The avian red nucleus: a comparative physiological and behavioural study on Columba livia

A. Johnston

Johnston, A. (1975) The avian red nucleus: a comparative physiological and behavioural study on Columba livia. Doctoral thesis, Durham University.

THE AVIAN RED NUCLEUS : A COMPARATIVE PHYSIOLOGICAL AND BEHAVIOURAL STUDY ON COLUMBA LIVIA

by A. Johnston

A Thesis Presented for the Degree
of Doctor of Philosophy

ACKNOWLEDGEMENTS

I gratefully acknowledge the help and encouragement of Dr. J.D. Delius without whom this research would not have been completed or indeed but for his constant cajolery and good humour, produced in written form.

I would like to record my appreciation of Professor F.V. Smith for making the facilities available for the carrying out of the experiments.

My thanks also to, the Technical Staff of the department especially Mr. A.M. Perry, Mr. D. Harper, Mr. M.G. Rollings and Mr. D.J. Barton for their assistance.

Lastly, I wish to thank my long-suffering wife, for putting up with years of financial deprivation to make this possible.

ABSTRACT

An anatomical and morphological study of the red nucleus of the pigeon showed that as in the mammal the nucleus is relatively highly vascularised and contains both large and small cells. The only relevant previous source indicated the existence of large cells only.

Lesions of the pigeon red nucleus (r.n.) effected a variety of symptoms including torticollis, abnormalities of leg muscle tone and movement, hyporeactivity, aphagia and asymmetry of pupil dilation although the latter was probably due to third nerve damage. Electrical stimulation of the r.n. produced contralateral circling and indications of ataxia.

Extensive investigation using electrical stimulation and evoked potential recording revealed telencephalic inputs to the r.n. from the archistriatum medialis, hyperstriatum ventrale, parolfactory lobe, olfactory bulb and neostriatum intermedium, diencephalic projections from the lateral hypothalamus and nucleus rotundus and a cerebellar link with the nucleus lateralis. Comparison with mammalian connections disclosed some similarities and in addition suggested that links with amygdala and septum may exist in the mammal. Evoked potential and single unit recordings following visual, somaesthetic and auditory stimulation demonstrated that the avian r.n. and individual cells respond in a very similar manner to that of the mammal. An olfactory projection was also demonstrated by nerve stimulation.

Investigation of the effects of r.n. lesions on previously established avoidance behaviour disclosed complete disruption of learning. Similar effects were shown on a pattern discrimination acquired under positive reinforcement, although brightness and colour discriminations were not affected.

Possible mechanisms involving the r.n. in behaviour control are discussed.

CONTENTS

PART 2

INTRODUCTION

The study of the red nucleus of the pigeon described herein is an attempt to clarify some recent observations by other workers which appear to implicate the mammalian red nucleus or red nucleus area in learning and memory.

To that date, the nucleus had been regarded as wholly motor in function, forming as it does, part of the classically defined extra-pyramidal motor system. In many ways these findings were a precursor of the now growing view that learning and memory systems may in fact be at least partially represented at midbrain or brain stem levels.

The pigeon was chosen as the subject of study for three reasons; firstly, because little was known about connections of the avian red nucleus, secondly, because the only available information suggested that the r.n. is phylogenetically an old structure and is of comparative interest in the bird, in that the nucleus consists only of large cells, whereas the mammalian nucleus consists of large and small cells which have different functional properties, perhaps leading to confusion in a lesion study, and thirdly because the laboratory in which the experiments were conducted had a considerable history and expertise in experimentation with pigeons.

The report is divided into two parts. Chapters 1, 2, 3, 4 and 5 forming a physiological study utilising a variety of techniques and 6, 7 and 8 making up a behavioural examination of the effects of lesions of the red nucleus on learning and memory. Specifically, Chapter 1, purely comparative, is a study of the anatomy and morphology of the pigeon red nucleus. In Chapters 2 and 3 detailed observations of gross behaviour are described following lesions and during electrical stimulation of the nucleus respectively. These two chapters describe attempts to gain information which would be relevant to the behavioural studies in Part 2. Chapters 4 and 5 contain reports of investigations of afferent linkages and sensory inputs to the nucleus, conducted in an effort to delineate connections possibly involved in learning and memory. Chapter 6 is a review of behavioural findings including an analysis of the experimental method adopted by other workers which perhaps contributed to their findings. Chapters 7 and 8 describe experiments suggested by the analysis aforementioned, both involving lesions of the r.n. Chapter 9, the overall conclusion, attempts to integrate the findings and compare the results with mammalian studies. Further introductory material has been included prefacing each chapter.

