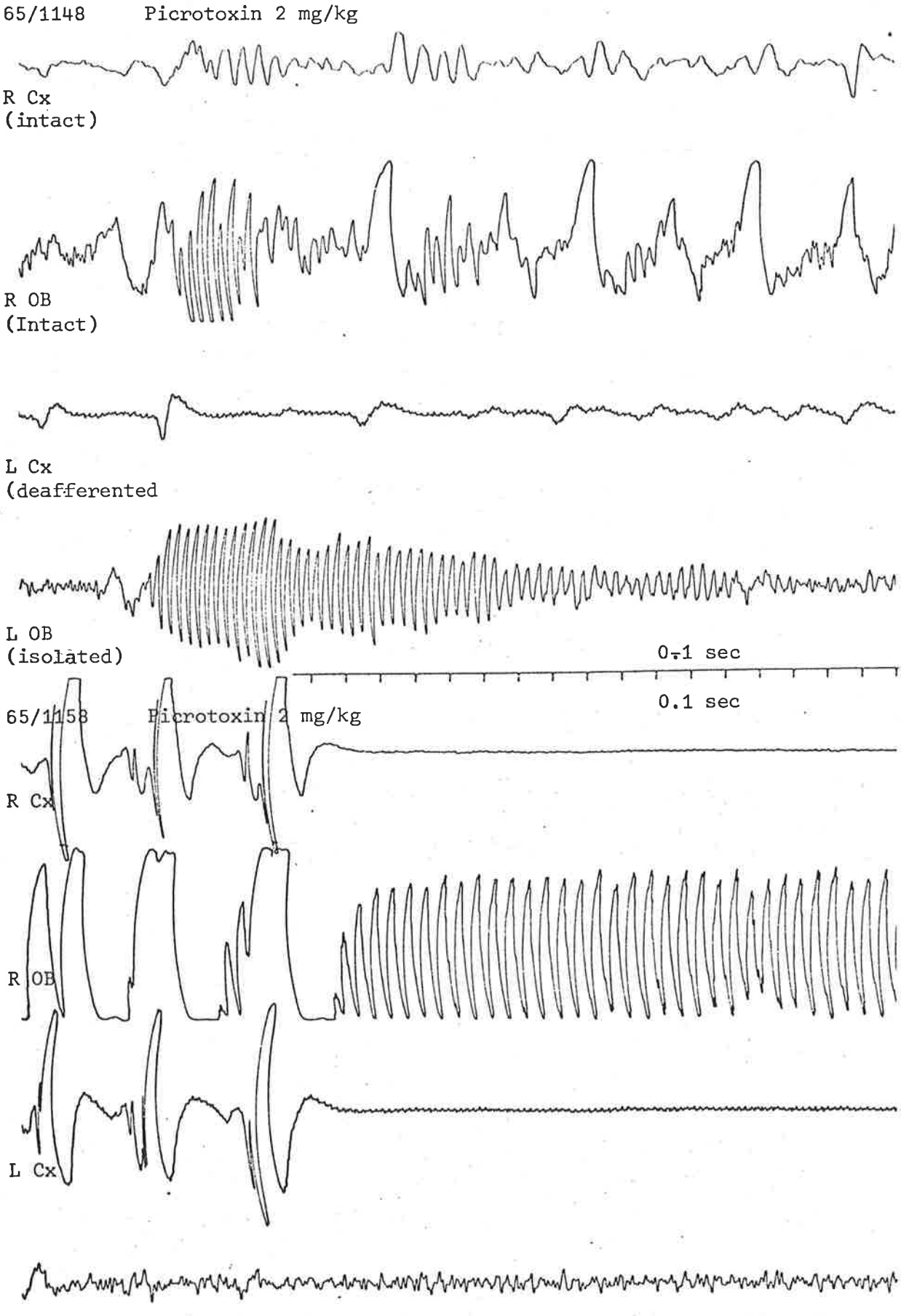


Fig. 5-4B Upper. An induced wave recorded from the four sites during a picrotoxin seizure.
Lower. Record of electrical activity with no induced activity 1 min after the upper record.

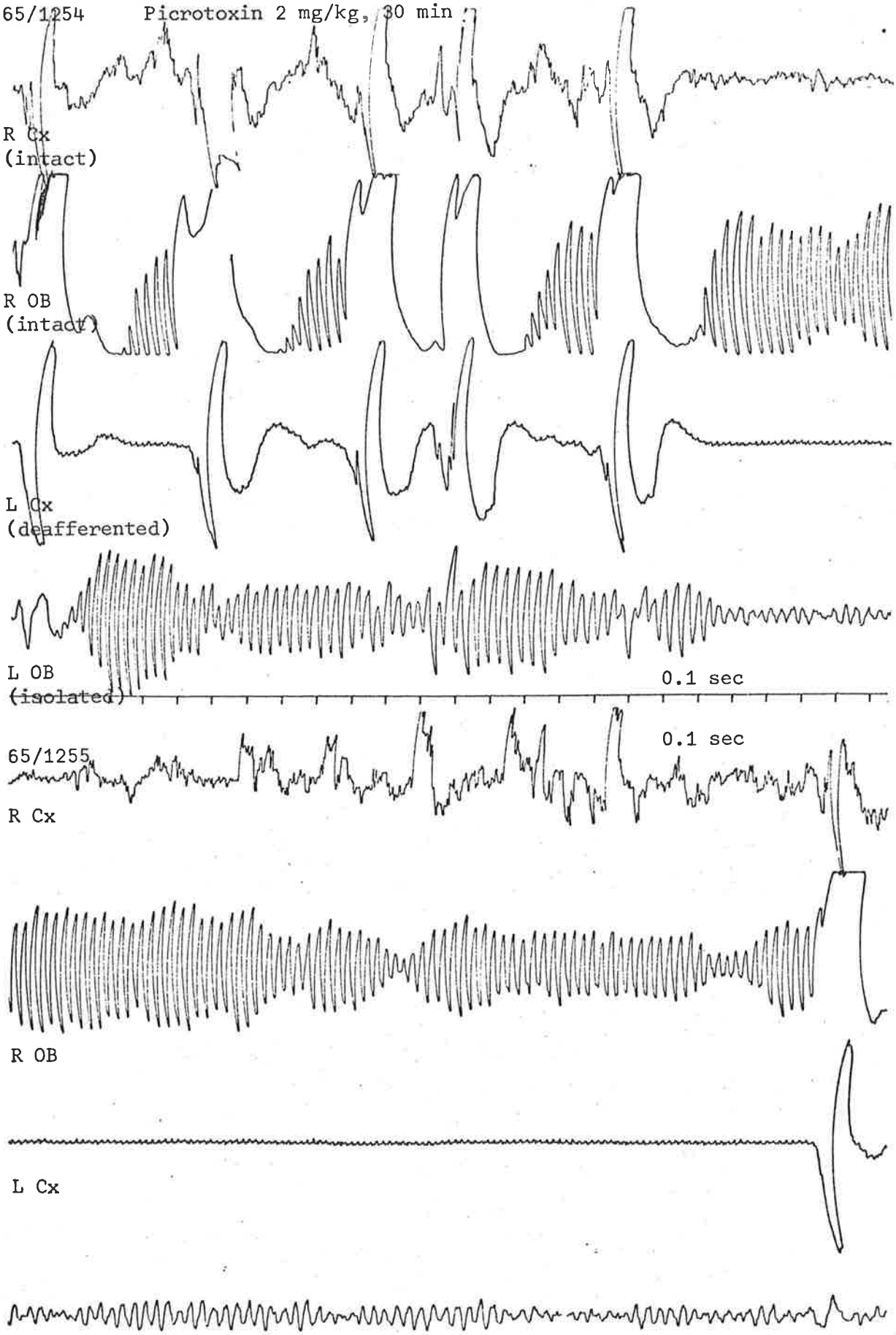


Fig. 5-4C Upper and lower records are continuous. Electrical activity (including an induced wave) 30 min after the injection of picrotoxin 2 mg/kg.

Bicuculline

Bicuculline caused generalized seizure activity at 0.2 mg/kg in two cases, and 0.3 mg/kg in the third. One of the former animals died after 30 min, of an unknown cause. No cardiovascular parameters were measured, but artificial ventilation was continued, and blood loss had been replaced. There was copious salivation in these three cases.

Fig. 5-5A shows induced waves recorded from intact and isolated olfactory bulbs, and the corresponding cortices of an immobilized phalanger. The first induced wave in the upper record (and lower 0.1 recording speed) shows an alerting response. The minimal induced wave in the deafferented cortex was probably the result of the incomplete peduncular transection. The high frequency component is not prominent in the intact bulb.

Fig. 5-5B shows the onset of seizure activity, and Fig. 5-5C is the record 1 min later, when the seizure was well established.

Isolated bulb: induced waves

There were minimal changes in the induced waves of the isolated bulb after bicuculline. The duration was not increased significantly, and the frequencies within each burst remained the same. Bursts began at about 50 Hz and terminated at 25 - 30 Hz. Intrinsic activity was increased in the examples shown in Fig. 5-5 (from experiment number W60, where peduncular transection was later shown to be incomplete), but not in the other two experiments in which bicuculline was used.

Intact bulb: induced waves

Induced waves in the intact bulb were masked by the seizure activity elsewhere. However, the intact bulb was still capable of initiating induced waves. Fig. 5-5C (lower) shows the record from an intact bulb (ROB, intact). The onset of a burst is buried between two spikes, but the burst continues during a period of post-ictal cortical depression.

Intact cortex: induced waves

No induced waves were recorded from the intact cortex during the seizure activity or during the periods of post-ictal depression. An induced wave was sometimes capable of initiating a seizure.

Deafferented cortex: electrical activity

The deafferented cortex convulsed synchronously with the intact cortex, with spike-and-waves at 3-5/sec. No induced waves were recorded if the transection was complete.

Discussion

Bicuculline has been shown by this study to have similar properties in the phalanger to those of picrotoxin. Such a similarity has been shown in other systems (for example, see Banna, Naccache & Jabbur, 1972). In particular, bicuculline did not significantly affect the induced waves recorded in the isolated bulb, at the convulsive doses used. On the other hand, the 35 Hz sine wave activity was not seen in the intact bulb after bicuculline.

This study does not necessarily imply that GABA is not being antagonized in the bulb, as discussed earlier. It does point out the necessity for further experiments with intact and isolated bulbs, with induced and evoked potentials. This study also does not clarify the origins or mechanism of the generalized seizures, but confirms the changes in the EEG reported by Florio & Longo (1972).

The structural similarities between bicuculline, picrotoxin and GABA are not clear, so it is difficult to specify the active part of the molecules. Manipulations of the molecules have not elucidated this problem. Picrotin, comprising 50% of picrotoxin, is inactive as a systemic convulsant, but has not been specifically tested for anti-GABA activity. Other derivatives of bicuculline (e.g. N-methylbicuculline, bicuculline methochloride) have also been shown to possess convulsant and anti-GABA activity.

In addition to the possibility there are two types of GABA receptors (straight and flexed), the possibility of a dual inhibitory system has to be considered. Meldrum & Horton (1971) raised such a query in connexion with a postulated dual GABA inhibitory system in the photosensitive baboon.

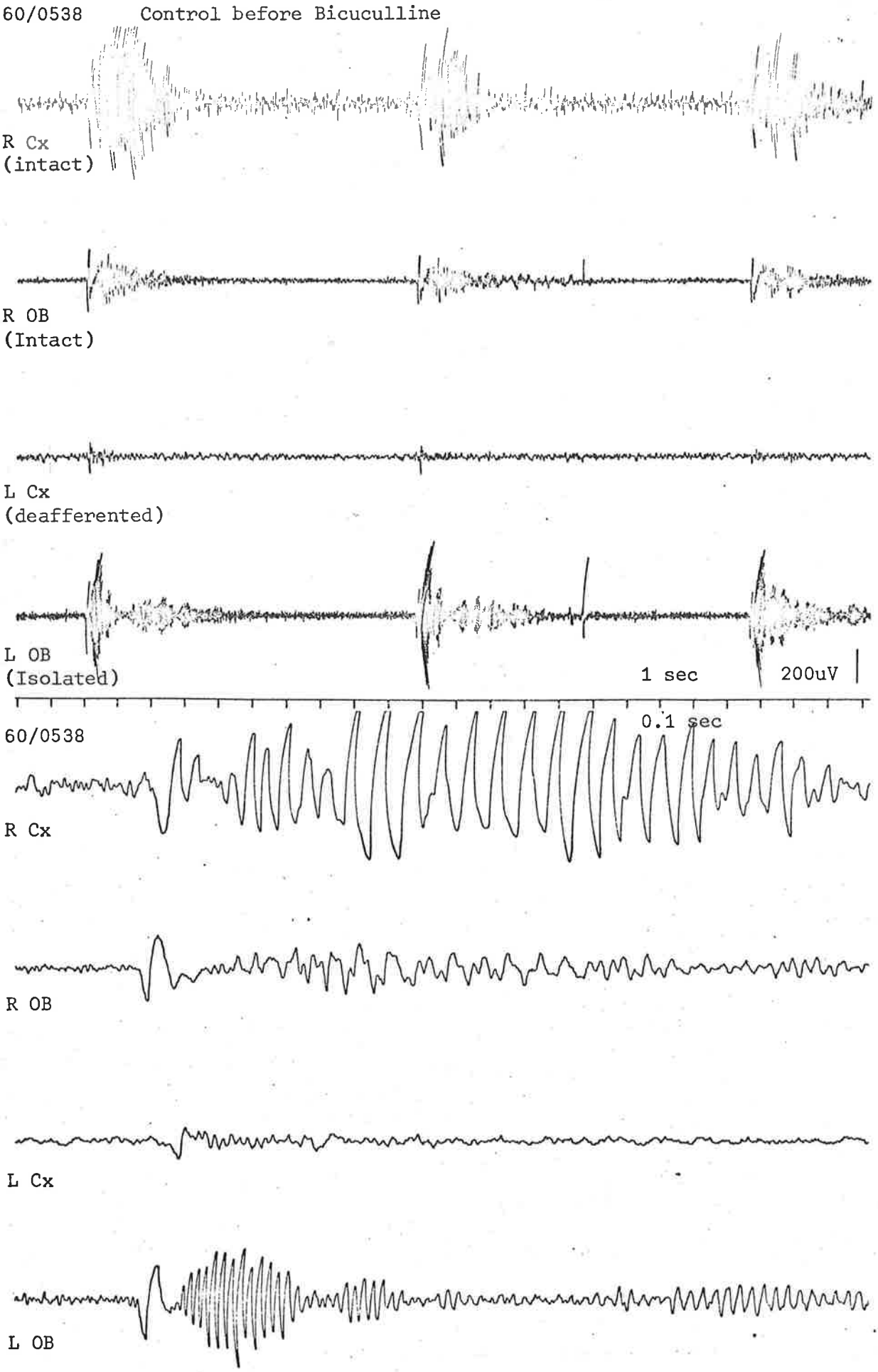


Fig. 5-5A Induced waves from intact and isolated olfactory bulbs and the corresponding cortices of the phalanger before bicuculline. The lower part shows the first induced wave at 0.1 speed

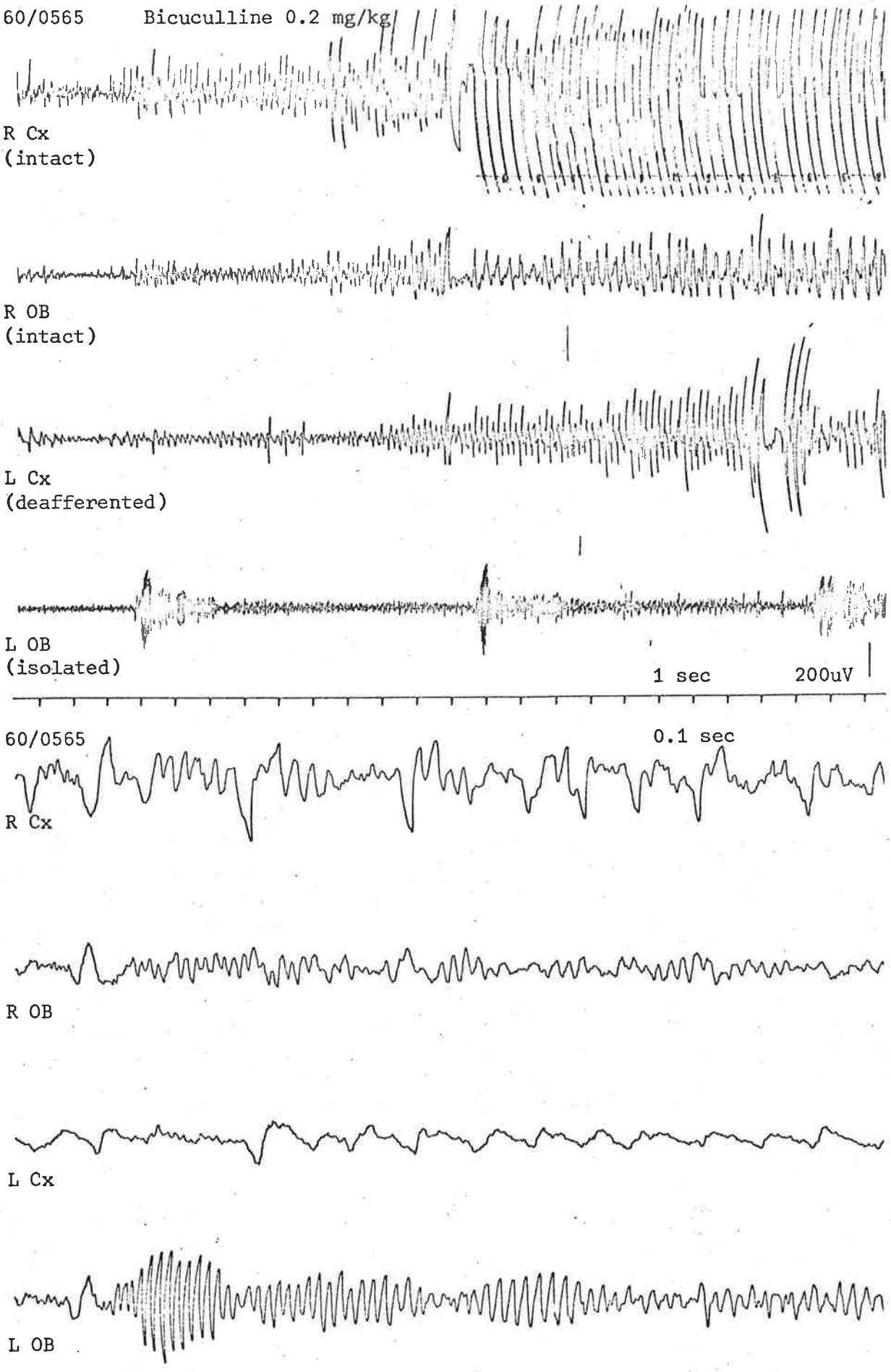


Fig. 5-5B Electrical activity and an induced wave recorded from the same sites after bicuculline, 0.2 mg/kg.

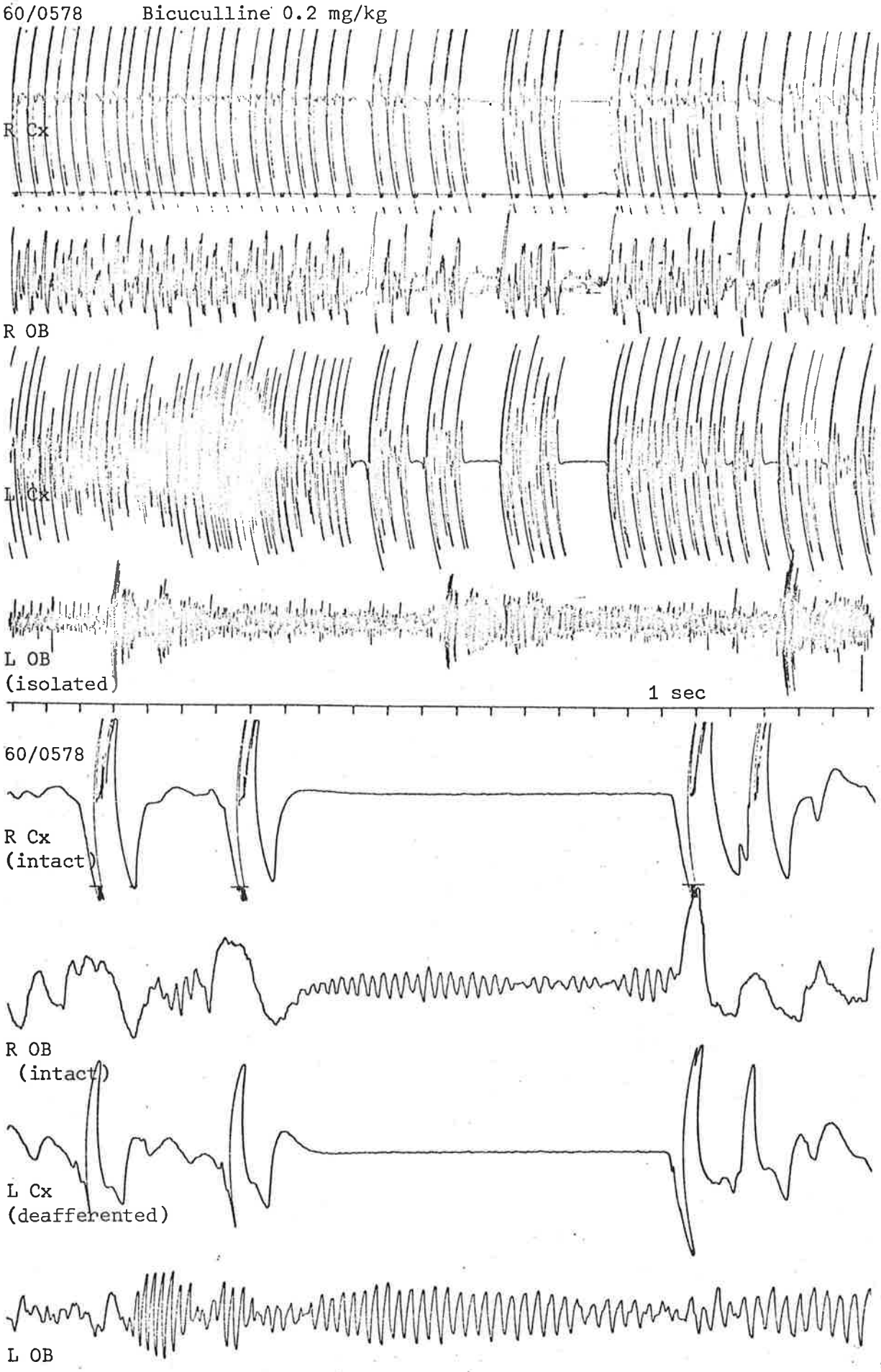


Fig. 5-5C Electrical activity 1 min after the preceding record from the same sites after bicuculline, 0.2 mg/kg.

Tetramine

Tetramine caused generalized seizure activity within 30 sec of injection of a second 0.1 mg/kg in the first two cases. In the third case, which had been pre-treated with diphenylhydantoin, 0.5 mg/kg was required to initiate seizure activity. Dimethylsulphoxide was inactive in the volumes used.

Fig. 5-6A shows the electrical activity recorded from the intact and isolated olfactory bulbs, the intact cortex and deafferented cortex. The upper part is an 0.1 speed record, 1 min after transection of the olfactory peduncle with the blunt spatula. The lower part is from the same sites half an hour later, still with no drugs. Fig. 5-6B is the record of electrical activity in intact and isolated olfactory bulbs and their respective cortices. The upper and lower records show consecutive induced waves, written out at the recording speed. Fig. 5-6C shows the first and fifth induced waves written out at 0.1 recording speed. Fig. 5-6D is the sixth induced wave, and an induced wave 10 min later, also written out at 0.1 recording speed.

Isolated bulb: induced waves

Immediately after transection of the left olfactory peduncle, the intrinsic activity of the isolated bulb became sinusoidal in nature, frequency 35 Hz (Fig. 5-6A, upper). This continued in the absence of any anaesthetic agent (apart from the local anaesthetic) for some five minutes. It gradually reverted to its usual high frequency low voltage activity. During the period of sinusoidal activity, induced waves were not seen. Within half an hour, induced waves were recorded again, and were apparently normal (Fig. 5-6A, lower). Tetramine had little effect on induced waves or intrinsic activity until the generalized seizures were well established (Fig. 5-6B, 5-6C, upper and lower). At this stage, intrinsic activity became more sinusoidal (Fig. 5-6D, upper), but the induced wave was still present. Ten minutes later (Fig. 5-6D, lower) intrinsic activity of the isolated bulb had returned to its usual appearance, but the induced wave was reduced in amplitude and duration. Despite these reductions, it contained the same frequencies as the first 300 msec of the induced wave before drugs (Fig. 5-6A, lower; by superimposition). The amplitude and duration of the induced wave in the isolated bulbs gradually returned to normal in the next half an hour (not illustrated), although the generalized seizures continued.

Intact bulb: induced waves

Induced waves were not able to be distinguished in the intact bulb during seizure activity. During this time, the intact bulb reflected the activity which was occurring in its cortex (Figs. 5-6C & D). Despite this, the intact bulb may have been active. On two of the waves during the induced wave of Fig. 5-6D (upper), an inflexion is seen, suggesting that bulbar activity may have been present, but masked. As the rate of spike activity slowed, the bulb began to produce high voltage, sinusoidal (20 - 35 Hz) activity in the interspike intervals (Fig. 5-6D, lower, continued onto Fig. 5-6E). This did not persist in either experiment; like the sinusoidal activity after bulbar transection, it lasted about 5 min. After this, in the periods of post-ictal depression, the induced waves were identical with those in the isolated bulb (see Fig. 5-3E, the same phenomenon after picrotoxin). Despite this sinusoidal activity, an induced wave was generated, and apparently terminated this sinusoidal wave (Fig. 5-6E, lower part).

Intact cortex: induced waves

Induced waves were recorded from the intact cortex until the generalized seizures began. They were then unable to be distinguished in the abnormal electrical activity. They were not able to be recorded during the periods of post-ictal depression. The duration of the tetramine seizures was not estimated: they were present for at least two hours in the first two experiments, until pentobarbitone was given.

Deafferented cortex: electrical activity

No induced activity was recorded from the cortex of the side of peduncular transection. The spontaneous activity was less than that of the intact cortex. The seizure in the experiment illustrated in Fig. 5-6B and D apparently had a different focus from the intact cortex. The spike frequency was 4-5/sec, compared with 6-7/sec of the intact side (Fig. 5-6D, upper). This difference was exaggerated later (Fig. 5-6D, lower) when the spikes in the deafferented cortex occurred at a rate of 0.5-1/sec, while those of the intact side were 3-5/sec.

Discussion

This study has shown that tetramine is a potent analeptic in the phalanger. It has some depressant effects on the induced waves of the isolated bulb. This was not expected. It also showed the ability to cause widespread seizures with apparently different sites of origin. This is not common in convulsants, and was only seen with tetramine in this study. The other analeptics had only caused synchronous

convulsions.

It is clear that tetramine should be studied further, not only in the olfactory system, but as a general analeptic.

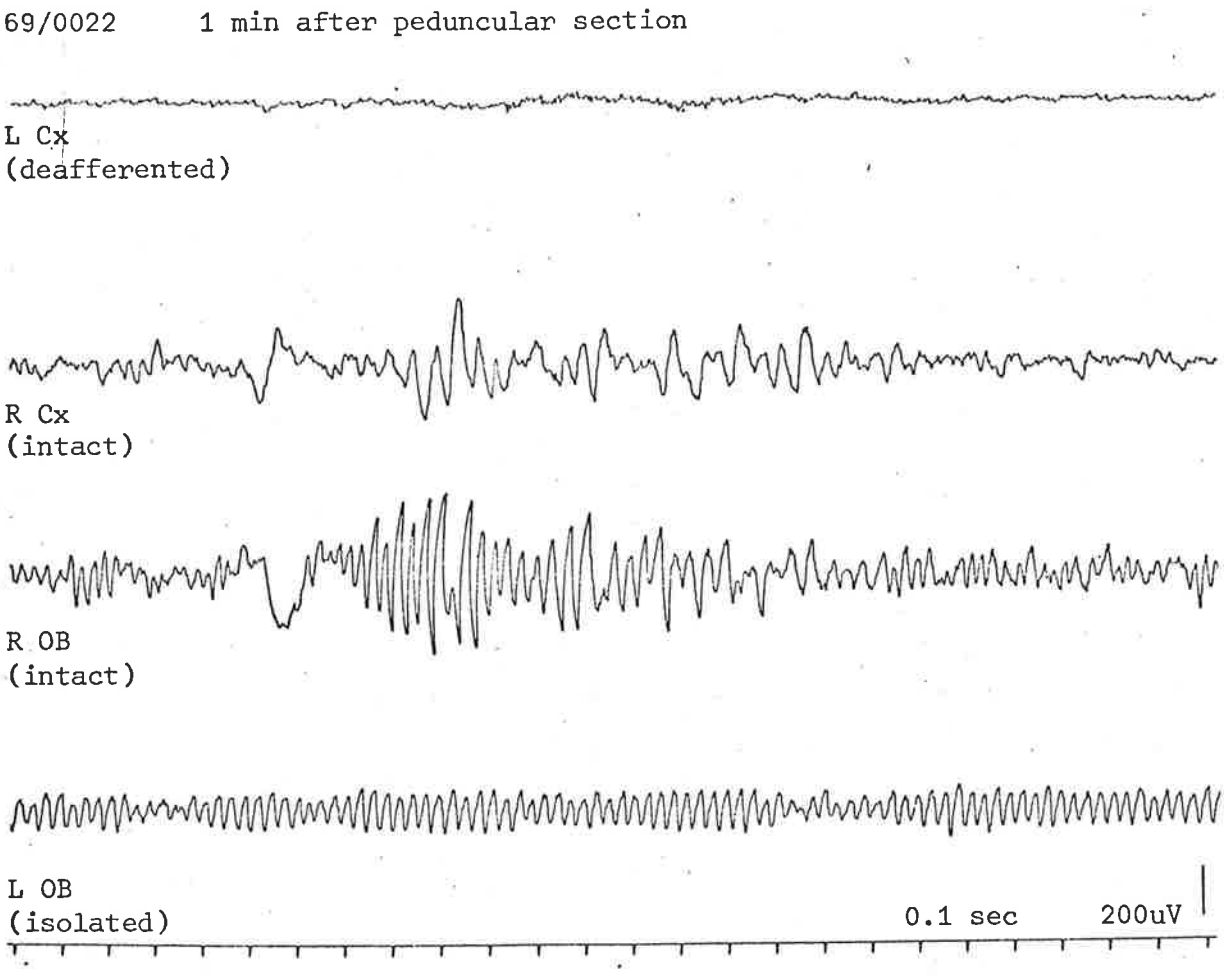


Fig. 5-6A Records from intact and isolated olfactory bulbs before tetramine, after peduncular transection.

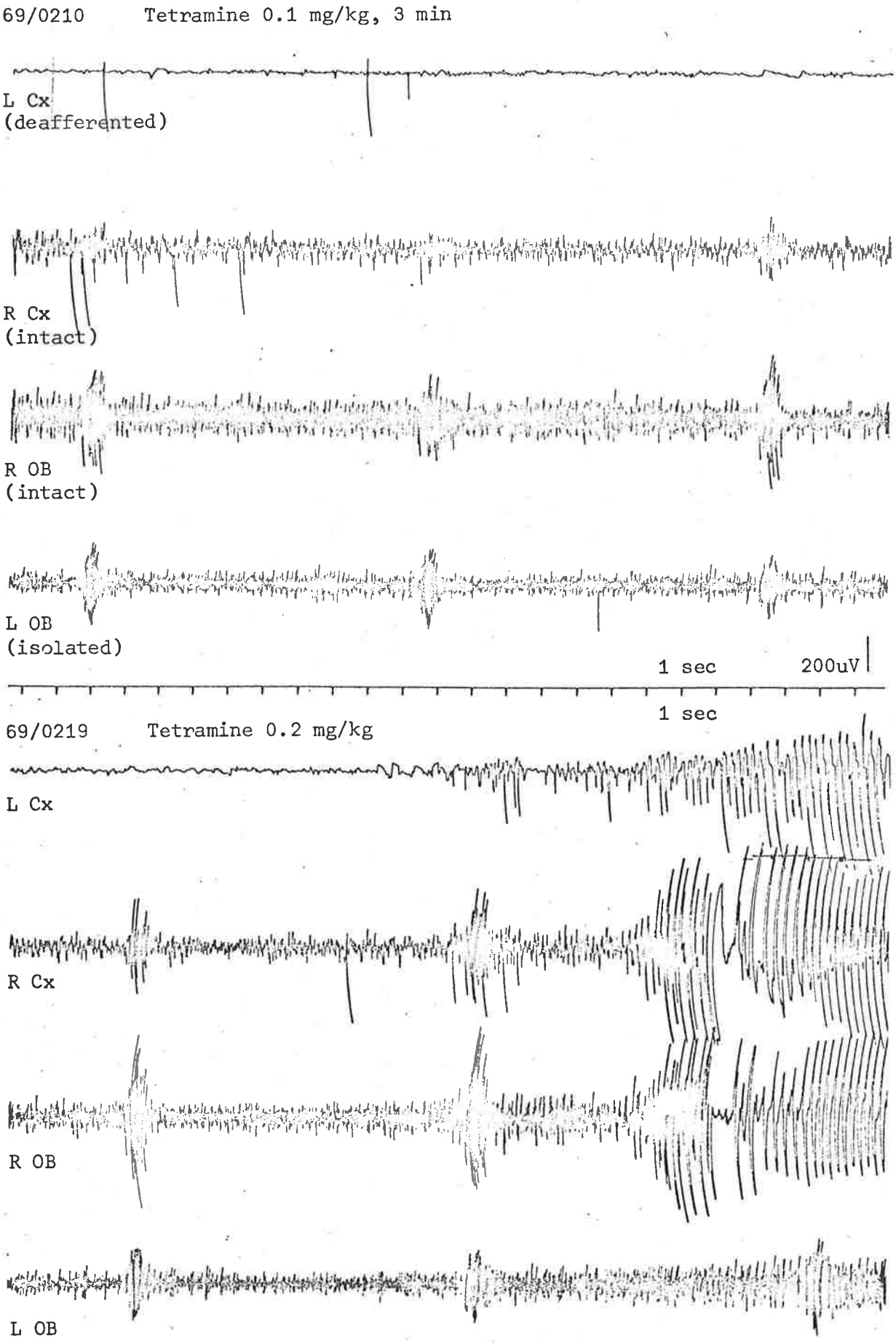


Fig. 5-6B Record of electrical activity in the four sites of the immobilized phalanger, after tetramine 0.1 mg/kg (upper) and an additional 0.1 mg/kg (between the records) had been given.

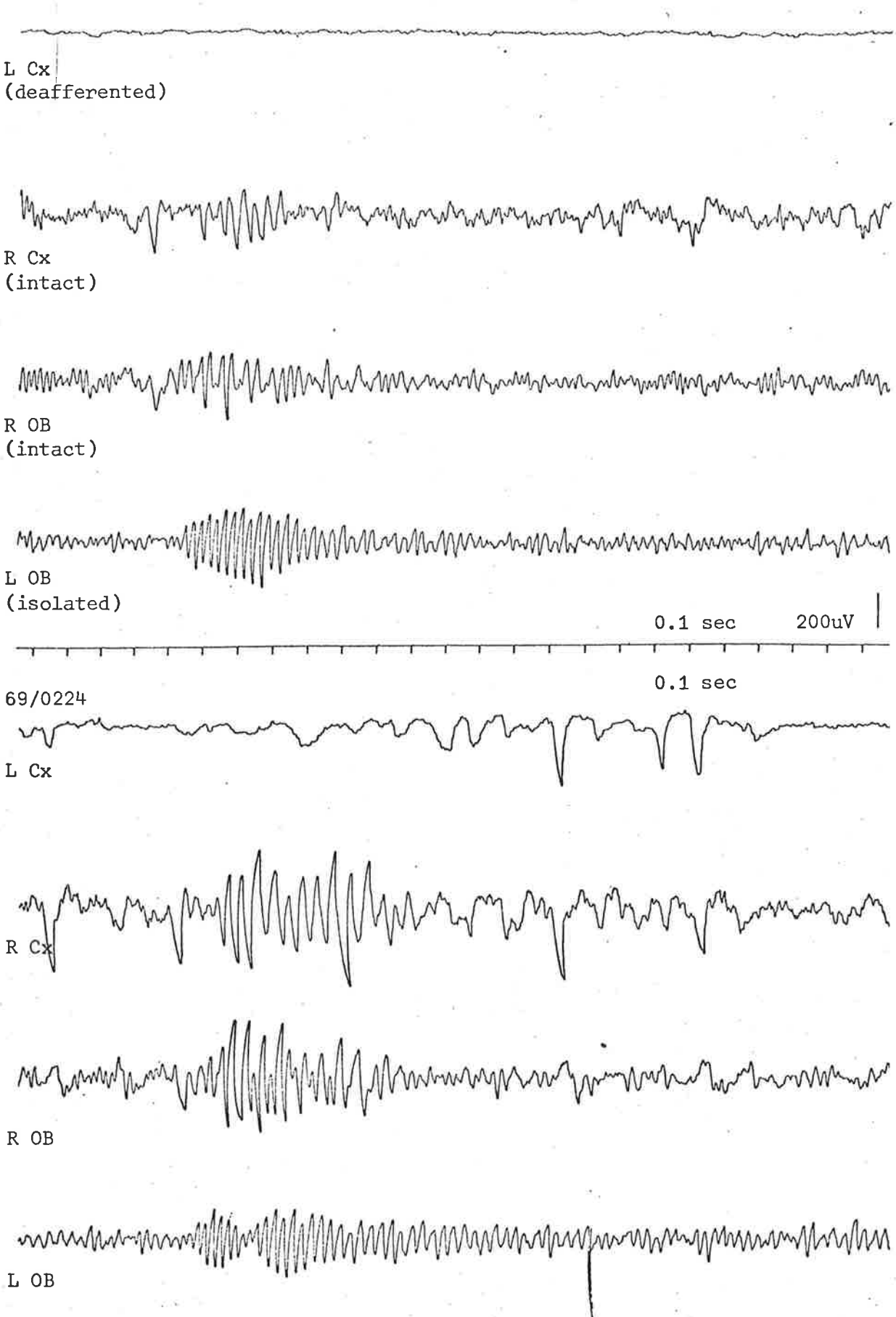


Fig. 5-6C Upper. The first induced wave of the preceding figure at 0.1 recording speed, after tetramine 0.1 mg/kg
Lower. The second induced wave of the preceding figure under the same conditions.

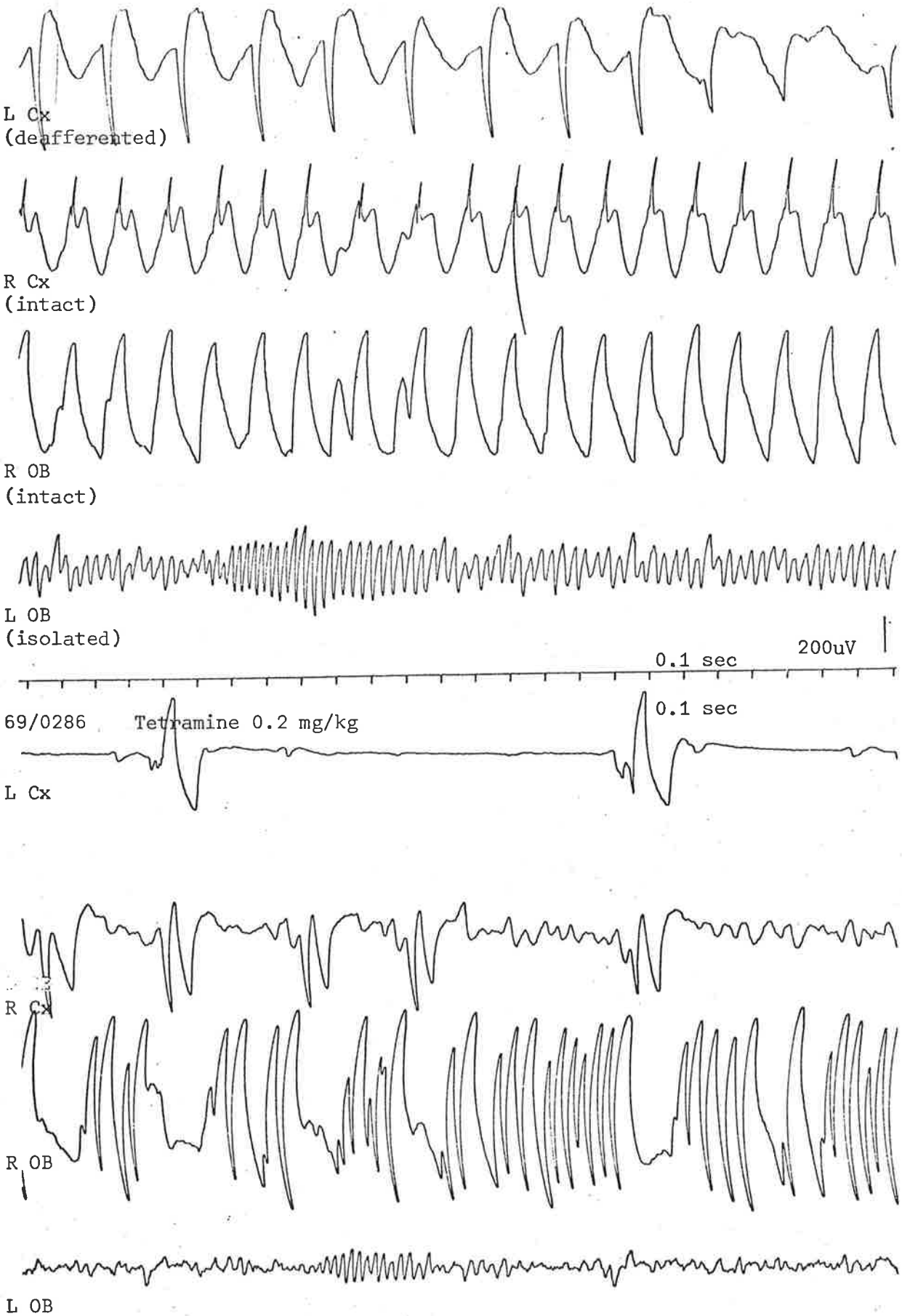


Fig. 5-6D Upper. The last induced wave of Fig. 5-6A at 0.1 recording speed after tetramine, 0.2 mg/kg.
Lower. Induced activity 10 min later, from the same four sites.

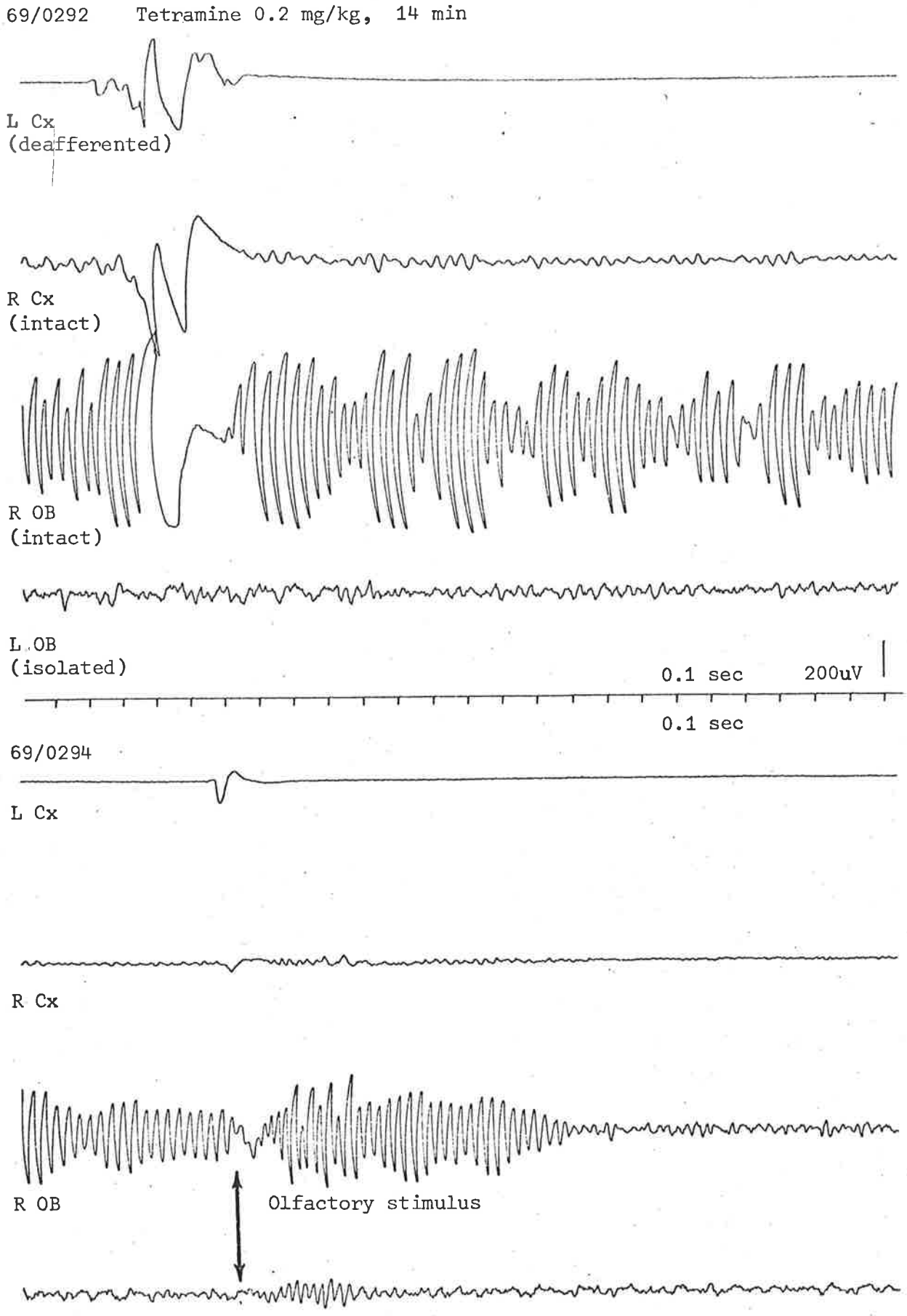


Fig. 5-6E Continuation of the lower part of the preceding figure under the same conditions (upper and lower parts of this are continuous) 14 min after tetramine, 0.2 mg/kg, during the period of post-ictal depression. The usual olfactory stimulus was applied where indicated by the arrow.

DISCUSSION

The experiments reported in this thesis have shown that the marsupial nervous system is sensitive to known analeptics in their usual doses. They also demonstrated the relative insensitivity of the isolated olfactory bulb to systemic convulsants. Despite doses which caused generalized seizures, induced waves were still able to be recorded. This observation has not been made previously; other workers have reported "seizures" in the isolated bulb.

Abnormal activity in the isolated bulb was not common, and consisted of the sinusoidal activity illustrated in Fig. 5-6A (upper) and 5-6D (upper). It was not possible to predict the occurrence of such activity, which was not related to a particular analeptic or anaesthetic agent. On one occasion, such activity was seen during electrically-induced seizures. This is illustrated in Fig. 5-7A & B. In this experiment, the animal had been prepared in the usual way, and repetitive (5/sec) trains (5 stimuli, 0.5 msec squarewaves 5 msec apart) of stimuli were applied to one of the cortical electrodes until self-sustaining seizure activity occurred. It was noticed that the intact bulbs reflected the cortical seizure activity. During the periods of inter-ictal cortical depression, both bulbs were seen to produce sinusoidal electrical activity. Such sinusoidal activity was inhibited for 200 msec or so by the positive waves of the seizure. The segment illustrated in Fig. 5-7B persisted for some 30 sec, when it resumed its usual appearance as the cortical activity recovered.

The significance of this sinusoidal activity is not clear. It might represent uninhibited mitral cell activity, but much more work, including unit studies, will be required. This will have to be done on intact and isolated bulbs, with and without drugs.

Freeman (1960*b*) has recorded sinusoidal activity in the olfactory cortex after electrical stimulation of the bulb. This activity resembled induced waves, and did not persist. He did not report generalized seizure activity.

It is not possible to deduce the fundamental action of convulsants from these experiments. Bicuculline and picrotoxin have been shown by other workers to be anti-GABA in some brain systems. Strychnine likewise has been shown to be a glycine antagonist. The basic actions of lignocaine and tetramine have not been determined. It is also not possible to deduce from their conventional structures (Fig. 5-8) a common active part of each molecule. All these drugs produced similar

63/0320 Electrically-induced seizure : no drug

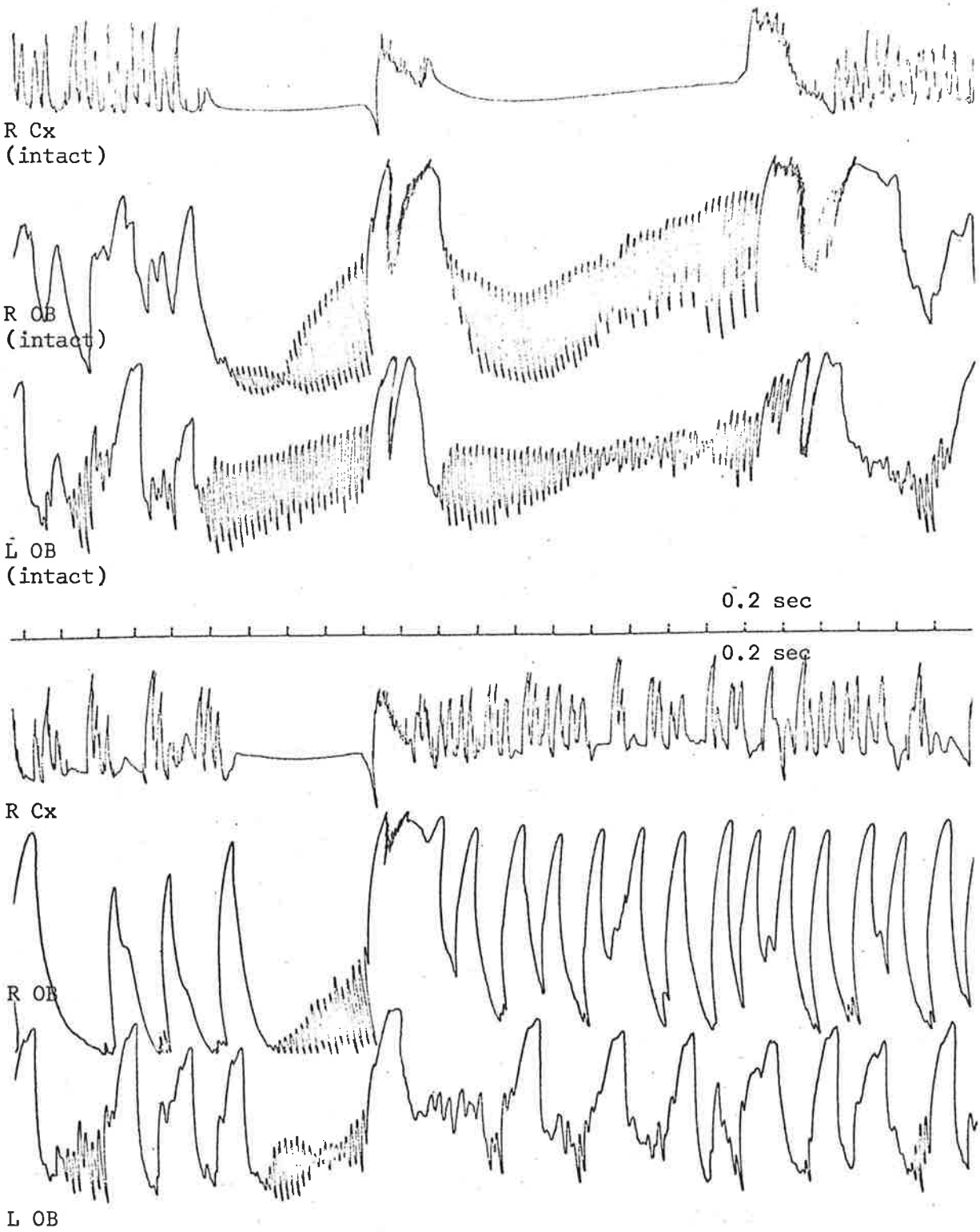


Fig. 5-7A Seizure activity recorded in the right olfactory cortex and both bulbs after electrical stimulation of the left olfactory cortex. There is sinusoidal activity in the bulbs during the periods of post-ictal cortical depression. (All sites are intact). The record is continued on the next page.