The studies reported have been placed not in historical order of completion but have been arranged to facilitate exposition. Apology is made for the inevitable small inconsistencies that such rearrangement has produced.

CHAPTER ONE

MORPHOLOGY AND ANATOMY OF THE RED NUCLEUS

Introduction

The casual observer examining a series of transverse sections through the midbrain of any vertebrate species will find that one of the most noticeable landmarks he can observe is a group of cells scattered around the midline at about the level of the emergent third nerve. The cells are conspicuous by their magnitude and are so dissimilar to the cells of the surrounding tissue as to be regarded as constituting a nucleus (see Fig.1·1). The anatomy and morphology of the red nucleus of many animals, including man, has been studied since the end of the last century. Massion (1967) has extensively reviewed the literature and for that reason only the salient points are outlined here.

The name red nucleus (nucleus ruber) derives from the fact that in fresh, unperfused tissue sections the nucleus discloses a pinkish red colour which distinguishes it from the surrounding mesencephalon. The colour is attributed to the high vascularity of the nucleus and Hough & Wolff (1939) found, in the cat, that the r.n. was unique in that it's vascularity was extraordinarily high in relation to the number of cell bodies.

Phylogenetic differences in terms of cellular composition of the nucleus are great. In general, the nucleus consists of large and small cells, the relative numbers of which differ in different mammalian species (see Massion 1967). Cajal (1909-1911) differentiated large, medium and small neurons in the r.n. According to Cajal's classification large neurons vary from 50 - 90 µ, medium cells 30 - 40 µ and small cells about 20 µ. In mammals the large cells tend to predominate in the caudal and small