63/0330 Electrically-induced seizure : no drug

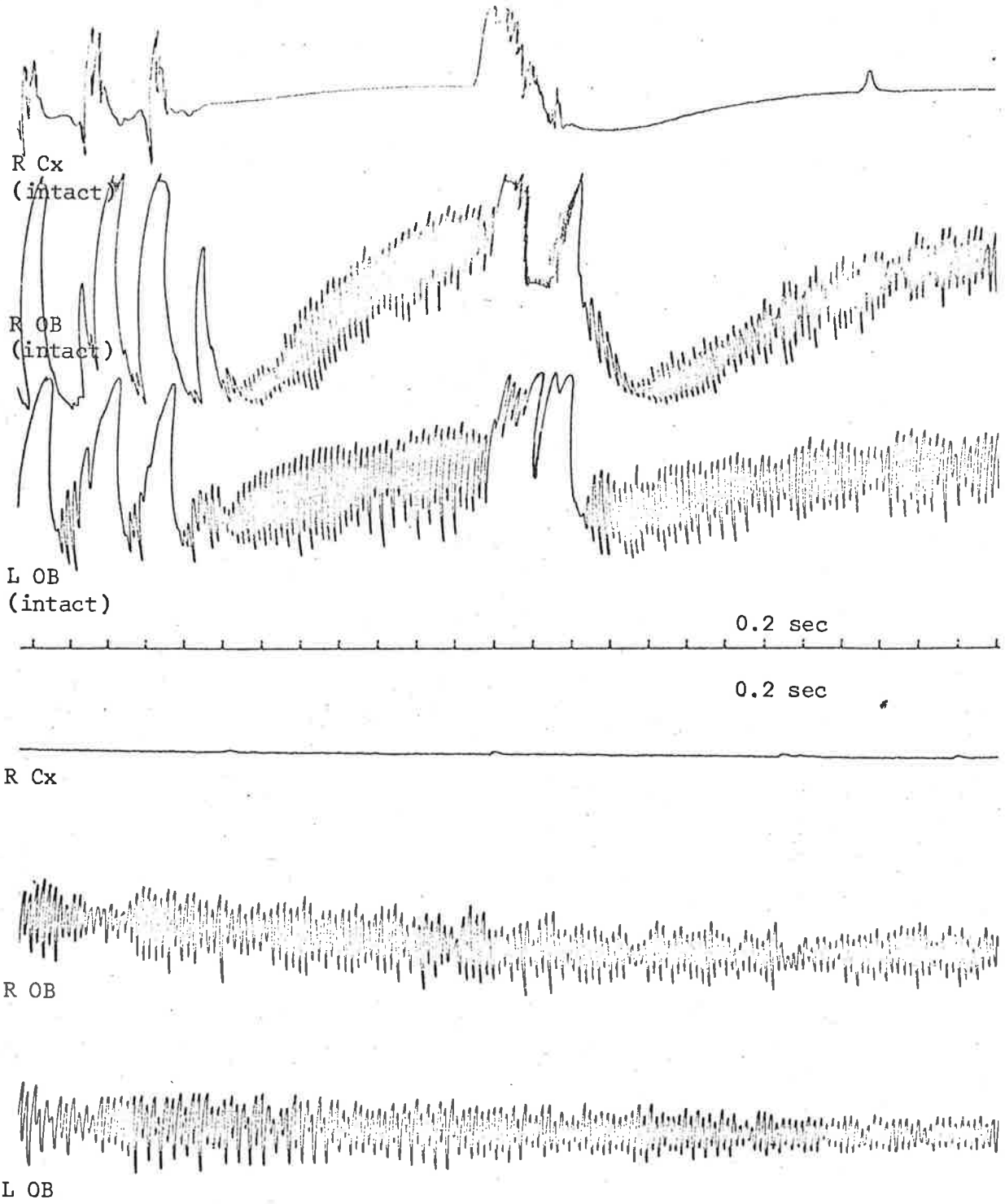


Fig. 5-7B Continuation of the preceding figure, showing electrically-induced seizures in the brain of the phalanger which was immobilized and without drugs other than the local anaesthetics.

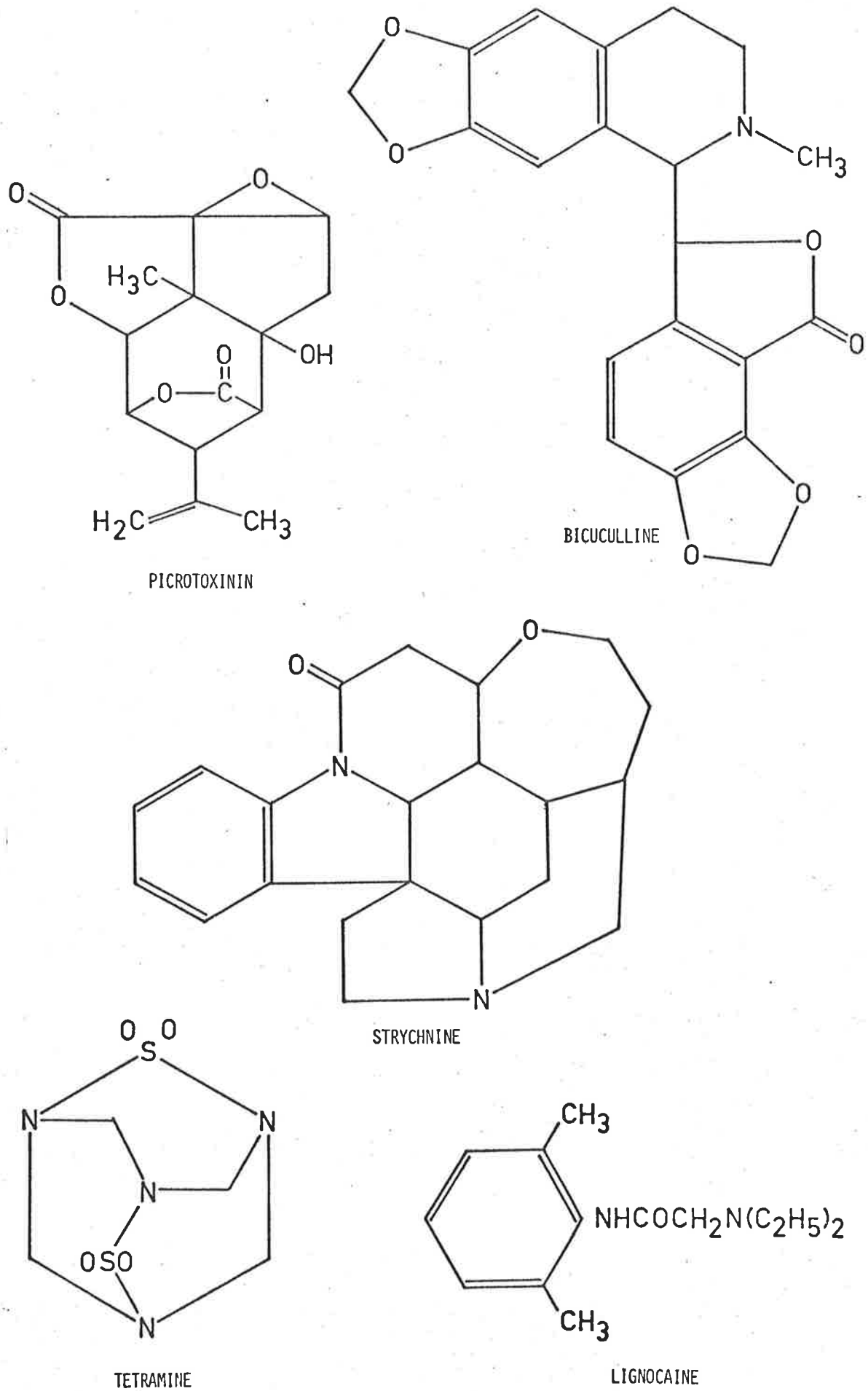


Fig. 5-8. Chemical structures of the analeptic agents used in this study.

changes in the phalanger.

Banerjee *et al.* (1970) postulated a common mechanism of action of tubocurarine, metrazole, strychnine and picrotoxin, namely disinhibition. They did not involve any possible common inhibitory transmitter.

Structure-action relationships of drugs can be used to imply the likely active site. The best-documented system is the neuromuscular junction. It may be possible to predict whether a substance is likely to stimulate or block the acetylcholine receptor. It is more difficult to apply this approach in the central nervous system with its increased complexity. However, it is known that some drugs antagonize the putative inhibitory transmitters, GABA and glycine. It is difficult to see the active site, for example, on the tetramine molecule. However, attempts are made to determine the active site by chemical manipulation of the molecule. For example, Johnston & Curtis (1972) have attempted to show structural similarities between bicuculline and other molecules.

The barbiturates comprise another group of drugs which have been structurally manipulated to produce either central nervous system depressants or excitants. Manipulation of the groups attached to the barbiturate nucleus, particularly if a C=C is close to C5, converts a depressant into an excitant. This group of convulsant barbiturates has not been extensively studied. (For a review see Prince, 1972.) Swanson (1934) reported the first such study. Six were described by Leonard & Harrison (1953), and Crawford (1969) described effects of two on single feline cortical neurones. Mechanisms of action were not known, but Crawford suggested that an explanation could be that they competed for muscarinic sites.

In these studies on the phalanger, it was noted in some cases that pentobarbitone caused irregular spiking activity in the cortical records. These did not become frank convulsions, but persisted as the dose was increased. It was assumed that such a response was peculiar to a particular animal, but its blood gas or pH status were not known.

Despite this observation, pentobarbitone was an effective anticonvulsant. Dose-response relationships were not constructed because of the small numbers of animals. The anticonvulsant dose was of the same order as in other species, 10 - 30 mg/kg. Duration of anticonvulsant activity was 1 - 2 hours in the case of tetramine, which is metabolized slowly (25% LD_{50} per day, Haskell & Voss, 1957).

Diphenylhydantoin (Dilantin, Parke-Davis) was shown in one case not to be an effective anticonvulsant at a dose of 80 mg/kg in tetramine

convulsions. Half an hour was allowed for an effect to be observed. After this, pentobarbitone 10 mg/kg was given, and the convulsion finished within one minute. This single observation suggests that there may be a species difference in diphenylhydantoin metabolism.

Lignocaine is different from the other convulsants, having an anti-convulsant effect at low levels. Such an action has not been described for the other analeptics used in these studies. It was not able to be seen because, apart from lignocaine, all were given by bolus injection of doses near to the convulsant dose. Lignocaine also produced a different type of seizure after gradually increasing frequency and amplitude of the intrinsic activity. Spike-and-wave seizures were not prominent, but post-ictal depression did occur.

During the periods of post-ictal depression, the intact and isolated bulbs both generated similar induced waves. This showed that the cortex modifies the bulbar wave, and also that the isolated bulb was not intrinsically damaged. Presence of induced waves during the post-ictal phase have been noted by Ueki, Tanaka & Sugano (1964; their p. 91, after chlorpromazine), and Ueki & Fukuda (1966; their p. 913). No comment is made in other reports about this phenomenon, which may be an area of fruitful research.

CONCLUSIONS

The analeptic agents strychnine, picrotoxin, bicuculline and tetramine have been shown to produce similar generalized electrical seizures in the phalanger. They have also been shown to have minimal effect on the intrinsic or induced activity of the isolated bulb.

Lignocaine in small doses enhanced the induced activity in some cases. It reduced the frequencies within the bursts of the isolated bulb. Large doses (15 mg/kg) caused generalized seizure activity, with some depression of the isolated bulbar induced wave.

Sinusoidal activity in intact and isolated bulbs was recorded in a few cases: after peduncular transection, after tetramine, and after electrically-induced seizures. The cause or significance of this activity is not known.

No simple structure-action relationships between the analeptics have been defined.

OVERVIEW

This final chapter is intended to summarize the findings of this thesis, and to point out deficiencies in knowledge. It is also intended to suggest possible areas of fruitful research in the light of the results presented here. Discussion will be carried out under the broad headings of neuroanatomy, evoked potentials, induced waves, anaesthetics and analeptics.

Neuroanatomy

The olfactory system of the phalanger has been shown in this thesis to have the same general arrangement as the olfactory system in other species and other marsupials.

Olfactory bulb

The olfactory bulb was shown to have the same lamination as in other species. There was a minor variation noted: tufted cells were more numerous in the outer half of the external plexiform layer (Fig. 1-3). There have been no quantitative studies of the components of the olfactory bulb of the phalanger. Switzer (1972) has reported such a study in the marsupial family *Dasyuridae*. His abstract did not comment on the distribution of cell types within the bulb. Marsupials also afford an opportunity to study the development of the components of the bulb. If structural studies were to be combined with functional ones (evoked potentials or induced activity), insight might be gained into bulb function. Such experiments would be fraught with technical difficulties. Developmental studies have been carried out in opossum (Morest, 1970; Ulinski, 1971), and mice, for example, by Kim (1972) and Hinds & Ruffett (1972, 1973); similar ones could be designed for the phalanger.

Histochemical studies of the phalanger bulb may also provide information about transmitter substances within that structure. It was noted in sections of bulb which I stained for cholinesterase, that the accessory bulb was deeply stained, but the main bulb only lightly. Such a difference was not noted by Shute & Lewis (1967); the significance of this observation is not known.

Degeneration within the bulb following peripheral deafferentation has been used to assess connexions within the bulb (Estable-Puig & de Estable, 1969; Berger, 1973). Ionizing radiation to the bulb also damages some of its components (de Estable Puig & Estable Puig, 1971). Specific volatile compounds inhaled may also cause selective damage in the bulb. Døving & Pinching (1973) have described regional changes in

the bulbs of rats after chronic (2 weeks to 11 months) exposure to cyclo-octanone. Such experiments could be combined with electrophysiological studies of field, induced and evoked potentials.

Formal ultrastructural studies have not been carried out. There was nothing to suggest that the marsupial olfactory bulb is different from that in other species in the two specimens examined for me by Dr Tulsi.

Anterior olfactory nucleus

Although the anterior olfactory nucleus was first described in opossum (Herrick, 1924), it has not been compared with that in the phalanger. There was a difference noted in the phalanger: pars externa apparently has two divisions (Fig. 1-5). The other parts have a similar general arrangement to opossum. This structure and its connexions require a formal comparative histological study.

Olfactory tubercle

The olfactory tubercle of the phalanger has been shown by light microscopy to have the same general structure as it has in other species. Its connexions in the phalanger have not been fully determined. It was seen to stain heavily for cholinesterase during this study. No other histochemical methods were used, but it would be desirable to examine it for the presence of other putative transmitters. The possibility of a geographical projection of bulb to tubercle has not been excluded by this work.

Olfactory cortex

Primary olfactory cortex is defined as the cortical areas which receive mitral cell axons. Adey (1953) made the simplest possible division of the rhinencephalon of the phalanger. His anterior (prepyriform) area had evidence of degenerating mitral cell axons after bulbar ablation. His posterior (entorhinal) area did not.

This study has shown that the primary olfactory cortex could be further divided into two areas on histological grounds. The anterior part has a thick layer of pyramidal cells, while the posterior part has only a single layer of such cells (Figs. 2-3 and 2-4). Fibre staining of such sections showed that the posterior extent of primary olfactory cortex was approximately marked by the radial arrangement of fine fibres in the angular (entorhinal) cortex. The lamina dissecans was present only beneath primary olfactory cortex. This description has defined its extent more precisely than before. It would now be valuable to make comparative studies, with or without histochemistry (for example,

with those of Geneser-Jensen & Blackstad, 1971).

Subcortical areas

Other areas of the olfactory system need further examination. The nucleus of the diagonal band is not prominent in the phalanger, and its connexions have not been described. Likewise, the nucleus of the lateral olfactory tract is not prominent in the phalanger. The amygdaloid complex and its connexions have not been adequately described in the phalanger.

Connexions of the olfactory system

No degeneration, histochemical or autoradiographic studies have been made in the phalanger in this series. Evidence has been presented in other species for connexions with the lateral hypothalamus (Scott & Pfaffman, 1967; Scott & Leonard, 1971; Scott, 1973) and through the habenular nuclei (Rausch & Long, 1971). The connexions of the stria terminalis have been re-evaluated recently (De Olmos & Ingram, 1972), as have those of the medial forebrain bundle (Morgane & Stern, 1972; histofluorescence and lesions). Other techniques to be used to study the olfactory system could include autoradiography following labelled amino acid injection (for example, see Krettek & Price, 1974), or horseradish peroxidase histochemistry (for example, see Lynch, Gall, Mensah & Cotman, 1974).

The anterior commissure of the phalanger (Figs. 1-6 and 1-7) needs to be compared with that of other marsupials, including opossum.

Stereotaxic atlas

The stereotaxic atlas produced for the present experiments was adequate for the purposes. It would not be profitable to publish it, because Warner's (1971) has already been accepted for publication. It will be necessary to apply the appropriate corrections to mine, bearing in mind the different ear bars. Warner's atlas was more concerned with diencephalon than other areas; mine was concerned with olfactory areas. For example, he dismissed the amygdaloid complex by labelling it "A", and did not comment on divisions of olfactory cortex or anterior olfactory nucleus.

Evoked potentials

Electrical potentials were evoked in the granule cell layer of the olfactory bulbs by electrical stimulation of more central structures. Positive potentials were the result of stimulation of mitral cell axons. Negative potentials were the result of stimulation of inhibitory efferent fibres. Mapping of the extent of mitral cell projection was carried out

by this method. The mitral cells projected to primary olfactory cortex (by definition), olfactory tubercle, nucleus of the lateral olfactory tract, and cortical and medial amygdaloid nuclei. Negative potentials were evoked in the bulb by stimulation of the primary olfactory cortex and underlying areas, anterior olfactory nucleus (caudal parts; the anterior areas were inaccessible to the exploring electrode used in these studies), olfactory tubercle, nucleus of the lateral olfactory tract, cortical and medial amygdaloid nuclei, and parts of the hippocampus.

These connexions are similar to those previously reported in the cat, guinea pig, rat and rabbit from this laboratory.

The question of a topographic projection of the bulb to lateral olfactory tract (suggested by Shepherd & Haberly, 1970), tubercle (Heimer, 1968), or olfactory cortex (no previous suggestion) has not been clarified. All records in this thesis were taken from the granule cell layer of the dorsal part of the bulb. It may be necessary to record from the ventral, and possibly lateral parts also. Polson (1971), however, showed spherical field symmetry of the bulb after stimulation of "prepyriform" and "periamygdaloid" cortex.

Accessory bulbar potentials were not examined in this thesis. It would require a similar set of experiments, recording from the accessory bulb, to determine its connexions. Field studies may be required to determine the accessory bulb's contribution to evoked bulbar potentials.

Inhibition of mitral cells by central stimulation can be studied by using pairs of electrical stimuli, a conditioning and a test stimulus. These can be applied through the same electrode, or through separate electrodes, a variable time apart. The duration and degree of inhibition caused by the conditioning stimulus can then be measured. The conditioning stimulus can be applied to any negative-producing area; the test stimulus to any part of the mitral cell projection. Such an experiment would give an indication of the nature and extent of the centrifugal systems. Polson (1971) examined the effects of anterior commissural and olfactory cortical conditioning stimulation on lateral olfactory tract stimulation. This experiment could also include conditioning stimuli applied to amygdala, anterior olfactory nucleus, olfactory tubercle, the Dennis & Kerr system and the Price & Powell diagonal band system.

Conditioning-test stimulus pairs can also be used to assess drug effects on the evoked potentials. Nicoll (1972*b*) has shown that it is

possible to measure the duration of mitral cell inhibition, before and after the drug in question, with this evoked potential method. Some of the experiments reported in this thesis have shown profound changes in evoked potentials during anaesthesia.

Evoked potentials should also be studied during the phase of post-ictal cortical depression. During this, intact bulbar induced bursts have the same appearance as those recorded from the isolated bulb. This implies that there is no inhibitory efferent activity. It would be possible to determine which component, if any, of the evoked response is depressed or obliterated.

The experiments reported here have relied on recording the responses in the bulbs only. If other recording sites were used simultaneously (e.g. stimulate the tract, and record in bulb, cortex, anterior commissure and amygdala), it might be possible to determine the sequence of excitation or inhibition, and thereby trace functional pathways.

Central connexions of the olfactory system have not been completely determined. Guevara-Aquilar *et al.* (1973) showed connexions between olfactory and respiratory areas of cat brain using evoked potentials. Motokizawa (1974) has shown these to be olfactory input to the thalamus, possibly *via* the cortical or medial amygdaloid nuclei. (Such connexions had been shown previously by Berry, Hagamen & Hinsey, 1952.) Further studies with evoked potentials will doubtless clarify connexions of and within the olfactory system.

Induced waves

Induced waves were recorded from the olfactory bulbs and cortices of immobilized phalangers. A standard olfactory stimulus was used. Although this stimulus did not resemble the natural olfactory stimuli in duration, air flow or repetition rate, it caused similar induced waves in different phalangers. These induced waves were also similar to those recorded from other marsupials and mammals in similar experiments. The bulbar waves in the phalanger began at a frequency of 50-60 Hz, had a duration of about 1 sec, and ended at a frequency of 20-30 Hz with the repetition rates used here (1 stimulus/5-10 sec). Similar waves were also able to be recorded from bulbs severed from their central connexions, implying that mechanisms intrinsic to the bulb controlled the duration and frequencies within each burst.

Induced waves were recorded also from olfactory cortex. These were characterized by a similar duration, but the predominant frequencies were approximately half those of the bulb. The lower cortical frequency

was imposed on the bulbar induced wave in the intact system, but not on the isolated bulb. The pathway for, or significance of this efferent activity was not able to be determined by this study.

The first olfactory stimulus to a single nostril after a quiescent period often induced an unusually prolonged burst in the stimulated bulb (an "alerting" response). A low-frequency burst could be recorded from the unstimulated bulb for the first few stimuli: this burst gradually disappeared. Such a phenomenon has not been described before. It is well-known that olfactory stimuli cause arousal (Arduini & Moruzzi, 1953; Yamamoto & Iwama, 1961). It has also been shown that arousal alters recorded olfactory activity (Komisaruk & Beyer, 1972). None of these papers illustrated the changes described in this thesis, the pathways and significance of which await clarification.

The effects of olfactory peduncular section on the function of the remaining parts of the system are not clear from the present study.

Affanni, Morita & Garcia Samartino (1968) described changes after peduncular section in the then isolated bulb of opossum, but not in other parts of the brain, or upon behaviour. Hara & Myers (1973) have recently described behavioural changes in opossum after bilateral olfactory bulbar ablation (*inter alii*), which increased "open mouth threat". (They noted that bilateral removal of amygdala or hippocampus reduced such threat. A similar effect in the phalanger was achieved by removal of entorhinal area, with no damage to hippocampus or amygdala, by Adey, Merrillees & Sunderland, 1956.) Another study on opossum behaviour (Berquist, 1970) has implicated the medial forebrain bundle as a component important in motivated behaviour.

The problems of odour coding have not been considered in the present study, but such experiments have been reported previously in opossum by Phillips & Michels (1964). These workers noted differences in activity of anterior and posterior parts of the bulbs with different odour stimulation. It is premature to speculate whether this observation and that of Døving & Pinching (1973) are related.

Microelectrode recordings were not made during this study, and it may be necessary to combine the macro- and microelectrode studies to determine the sequence of excitation of bulb and cortex. A bulbar signal is required to trigger the cortical burst, which is apparently fed back onto the bulbar response. The efferent pathways for this are not known. Selective ablation studies may be required to determine

these, but on the present evidence the system most likely to be implicated would seem to be that of Dennis & Kerr.

Anaesthetics

Induced waves and evoked potentials were modified by various central nervous system depressants.

Nitrous oxide increased background activity and reduced the duration of the induced bursts. It produced minimal changes in the electrical potentials evoked in the bulb by olfactory cortical stimulation. The mechanisms producing the presumed non-specific depression of excitatory and inhibitory influences were unable to be determined by the present study.

Induced waves were unable to be recorded after chloroform had been used as the initial general anaesthetic, presumably due to an action of the olfactory mucosa. It had been shown previously that chloroform interfered with the ciliary action of the olfactory epithelium (Ai & Takagi, 1963). The present study showed central effects in addition to peripheral ones. Chloroform depressed the cortical induced waves before the bulbar ones during induction. The cortical bursts, however, returned before the bulbar ones during recovery from chloroform.

Halothane also depressed induced waves and caused cortical spike activity. The high frequency bulbar burst was apparently depressed earlier by halothane than by chloroform, comparing it with the olfactory cortical burst. At low (2 mg/kg) levels, barbiturates (pentobarbitone and thiopentone) caused an increase in the amplitude and duration of the induced waves recorded in the intact bulb and cortex. This increase was of the low frequency (cortical) component. The isolated bulb, however, was depressed. Higher doses of barbiturate eventually caused generalized depression of brain activity, sometimes with transient spike activity, in all sites but the isolated bulb. Evoked potentials were altered by these drugs, with increase in the duration of inhibition of mitral cells by a conditioning electrical stimulus to LOT. But the question of whether barbiturates are GABA agonists was unable to be clarified.

The study showed that barbiturates are unsuitable anaesthetics for olfactory physiology.

Propanidid had similar effects to pentobarbitone at low and high doses. From the structure-action point of view, a common active site was not apparent either in the conventional structure or Catalin models of the molecules, and the basis of the similarity of action is

undetermined. Similar experiments could be performed with propanidid as with the barbiturates, but with the added difficulty imposed by its evanescent action.

Diazepam in small doses caused an initial increase in the cortical component of the induced waves. Unlike pentobarbitone, there were minimal changes in the conditioning-test evoked responses at doses of diazepam which abolished the induced bursts (3 mg diazepam/kg). This suggests that diazepam has a different mechanism of action to the barbiturates, but offers little to the elucidation of the mechanism.

Analeptics

Various analeptic agents were used to modify induced olfactory activity. The common finding was that these drugs had minimal effect on the isolated bulb, despite their marked effect on the rest of the brain.

During seizure activity, no induced waves were recorded from the intact sites. In the periods of post-ictal cortical depression, however, induced waves in the intact bulb were similar to those in the isolated bulb. This implied that all efferent activity to the bulb had been removed. It also implied that the convulsants used, including bicuculline (a GABA antagonist), did not alter the intrinsic mechanisms of the bulb, which was surprising in view of the implication of GABA in bulbar dendro-dendritic inhibition.

Another significant observation was that the isolated bulb did not generate spike-and-wave activity at the drug levels used in this study. It was noted on some occasions, however, that both isolated and intact bulbs could generate "sinusoidal activity" in the absence of olfactory stimuli. Such activity was not related to the analeptic agent, having also been recorded after an electrically-induced seizure. Its mode of generation and significance are unknown.

It would be fruitful to study evoked responses within the olfactory system during the periods of post-ictal cortical depression. It could be assumed that efferent activity would be minimal during these periods. Discrete stimulation of different areas, with interaction, might reveal their function more clearly.

No simple structure-action relationships were noted among the analeptics. Such studies as these are not sensitive or specific enough to uncover similarities, common final results being expressed for activation of various possible sites more centrally.

Note added in proof :

A recent review of the convulsants and their postulated modes of action places emphasis on anti-GABA and anti-glycine activity. Molecular models have been used to show interaction of the analeptics with receptor sites. Particularly interesting is the model showing that tetramine is anti-GABA (his Fig. 8a & b).

Smythies, J.R. (1974)
Relationships between the chemical structure and biological activity of convulsants.
Pharmacol. Rev. 14:9-21

APPENDIX

Data collection and analysis

Records obtained during the studies reported here were all assessed visually in the first instance. In some cases, a crude zero-crossing analysis was carried out to estimate major frequency components. It was decided to undertake numerical analysis of some induced waves to attempt to describe them more fully, and uncover relationships between the bulbar and cortical components.

Spectral analysis had been used by Polson (1971) and Coutts (1972) in analysis of olfactory induced waves. They had different approaches to the problem of the window function, however.

Periodicities in sets of data may be uncovered by estimating energy density (power) with respect to frequency. An assumption which must be made is that the signal being analyzed is stationary; its characteristics do not change with time. It is clear, however, that induced waves (and other EEG signals) vary with time. A common approach to this problem is to perform conventional spectral analysis over consecutive discrete time segments (Walter, 1963; Priestly, 1967). Power spectrum analyses have been discussed with reference to EEG data by such workers as Dumermuth, Huber, Kleiner & Gasser (1970); Kleiner, Flühler, Huber & Dumermuth (1970); Dumermuth (1971); Cox, Nolle & Arthur (1972); and Kawabata (1973).

The fast Fourier transform requires 2^n data points to operate upon. There follows a description of the method which had to be used in this study to achieve this.

Typical induced waves were selected by eye from the polygraph record, the magnetic tape replaying at 0.1 recording speed. Analogue-to-digital conversion was carried out also at 0.1 real time by the Computer of Average Transients (Mnemetron CAT 400-C). With tape and polygraph running, the CAT was manually triggered at an appropriate part of the induced wave. Induced waves from a single channel were stored for 16 sec (1.6 sec real time). If two channels were to be digitized simultaneously, CAT sweep speed was set at 8 sec (0.8 sec real time), each channel being stored in 200 locations. Each method gave the same sampling rate, 250/sec (Nyquist frequency 125 Hz).

The store of the CAT was then plotted on the X-Y plotter, as a visual check on the data actually digitized. The store was printed out on the CAT line printer if it was the data intended. A suitably-

interfaced paper-tape punch was available, and the CAT store, suitably identified, was also read onto paper tape.

These data were to be manipulated by the University of Adelaide CDC-6400 computer. However, its paper-tape reader had been salvaged from the depths of the Electrical Engineering Department, and was subject to mechanical idiosyncrasies. It had an intermittent fault which caused it to mutilate the paper tape passing through it. It was also able to insert sporadic zeros into the data, with resulting parity chaos.

Because of the potential unreliability of this method, the values recorded by the line printer were manually transferred (by me) to 80-column cards. The data were then checked for errors by printing and plotting the values on the cards.

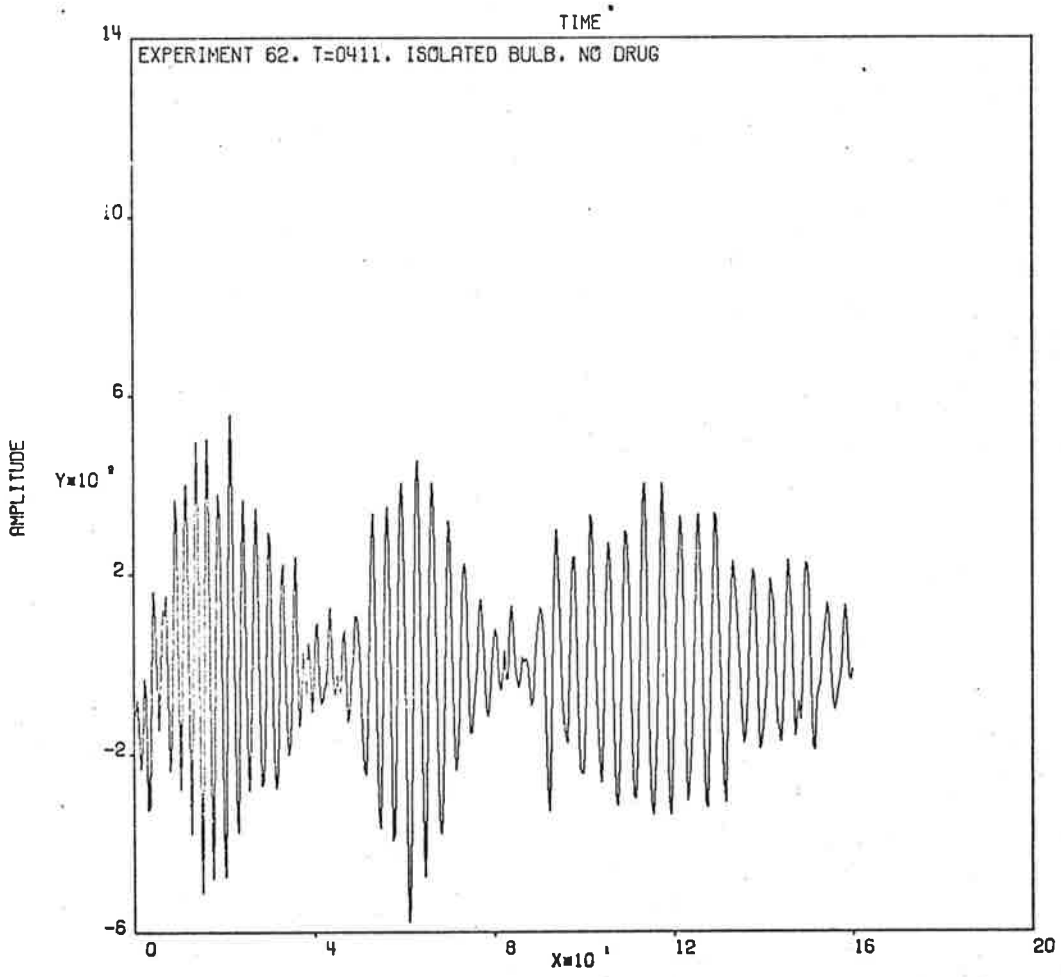
Spectral analysis was performed after normalizing the data and removing the mean to reduce the effects of DC components. The algorithm was based on a subroutine originally obtained from U.C.L.A. modified for this machine.

Epochs of 64 data points were analyzed (0.256 sec of the 1.6 sec period), and the resulting time-varying power spectra and their logarithms plotted by QIKPLT on the line printer, or AUTPLT on the drum plotter. When simultaneous bulbar and cortical records were analyzed, the cross spectrum was also plotted.

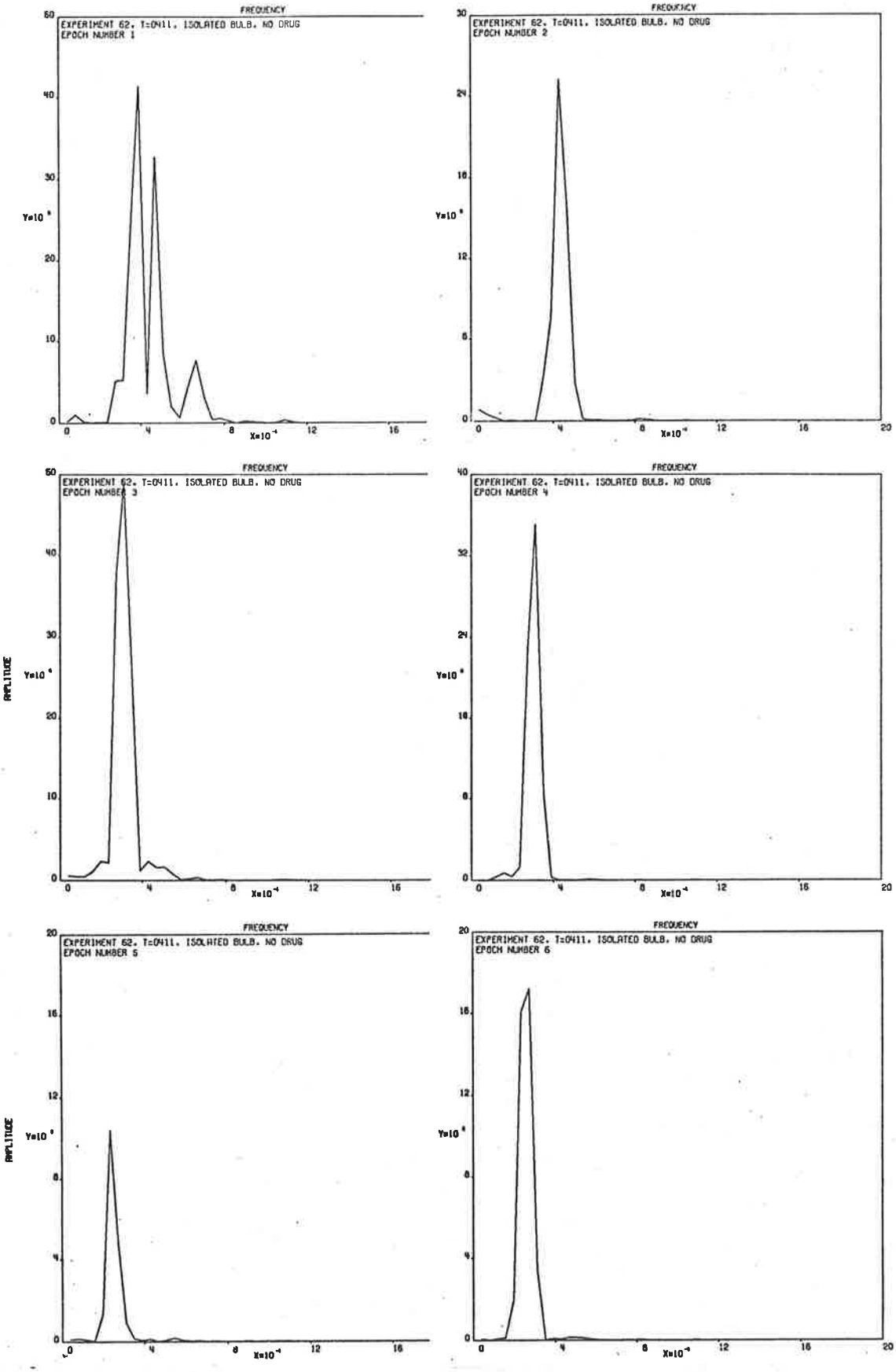
Examples of input data and results of spectral analysis are shown on the following pages.

Because of the temporal hardship imposed by this method of analysis, it was not used as much as it could have been. It has been used by other workers to study drug effects, for example, Eidelberg, Long & Miller (1965); Schallek, Lewison & Thomas (1967, 1968), and Itil, Keskiner, Han, Hsu & Ulett (1971).

Improved hardware will enable time-varying power spectral analysis to be more widely used. It has applications not only in EEG analysis, but also in analysis of evoked responses (Freeman, 1970; Davis, 1973; Nunez, 1973). Analysis of EEG by bispectrum (for example, Dumermuth, Huber, Kleiner & Gasser, 1971) or complex demodulation (for example, Levine, Elashoff, Calloway, Payne & Jones, 1972) are outside the scope of this appendix.



AN INDUCED WAVE RECORDED FROM AN ISOLATED BULB, DIGITIZED IN THE CAT 400C, MANUALLY TRANSFERRED TO 80-COLUMN CARDS, AND PROCESSED IN THE CDC-6400. THE DATA WERE NORMALIZED, HAD THE MEAN REMOVED, AND WAS PLOTTED ON THE 10" PLOTTER AS A VISUAL CHECK THAT NO ERRORS HAD BEEN MADE. THE DATA WERE DIVIDED INTO BLOCKS OF 64 POINTS (0.256 SEC) FOR THE SPECTRAL ANALYSIS.



THE POWER SPECTRA OF THE SIX SEGMENTS OF THE PRECEDING INDUCED WAVE, PLOTTED ON THE 10" PLOTTER. RESOLUTION IS LIMITED TO 4 HZ. THE INDUCED WAVE CAN BE SHOWN TO SLOW IN FREQUENCY WITH TIME.

1 62 411 10 1600 2 2 0 9 1

EXPERIMENT 62, T=0411, ISOLATED BULB, NO DRUG

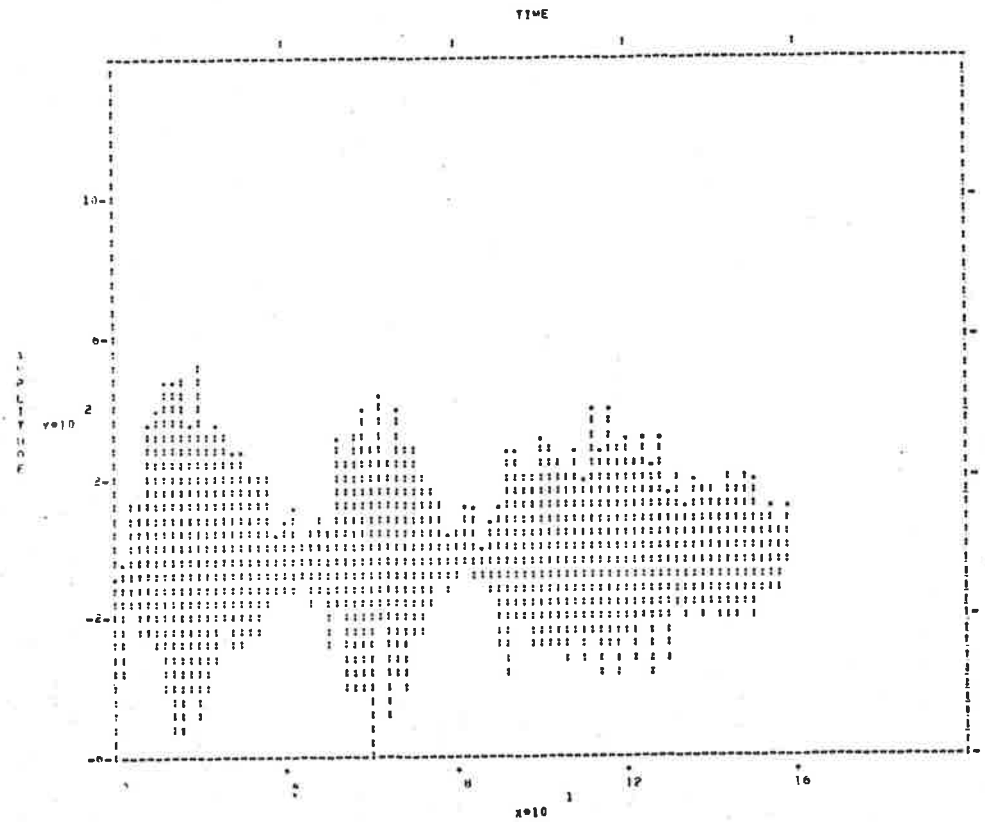
EXPERIMENTAL ANIMAL POSSUM

EXPERIMENT NUMBER 62

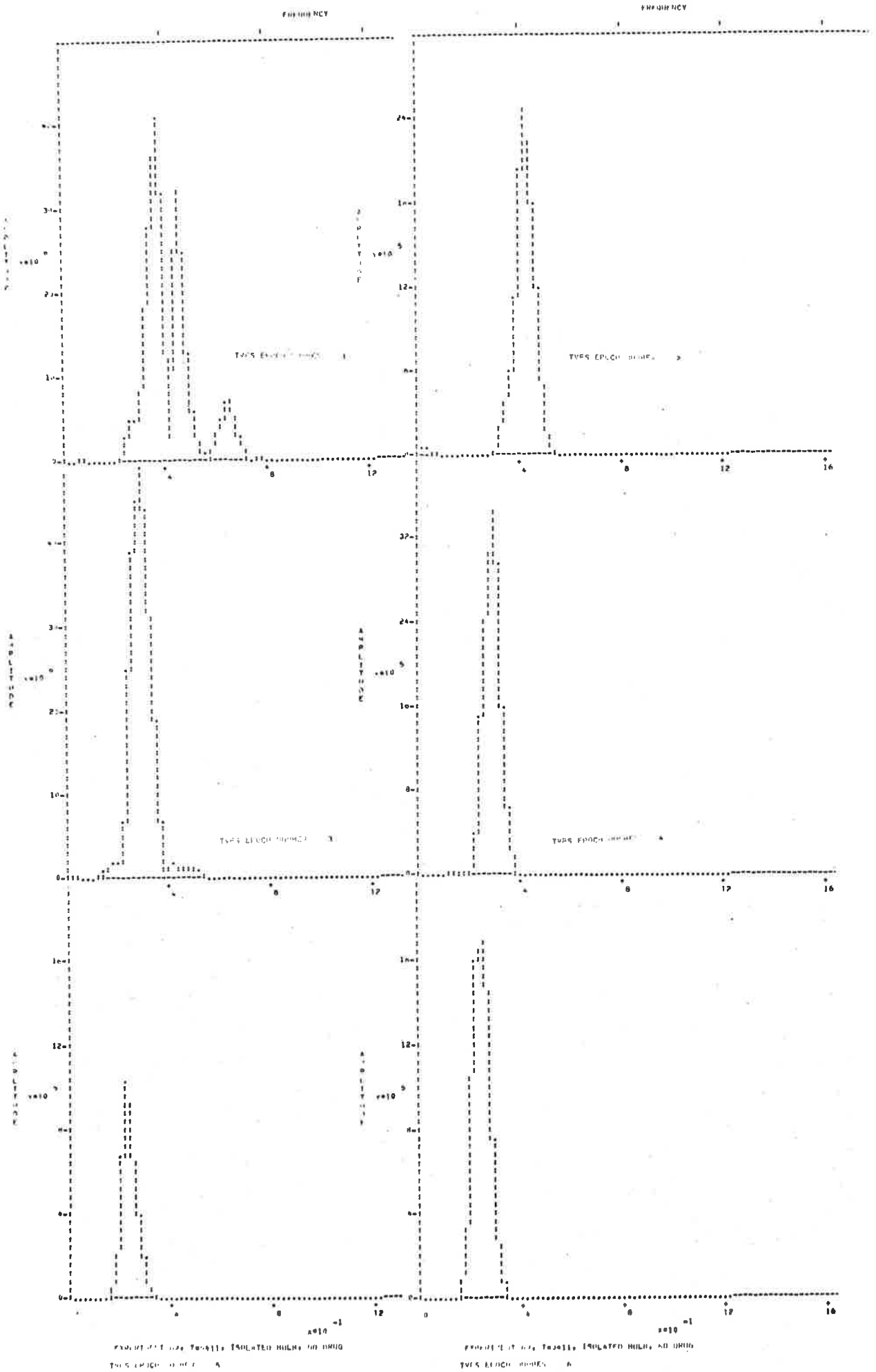
TAPE COUNTER 411 FEET.

DRUG USED NO DRUG

19688	18946	18831	18633	18727	19042	18954	18452	18463	19018	19429	19292	19036	18812	19064	19364
19325	19499	19094	18630	18767	19272	19645	19742	18943	18542	18956	19659	19918	19700	18850	18337
18679	19671	20111	19742	18688	18670	18709	19761	20122	19682	18644	18133	18723	19462	19873	19760
19296	18394	18145	18905	19409	20230	19807	19197	18522	18346	18915	19598	19847	19421	18789	18534
18742	19142	19609	19406	19930	18437	18561	18576	18777	19263	19697	19643	19165	18704	18547	18621
19036	19409	19557	19190	18843	18697	18753	19001	19396	19588	19132	18828	18942	19156	19055	18969
19134	19100	18892	19028	19252	19290	19036	18920	18942	19000	19015	19230	19357	19175	18995	18967
19055	19491	18980	19206	19254	19082	18846	18886	19065	19110	19321	19318	19234	19073	18794	18634
[illegible]	18834	19273	19650	19782	19397	18914	18452	18361	18739	19244	19654	19816	19616	18941	18305
18344	18766	19358	19784	19424	19722	19125	18408	17978	18144	18727	19399	19911	20023	19687	19017
18476	19147	18485	19196	19659	19623	19744	19368	18896	18413	18337	18521	19030	19476	19751	19620
19294	19832	18629	19691	18671	19206	19501	19554	19479	19111	18794	18797	18899	18995	19092	19335
19332	19294	19139	18907	18870	18948	19091	19206	19258	19220	19033	18990	19046	19164	19040	19039
19712	19364	19265	19100	19040	19002	19039	19134	19119	19125	19116	19020	18920	18940	19108	19228
19316	19355	19329	19227	18869	19563	18444	18791	19264	19566	19707	19550	19289	18968	18853	18782
18749	19963	19391	19578	19588	19312	16935	18640	18614	18612	18835	19116	19445	19774	19726	19440
19091	18856	18685	18570	18781	19192	19512	19647	19557	19256	18827	18516	18466	18656	19019	19381
19791	19686	19532	19245	18756	18513	18449	18671	19013	19422	19821	19921	19712	19130	18703	18481
19427	18573	18826	19207	19584	19920	19746	19276	18857	18496	18425	18689	19042	19363	19561	19769
19666	19303	18804	18486	18576	18714	18917	19290	19667	19776	19604	19283	18795	18485	18457	18772
19140	19457	19791	19749	19456	19163	18664	18578	18587	18845	19219	19455	19568	19467	19373	19202
19043	18859	18757	18860	18960	19225	19312	19528	19501	19267	18967	18720	18746	18822	18924	19022
19233	19685	19427	19375	19116	18842	18824	18755	18867	19132	19377	19572	19478	19327	18997	18782
18875	18936	18854	19402	19425	19559	19940	19408	19035	18742	18719	18939	18993	19011	19158	19215
19715	[illegible]	[illegible]	[illegible]	[illegible]	18901	18932	18997	19020	19102	19266	19363	19294	19657	19635	19077



THE INPUT DATA, PRINTED OUT, AND PLOTTED WITH QUIPLT, AS A CHECK ON THE ACCURACY OF THE MANUAL HANDLING. THE INDUCED WAVE WAS DIVIDED INTO 64 DATA POINTS FOR SPECTRAL ANALYSIS OF EACH. IN THE CASE ILLUSTRATED HERE, IT WAS FROM AN ISOLATED BULB, DRUG FREE.



SPECTRAL ANALYSIS OF THE SIX EPOCHS OF THE INDUCED WAVE ILLUSTRATED ON THE RRECEDING PAGE

1 62 456 10 1600 2 2 0 9 1

EXPERIMENT 62, TAPE 0456, ISOLATED BULB, NO DRUG

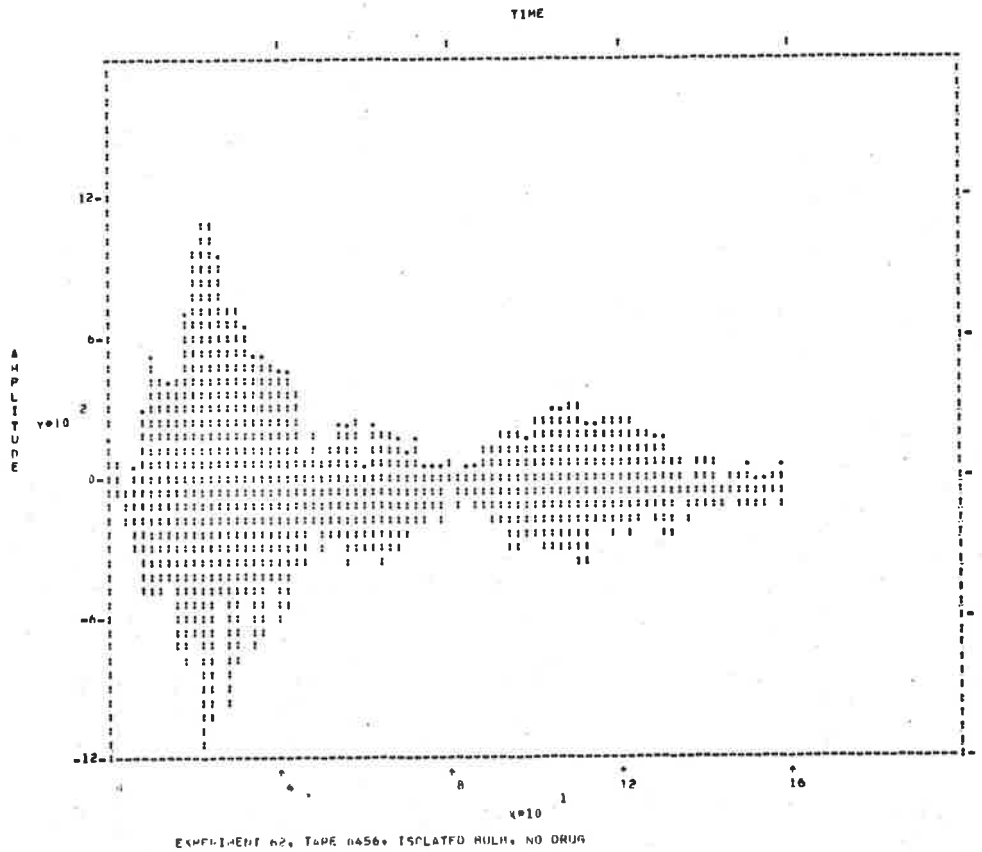
EXPERIMENTAL ANIMAL POSSUM

EXPERIMENT NUMBER 62

TAPE COUNTER 456 FEET

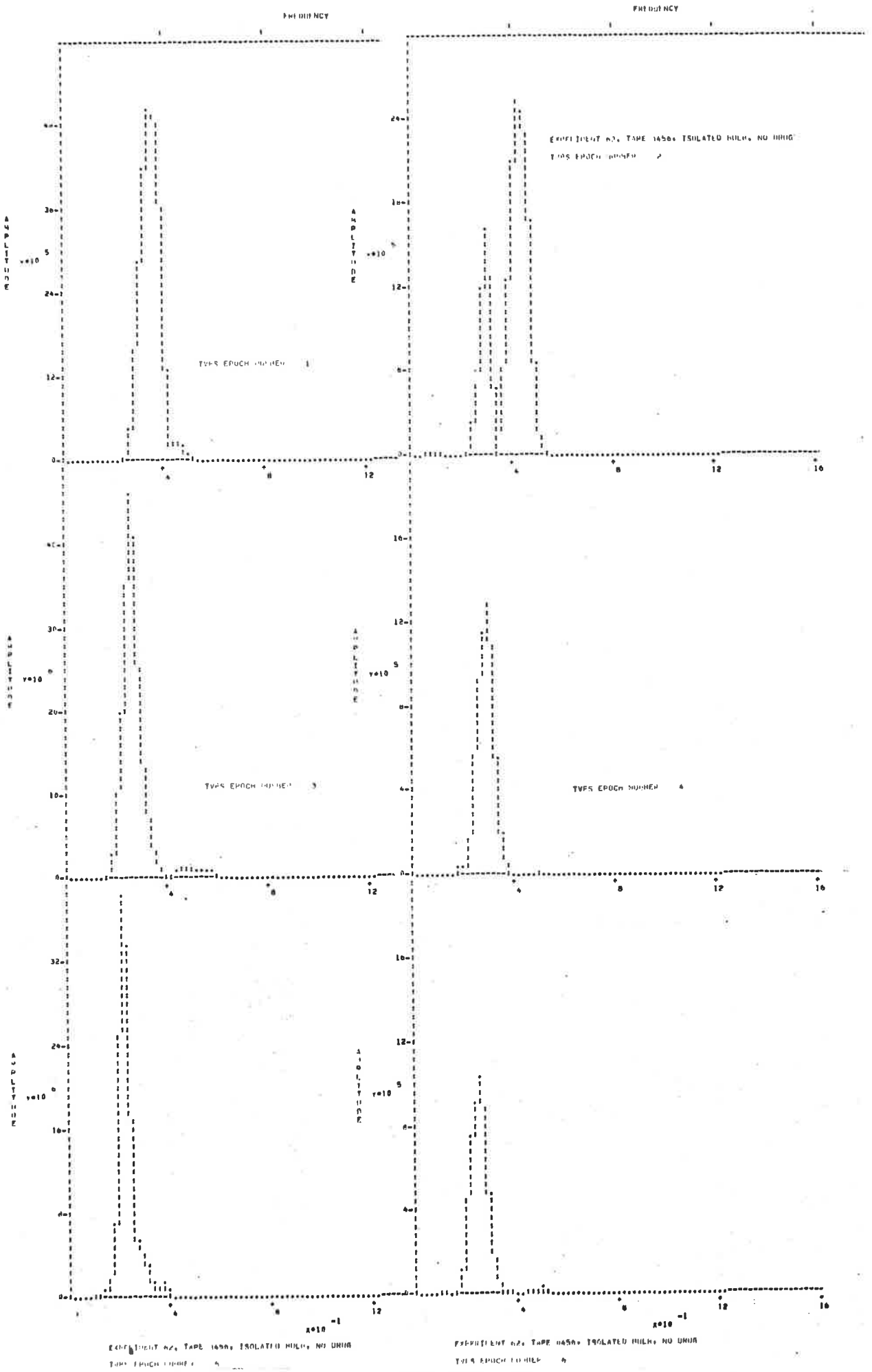
DRUG USED NO DRUG

19675 19670 19620 19512 19513 19404 19309 19250 19213 19244 19150 19045 18971 19052 19124 19328
19416 19068 18662 18834 19353 19367 19935 19046 18340 18756 19786 20472 20322 19168 18304 18726
19068 20271 20112 19186 18769 18850 19858 20214 19711 18396 17781 18847 20297 20477 20184 18908
17618 17434 19634 21108 21429 20075 17960 16835 17706 19695 21254 21706 20772 18861 17181 17237
18822 20436 21404 21113 19913 18244 17253 18024 19492 20673 20967 20262 18943 17657 17656 18828
20056 20726 20491 19575 18282 17734 18305 19343 20231 20453 19968 18822 17963 18068 18964 19971
20332 20196 19404 18425 18002 18124 19149 19963 20329 20003 19187 18462 18164 18615 19408 20002
20137 19975 19527 18904 18586 18549 18940 19434 19805 19771 19550 19291 18923 18658 18742 18994
19247 19504 19663 19608 19351 19044 18787 18828 19037 19342 19737 19823 19669 19201 18720 18505
18755 19347 19704 19906 19783 19453 19045 18693 18731 18979 19397 19739 19840 19644 19127 18748
18624 18840 19218 19542 19773 19764 19592 19102 18872 18689 18926 19384 19650 19708 19592 19401
19214 19009 18883 18831 19189 19540 19841 19862 19435 19313 19199 18956 18948 19042 19262 19402
19444 19446 19400 19277 19036 18951 19107 19419 19521 19398 19417 19427 19389 19241 19071 19043
19198 19244 19312 19297 19353 19401 19467 19372 19325 19214 19082 19197 19125 19071 19171 19395
19630 19658 19451 19103 19015 19008 18915 18972 19233 19654 19782 19683 19512 19217 18960 18743
18744 19074 19505 19761 19729 19451 19425 19068 18817 18803 18891 18994 19348 19710 19927 19858
19514 19050 18639 18630 18902 19118 19422 19728 19961 19832 19464 18969 18678 18735 18763 19009
19473 19830 20007 19426 19478 19028 18711 18583 18636 19057 19452 19733 19819 19682 19276 18913
18607 18946 18996 19112 19367 19714 19903 19892 19571 19145 18849 18812 19095 19031 19151 19582
19880 19809 19347 18994 18863 18926 18960 18954 19206 19583 19772 19592 19389 19199 19100 19050
18913 18975 19254 19573 19720 19633 19461 19203 18930 18881 19074 19251 19221 19362 19477 19464
19306 19344 19318 19218 19112 18953 18997 19136 19224 19463 19530 19466 19316 19099 19105 19070
19223 19401 19505 19487 19365 19280 19168 19075 19131 19239 19247 19126 19218 19325 19408 19339
19474 19256 19267 19390 19368 19173 19036 19106 19365 19417 19384 19284 19156 19206 19223 19176
19151 19147 19295 19248 19237 19250 19270 19384 19388 19314 19234 19102 19067 19179 19353 19428