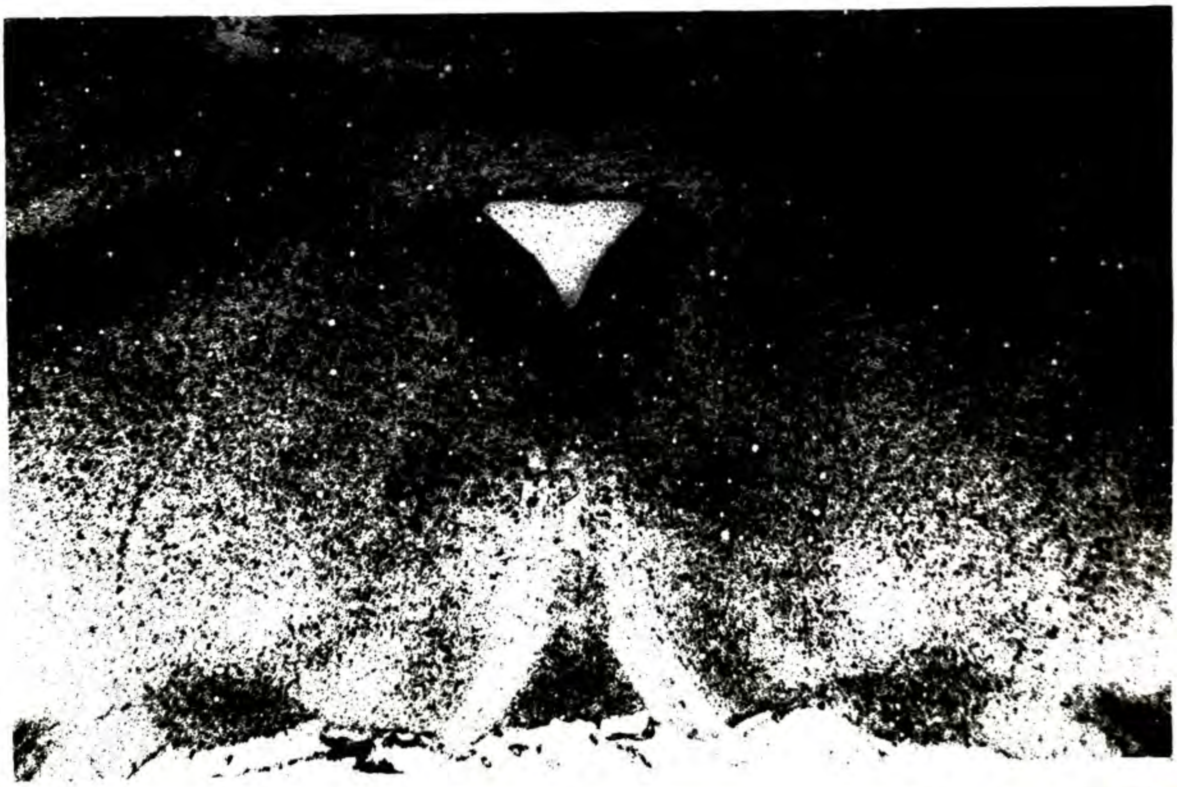


Fig. 1.1 Photomicrograph of a transverse section of the brain of the pigeon showing the red nuclei each side of the midline just below the centre of the Fig. Note the large cells of the nuclei relative to surrounding areas.

cells in the rostral part of the nucleus. Although there are exceptions it seems that the relative proportions of large and small cells change in favour of the small as one ascends the evolutionary scale - viz Ariens-Kappers et al (1960):-

"....Thus the parvocellular portion has become a conspicuous portion in the higher mammals. Its development is associated with a gradual increase in the range of functional activity of the red nucleus associated with the progressively greater development of forebrain regions in higher forms".

The position in sub-mammalian species has received little investigation, although Arien-Kappers suggests that the r.n. of the bird and reptile contains large cells only viz:-

"....the avian red nucleus is the homologue of that of reptiles and <u>is comparable in a general way with the magnocellular portion of the mammalian red nucleus</u>."

Some authors, notably Hatschek (1907), Von-Monakow (1909), Fuse (1937) and Otabe and Horowitz (1970), have further sub-divided the mammalian r.n. into cellular groupings, although there is some doubt whether these groupings hold true in all species e.g. Brodal and Gogstad (1954) could find no evidence for groupings in the cat and furthermore doubt whether the nucleus could be divided into magnocellular and parvocellular areas.

The large cells of the r.n. lend themselves readily to a count and this has been done for man (Olszewski and Baxter 1954), cat (Grofova and Marsala 1961) and domestic pig (Otabe and Horowitz 1970) with numbers of 100, 450 and 200 respectively. Clearly there is a marked regression of large cells in man. In view of the lack of data regarding the bird, it was considered necessary, in order to lend credence to the view that the avian r.n. was homologous to that of the mammal, to look closely at:-

(1) Vascularity

(2) The number and size of cells

and (3) The structure of the nucleus

Materials and Method

(1) Cell Count and Measurement

Five mature pigeons of mixed strain were used in the study. Each bird was sacrificed by an overdose of Nembutal and perfused through the carotid arteries with 5% saline followed by 10% formalin. The brain was then fixed in 10% formalin for several weeks and serial paraffin sections were taken at 15 µ. Every fifth section through the r.n. area was taken and stained with cresyl violet.

The cell count was accomplished by means of an ocular reticule and the size of cell and cell nucleus measured by means of a micrometer fitted to the microscope platform. The cells were classified as large, medium or small in accordance with the classification of Ramon y Cajal:- large cells - more than 50 µ in diameter, medium - 26 - 49 µ and small - less than 26 µ.

Counting Procedure

Large, medium and small cells which exhibited a nucleus were counted. The number of cells in each category was estimated using the method adopted by Otabe and Horowitz (1970). Tissue shrinkage due to the histological procedure was estimated at 25% although the initial count of cells did not take this into account. Each section of 15 µ represented a piece of tissue 75 µ thick. The nuclei of the large cells measured 22 µ ± 2, therefore each 15 µ section contained all, or a substantial part of the nuclei in 51 µ thickness of tissue (see Fig.1.2). The number of large cell nuclei counted in the section was multiplied by 1.5 $\left(\frac{75\mu}{51\mu} \simeq 1.5\right)$ to estimate the number in 75 µ thickness of the r.n.