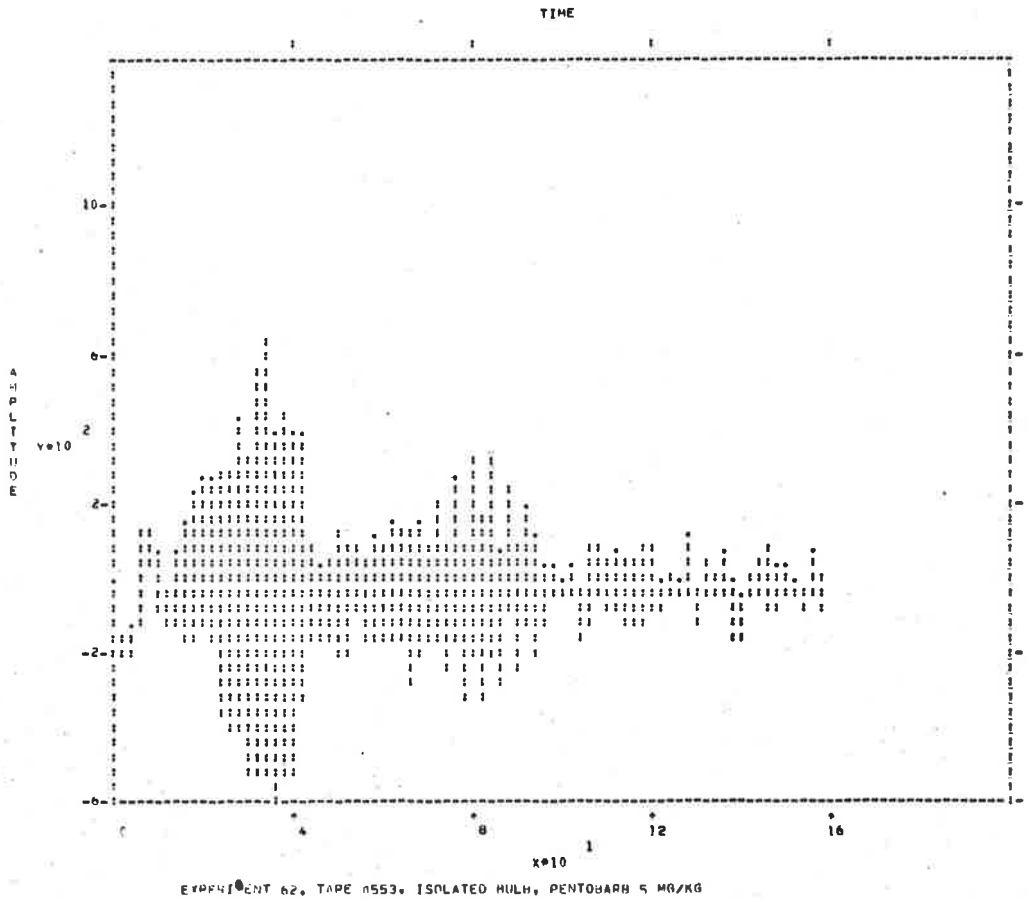
EXPERIMENT 62, TAPE 0456, ISOLATED BULB, NO DRUG

INPUT DATA FROM AN ISOLATED BULB BEFORE PENTOBARB WAS GIVEN, WITH THE WAVE QIKPLTTED AFTER NORMALIZING AND REMOVING THE MEAN. A COS-SQUARED DATA WINDOW WAS APPLIED TO THE SIX BLOCKS OF 64 DATA POINTS, AND THE SPECTRAL ANALYSIS WAS PERFORMED ON EACH SET OF DATA. THE SPECTRAL ESTIMATES WERE ALSO QIKPLTTED AND APPEAR ON THE NEXT PAGE.



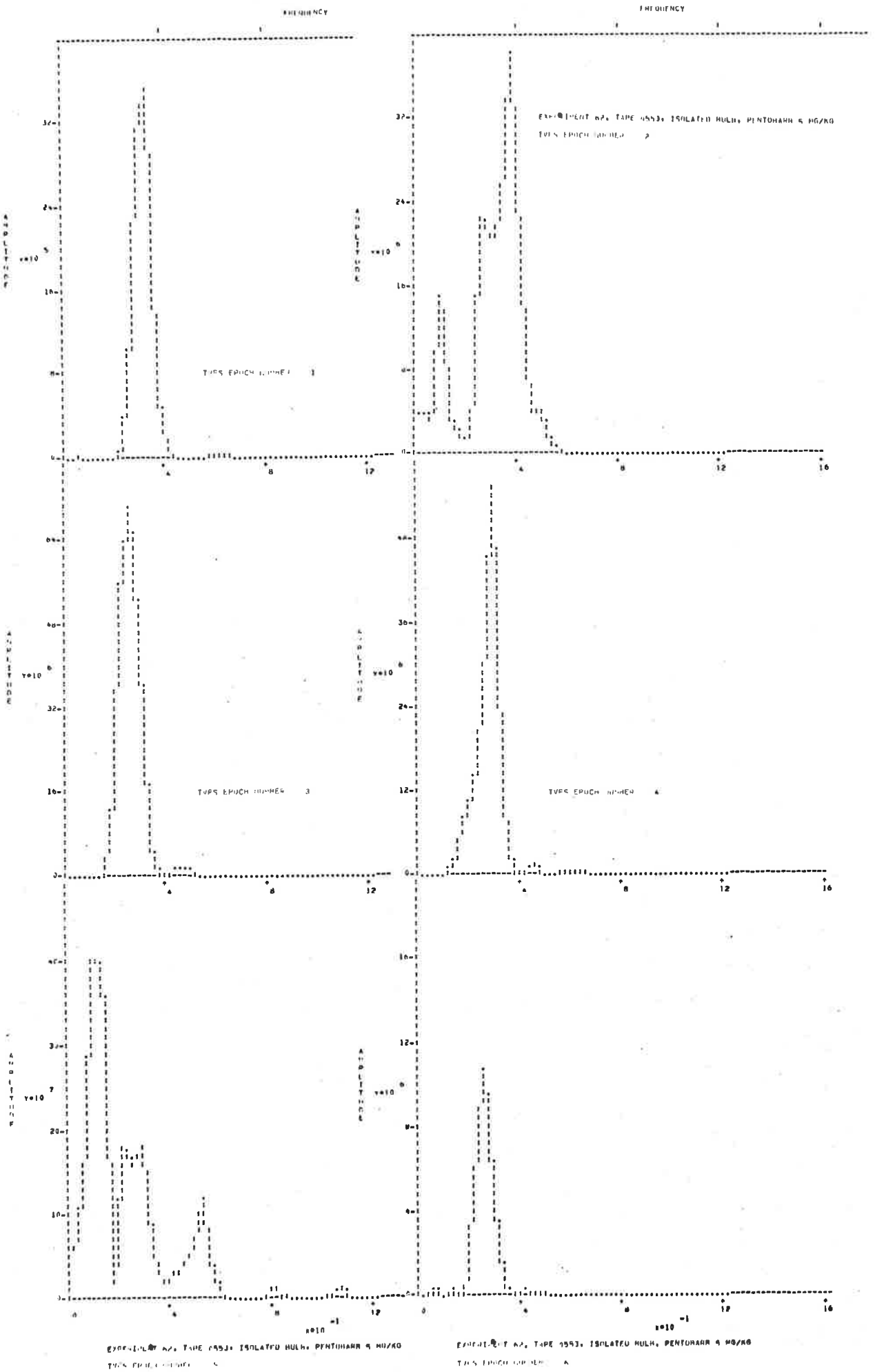
SPECTRAL ESTIMATED OF THE PRECEDING DRUG-FREE INDUCED WAVE

```
    1    62   553    10  1600     2     2     0    20     1

EXPERIMENT 62, TAPE 0553, ISOLATED HULH, PENTOHARB 5 MG/KG
EXPERIMENTAL ANIMAL  POSSUM
EXPERIMENT NUMBER    62
TAPE COUNTER   553 FEET
DRUG USED  PENTOHARB

19274 19221 19175 19135 18976 18899 18835 18915 18890 18891 18933 18874 18921 18929 19010 19145
19338 19481 19472 19535 19526 19449 19398 19340 19358 19348 19410 19368 19228 19126 19132 19231
19139 19031 19024 19136 19418 19391 19156 19043 19289 19599 19495 19210 18986 19176 19510 19701
19746 19431 19058 18996 19386 19536 19043 19466 19982 19958 19287 19708 19884 19396 18711 18506
18974 19557 19893 19036 19050 18467 18617 19214 19843 20177 19958 18987 18199 18355 19074 19961
20041 20399 19851 18549 18197 18626 19407 20272 20623 20068 18860 18029 18194 18864 19487 20020
20222 19883 19092 18189 18240 18859 19426 19866 20102 19961 19301 18680 18616 18913 19232 19357
19481 19474 19257 18984 18971 19167 19260 19328 19397 19334 19188 19065 18976 18897 19186 19492
19546 19473 19243 19000 18869 18977 19301 19463 19444 19268 19206 19105 18968 18929 19008 19222
19431 19519 19469 19244 19049 18948 19029 19084 19115 19295 19481 19599 19370 19109 18967 18975
19188 19174 19369 19547 19557 19386 19129 18893 18744 18841 19070 19367 19494 19572 19470 19250
19138 18971 18960 18972 19128 19464 19716 19720 19463 19078 18885 18771 18813 18989 19312 19706
19648 19857 19662 19007 18758 18599 18700 18857 19205 19703 19950 19866 19414 19025 18777 19651
19878 19855 19337 19727 19976 19849 19432 18990 18734 18730 18846 19105 19379 19622 19784 19476
19488 19147 18835 18776 18846 19006 19263 19537 19660 19656 19530 19273 19033 18882 18949 19012
19047 19200 19260 19335 19341 19349 19240 19167 19214 19234 19259 19247 19162 19205 19274 19345
19341 19315 19272 19214 19197 19063 19018 18970 19001 19202 19378 19477 19474 19487 19393 19278
19241 19161 19164 19106 19081 19146 19279 19391 19412 19401 19250 19140 19088 19071 19214 19284
19355 19398 19342 19271 19180 19160 19018 19002 19130 19201 19386 19484 19416 19322 19242 19182
19121 19176 19159 19175 19238 19245 19306 19288 19253 19191 19169 19199 19192 19263 19239 19276
19329 19395 19432 19338 19164 19067 19063 19118 19149 19241 19283 19398 19401 19229 19227 19294
19258 19213 19202 19208 19263 19417 19372 19280 19253 19279 19273 19214 19122 18956 19069 19138
19135 19158 19169 19272 19295 19232 19194 19224 19298 19283 19307 19292 19373 19484 19369 19338
19121 19187 19209 19105 19191 19260 19349 19272 19228 19265 19189 19242 19257 19216 19228 19231
19146 19111 19184 19218 19207 19223 19244 19423 19373 19360 19300 19269 19214 19131 19169 19184
```



EXPERIMENT 62, TAPE 0553, ISOLATED HULH, PENTOBARB 5 MG/KG

INDUCED WAVE FROM THE SAME SITE AS THE PRECEDING ONE, AFTER PENTOBARBITONE, 5 MG/KG. THE SPECTRAL ANALYSIS APPEARS ON THE NEXT PAGE.



SPECTRAL ESTIMATES OF THE SIX EPOCHS OF AN INDUCED WAVE RECORDED FROM AN ISOLATED OLFACTORY BULB AFTER PENTOBARBITONE, 5 MG/KG

1 62 642 10 1600 2 2 0 20 1

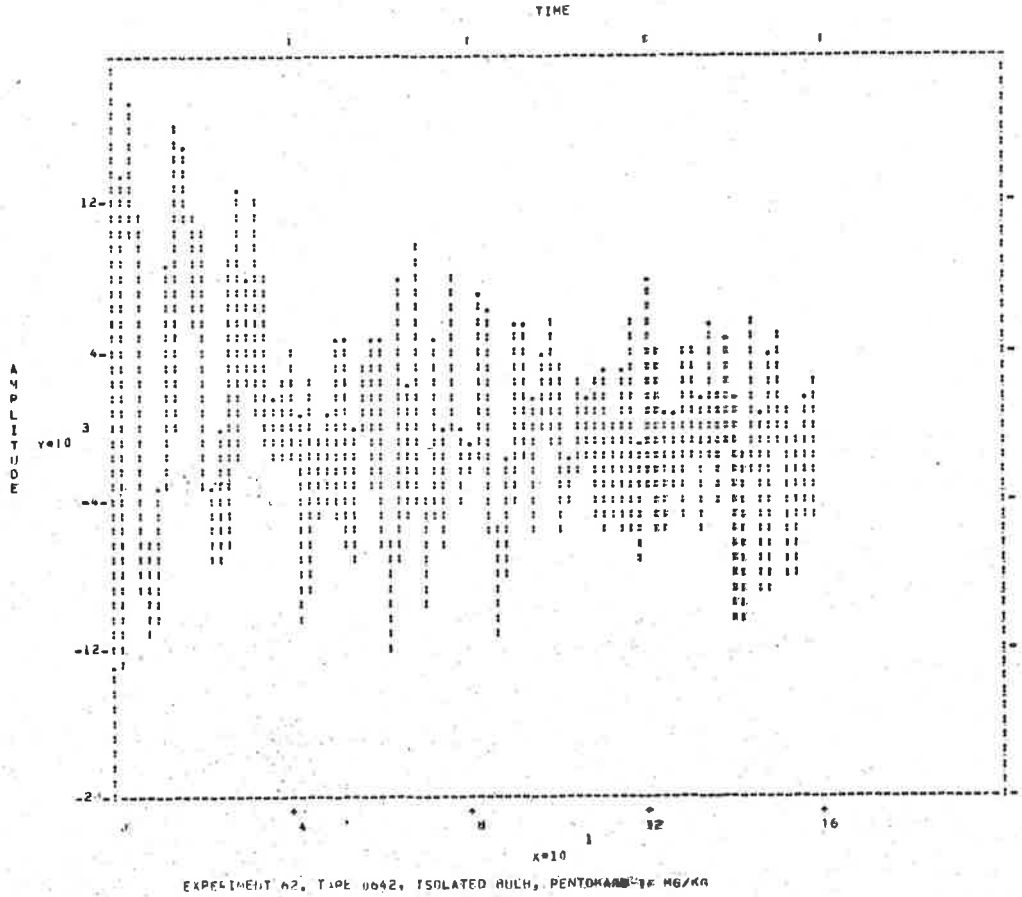
EXPERIMENT 62, TYPE 0642, ISOLATED BULB, PENTOBARB 10 MG/KG

EXPERIMENTAL ANIMAL POSSUM

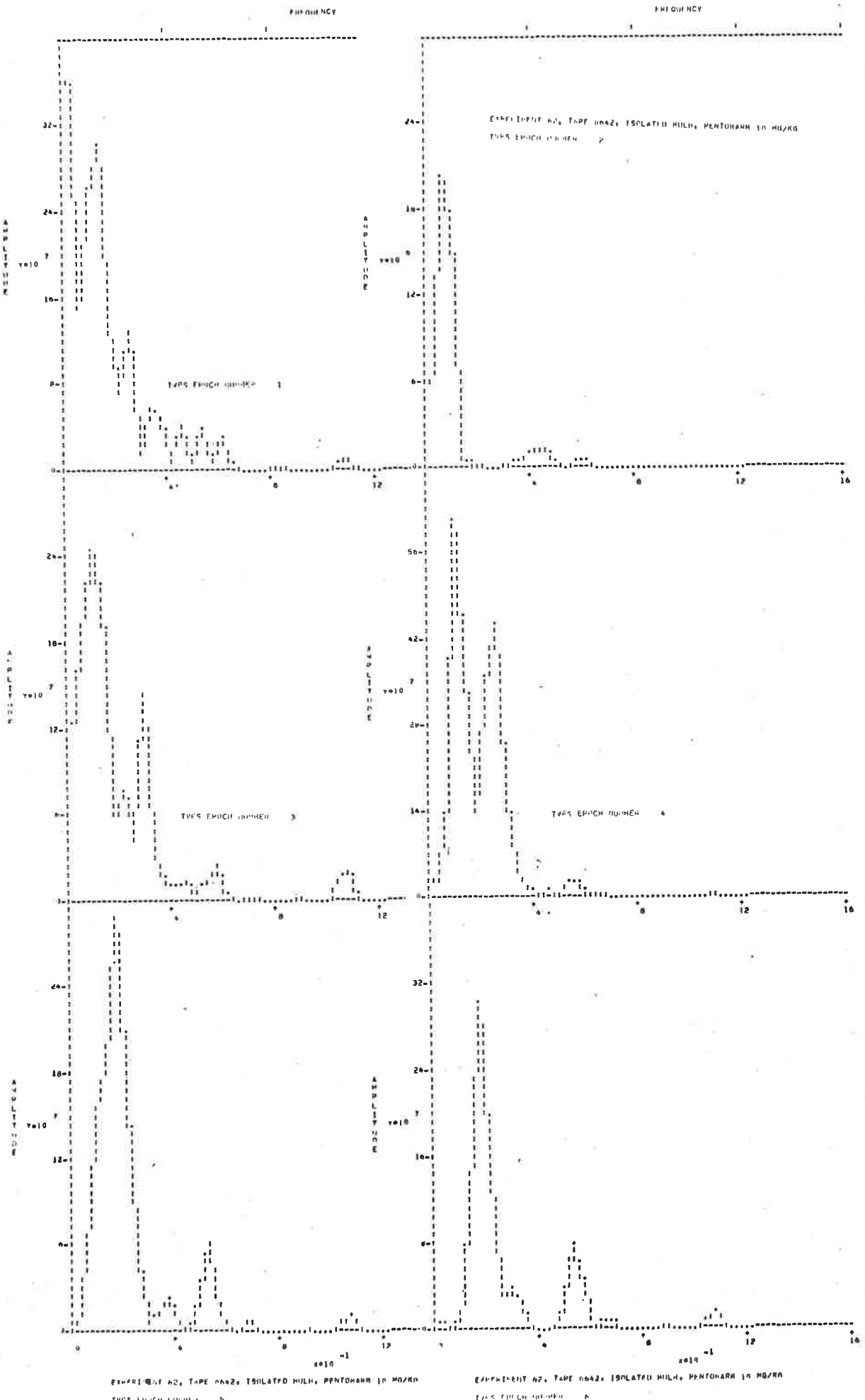
EXPERIMENT NUMBER 62

TAPE COUNTER 642 FEET

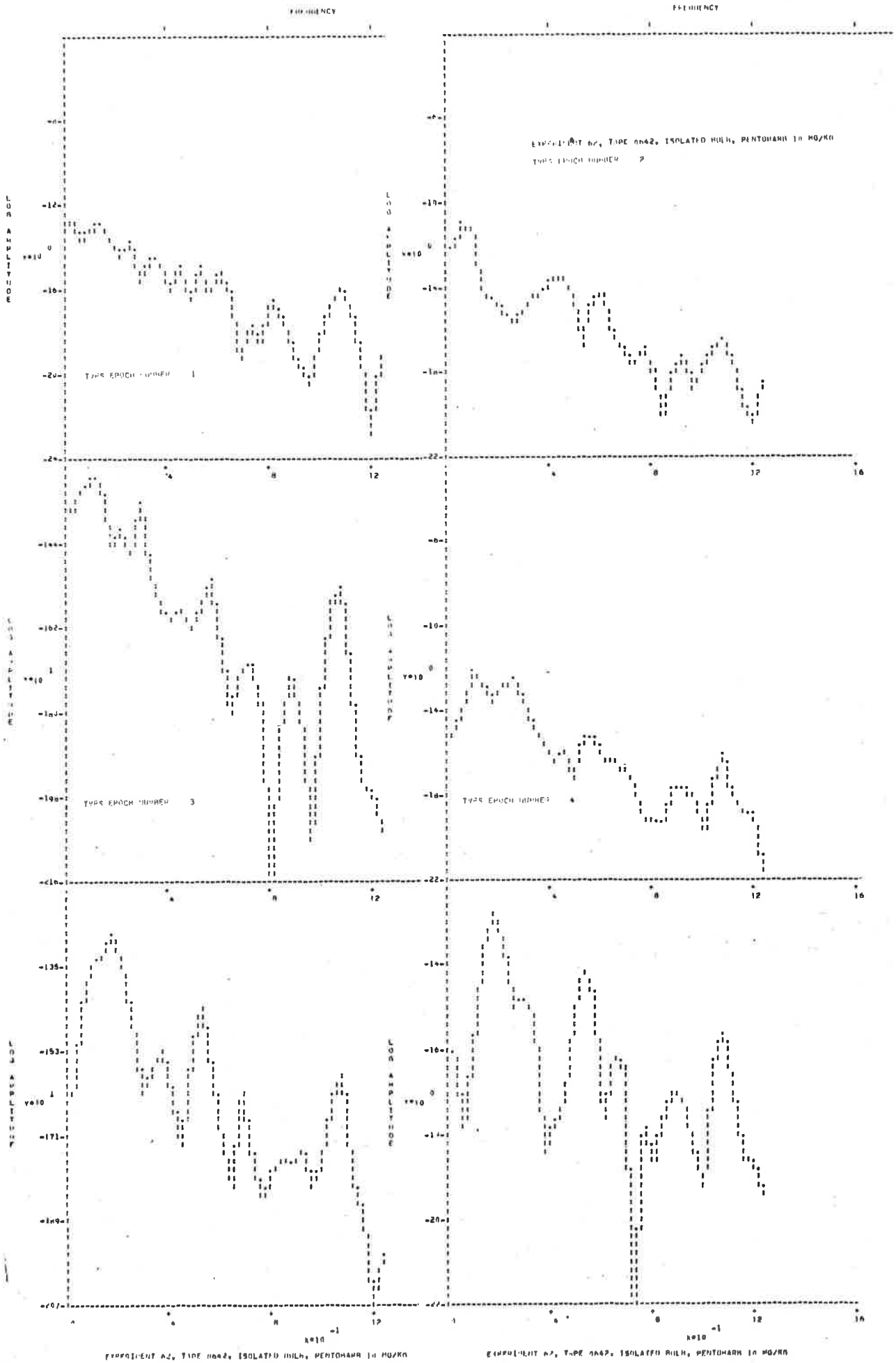
DRUG USED PENTOBARB



INDUCED WAVE FROM THE ISOLATED BULB; PENTOBARBITONE 10 MG/KG



SPECTRAL ESTIMATES OF THE SIX EPOCHS OF THE PRECEDING INDUCED WAVE, PENTOBARBITONE 10 MG/KG



LOGARITHMIC PLOTS OF THE POWER SPECTRA OF THE SIX EPOCHS, PENTOBARBITONE 10 MG/KG

BIBLIOGRAPHY

The numbers in brackets in this bibliography indicate the pages of the text on which the reference is cited.

Abbreviations of journal names in this bibliography will be given, as far as possible, in accordance with Index Medicus.

A "literature search" was carried out by the MEDLARS Service of the National Library, and the search generator appears on the next page. The assistance of S. Harvey is gratefully acknowledged.

MEDLARS SEARCH INPUT GENERATOR

```
1 246NO 0010  *     OLFACTORY SYSTEM OF MARSUPIALS
1 246NO 0020  *     P R WILSON
1 246NO 0030  *     HUMAN PHYSIOL UNI OF ADELAIDE
1 246NO 0040  *     FORMULATED BY S HARVEY
1 246NO 0050  M05   OLFACTORY BULB
1 246NO 0060  M06   OLFACTORY MUCOSA
1 246NO 0070  M07   OLFACTORY NERVE
1 246NO 0080  M08   OLFACTORY PATHWAYS
1 246NO 0090  M09   LIMBIC SYSTEM
1 246NO 0100  C10   E5.075 MICROSCOPY
1 246NO 0110  M11   HISTOLOGY, COMPARATIVE
1 246NO 0120  M12   ANATOMY, COMPARATIVE
1 246NO 0130  S13   CYTOLOGY
1 246NO 0140  S14   PATHOLOGY
1 246NO 0150  S15   ANATOMY + HISTOLOGY
1 246NO 0160  S16   EMBRYOLOGY
1 246NO 0170  S17   PHYSIOPATHOLOGY
1 246NO 0180  S18   PHYSIOLOGY
1 246NO 0190  S19   GROWTH + DEVELOPMENT
1 246NO 0200  M20   SYNAPSES
1 246NO 0210  M21   SYNAPTOSOMES
1 246NO 0220  C21   A11.025.26 SYNAPTIC MEMBRANES
1 246NO 0230  M22   SYNAPTIC VESICLES
1 246NO 0240  M23   PHYSIOLOGY, COMPARATIVE
1 246NO 0250  M24   NEUROPHYSIOLOGY
1 246NO 0260  M25   NERVE DEGENERATION
1 246NO 0270  M26   NERVE ENDINGS
1 246NO 0280  M27   NERVE NET
1 246NO 0290  M28   NERVE REGENERATION
1 246NO 0300  C29   G1.044 ELECTROPHYSIOLOGY
1 246NO 0310  M30   ELECTRIC STIMULATION
1 246NO 0320  C32   G1.077.34 NEURAL CONDUCTION
1 246NO 0330  C33   G1.077.35 NEURAL TRANSMISSION
1 246NO 0340  M34   ODORS
1 246NO 0350  C35   A8.030.13 BRAIN
1 246NO 0360  C36   D6.036 ANESTHETICS
1 246NO 0370  C37   D6.042 ANESTHETICS, LOCAL
1 246NO 0380  C38   D6.006.06 ANALEPTICS
1 246NO 0390  M39   HUMAN
1 246NO 0400  C40   B2 VERTEBRATES
1 246NO 0410  C41   B2.072.36 MARSUPALIA
1 246NO 0420  M42+  M05M09
1 246NO 0430  I43+  C10M12
1 246NO 0440  S44+  S13S19
1 246NO 0450  U45+  M42/S44
1 246NO 0460  I48+  M20M28
1 246NO 0470  I49+  C29C33
1 246NO 0480  C50+  C36C38
1 246NO 0490  I51+  M39C40
1 246NO 0500  R01   (M42*(I43+I48+I49*(C50+M34))+
1 246NO 0510  R01   U45+C35*I49*C50)*I51
1 246NO 0520  R02   C35*I49*C50+M34+C41
1 246NO 0530  R03   C35*I49*C50+C41
1 246NO 0540  R04   C41
```

LAST TAPE SEARCHED

MAR 1974

Abbie, A. A. (1939). The origin of the corpus callosum and the fate of structures related to it. J. Comp. Neurol. 70: 9-44 (1-5,23).

Abbie, A. A. (1940). Cortical lamination in the monotremata. J. Comp. Neurol. 72: 429-467 (1-16).

Abbie, A. A. (1942). Cortical lamination in a polyprotodont marsupial, *Perameles nasuta*. J. Comp. Neurol. 76: 509-536 (1-5,15,16).

Adey, W. R. (1953). An experimental study of the central olfactory connexions in a marsupial (*Trichosurus vulpecula*). Brain 76: 311-330 (1-5,15,16,18,20,23,24; 2-2,21,22,23,24,26,29; 6-2).

Adey, W. R. (1957). The organization of the rhinencephalon. In: The Reticular Formation of the Brain. An International Symposium, Vol. 31, pp.776. Boston. Little, Brown (1-24).

Adey, W. R. (1970). Higher olfactory centres. In: Taste and Smell in Vertebrates. Ciba Foundation Symposium, pp.357-376. Eds. Wolstenholme, G. E. W. & Knight, J. London. Churchill (1-1,24).

Adey, W. R. & Dunlop, C. W. (1960). The action of certain cyclohexamines on hippocampal system during approach performance in the cat. J. Pharmacol. Exp. Ther. 130: 418-426 (4-7).

Adey, W. R., Dunlop, C. W. & Sunderland, S. (1958). A survey of rhinencephalic interconnections with the brain stem. J. Comp. Neurol. 110: 173-198 (2-2).

Adey, W. R., Merrillees, N. C. R. & Sunderland, S. (1956). The entorhinal area; behavioural, evoked potential and histological studies of its relationships with brain-stem regions. Brain 79: 414-439 (2-26; 6-6).

Adey, W. R., Sunderland, S. & Dunlop, C. W. (1957). The entorhinal area; electrophysiological studies of its interrelations with rhinencephalic structures and the brainstem. Electroencephalogr. Clin. Neurophysiol. 9: 309-324 (2-2).

Adrian, E. D. (1942). Olfactory reactions in the brain of the hedgehog. J. Physiol. (Lond.) 100: 459-473 (3-1; 4-4,6)

Adrian, E. D. (1950). The electrical activity of the mammalian olfactory bulb. Electroencephalogr. Clin. Neurophysiol. 2: 377-388 (3-1,26; 4-7).

Affanni, J. M., Morita, E. & García Samartino, L. (1968). Effecto de la seccion de los pedúnculos olfatorios y de la comisura anterior sobre la actividad del bulbo olfatorio del marsupial *Didelphis azarae*. Rev. Soc. Argent. Biol. 44: 183-188 (3-7,8,26; 6-6).

Ai, N. & Takagi, S. F. (1963). The effect of ether and chloroform on the olfactory epithelium. Jap. J. Physiol. 13: 454-465 (6-7).

Ajmone-Marsan, C. (1969). Acute effects of topical epileptogenic agents. In: Basic Mechanisms of the Epilepsies. First edition, pp.299-319. Eds. Jasper, H. H., Ward, A. A. & Pope, A. Boston. Little, Brown & Co. (5-2).

Allison, A. C. (1950). An investigation of the morphology of the mammalian olfactory system. Unpublished thesis. University of Oxford. Cited by Allison, 1953 (1-7).

Allison, A. C. (1953). The morphology of the olfactory system in the vertebrates. Biol. Rev. 28: 195-244 (1-7,20,24).

Allison, A. C. & Warwick, R. T. T. (1949). Quantitative observations on the olfactory system of the rabbit. Brain 72: 186-197 (1-7).

Anderson, C. A. & Westrum, L. E. (1972). An electronmicroscopic study of the normal synaptic relationships and early degenerative changes in the rat olfactory tubercle. Z. Zellforsch. Mikrosk. Anat. 127: 462-482 (1-18).

Andersen, R. A., Barstad, J. A. B. & Laake, K. (1972). Access of quaternary drugs to the central nervous system. Progr. Brain. Res. 36: 189-194 (3-23).

Araki, Y. & Ueki, S. (1972). Changes in sensitivity to convulsion in mice with olfactory bulb ablation. Jap. J. Pharmacol. 22: 447-456 (5-12).

Arduini, A. & Moruzzi, G. (1953). Olfactory arousal reactions in the "cerveau isolé" cat. Electroencephalogr. Clin. Neurophysiol. 5: 243-250 (6-6).

Banerjee, V., Feldberg, W. & Georgiev, V. P. (1970). Microinjections of tubocurarine, leptazol, strychnine and picrotoxin into the cerebral cortex of anaesthetized cats. Br. J. Pharmacol. 40: 6-22 (5-11,29).

Banna, N. R., Naccache, A. & Jabbur, S. J. (1972). Picrotoxin-like action of bicuculline. Eur. J. Pharmacol. 17: 301-302 (5-24).

Baumgarten, R. von, Bloom, F. E., Oliver, A. P. & Salmoiraghi, G. C. (1963). Response of individual olfactory nerve cells to microelectrophoretically administered chemical substances. Pfluegers Archiv. 277: 125-140 (3-30).

Baumgarten, R. von, Green, J. D. & Mancia, M. (1962*a*). Recurrent inhibition in the olfactory bulb. II. Effects of antidromic stimulation of commissural fibers. J. Neurophysiol. 25: 489-500 (3-3; 4-11; 5-8).

Beart, P. M. & Johnston, G. A. R. (1973). GABA uptake in rat brain slices: inhibition by GABA analogues and by various drugs. J. Neurochem. 20: 319-324 (5-11,13).

Becker, C. J. & Freeman, W. J. (1968). Prepyriform electrical activity after loss of peripheral or central input, or both. Physiol. Behav. 3: 597-599 (3-5,12,28; 4-13).

Berger, B. (1973). Dégénérescence transsynaptique dans le bulbe olfactif du lapin, après dés afférentation périphérique. Observation ultrastructurale. Acta. Neuropathol. (Berl.) 24: 128-152 (6-1).

Bergquist, E. H. (1970). Output pathways of hypothalamic mechanisms for sexual, aggressive, and other motivated behaviors in opossum. J. comp. Physiol. Psychol. 70: 389-398 (6-6).

Bernhard, C. G. & Bohm, E. (1955). The action of local anaesthetics on experimental epilepsy in cats and monkeys. Br. J. Pharmacol. 10: 288-295 (5-3).

Bernhard, C. G., Bohm, E. & Wiesel, T. (1956). On the evaluation of the anticonvulsive effect of local anaesthetics. Arch. Int. Pharmacodyn. Ther. 108: 392-407 (5-3).

Berry, C. M., Hagamen, W. D. & Hinsey, J. C. (1952). Distribution of potentials following stimulation of olfactory bulb in cat. J. Neurophysiol. 15: 139-148 (6-5).

Biedenbach, M. A. (1964). A pharmacological study of prepyriform potentials with parametric controls. Dissertation for the degree of Doctor of Philosophy in Physiology, University of California, Berkeley. (University Microfilms, Inc., Ann Arbor, Michigan, No. 65-2951.) (3-25,28; 4-5,7,10; 5-8)

Biedenbach, M. A. (1966). Effects of anesthetics and cholinergic drugs on prepyriform electrical activity in cats. Exp. Neurol. 16: 464-479 (5-8).

Biedenbach, M. A. & Stevens, C. F. (1969*a*). Electrical activity in cat olfactory cortex produced by synchronous orthodromic volleys. J. Neurophysiol. 32: 193-203 (1-25; 2-2)

Biedenbach, M. A. & Stevens, C. F. (1969*b*). Synaptic organization of cat olfactory cortex as revealed by intracellular recording. J. Neurophysiol. 32: 204-214 (1-25; 2-2).

Biedler, L. M. & Tucker, D. (1955). Response of nasal epithelium to odor stimulation. Science 76: 122 (3-8).

Bimar, J. & Naquet, R. (1972). Mechanism of anesthesia. 6.2. Neurophysiological aspects. Handbuch Exp. Pharmakol. 30: 364-386 (4-23).

Blackstad, T. W. (1956). Commissural connections of the hippocampal region in the rat, with special reference to their mode of termination. J. Comp. Neurol. 105: 417-537 (2-25).

Bloom, F. E., Costa, E. & Salmoiraghi, G. C. (1964). Analysis of individual rabbit olfactory bulb responses to the microelectrophoresis of acetylcholine, norepinephrine and serotonin synergists and antagonists. J. Pharmacol. Exp. Ther. 146: 16-23 (4-11).

Boudreau, J. C. (1963). Behavioral correlates of electrical activity in the olfactory system of the cat. Ph.D. Thesis. University of California, Berkeley. (University Microfilms, Inc., Ann Arbor, Michigan, No. 64-2029.) (3-4; 4-34)

Boudreau, J. C. (1964). Computer analysis of electrical activity in the olfactory system of the cat. Nature 201: 155-158 (3-4).

Boudreau, J. C. & Freeman, W. J. (1963). Spectral analysis of electrical activity in the prepyriform cortex of the cat. Exp. Neurol. 8: 423-439 (3-4; 3-25).

Brownson, R. H., McDougal, H. D. & Suter, D. B. (1972). Parathion-induced changes in acetylcholinesterase of the olfactory bulb in the immature rat. Anat. Rec. 172: 278 (5-11).

Burkhardt, D. A. (1972). Effects of picrotoxin and strychnine upon electrical activity of the proximal retina. Brain Res. 43: 246-249 (5-21).

Burton, P. T. C. (1958). Halothane concentration from a Rowbotham's bottle in a circle absorption system. Br. J. Anaesth. 30: 312-316 (4-23).

Cain, D. P. & Bindra, D. (1972). Responses of amygdala single units to odors in the rat. Exp. Neurol. 35: 98-110 (1-20).

Cajal, S. Ramón y. (1909). Histologie du Système Nerveaux de l'homme et des Vertebrès. Vol. II. Paris. A. Maloine. Reprinted by Consijo Superior de Investigaciones Cientificas. Madrid. 1955. (1-1,2,19,22).

Cajal, S. Ramón y. (1911). Studies on the cerebral cortex (limbic structures). Translated from the Spanish by Lisbeth M. Kraft, pp.179. London. Lloyd-Luke. 1955.

Caton, R. (1875). The electric currents of the brain. Br. Med. J. 2: 278 (1-1; 3-1; 4-8).

Chenoweth, M. B. (1972). Foreword and commentary. Handbuch Exp. Pharmakol. 30: 1-3 (4-23).

Clark, D. L. & Rosner, B. S. (1973). Neurophysiologic effects of general anesthetics: I. The electroencephalogram and sensory evoked responses in man. Anesthesiology 38: 564-582 (4-16).

Conway, C. M. & Ellis, D. B. (1970). Propanidid. Br. J. Anaesth. 42: 249-254 (4-32).

Coutts, R. P. (1972). Spectral analysis of a biological system. Project Report: Department of Electrical Engineering, University of Adelaide (A-1).

Cowan, W. M., Gottlieb, D. I., Hendrickson, A. E., Price, J. L. & Woolsey, T. A. (1972). The autoradiographic demonstration of axonal connections in the central nervous system. Brain Res. 37: 21-51 (1-18).

Cowan, W. M., Raisman, G. & Powell, T. P. S. (1965). The connexions of the amygdala. J. Neurol. Neurosurg. Psychiatry 28: 137-151 (1-19; 2-21).

Cox, J. R. Jr, Nolle, F. M. & Arthur, R. M. (1972). Digital analysis of the electroencephalogram, the blood pressure wave, and the electrocardiogram. Proc. IEEE 60: 1137-1164 (A-1).

Crankshaw, D. P. & Raper, C. (1971). The effect of solvents on the potency of chlordiazepoxide, diazepam, medazepam and nitrazepam. J. Pharm. Pharmacol. 23: 313-321 (4-37).

Crawford, J. M. (1969). Effects of convulsant barbiturates on cortical neurones. Brain Res. 12: 485-489 (5-29).

Crosby, E. C. & Humphrey, T. (1939). Studies of the vertebrate telencephalon. I. The nuclear configuration of the olfactory and accessory olfactory formations and of the nucleus olfactorius anterior of certain reptiles, birds, and mammals. J. Comp. Neurol. 71: 121-213 (1-5,13).

Crosby, E. C. & Humphrey, T. (1941). Studies of the vertebrate telencephalon. II. The nuclear pattern of the anterior olfactory nucleus, tuberculum olfactorium, and the amygdaloid complex in adult man. J. Comp. Neurol. 74: 309-352 (1-17).

Curtis, D. R. (1959). Pharmacological investigations upon inhibition of spinal motoneurones. J. Physiol. (Lond.) 145: 175-192 (5-1).

Curtis, D. R. (1970). Amino acid transmitters in the mammalian central nervous system. Proc. 4th Int. Congress Pharmacol. Vol. I, pp.9-31. Basel. Schwabe & Co. (5-12).

Curtis, D. R. (1971). Neutral amino acids as central transmitters. The use of amino acid antagonists. In: Research in Physiology. pp.403-411. Eds. Kao, F. F., Koizumi, K. & Vassalle, M. Bologna. Aulo Gaggi (5-12).

Curtis, D. R. & Crawford, J. M. (1969). Central synaptic transmission: Microelectrophoretic studies. Annu. Rev. Pharmacol. 9: 209-240 (5-12).

Curtis, D. R. & Eccles, R. M. (1958). The excitation of Renshaw cells by pharmacological agents applied electrophoretically. J. Physiol. (Lond.) 141: 435-445 (4-1).

Dale, H. H. & Dudley, H. W. (1929). The presence of histamine and acetylcholine in the spleen of the ox and the horse. J. Physiol. (Lond.) 67: 97-123 (1-1).

Darbinjan, T. M., Golovchinsky, V. B. & Plehotkina, S. I. (1971). The effects of anesthetics on reticular and cortical activity. Anesthesiology 34: 219-229 (4-32).

Davis, A. E. (1973). Power spectral analysis of flash and click evoked responses. Electroencephalogr. Clin. Neurophysiol. 35: 287-291 (A-2).

Davy, H. (1800). Researches, chemical and philosophical; chiefly concerning nitrous oxide, or dephlogisticated nitrous air, and its respiration. pp.580. Facsimile Reproduction, 1972. London. Butterworths. (4-16).

Dawson, T. J. (1969). Temperature regulation and evaporative water loss in the brush-tailed possum *Trichosurus vulpecula*. Comp. Biochem. Physiol. (A) 28: 401-407 (2-3).

de Blainville (1816). Cited by Jones, 1924*a*. (1-2).

De Estable Puig, R. F. & Estable Puig, J. F. (1971). Cell response of the olfactory bulb to ionizing radiation injury. An electron microscopical study. Acta. Neuropath. (Berl.) 17: 287-301 (6-1).

DeFrance, J. F. & Hutchinson, R. R. (1972). Electrographic changes in the amygdala and hippocampus associated with biting attack. Physiol. Behav. 9: 83-88 (1-20).

DeGroat, W. C. (1972). GABA-depolarization of a sensory ganglion: antagonism by picrotoxin and bicuculline. Brain Res. 38: 429-432 (5-21).

de Jong, R. H. & Heavner, J. E. (1971). Diazepam prevents local anesthetic convulsions. Anesthesiology 34: 523-531 (4-6; 5-5).

de Jong, R. H. & Heavner, J. E. (1972). Local anesthetic seizure prevention: diazepam versus pentobarbital. Anesthesiology 36: 449-457 (4-6,11; 5-5).

de Jong, R. H., Heavner, J. E. & de Oliveira, L. F. (1972*a*). Effects of nitrous oxide on the lidocaine seizure threshold and diazepam protection. Anesthesiology 37: 299-303 (4-2; 5-5).

de Jong, R. H., Heavner, J. E. & de Oliveira, L. F. (1972*b*). Nitrous oxide elevates local anesthetic seizure threshold. Exp. Neurol. 35: 558-564 (5-5).

de Jong, R. H., Robles, R. & Heavner, J. E. (1970). Suppression of impulse transmission in the cat's dorsal horn by inhalational anesthetics . Anesthesiology 32: 440-445 (4-16).

de Jong, R. H. & Wagman, I. H. (1963). Cortical and subcortical electrical effects of I.V. lidocaine and inhalational anesthetics. Fed. Proc. 22: 187 (4-2; 5-3).

Dennis, B. J. (1965). Characteristics and connections of the olfactory system with special emphasis on the derivation of centrifugal components. Ph.D. Thesis, University of Adelaide (1-24; 2-1,15).

Dennis, B. J. & Kerr, D. I. B. (1968). An evoked potential study of centripetal and centrifugal connections of the olfactory bulb in the cat. Brain Res. 11: 373-396 (1-2,13,18,20,24,26,27; 2-1,2,5,6,7,9, 12,15,16,18,19,20,22,28).

De Olmos, J. S. (1972). Some connexions of the stria terminalis in the guinea pig brain. Anat. Rec. 172: 300 (1-18).

De Olmos, J. S. & Ingram, W. R. (1972). The projection field of the stria terminalis in the rat brain. An experimental study. J. Comp. Neurol. 146: 303-334 (6-3).

Doenicke, A., Kugler, J., Schellenberger, A. & Gurtner, T. (1966). The use of electroencephalography to measure recovery time after intravenous anaesthesia. Br. J. Anaesth. 38: 580-590 (4-32).

Domino, E. F. (1962). Sites of action of some CNS-depressants. Annu. Rev. Pharmacol. 2: 215-250 (4-5).

Domino, E. F. (1964). Neurobiology of phencyclidine (sernyl), a drug with an unusual spectrum of pharmacological activity. Int. Rev. Neurobiol. 6: 303-347 (4-7).

Domino, E. F. & Ueki, S. (1959). Electrical "seizure-like" discharge in the amygdala of dogs following general anesthetics. Fed. Proc. 18: 385 (4-2,3,4,5,19,22,25,26).

Domino, E. F. & Ueki, S. (1960). An analysis of the electrical burst phenomenon in some rhinencephalic structures of the dog and monkey. Electroencephalogr. Clin. Neurophysiol. 12: 635-648 (3-5,25; 4-7,8).

Døving, K. B. & Pinching, A. J. (1973). Selective degeneration of neurones in the olfactory bulb following prolonged odour exposure. Brain Res. 52: 115-129 (6-1,6).

Dumermuth, G. (1971). Electronic data processing in pediatric EEG research. Neuropaediatrie 2: 349-374 (A-1).

Dumermuth, G., Huber, P. J., Kleiner, B. & Gasser, T. (1970). Numerical analysis of electroencephalographic data. IEEE Trans. Audio. Electroacoust. AU-18: 404-411 (A-1).

Dumermuth, G., Huber, P. J., Kleiner, B. & Gasser, T. H. (1971). Analysis of the interrelations between frequency bands of the EEG by means of the bispectrum. A preliminary study. Electroencephalogr. Clin. Neurophysiol. 31: 137-148 (A-2).

Dundee, J. W. (1971). Comparative analysis of intravenous anesthetics. Anesthesiology 35: 137-148 (4-27).

Dundee, J. W. & Clarke, R. S. J. (1964). Clinical studies of induction agents. IX. A comparative study of a new eugenol derivative, FBA. 1420, with G.29.505 and standard barbiturates. Br. J. Anaesth. 36: 100-105 (4-32).

Dundee, J. W. & Clark, R. S. J. (1971). Non-barbiturate intravenous anaesthetics. In: General Anaesthesia. 3rd edn., Vol. 1. Basic Sciences pp.513-529. Eds. Gray, T. C. & Nunn, J. F. London. Butterworths (4-32).

Dundee, J. W. & Haslett, W. H. K. (1970). The benzodiazepines. A review of their actions and uses relative to anaesthetic practice. Br. J. Anaesth. 42: 217-234 (4-36).

Ebner, F. F. (1969). A comparison of primitive forebrain organization in metatherian and eutherian mammals. Ann. N.Y. Acad. Sci. 167: 241-257 (1-5).

Eger, E. I. II (1971). Inhalational anaesthesia: Pharmacokinetics. In: General Anaesthesia. 3rd edn. Vol. 1. pp.439-464. Ed. Gray, T. C. & Nunn, J. F. London. Butterworths.

Eidelberg, E., Lesse, H. & Gault, F. P. (1961). Convulsant effects of cocaine. Fed. Proc. 20: 322 (5-3).

Eidelberg, E., Lond, M. & Miller, M. K. (1965). Spectrum analysis of EEG changes induced by psychotomimetic agents. (Int. J.) Neuropharmacol. 4: 255-264 (A-2).

Eisenberg, J. F. & Kleiman, D. G. (1972). Olfactory communication in mammals. Annu. Rev. Ecol. Systematics 3: 1-32 (1-5; 3-6).

Ellinwood, E. H. Jr & Escalante, O. (1970). Chronic amphetamine effect on the olfactory forebrain. Biol. Psychiatry. 2: 189-204 (5-12).

Elsner, R., Franklin, D. L., VanCitters, R. L. & Kenney, D. W. (1966). Cardiovascular defense against asphyxia. Science 153: 941-949 (3-6).

Esplin, D. W. & Zablocka-Esplin, B. (1969). Mechanisms of action of convulsants. In: Basic Mechanisms of the Epilepsies. 1st edn. pp.167-183, Discussion 184-193. Eds. Jasper, H. H., Ward, A. A. & Pope, A. Boston. Little, Brown & Co. (5-1).

Estable-Puig, J. F. & De Estable, R. F. (1969). Acute ultrastructural changes in the rat olfactory glomeruli after peripheral deafferentation. Exp. Neurol. 24: 592-602 (6-1).

Fanning, J. C. & Wilson, P. R. (1971). Observations on the structure of the trachea and lungs of the adult brush-tailed possum (*Trichosurus vulpecula*). J. Anat. 108: 214 (3-22).

Feldberg, W. & Vogt, M. (1948). Acetylcholine synthesis in different regions of the central nervous system. J. Physiol. (Lond.) 107: 372-381 (5-11).

Felix, D. & McLennan, H. (1971). The effect of bicuculline on the inhibition of mitral cells of the olfactory bulb. Brain Res. 25: 661-664 (4-12; 5-8).

Ferrer, N. G. (1969). Efferent projections of the anterior olfactory nucleus. J. Comp. Neurol. 137: 309-320 (1-14,15).

Ferrer, N. G. (1972). Projections of the olfactory tubercle in the golden hamster (*Mesocricetus auratus*). J. Hirnforsch 13: 203-210 (1-18).

Fink, R. P. & Heimer, L. (1967). Two methods for selective silver impregnation of degenerating axons and their synaptic endings in the central nervous system. Brain Res. 4: 369-374 (2-25).

Florio, V. & Longo, V. G. (1972). Electroencephalographic effects of bicuculline. Physiol. Behav. 9: 283-285 (5-24).

Frankenhaeuser, B. (1951). Limitations of method of strychnine neuronography. J. Neurophysiol. 14: 73-79 (5-2,18).

Freeman, W. J. (1959). Distribution in time and space of prepyriform electrical activity. J. Neurophysiol. 22: 644-665 (3-2).

Freeman, W. J. (1960*a*). Correlation of electrical activity of prepyriform cortex and behavior in cat. J. Neurophysiol. 23: 111-131 (3-2).

Freeman, W. J. (1960*b*). Repetitive electrical stimulation of prepyriform cortex in cat. J. Neurophysiol. 23: 383-396 (3-2; 4-31; 5-28).

Freeman, W. J. (1963). The electrical activity of a primary sensory cortex: analysis of EEG waves. Int. Rev. Neurobiol. 5: 53-119 (3-3,4).

Freeman, W. J. (1968*a*). Relations between unit activity and evoked potentials in prepyriform cortex of cats. J. Neurophysiol. 31: 337-348 (4-10).

Freeman, W. J. (1968). Effects of surgical isolation and tetanization on prepyriform cortex in cats. J. Neurophysiol. 31: 349-357 (3-28; 4-10).

Freeman, W. J. (1968*b*). Patterns of variation in waveform of averaged evoked potentials from prepyriform cortex of cats. J. Neurophysiol. 31: 1-13 (4-13).

Freeman, W. J. (1970). Spectral analysis of prepyriform averaged evoked potentials in cats. J. Biomed. Systems 1: 3-22 (A-2).

Freeman, W. J. (1972*a*). Spatial divergence and temporal dispersion in primary olfactory nerve of cat. J. Neurophysiol. 35: 733-744 (2-15).

Freeman, W. J. (1972*b*). Measurement of open-loop responses to electrical stimulation in olfactory bulb of cat. J. Neurophysiol. 35: 745-761 (2-15).

Freeman, W. J. (1972*c*). Measurement of oscillatory responses to electrical stimulation in olfactory bulb of cat. J. Neurophysiol. 35: 762-779. (2-15).