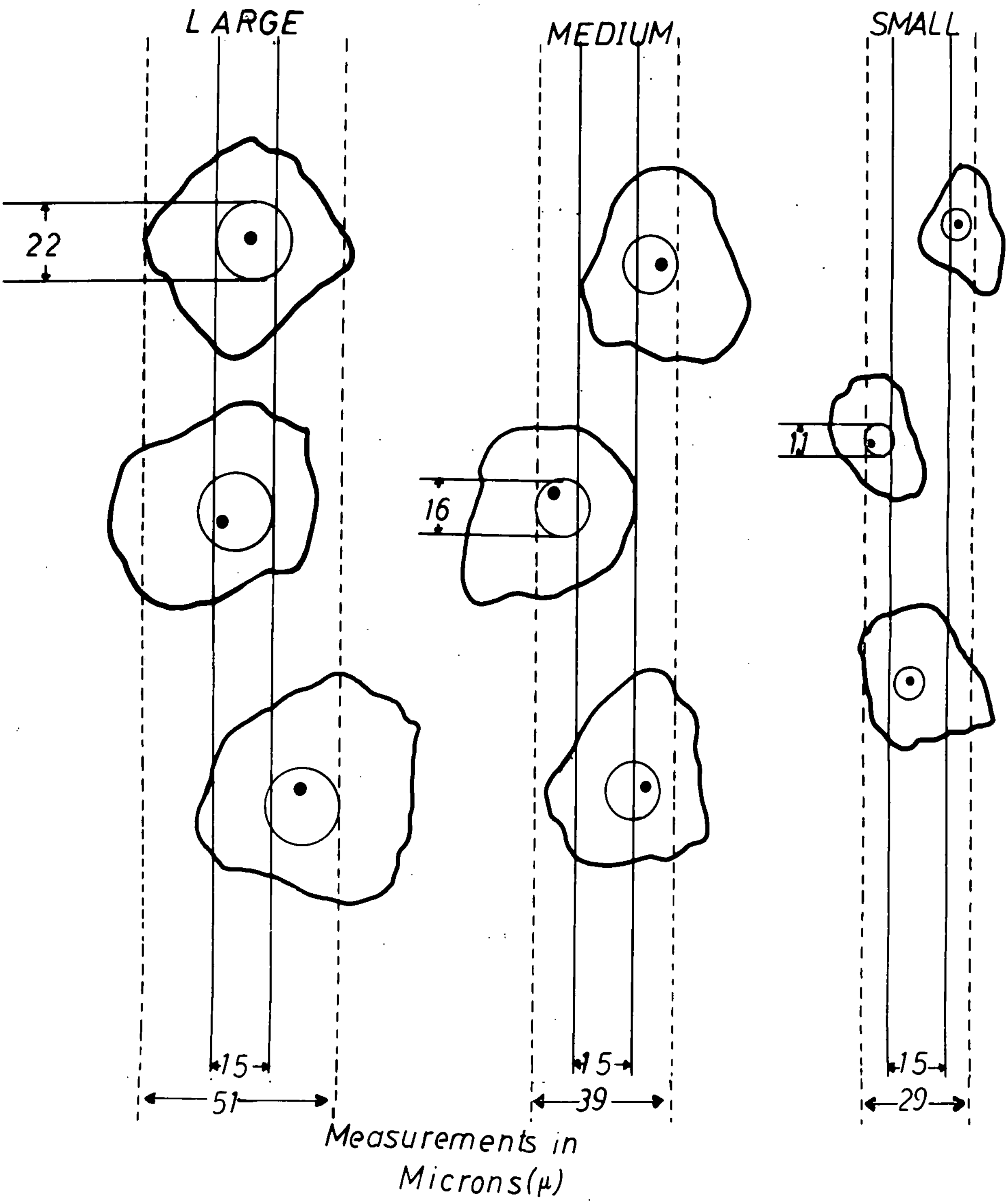


Fig.1·2 Schematic illustration of the three types of neurons and their nuclei, and the measurements used for estimating cell population.

The nuclei of medium cells measured 16 μ ± 2. Each section covered all medium cell nuclei in a tissue thickness of 39 μ and the number of cells counted in one section was multiplied by 2 $\left(\frac{75\ \mu}{39\ \mu} \simeq 2\right)$.

The nuclei of small cells had a diameter of 11 μ ± 2. Each section covered all small cell nuclei in approximately 29 μ thickness of tissue and the number of small cells counted was multiplied by 2.6 $\left(\frac{75\ \mu}{29\ \mu} \simeq 2.6\right)$ to estimate the population in 75 μ thickness of tissue.

(2) Vascularity

A single bird was used. Following sacrifice the unperfused brain was fixed for several days in 10% formalin and 200 μ slices of tissue taken through the r.n. after blocking in paraffin wax. The stain used was the "Benzidine method for Hæmoglobin" (McManus and Mowray 1960).

OBSERVATIONS

(1) Vascularity

Fig. 1·3 clearly shows that the r.n. area of the pigeon, as the mammal, has a high vascularity. The Fig. shows the complex network of very fine capillaries serving the nucleus.

(2) Cell Count and Measurement

It was found that little difference in cell count occurred from one bird to another and for that reason Table 1·1 shows the data for one bird only.

Table 1·1

Estimated Cell Count

Cell Count	Correction	Est. Population
Large 103	x 1.5	155
Medium 366	x 2.0	732
Small 364	x 2.6	946

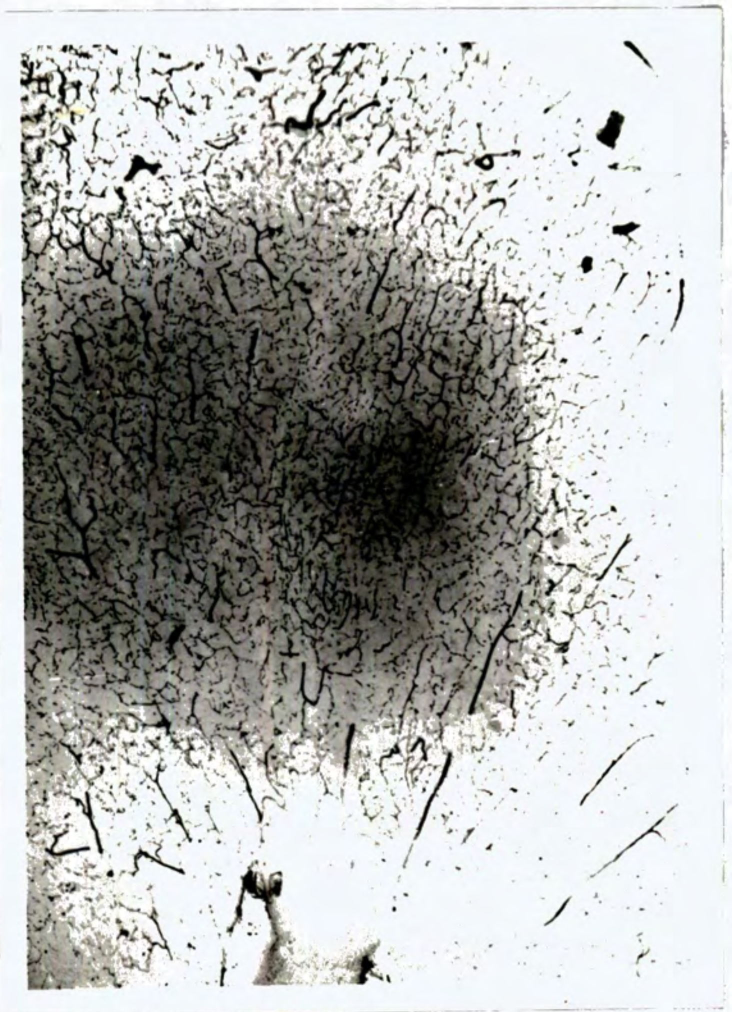


Fig. 1·3. Microphotograph of the r.n. area to show the vascularity of the r.n. The relatively greater supply of fine capillaries contained within the nucleus is apparent in the centre of the photograph. Magnification - x 25.