Freeman, W. J. (1972*d*). Depth recording of average evoked potential of olfactory bulb. J. Neurophysiol. 35: 780-796 (2-15).

Freeman, W. J. (1974). Topographic organization of primary olfactory nerve in cat and rabbit as shown by evoked potentials. Electroencephalogr. Clin. Neurophysiol. 36: 33-45 (1-7; 2-15).

Fujita, T., Ishikura, H. & Kitana, Y. (1972). The analgesic action and neuronal mechanism of propanidid. (Human and animal studies.) Br. J. Anaesth. 44: 809-816 (4-10).

Fukuda, T. (1966). Effects of stimulations of brain structures on the induced waves of the olfactory bulb and influences of drugs upon them. Fukuoka Acta. Med. 57: 916-929 (4-6,7,8,37; 5-2).

Gault, F. P. & Coustan, D. R. (1965). Nasal air flow and rhinencephalic activity. Electroencephalogr. Clin. Neurophysiol. 18: 617-624 (3-5).

Gault, F. P. & Leaton, R. N. (1963). Electrical activity of the olfactory system. Electroencephalogr. Clin. Neurophysiol. 15: 299-304 (3-5,28).

Gellhorn, E. (1953). On the physiological action of carbon dioxide on cortex and hypothalamus. Electroencephalogr. Clin. Neurophysiol. 5: 401-413 (3-30).

Geneser-Jensen, F. A. & Blackstad, T. W. (1971). Distribution of acetylcholinesterase in the hippocampal region of the guinea pig. I. Entorhinal area, parasubiculum, and presubiculum. Z. Zellforsch. Mikrosk. Anat. 114: 460-481 (6-3).

Gillilan, L. A. (1972). Blood supply to primitive mammalian brains. J. Comp. Neurol. 145: 209-222 (3-26).

Goldring, S., O'Leary, J. L., Holmes, T. G. & Jerva, M. J. (1961). Direct response of isolated cerebral cortex of cat. J. Neurophysiol. 24: 633-650 (4-13).

Golgi, C. (1873). Sulla struttura della sostanza grigia del cervello. Gazz. Med. Ital. 31: 244-246 (1-1).

Golgi, C. (1875). Sulla Fina Anatomia dei Bulbi Olfactorii. Reggio-Emilia. Cited by Cajal, 1911 (1-1,20).

Gordon, E. (1971). The acid-base balance and oxygen tension of the cerebrospinal fluid, and their implications for the treatment of patients with brain lesions. Acta. Anaesthiol. Scand. Suppl. 39: 1-36 (3-22).

Graham, L. T. Jr (1973). Distribution of glutamic acid decarboxylase activity and GABA content in the olfactory bulb. Life Sci. (1) 12: 443-447 (5-11).

Gray, P. A. Jr (1924). The cortical lamination pattern of the opossum, *Didelphys virginiana*. J. Comp. Neurol. 37: 221-263 (1-15,24).

Green, J. D., Mancia, M. & Baumgarten, R. von (1962). Recurrent inhibition in the olfactory bulb. I. Effects of antidromic stimulation of the lateral olfactory tract. J. Neurophysiol. 25: 461-488 (4-11; 5-7).

Gudden, B. A. (1870). Cited by Meyer, A., 1971 (1-24).

Guevara-Aguilar, R., Aguilar-Baturoni, H. U., Aréchiga, H. & Alcocer-Cuarón, C. (1973). Efferent evoked responses in the olfactory pathway of the cat. Electroencephalogr. Clin. Neurophysiol. 34: 23-32 (2-21,26; 6-5).

Haberly, L. B. (1973*a*). Unitary analysis of opossum prepyriform cortex. J. Neurophysiol. 36: 762-774 (2-2,12).

Haberly, L. B. (1973*b*). Summed potentials evoked in opossum prepyriform cortex. J. Neurophysiol. 36: 775-788 (2-2,12).

Haberly, L. B. & Shepherd, G. M. (1973). Current-density analysis of summed evoked potentials in opossum prepyriform cortex. J. Neurophysiol. 36: 789-802 (2-2,12).

Hagbarth, K-E. & Kerr, D. I. B. (1954). Central influences on spinal efferent conduction. J. Neurophysiol. 17: 295-307 (1-2,24; 3-2).

Hahn, F. (1962). Analeptics. Pharmacol. Rev. 12: 447-530.

Hall, E. (1972). The amygdala of the cat: a Golgi study. Z. Zellforsch. Mikrosk. Anat. 134: 439-458 (1-19).

Halpern, L. M. & Black, R. G. (1968). Gallamine triethiodide facilitation of local cortical excitability compared with other neuromuscular blocking agents. J. Pharmacol. Exp. Ther. 162: 166-173 (3-23).

Halpern, M. (1973). Olfactory bulb and accessory olfactory bulb projections in the snake, *Thamnophis sirtalis*. Anat. Rec. 175: 337 (1-13).

Hara, K. & Myers, R. E. (1973). Role of forebrain structures in emotional expression in opossum. Brain Res. 52: 131-144 (6-6).

Harrison, G. A. (1964). Ayre's T piece: a review of its modifications. Br. J. Anaesth. 36: 115 (2-3).

Haskell, A. R. & Voss, E. (1957). The pharmacology of tetramine (tetraethylenedisulphotetramine). J. Pharm. Sci. 46: 239-242 (5-29).

Haugen, F. P. & Melzack, R. (1957). The effects of nitrous oxide on responses evoked in the brain stem by tooth stimulation. Anesthesiology 18: 183-195 (4-16).

Heath, C. J. & Jones, E. G. (1971). Interhemispheric pathways in the absence of a corpus callosum. An experimental study of commissural connexions in the marsupial phalanger. J. Anat. 109: 253-270 (1-5,23; 2-21,25,26).

Heimer, L. (1968). Synaptic distribution of centripetal and centrifugal nerve fibres in the olfactory system of the rat. An experimental anatomical study. J. Anat. 103: 413-432 (1-13,14,18,20; 2-17; 6-4).

Heimer, L. (1969). The secondary olfactory connections in mammals, reptiles and sharks. Ann. N.Y. Acad. Sci. 167: 129-146 (1-12).

Hernández-Peón, R., Lavin, A., Alcocer-Cuarón, C. & Marcelin, J. P. (1960). Electrical activity of the olfactory bulb during wakefulness and sleep. Electroencephalogr. Clin. Neurophysiol. 12: 41-58 (2-28; 3-4,27,28; 4-3,5).

Herrick, C. L. (1892). The cerebrum and olfactories of the opossum *Didelphys virginica*. J. Comp. Neurol. 2: 1-20 (1-5,6).

Herrick, C. L. (1893). Laboratory notes from Denison University. VII. The callosum and hippocampal region in marsupial and lower brains. J. Comp. Neurol. 3: 176-182 (1-5).

Herrick, C. J. (1910). The morphology of the forebrain in amphibia and reptilia. J. Comp. Neurol. 20: 413-547 (1-13).

Herrick, C. J. (1924). The nucleus olfactorius anterior of the opossum. J. Comp. Neurol. 37: 317-359 (1-5,6,13,21,23; 6-2).

Hill, R. G., Simmonds, M. A. & Straughan, D. W. (1972). Convulsive properties of d-tubocurarine and cortical inhibition. Nature 240: 51-52 (5-21).

Hinds, J. W. (1968*a*). Autoradiographic study of histogenesis in the mouse olfactory bulb. I. Time of origin of neurons and neuroglia. J. Comp. Neurol. 134: 287-304 (1-6,7).

Hinds, J. W. (1968*b*). Autoradiographic study of histogenesis in the mouse olfactory bulb. II. Cell proliferation and migration. J. Comp. Neurol. 146: 253-276 (1-6,7).

Hinds, J. W. (1970). Reciprocal and serial dendrodendritic synapses in the glomerular layer of the rat olfactory bulb. Brain Res. 17: 530-534 (1-7).

Hinds, J. W. (1972*a*). Early neuron differentiation in the mouse olfactory bulb. I. Light microscopy. J. Comp. Neurol. 146: 233-252 (1-6,7).

Hinds, J. W. (1972*b*). Early neuron differentiation in the mouse olfactory bulb. II. Electron microscopy. J. Comp. Neurol. 146: 253-276 (1-6,7).

Hinds, J. W. & Ruffett, T. L. (1972). The development of the axon initial segment in mitral cells of the mouse olfactory bulb. Anat. Rec. 172: 330-331 (6-1).

Hinds, J. W. & Ruffett, T. L. (1973). Mitral cell development in the mouse olfactory bulb: reorientation of the perikaryon and maturation of the axon initial segment. J. Comp. Neurol. 151: 281-306 (6-1).

Hori, N. & Katsuda, N. (1972). Involvement of inhibitory mechanisms in the electrical activity of guinea pig olfactory cortex *in vitro*. Jap. J. Pharmacol. Suppl. 22: 71 (5-11).

Hosoya, Y. (1973). Electron microscopic observations of the granule cells (Calleja's island) in the olfactory tubercle of rats. Brain Res. 54: 330-334 (1-18).

Huggins, S. E., Parsons, L. C. & Pẽna, R. V. (1968). Further study of the spontaneous electrical activity of the brain of *Caiman sclerops:* olfactory lobes. Physiol. Zool. 41: 371-383 (3-22,30; 4-3,6).

Hughes, J. R., Andy, O. J., Hendrix D. E., Wang, C., Wetzel, N. & Peeler, D. (1972). Correlations between perceptual and electrophysiological responses from the human amygdala, olfactory bulb and tract. Electroencephalogr. Clin. Neurophysiol. 33: 241 (1-20).

Hughes, J. R. & Hendrix, D. E. (1967). The frequency component hypothesis in relation to the coding mechanism in the olfactory bulb. In: Olfaction and Taste II. pp.51-87. Ed. Hayashi, T. Wenner-Gren Symposium Series. Oxford. Pergamon Press (3-7).

Hughes, J. R. & Mazurowski, J. A. (1962*a*). Studies on the supracallosal mesial cortex of unanesthetized, conscious mammals. II. Monkey. B. Responses from the olfactory bulb. Electroencephalogr. Clin. Neurophysiol. 14: 635-645 (4-8).

Hughes, J. R. & Mazurowski, J. A. (1962*b*). Studies on the supracallosal mesial cortex of unanesthetized, conscious mammals. II. Monkey. C. Frequency analysis of responses from the olfactory bulb. Electroencephalogr. Clin. Neurophysiol. 14: 646-653 (4-5).

Hughes, J. R. & Mazurowski, J. A. (1964). Comparative studies on the frequency analysis of responses from the olfactory bulb of unanesthetized monkeys and rabbits. In: Proc. Conf. Data Acquisition and Processing in Biology and Medicine. Vol. 6. pp. 243-257. New York. Pergamon (4-5).

Huxley (1880). Cited by Jones, 1924*a* (1-2).

Itil, T., Keskiner, A., Han, H., Hsu, W. & Ulett, G. (1971). EEG changes after fluphenazine enanthate and decanoate based on analog power spectra and digital computer period analysis. Psychopharmacologia 20: 230-241 (A-2).

Iwase, Y., Uruha, M. & Ochi, J. (1961). Analysis of the olfactory bulb response induced by direct electrical stimulation in the rabbit. Jap. J. Physiol. 11: 507-519 (5-7).

Jalfre, M., Monachon, M. A. & Haefely, W. (1971). Effects on the amygdalo-hippocampal evoked potential in the cat of four benzodiazepines and some other psychotropic drugs. Arch. Pharmakol. 270: 180-191 (4-10,11).

Johnston, G. A. R. & Curtis, D. R. (1972). Y-aminobutyrylcholine and central inhibition. J. Pharm. Pharmacol. 24: 251-252 (5-29).

Johnston, J. B. (1913). The morphology of the septum, hippocampus, and pallial commissures in reptiles and mammals. J. Comp. Neurol. 23: 371-478 (1-5,23).

Johnston, J. B. (1923). Further contributions to the study of the evolution of the forebrain. Parts I-IV. J. Comp. Neurol. 35: 337-481. Part V. J. Comp. Neurol. 36: 143-192 (1-5,17,18,19,22; 2-26).

Jones, F. W. (1924). Unscientific Essays. XXI. Of Sights and Scents. pp. 190-197. London. Edward Arnold & Co. (1-1; 3-6).

Jones, F. W. (1924*a*). The Mammals of South Australia. Part II. The Bandicoots and the Herbivorous Marsupials. pp.133-270. Adelaide. Government Printer (1-2,3,4; 3-6).

Joy, R. M., Hance, A. J. & Killam, K. F. Jr (1971). Spectral analysis of long EEG samples for comparative purposes. Neuropharmacol. 10: 471-481 (4-38).

Julien, R. M. (1973). Lidocaine in experimental epilepsy: correlation of anticonvulsant effect with blood concentrations. Electroencephalogr. Clin. Neurophysiol. 34: 639-645 (5-3).

Katsuda, N. & Hori, N. (1972). Effect of procaine, lidocaine and pentobarbital on electrical activity of guinea-pig olfactory cortex *in vitro*. Jap. J. Pharmacol. Suppl. 22: 70 (5-11).

Kavan, E. M., Besson, J.-M. & Aleonard, P. (1971). Effect of propanidid on evoked cortical and subcortical responses in the cat. Acta. Anaesthesiol. Scand. 15: 267-276 (4-32).

Kawabata, N. (1973). A nonstationary analysis of the electroencephalogram. IEEE Trans. Biomed. Eng. BME-20: 444-452 (A-1).

Kerr, D. I. B. (1960). Properties of the olfactory efferent system. Aust. J. Exp. Biol. Med. Sci. 38: 29-36 (3-2,27).

Kerr, D. I. B. & Hagbarth, K-E. (1955). An investigation of olfactory centrifugal fiber system. J. Neurophysiol. 18: 362-374 (1-26; 2-1; 3-2; 4-4,6,9,10,30).

Khazan, N., Kandalaft, I. & Sulman, F. G. (1967). The EEG of the olfactory bulb of the rabbit and its reaction to psychopharmacological agents. Psychopharmacologia 10: 226-236 (4-6,7; 5-7).

Kim, S. U. (1972). Light and electron microscope study of neurons and synapses in neonatal mouse olfactory bulb cultured *in vitro*. Exp. Neurol. 36: 336-349 (6-1).

Kitahata, L. M., McAllister, R. G. & Taub, A. (1973). Identification of central trigeminal nociceptors and the effects of nitrous oxide. Anesthesiology 38: 12-19 (4-16).

Kleiner, B., Flühler, H., Huber, P. J. & Dumermuth, G. (1970). Spectrum analysis of the electroencephalogram. Comput. Programs Biomed. 1: 183-197 (A-1).

Komisaruk, B. R. & Beyer, C. (1972). Responses of diencephalic neurons to olfactory bulb stimulation, odor, and arousal. Brain Res. 36: 153-170 (6-6).

Krettek, J. E. & Price, J. L. (1974). A direct input from the amygdala to the thalamus and the cerebral cortex. Brain Res. 67: 169-174 (6-3).

Land, L. J. (1973). Localized projection of olfactory nerves to rabbit olfactory bulb. Brain Res. 63: 153-166 (1-7).

Land, L. J., Eager, R. P. & Shepherd, G. M. (1970). Olfactory nerve projections to the olfactory bulb in rabbit: demonstration by means of a simplified ammoniacal silver degeneration method. Brain Res. 23: 250-254 (1-7).

Lavin, A., Alcocer-Cuarón, C. & Hernández-Peón, R. (1959). Centrifugal arousal in the olfactory bulb. Science 129: 332-333 (2-28; 3-5).

Lee, J. A. & Atkinson, R. S. (1973). A Synopsis of Anaesthesia. 7th edn. pp.991. Bristol. John Wright & Sons.

Legge, K. F., Randić, M. & Straughan, D. W. (1966). The pharmacology of neurones in the pyriform cortex. Br. J. Pharmacol. 26: 87-107 (4-12).

Le Gros Clark, W. E. & Meyer, M. (1947). The terminal connections of the olfactory tract in the rabbit. Brain 70: 304-328 (1-18).

Leonard, C. A. & Harrison, J. W. E. (1953). The analeptic activity of 6 convulsant barbiturates in acute barbiturate poisoning in mice. Am. J. Pharm. 125: 157-162 (4-31; 5-29).

Leonard, C. M. & Scott, J. W. (1971). Origin and distribution of the amygdalofugal pathways in the rat: an experimental neuroanatomical study. J. Comp. Neurol. 141: 313-330 (1-19).

Levine, D. A., Elashoff, R., Callaway, E. III, Payne, D. & Jones, R. T. (1972). Evoked potential analysis by complex demodulation. Electroencephalogr. Clin. Neurophysiol. 32: 513-520 (A-2).

Levy, R. A. & Anderson, E. G. (1973). Bicuculline and picrotoxin blockade of positive dorsal root potentials. Nature (New Biol.) 241: 156-158 (5-21).

Lohman, A. H. M. (1963). The anterior olfactory lobe of the guinea pig. A descriptive and experimental anatomical study. Acta. Anat. (Basel) 53 Suppl. 49: 1-109 (1-13,14,18,20,24).

Lohman, A. H. & Lammers, H. J. (1967). On the structure and fibre connections of the olfactory centres in mammals. Progr. Brain Res. 23: 65-81 (1-14).

Lohman, A. H. M. & Mentink, G. M. (1969). The lateral olfactory tract, the anterior commissure, and the cells of the olfactory bulb. Brain Res. 12: 396-413 (1-12,13,14).

Loo, Y. T. (1931). The forebrain of the opossum, *Didelphis virginiana*. Part II. Histology. J. Comp. Neurol. 52: 1-148 (1-5,20,22,23; 2-26).

Lynch, G., Gall, C., Mensah, P. & Cotman, C. W. (1974). Horseradish peroxidase histochemistry: a new method for tracing efferent projections in the central nervous system. Brain Res. 65: 373-380 (6-3).

McCotter, R. E. (1912). The connection of the vomeronasal nerves with the accessory olfactory bulb in the opossum and other mammals. Anat. Rec. 6: 299-318 (1-5,6,12).

MacLean, P. D. & Delgado, J. M. R. (1953). Electrical and chemical stimulation of fronto temporal portion of limbic system in the waking animal. Electroencephalogr. Clin. Neurophysiol. 5: 91-100 (4-12).

MacLean, P. D., Rosner, B. S. & Robinson, F. (1957). Pyriform responses to electrical stimulation of olfactory fila, bulb and tract. Am. J. Physiol. 189: 395-400 (5-7).

McLennan, H. (1971). The pharmacology of inhibition of mitral cells in the olfactory bulb. Brain Res. 29: 177-184 (5-9).

McLennan, H. & Graystone, P. (1965). The electrical activity of the amygdala, and its relationship to that of the olfactory bulb. Can. J. Physiol. Pharmacol. 43: 1009-1017 (3-25).

MacLeod, P. (1971). Structure and Function of Higher Olfactory Centers. In: Handbook of Sensory Physiology IV. Chemical Senses, 1. Olfaction. pp.152-204. Ed. Beidler, L. M. Berlin. Springer Verlag (1-24,26).

Marshall, E. K. Jr, Walzl, E. M. & LeMessurier, D. H. (1937). Picrotoxin as a respiratory stimulant. J. Pharmacol. Exp. Ther. 60: 472-486 (5-21).

Mechelse, K. & Lieuwens, W. H. G. (1969). Vigilance and rhinencephalic burst activity. Psychiatr. Neurol. Neurochir. 72: 97-108 (3-4,5).

Meldrum, B. S. & Horton, R. W. (1971). Convulsive effects of 4-deoxypyridoxine and of bicuculline in photosensitive baboons (*Papio papio*) and in rhesus monkeys (*Macaca umlatta*). Brain Res. 35: 419-436 (5-24).

Meyer, A. (1971). Historical Aspects of Cerebral Anatomy. Part 2. The 'Olfactory Brain'. pp.75-118. London. Oxford University Press.

Morest, D. K. (1970). A study of neurogenesis in the forebrain of opossum pouch young. Z. Anat. Entwicklungsgesch. 130: 265-305 (1-6; 3-7; 6-1).

Morest, D. K. & Morest, R. R. (1966). Perfusion-fixation of the brain with chrome-osmium solutions for the rapid Golgi method. Am. J. Anat. 118: 811-832 (1-6).

Morgane, P. J. & Stern, W. C. (1972). The chemistry of the medial forebrain bundle. Anat. Rec. 172: 369 (6-3).

Mori, K., Kawamata, M., Miyajima, S. & Fujita, M. (1972). The effects of several anesthetic agents on the neuronal reactive properties of thalamic relay nuclei in the cat. Anesthesiology 36: 550-557 (4-16).

Morris, M. E. (1971). The action of carbon dioxide on afferent transmission in the dorsal column-medial lemniscal system. J. Physiol. (Lond.) 218: 651-669 (3-30).

Morrison, R. G. B. (1969). Comparative studies on the olfactory system of the mammal. Ph.D. Thesis. Department of Human Physiology, University of Adelaide (1-13,20,24; 2-1,15,19).

Motokizawa, F. (1974). Olfactory input to the thalamus: electrophysiological evidence. Brain Res. 67: 334-337 (6-5).

Motokizawa, F. & Furuya, N. (1973). Neural pathway associated with the EEG arousal response by olfactory stimulation. Electroencephalogr. Clin. Neurophysiol. 35: 83-91 (3-5).

Moulton, D. G. (1963). Electrical activity in the olfactory system of rabbits with indwelling electrodes. In: Olfaction and Taste I. pp.71-84. Ed. Zotterman, Y. Wenner-Gren Symposium Series. Oxford. Pergamon Press (3-3).

Moulton, D. G. (1967). Olfaction in mammals. Am. Zoologist 7: 421-429 (1-5).

Moulton, D. G. & Beidler, L. M. (1967). Structure and function in the peripheral olfactory system. Physiol. Rev. 47: 1-52 (1-24,25).

Mozell, M. M. (1962). Olfactory and neural responses in the frog. Am. J. Physiol. 203: 353-358 (1-25).

Munson, E. S., Martucci, R. W. & Wagman, I. H. (1973). Bupivicaine and lignocaine induced seizures in rhesus monkeys. Br. J. Anaesth. 44: 1025-1029 (5-5).

Munson, E. S., Pugno, P. A. & Wagman, I. H. (1972). Does oxygen protect against local anesthetic toxicity? Anesth. Analg. (Cleve.) 51: 422-427 (5-6).

Munson, E. S. & Wagman, I. H. (1973). Elevation of lidocaine seizure threshold by gallamine in rhesus monkeys. Arch. Neurol. 28: 329-333 (3-30; 5-6).

Nauta, W. J. H. & Gygax, P. A. (1954). Silver impregnation of degenerating axons in the central nervous system: a modified technic. Stain Technol. 29: 91-93 (2-25).

Neidle, A., Kandera, J. & Lajtha, A. (1973). The uptake of amino acids by the intact olfactory bulb of the mouse: a comparison with tissue slice preparations. J. Neurochem. 20: 1181-1193 (5-11).

Ngai, S. H. (1972). Clinical monographs. 3.1. Halothane. Handbuch Exp. Pharmakol. 30: 33-76 (4-23).

Nicoll, R. A. (1969). Inhibitory mechanisms in the rabbit olfactory bulb: Dendrodendritic mechanisms. Brain Res. 14: 157-172 (2-10,12; 4-8,9; 5-7).

Nicoll, R. A. (1970*a*). Recurrent excitatory pathways of anterior commissure and mitral cell axons in the olfactory bulb. Brain Res. 19: 491-493 (2-12).

Nicoll, R. A. (1970*b*). Identification of tufted cells in the olfactory bulb. Nature 227: 623-625 (2-12; 4-30).

Nicoll, R. A. (1970*c*). GABA and dendrodendritic inhibition in the olfactory bulb. Pharmacologist 12: 236 (4-12; 5-22).

Nicoll, R. A. (1971*b*). Pharmacological evidence for GABA as the transmitter in granule cell inhibition in the olfactory bulb. Brain Res. 35: 137-149 (4-12; 5-8,10,19,22).

Nicoll, R. A. (1972*a*). Olfactory nerves and their excitatory action in the olfactory bulb. Exp. Brain Res. 14: 185-197 (4-9).

Nicoll, R. A. (1972*b*). The effects of anaesthetics on synaptic excitation and inhibition in the olfactory bulb. J. Physiol. (Lond.) 223: 803-814 (4-9,31,38; 6-4).

Nieuwenhuys, R. (1967). Comparative anatomy of olfactory centres and tracts. Prog. Brain Res. 23: 1-64 (1-6).

Nunuz, P. L. (1973). Representation of evoked potentials by Fourier-Bessel expansions. IEEE Trans. Biomed. Eng. BME-20: 372-374 (A-2).

Obenchain, J. B. (1925). The brains of the South American marsupials *Caenolestes* and *Orolestes*. Field Museum Natural History 14: 175-232 (Pub. 224, Zool. series). (1-5,19).

Ochi, J. (1963). Olfactory bulb response to antidromic olfactory tract stimulation in the rabbit. Jap. J. Physiol. 13: 113-128 (4-11).

O'Leary, J. L. (1937). Structure of the primary olfactory cortex of the mouse. J. Comp. Neurol. 67: 1-31 (1-15).

Oswaldo-Cruz, E. & Rocha-Miranda, C. E. (1968). The brain of the opossum (*Didelphis marsupialis*). A cytoarchetectonic atlas in stereotaxic coordinates. pp.99. Rio de Janeiro. Instituto de Biofisica (1-18,19).

Ottoson, D. (1954). Sustained potentials evoked by olfactory stimulation. Acta. Physiol. Scand. 32: 384-386 (3-1,16).

Ottoson, D. (1956). Analysis of the electrical activity of the olfactory epithelium. Acta. Physiol. Scand. 35; Suppl. 122: 1-83 (4-3).

Ottoson, D. (1959*a*). Studies on slow potentials in the rabbit's olfactory bulb and nasal mucosa. Acta. Physiol. Scand. 47: 136-148 (4-7).

Ottoson, D. & Shepherd, G. M. (1967). Experiments and concepts in olfactory physiology. Progr. Brain Res. 23: 83-138 (1-24).

Pagano, R. R. (1966). The effects of central stimulation and nasal air flow on induced activity of olfactory structures. Electroencephalogr. Clin. Neurophysiol. 21: 269-277 (3-5,21).

Pagano, R. R. & Gault, F. P. (1964). Amygdala activity: a central measure of arousal. Electroencephalogr. Clin. Neurophysiol. 17: 255-260 (1-20).

Parbrook, G. D. (1967). The levels of nitrous oxide analgesia. Br. J. Anaesth. 39: 974-982 (4-16).

Peñaloza-Rojas, J. H. & Alcocer-Cuarón, C. (1967). The electrical activity of the olfactory bulb in cats with nasal and tracheal breathing. Electroencephalogr. Clin. Neurophysiol. 22: 468-472 (3-5).

Peñaloza-Rojas, J. H. & Zeidenweber, J. (1965). Local and EEG effects of adrenaline and acetylcholine within the olfactory bulb. Electroencephalogr. Clin. Neurophysiol. 19: 88-90 (4-12).

Petsche, H. (1970). The quantitative analysis of EEG data. Prog. Brain Res. 33: 63-86 (2-14).