Correction for shrinkage:- x 25%

Large	155 + 25% x 732	338
Medium	732 - 183 + 25% x 946	786
Small	946 - 237	709

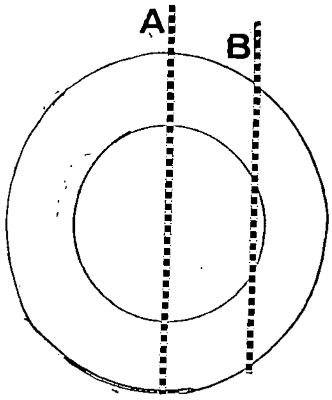
It will be recalled that tissue shrinkage was estimated to be 25%. It follows that to fit Cajal's classification the totals require adjustment for this. The adjusted totals are shown above:- i.e. - 25% of counted medium cells have been assumed to be large and 25% of small assumed to be medium. (Cell sizes were distributed fairly evenly within each category).

(3) General Structure of the Nucleus

The nucleus was found to be oval in shape with the longest axis situated in a rostral-caudal plane roughly parallel to the midline and about 0.5 mm lateral. The rostro-caudal extent measured 2.8 mm and the maximum transverse diameter 2.5 mm. The nucleus extends rostrally from the level of the mammillary bodies down to the level of the decussation of the brachium conjunctivum.

Large, medium and small cells appeared to be equally distributed throughout each level of the nucleus. No noticeable sub grouping of cells, such as to constitute the sub groupings delineated in some mammals, was observed.

One methodological point should be made at this juncture. It occurred to the author that a section through the cell, exhibiting a nucleus, may not in fact give true cell body and nucleus diameters e.g.:-



Section A, the true diameter of cell and nucleus, would give different figures from section B and yet represent the same or similar cells.

An indication of cell group differences would be given by the ratio of cell to nucleus. One would expect, where the section is of type B, to obtain a higher ratio than in type A. Fig.1·4 graphs the relationship between cell and nucleus diameters for a random sample of cells falling within each category. It is possible that the tentative functions drawn may represent 3 populations of cells. Table1·2 gives cell diameters, nucleus diameters and cell/nucleus ratios (corrected for shrinkage) for the sample of cells used in Fig.1·4. It can be seen that there do appear to be three cell size categories of which the rough divisions are shown under hypothesis (1). The mean cell/nucleus ratio for the assumed magnocellular = 2.29, and for the parvocellular 1.64. Within the assumed medium range there are several obvious extreme values (ringed) which may well represent the type B section hypothesis outlined above. The point will not be laboured, for it is possible to argue in many different ways e.g.:- that one could divide all the cells (in ratio terms) into only large and small - see Table1·2 - hypothesis 2.

What does emerge however from the ratio method is that there is clearly a distinct cell type with a lower ratio value than the large cell which must surely be indicative of a true small cell population contrary to the assertion of Ariens-Kappers.