Phillips, D. S. & Michels, K. M. (1964). Selective stimulation and electrophysiological responses of the olfactory bulb of the opossum. Percept. Mot. Skills 18: 63-69 (6-6).

Phillips, C. G., Powell, T. P. S. & Shepherd, G. M. (1963). Responses of mitral cells to stimulation of the lateral olfactory tract in the rabbit. J. Physiol. (Lond.) 168: 65-88 (2-13).

Pigache, R. M. (1970). The anatomy of "Paleocortex". A critical review. Ergeb. Anat. Entwicklungsgesch 43: 7-62 (1-4,13,17,18,20,24,26; 2-15; 3-5).

Pinching, A. J. & Brooke, R. N. L. (1973). Electron microscopy of single cells in the olfactory bulb using Golgi impregnation. J. Neurocytol. 2: 157-170 (1-10).

Pinching, A. J. & Powell, T. P. S. (1971*a*). Ultrastructural features of transneuronal cell degeneration in the olfactory system. J. Cell. Sci. 8: 253-287 (1-6,7,8,9,10,27).

Pinching, A. J. & Powell, T. P. S. (1971*b*). The neuron types of the glomerular layer of the olfactory bulb. J. Cell. Sci. 9: 305-345 (1-6,7,8,9,27).

Pinching, A. J. & Powell, T. P. S. (1971*c*). The neuropil of the glomeruli of the olfactory bulb. J. Cell. Sci. 9: 347-377 (1-6,7, 9,27).

Pinching, A. J. & Powell, T. P. S. (1971*d*). The neuropil of the periglomerular region of the olfactory bulb. J. Cell. Sci. 9: 379-409 (1-6,7).

Pinching, A. J. & Powell, T. P. S. (1972*a*). A study of terminal degeneration in the olfactory bulb of the rat. J. Cell. Sci. 10: 585-619 (1-7).

Pinching, A. J. & Powell, T. P. S. (1972*b*). The termination of centrifugal fibres in the glomerular layer of the olfactory bulb. J. Cell. Sci. 10: 621-635 (1-7).

Pinching, A. J. & Powell, T. P. S. (1972*c*). Experimental studies on the axons intrinsic to the glomerular layer of the olfactory bulb. J. Cell. Sci. 10: 637-655 (1-7).

Polson, P. (1971). An analysis of the electrical activity of the mammalian olfactory system. Ph.D. Thesis. Department of Electrical Engineering, University of Adelaide. (1-24,26,27; 2-2,5,7,9,10,12,13, 14,15,16; 3-25,29; 4-35; 6-4; A-1).

Popper, K. R. (1963). Science: Problems, aims, responsibilities. Fed. Proc. 22: 961-972 (Intro).

Powell, T. P. S. Cowan, W. M. & Raisman, G. (1965). The central olfactory connexions. J. Anat. (Lond.) 99: 791-813 (1-18,20,25).

Price, J. L. (1968*a*). The termination of centrifugal fibres in the olfactory bulb. Brain Res. 7: 483-486 (1-17).

Price, J. L. (1969*a*). The origin of the centrifugal fibres to the olfactory bulb. Brain Res. 14: 542-545 (1-17,26).

Price, J. L. & Powell, T. P. S. (1970*a*). The morphology of the granule cells of the olfactory bulb. J. Cell. Sci. 7: 91-123 (1-6,24,27).

Price, J. L. & Powell, T. P. S. (1970*b*). The synaptology of the granule cells of the olfactory bulb. J. Cell. Sci. 7: 125-155 (1-6,11,24,27).

Price, J. L. & Powell, T. P. S. (1970*c*). An electron-microscopic study of the termination of the afferent fibres to the olfactory bulb from the cerebral hemisphere. J. Cell. Sci. 7: 157-187 (1-6,8,14,24,26, 27; 2-19).

Price, J. L. & Powell, T. P. S. (1970*d*). The mitral and short axon cells of the olfactory bulb. J. Cell. Sci. 7: 631-651 (1-6,10,24,27).

Price, J. L. & Powell, T. P. S. (1970*e*). An experimental study of the origin and the course of the centrifugal fibres to the olfactory bulb. J. Anat. 107: 215-237 (1-6,14,17,24,26,27; 2-19).

Price, J. L. & Powell, T. P. S. (1970*f*). The afferent connexions of the nucleus of the horizontal limb of the diagonal band. J. Anat. 107: 239-256 (1-6,17).

Price, J. L. & Powell, T. P. S. (1971). Certain observations on the olfactory pathway. J. Anat. 110: 105-126 (1-6,18).

Priestly, M. B. (1967). Power spectral analysis of non-stationary random processes. J. Sound Vibrat. 6: 86-97 (A-1).

Prince, D. A. (1972). Topical convulsant drugs and metabolic antagonists. In: Experimental Models of Epilepsy - A Manual for the Laboratory Worker. pp.51-83. Ed. Purpura, D. P., Penry, J. K., Tower, D. B., Woodbury, D. M. & Walter, R. D. New York. Raven Press. (5-2,21,29).

Prince, D. A. & Wagman, I. H. (1966). Activation of limbic system epileptogenic foci with intravenous lidocaine. Electroencephalogr. Clin. Neurophysiol. 21: 416 (5-3).

Purpura, D. P., Penry, J. K., Tower, D. B., Woodbury, D. M. & Walter, R. D. (1972). Editorial Preface. Experimental Models of Epilepsy - A Manual for the Laboratory Worker. New York. Raven Press (5-1).

Putnam, S. J. & Cone, D. M. (1966). Terminal connections of the olfactory tract fibers in the opossum, *Didelphis virginiana*. Anat. Rec. 154: 405. (1-5; 2-21,24).

Raisman, G. (1972). An experimental study of the projection of the amygdala to the accessory olfactory bulb and its relationship to the concept of a dual olfactory system. Exp. Brain Res. 14: 395-408 (1-12; 2-17).

Raisman, G., Cowan, W. M. & Powell, T. P. S. (1965). The extrinsic afferent, commissural and association fibres of the hippocampus. Brain 88: 963-996 (2-25).

Rall, W. & Shepherd, G. M. (1968). Theoretical reconstruction of field potentials and dendrodendritic synaptic interactions in olfactory bulb. J. Neurophysiol. 31: 884-915 (1-27; 2-10,12,13,14; 4-8).

Rall, W., Shepherd, G. M., Reese, T. S. & Brightman, M. W. (1966). Dendro-dendritic synaptic pathway for inhibition in the olfactory bulb. Exp. Neurol. 14: 44-56 (1-11; 2-12; 4-8,11).

Ralls, K. (1971). Mammalian scent marking. Science 171: 443-449 (1-5).

Ramon-Moliner, E. (1973). Presynaptic perikarya in olfactory bulb of guinea pig. Brain Res. 63: 351-356 (1-11).

Randić, M. & Straughan, D. W. (1965). Iontophoretic study of palaeocortical neurones. J. Physiol. (Lond.) 177: 67-68 (4-12).

Ratliff, F. (1972). Contour and contrast. Sci. Am. 226: 90-101 (3-14).

Rausch, L. J. & Long, C. J. (1971). Habenular nuclei: a crucial link between the olfactory and motor systems. Brain Res. 29: 146-150 (6-3).

Reese, T. S. & Brightman, M. W. (1965). Electronmicroscopic studies on the rat olfactory bulb. Anat. Rec. 151: 492 (1-7).

Reese, T. S. & Brightman, M. W. (1970). Olfactory surface and central olfactory connections in some vertebrates. In: Taste and Smell in Vertebrates. Ciba Foundation Symposium.
Eds Wolstenholme, G. E. W. & Knight, J. London. Churchill (1-7).

Riblet, L. A. & Tuttle, W. W. (1970). Investigation of the amygdaloid and olfactory electrographic response in the cat after toxic dosage of lidocaine. Electroencephalogr. Clin. Neurophysiol. 28: 601-608 (5-5).

Richards, C. D. (1972*a*). Potentiation and depression of synaptic transmission in the olfactory cortex of the guinea-pig. J. Physiol. (Lond.) 222: 209-231 (4-12).

Richards, C. D. (1972*b*). On the mechanism of barbiturate anaesthesia. J. Physiol. (Lond.) 227: 749-767 (4-13,27,38).

Richards, C. D. (1973). On the mechanism of halothane anaesthesia. J. Physiol. (Lond.) 233: 439-456 (4-13).

Richards, C. D. & Sercombe, R. (1968). Electrical activity observed in guinea-pig olfactory cortex maintained *in vitro*. J. Physiol. (Lond.) 197: 667-683 (4-12).

Richards, C. D. & Sercombe, R. (1970). Calcium, magnesium, and the electrical activity of guinea-pig olfactory cortex *in vitro*. J. Physiol. (Lond.) 211: 571-584 (4-12).

Rolls, E. T. (1972). Activation of amygdaloid neurones in reward, eating and drinking elicited by electrical stimulation of the brain. Brain Res. 45: 365-381 (1-20).

Rose, M. (1912). Histologische Lokalisation der Grosshirnrinde bei kleinen Säugetieren (Rodentia, Insectivora, Chiroptera). J. Psychol. Neurol. 19: 391-479 (2-24).

Rose, M. (1926). Über das histogenetische Prinzip der Einteilung der Grosshirnrinde. J. Psychol. Neurol. 32: 97-160 (1-6).

Rosner, B. S. & Clark, D. L. (1973). Neurophysiologic effects of general anesthetics: II. Sequential regional actions in the brain. Anesthesiology 39: 59-81 (4-16).

Salmoiraghi, G. C. & Nicoll, R. A. (1968). Effects of drugs on responses in the olfactory bulb. In: Drugs and Sensory Function. pp.73-89. Ed. A. Herxheimer. London. Churchill. (5-8).

Sasa, M., Nakai, Y. & Takaori, S. (1967). Effects of volatile anesthetics on the evoked potentials and unitary discharges in the central auditory system caused by click stimuli in cats. Jap. J. Pharmacol. 17: 364-380 (4-3,16).

Scalia, F. (1968). A review of recent experimental studies on the distribution of the olfactory tracts in mammals. Brain Behav. Evol. 1: 101-123 (1-24).

Schallek, W., Lewinson, T. & Thomas, J. (1967). Power spectrum analysis of drug effects on electroencephalogram of cat. (Int. J.) Neuropharmacol. 6: 253-264 (A-2).

Schallek, W., Lewinson, T. & Thomas, J. (1968). Power spectrum analysis as a tool for statistical evaluation of drug effects on electrical activity of brain. (Int. J.) Neuropharmacol. 7: 35-46 (A-2).

Schneider, R. A. (1971). The sense of smell and human sexuality. Med. Aspects Human Sexuality May 1971: 156-168 (1-5; 3-6).

Scholander, P. F. (1947). Analyzer for accurate estimation of respiratory gases in one-half cubic centimetre samples. J. Biol. Chem. 167: 235-250 (3-13).

Schwartze, P. (1970*a*). Beiträge zur Steurung der Bursttätigkeit des Bulbus olfactorius. I. Die Olfactoriusbursts unter Urethannarkose und nach sensorischen Reizen beim Kaninchen. Acta. Biol. Med. Ger. 25: 127-138 (3-4; 4-7).

Schwartze, P. (1970*b*). Beiträge zur Steurung der Bursttätigkeit des Bulbus olfactorius. II. Die Olfactoriusbursts unter Hypoxie, nach Hirnreizungen und nach Durchschneidung des Gehirns in verscheidenen Ebenen beim Kaninchen. Acta. Biol. Med. Ger. 25: 139-150 (3-4,29).

Schwartze, P. & Schönfelder, J. (1973). Beiträge zur Stenerung der Bursttätigkeit des Bulbus olfactorius. IV. Die Ontogenese der Bulbus-olfactorius. Bursttätigkeit bei Katze und Meerschweinchen. Acta. Biol. Med. Ger. 30: 709-717 (3-7) (appearing incorrectly in the text as "Schwartze, 1973").

Scott, J. W. (1973). Analysis of the responses of lateral hypothalamic neurons to olfactory bulb shock. Anat. Rec. 175: 438-439 (6-3).

Scott, J. W. & Leonard, C. M. (1971). The olfactory connections of the lateral hypothalamus in the rat, mouse and hamster. J. Comp. Neurol. 141: 331-344 (6-3).

Scott, J. W. & Pfaffmann, C. (1967). Olfactory inputto the hypothalamus: electrophysiological evidence. Science 158: 1592-1594 (6-3).

Secher, O. (1971). Physical and chemical data on anaesthetics. Acta. Anaesth. Scand. Suppl. 42: 1-95 (3-21).

Sharer, L. & Kutt, H. (1971). Intravenous administration of diazepam. Effects on penicillin-induced focal seizures in the cat. Arch. Neurol. 24: 169-175 (4-11,38).

Sharpless, S. K. (1970). Hypnotics and Sedatives. 1. The barbiturates. In: The Pharmacological Basis of Therapeutics. 4th edn. pp.98-120. Eds. Goodman, L. S. & Gilman, A. London. Collier-Macmillan (4-27).

Shepherd, G. M. (1966). The orientation of mitral cell dendrites. Exp. Neurol. 14: 390-395 (1-10).

Shepherd, G. M. (1971). Physiological evidence for dendrodendritic synaptic interactions in the rabbit's olfactory glomerulus. Brain Res. 32: 212-217 (1-9).

Shepherd, G. M. (1972). Synaptic organization of the mammalian olfactory bulb. Physiol. Rev. 52: 864-917 (1-6,20,24,27).

Shepherd, G. M. & Haberly, L. B. (1970). Partial activation of olfactory bulb: Analysis of field potentials and topographic relation between bulb and lateral olfactory tract. J. Neurophysiol. 33: 643-653 (2-2,14,17; 6-4).

Shimoji, K. & Bickford, R. G. (1971). Differential effects of anesthetics on mesencephalic reticular neurons: II. Responses to repetitive somatosensory electrical stimulation. Anesthesiology 35: 76-80 (4-16).

Shute, C. C. D. & Lewis, P. R. (1967). The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections. Brain 90: 497-520 (6-1).

Simpson, J. Y. (1847). Cited by Lee & Atkinson, 1973 (4-20).

Smith, G. E. (1894). A preliminary communication upon the cerebral commissures of the mammalia, with special reference to the monotremata and marsupialia (with 5 figures). Proc. Linn. Soc. New S. Wales 9: 635-657 (1-5).

Smith, G. E. (1895). The comparative anatomy of the cerebrum of *Notoryctes typhlops*. Trans. R. Soc. S. Aust. 1895: 167-193 (1-5).

Smith, G. E. (1902). On a peculiarity of the cerebral commissures in certain marsupialia, not hitherto recognised as a distinctive feature of the Diprotodontia. Proc. R. Soc. Lond. (Biol.) 70: 226-231 (1-3,5,23).

Smith, W. D. A. (1971). Pharmacology of nitrous oxide. Int. Anesth. Clin. 9(3): 91-123 (4-16).

Spencer, G. T. (1971). Tracheostomy and endotracheal intubation in the intensive care unit. In: General Anaesthesia. 3rd edn. Vol. 2. pp.553-572. Eds. T. C. Gray & J. F. Nunn. London. Butterworths (3-23).

Sprenkel, H. B. van der (1926). Stria terminalis and amygdala in the brain of the opossum (*Didelphis virginiana*). J. Comp. Neurol. 42: 211-254 (1-5).

Sternbach, L. H., Randall, L. O., Banziger, R. & Lehr, H. (1968). Structure-activity relationships in the 1,4-benzodiazepine series. In: Drugs Affecting the Central Nervous System. Vol. 2. pp.237-264. Ed. Burger, A. London. Edward Arnold (4-36).

Stevens, C. F. (1969). Structure of cat frontal olfactory cortex. J. Neurophysiol. 32: 184-192 (1-25).

Stoelting, R. K. & Eger, E. I. II (1969). An additional explanation for the second gas effect: a concentrating effect. Anesthesiology 30: 273-277 (2-3).

Stone, H., Carregal, E. J. A. & Williams, B. (1966). The olfactory-trigeminal response to odorants. Life Sci. (1) 5: 2195-2201 (3-6).

Stone, H., Williams, B. & Carregal, E. J. A. (1968). The role of the trigeminal nerve in olfaction. Exp. Neurol. 21: 11-19 (3-6).

Stratten, W. P. & Barnes, C. D. (1971). Diazepam and presynaptic inhibition. Neuropharmacol. 10: 685-696 (4-11).

Straw, R. N. (1969). Lack of effect of paralyzation with gallamine and decamethonium on duration of hippocampal and amygdaloid after-discharge. Proc. Soc. Exp. Biol. Med. 130: 291-294 (3-23).

Swanson, E. E. (1934). Short acting barbituric acid and derivatives. Proc. Soc. Exp. Biol. Med. 31: 963-964 (5-29).

Swanson, E. E. & Chen, K. K. (1939). The aberrant action of sodium 1:3-dimethylbutylethylbarbiturate. J. Pharm. Pharmacol. 12: 657-660 (4-27).

Switzer, R. C. (1972). Quantitative relationships of the tufted cells, mitral cells, discoids of internal granule cells and subvolumes of olfactory bulbs in the marsupial family Dasyuridae. Anat. Rec. 172: 415 (6-1).

Thompson, J. A. & Pears, F. N. (1962). The functions of the anal glands of the brushtail possum. Victorian Naturalist 78: 306-308 (1-4).

Timiras, P. S., Woolley, D. E., Silva, A. J. & Williams, B. (1967). Changes in electrical activity of the olfactory cortex induced by radiation and drugs. Radiat. Res. 30: 391-403 (4-6; 5-6).

Torda, T. A. G. & O'Brien, D. (1971). Electroencephalographic and electrocardiographic effects of propanidid in man. Anaesthesia 26: 429-435 (4-32).

Tsai, C. (1925). The optic tracts and centers of the opossum, *Didelphis virginiana*. J. Comp. Neurol. 39: 173-216 (1-23).

Tucker, D. (1963*a*). Physical variables in the olfactory stimulation process. J. Gen. Physiol. 46: 453-489 (3-6,23).

Tucker, D. (1963*b*). Olfactory, vomeronasal and trigeminal receptor responses to odorants. In: Olfaction and Taste. I. pp.45-69. Ed. Zotterman, Y. Wenner-Gren Symposium Series. Oxford. Pergamon Press (3-6).

Tucker, D. (1971). Nonolfactory responses from the nasal cavity: Jacobson's organ and the trigeminal system. In: Handbook of Sensory Physiology. Vol. IV. Chemical Senses. Part 1. Olfaction. pp.151-181. Ed. L. M. Biedler. Berlin. Springer-Verlag (3-6,31).

Tuttle, W. W. & Elliott, H. W. (1969). Electrographic and behavioural study of convulsants in the cat. Anesthesiology 30: 48-64 (5-3,5,12).

Ueki, S. & Domino, E. F. (1961). Some evidence for a mechanical receptor in olfactory function. J. Neurophysiol. 24: 12-25 (3-5,23,25).

Ueki, S. & Fukuda, T. (1966). Effects of central nervous stimulants on the electrical activity of the olfactory bulb in rabbits. Fukuoka Acta. Med. 57: 909-915 (4-5; 5-2,6,18,30).

Ueki, S., Tanaka, K. & Sugano, H. (1964). Pharmacological studies on the electrical activities of the olfactory bulb. Jap. J. Pharmacol. 14: 80-93 (4-5,7; 5-6,30).

Ulinski, P. S. (1971). External morphology of pouch young opossum brains: a profile of opossum neurogenesis. J. Comp. Neurol. 142: 33-58 (3-7; 6-1).

Vaccarezza, O. L. & Affanni, J. M. (1964). Actividad bioelectrica del bulbo olfactorio del marsupial *Didelphis azarae*. Rev. Soc. Argent. Biol. 40: 9-13 (3-7,26).

Vaccarezza, O. L. & Affanni, J. M. (1966). Activite bioelectrique du bulbe olfactif de l'Opossum, *Didelphis azarae*. C.R. Soc. Biol. (Paris) 160: 691-693 (3-7,8).

Valverde, F. (1965). Studies on the Piriform Lobe. Cambridge, Mass. Harvard University Press (1-10,14; 2-13,20).

Van Twyver, H. B. & Allison, T. (1970). Sleep in the opossum *Didelphis marsupialis*. Electroencephalogr. Clin. Neurophysiol. 29: 181-189 (Table 3-1).

Viukari, M. (1970-71). Brain function, carbon dioxide, anticonvulsants, and tranquillizers. Spinal fluid pH in brain diseases. Behav. Neuropsychiatry 2 (9-10): 2-5 (3-29).

Wagman, I. H. & de Jong, R. H. (1964). Relation between pCO_2 and electrical seizure activity induced by lidocaine. Fed. Proc. 23: 348 (5-3).

Wagman, I. H., de Jong, R. H. & Prince, D. (1964). Effects of lidocaine upon spontaneous and evoked activity within the limbic system. Electroencephalogr. Clin. Neurophysiol. 17: 453 (5-3).

Wagman, I. H., de Jong, R. H. & Prince, D. (1967). Effects of lidocaine on the central nervous system. Anesthesiology 28: 155-172 (5-3).

Wagman, I. H., de Jong, R. H. & Prince, D. A. (1968). Effects of lidocaine on spontaneous cortical and subcortical electrical activity. Production of seizure discharges. Arch. Neurol. 18: 277-290 (5-4).

Wale, N. & Jenkins, L. C. (1973). Site of action of diazepam in the prevention of lidocaine induced seizure activity in cats. Can. Anaesth. Soc. J. 20: 146-152 (4-6; 5-5).

Walsh, R. R. (1959). Olfactory bulb potentials evoked by electrical stimulation of the contralateral bulb. Am. J. Physiol. 196: 327-329 (3-29).

Walter, D. O. (1963). Spectral analysis for electroencephalograms: mathematical determination of neurophysiological relationships from records of limited duration. Exp. Neurol. 8: 155-181 (A-1).

Ward, A. A. (1972). Topical convulsant metals. In: Experimental Models of Epilepsy - A Manual for the Laboratory Worker. pp.13-25. Ed. Purpura, D. P., Penry, J. K., Tower, D. B., Woodbury, D. M. & Walter, R. D. New York. Raven Press (5-2).

Warner, G. (1971). The rubrospinal tract of the possum. Appendix II. A stereotaxic atlas of the brain of the brush-tailed possum (*Trichosurus vulpecula*). B.Sc.(Med.) Thesis. School of Anatomy, University of N.S.W. (See also Warner, Watson & Ward, 1973) (2-3; 6-3).

Warner, G., Watson, C. R. R. & Ward, L. A. (1973). A stereotaxic atlas of the brain of the brush-tailed possum (*Trichosurus vulpecula*). J. Anat. 114: 152 (2-3).

Wenzel, B. M. & Sieck, M. H. (1966). Olfaction. Annu. Rev. Physiol. 28: 381-434 (1-5).

Werner, H. W. & Tatum, A. L. (1939). A comparative study of the stimulant analeptics picrotoxin, metrazol and coramine. J. Pharmacol. Exp. Ther. 66: 260-278 (5-21).

Westecker, M. E. (1969). The time course of facilitation and inhibition in the olfactory bulb, investigated with double pulse stimulation of the lateral olfactory tract. Brain Res. 16: 527-529 (2-12).

Westecker, M. E. (1970*a*). Responses of single cells in the olfactory bulb of rabbits to air flow. Pfleugers Arch. 315: 93-104 (2-12; 3-28).

Westecker, M. E. (1970*b*). Alternating characteristics of the evoked potentials in the olfactory bulb in response to repetitive stimulation of the lateral olfactory tract. Brain Res. 17: 142-144 (2-12).

Westecker, M. E. (1970*c*). Excitatory and inhibitory interactions in the olfactory bulb involving dendrodendritic synapses between mitral cells and granular cells. Pfleugers Arch. 317: 173-186 (2-10,12).

Westrum, L. E. (1969). Electron microscopy of degeneration in the lateral olfactory tract and plexiform layer of the prepyriform cortex of the rat. Z. Zellforsch. Mikrosk. Anat. 98: 157-187 (1-24,25).

White, E. L. (1972*a*). Synaptic organization of the glomerulus in the mouse olfactory bulb. Anat. Rec. 172: 426-427 (1-7,9).

White, E. L. (1972*b*). Synaptic organization in the olfactory glomerulus of the mouse. Brain Res. 37: 69-80 (1-7,8,9,27).

White, E. L. (1973*a*). Strain differences in the synaptic connections of the olfactory glomerulus. Anat. Rec. 175: 493 (1-7,8,9).

White, E. L. (1973*b*). Synaptic organization of the mammalian olfactory glomerulus: new findings including an intraspecific variation. Brain Res. 60: 299-313 (1-8,9).

White, L. E. Jr (1965). Olfactory bulb projections of the rat. Anat. Rec. 152: 465-480 (1-18,20,25).

Willey, T. J. & Freeman, W. J. (1968). Alteration of prepyriform evoked response following prolonged electrical stimulation. Am. J. Physiol. 215: 1435-1441 (4-10).

Winans, S. S. & Scalia, F. (1970). Amygdaloid nucleus: new afferent input from the vomeronasal organ. Science 170: 330-332 (1-12; 2-17).

Winter, J. W. (1971). Olfaction in *Trichosurus vulpecula*. Personal communication.

Winter, J. W. (1972). Olfactory behaviour of *Trichosurus vulpecula*. Aust. Mammal. 1: 72 (1-4).

Wood, F. C. & Battye, A. E. (1933). Cited by Haskell & Voss, 1957 (5-13).

Yamaguchi, T., Regli, F. & Waltz, A. G. (1972). Effects of hyper-ventilation with and without carbon dioxide on experimental cerebral ischaemia and infarction. Brain 95: 123-132 (3-22).

Yamamoto, C. & Iwama, K. (1961). Arousal reaction of the olfactory bulb. Jap. J. Physiol. 11: 335-345 (3-27; 6-6).

Yamamoto, C. & Kurokawa, M. (1970). Synaptic potentials recorded in brain slices and their modification by changes in the level of tissue ATP. Exp. Brain Res. 10: 159-170 (3-29).

Yamamoto, C. & McIlwain, H. (1966). Electrical activities in thin sections from the mammalian brain maintained in chemically-defined media *in vitro*. J. Neurochem. 13: 1333-1343 (4-12).

Yamamoto, C. & Yamamoto, T. (1962). Oscillation potential in strychninized olfactory bulb. Jap. J. Physiol. 12: 14-24 (4-5; 5-7,18).

Yamamoto, C., Yamamoto, T. & Iwama, K. (1963). The inhibitory systems in the olfactory bulb studied by intracellular recording. J. Neurophysiol. 26: 403-415 (1-27; 4-11).

Yamamura, H. & Kato, S. (1970). Effects of anesthetics on the various cortical systems. Int. Anesth. Clin. 8(1): 85-102 (4-16).

Zernicki, B. (1968). Pretrigeminal cat. Brain Res. 9: 1-14 (3-29).

Ziehen, T. H. (1897). Semon: Zoologische Forschungsreisen Bd. 3. Monotremen und Marsupials, 2 S. 726. Cited by McCotter (1912) (1-12).

I'd like to know

What this whole show

Is all about

Before it's out

Piet Hein