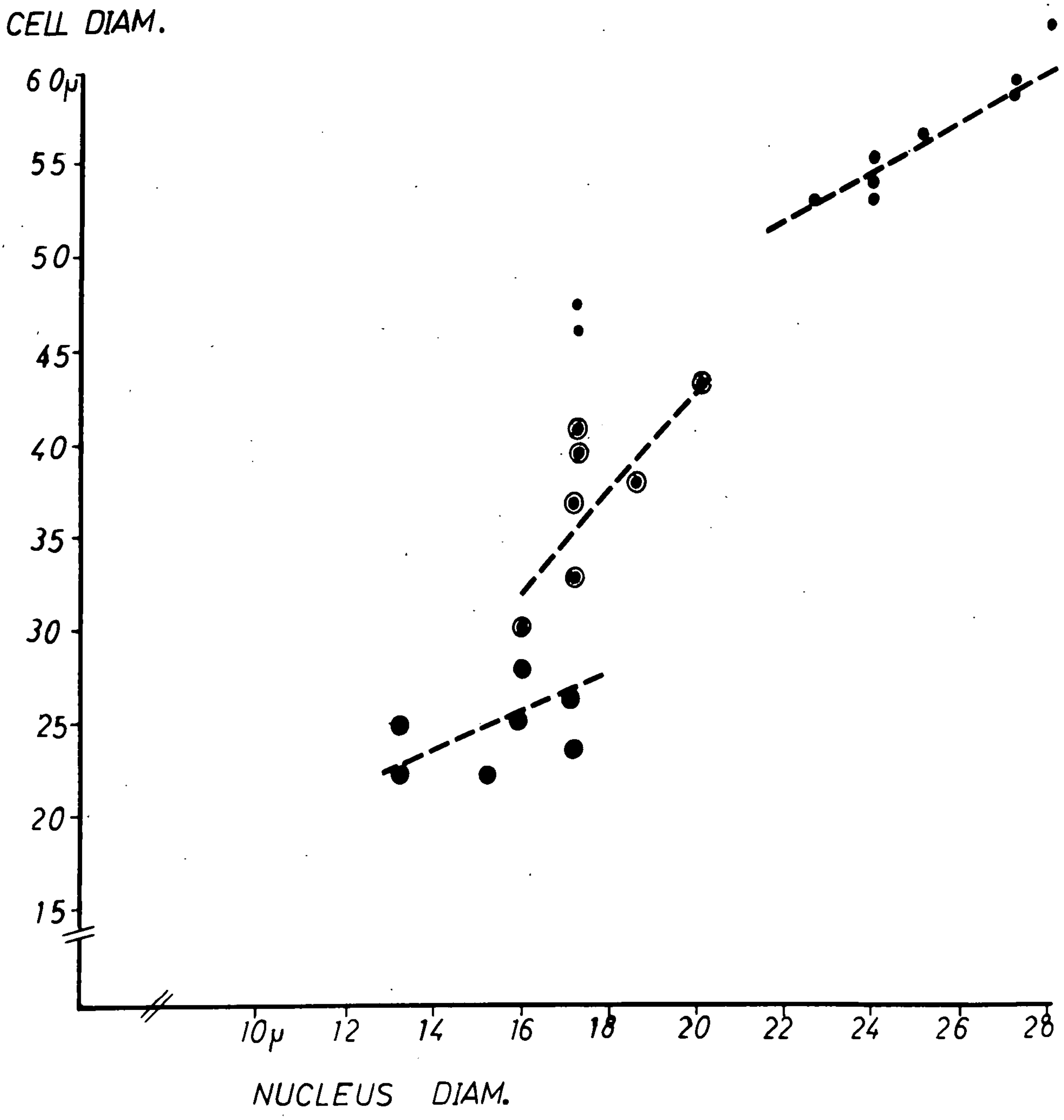


Fig 1·4 The relationship between cell and nucleus diameters. The functions may represent three different types of cell.

TABLE 1.2

Diameters of cell bodies, nuclei and ratios of cell to necleus measurements.

Cell Diameters	Nucleus Diameters	Ratio Cell/Nucleus	hyp. 1	hyp 2
66.6 u	28.0 u	2.4	LARGE	LARGE
60.0	26.6	2.3		
58.6	26.6	2.2		
57.3	25.3	2.3		
57.3	25.3	2.3		
56.0	24.0	2.3		
54.6	24.0	2.3		
53.3	24.0	2.2		
53.3	22.6	2.4		
53.3	22.6	2.4		
48.0	17.3	2.8	MEDIUM	
46.6	17.3	2.5		
41.3	17.3	2.4		
40.0	17.3	2.3		
38.6	18.6	2.1		SMALL
37.3	17.3	2.0		
37.3	17.3	2.1		
33.3	17.3	2.0		
30.6	16.0	1.9		
28.0	16.0	1.75	SMALL	
26.6	17.3	1.6		
25.3	16.0	1.6		
25.3	13.2	1.9		
25.3	16.0	1.6		
22.6	13.2	1.7		
22.6	14.6	1.6		

CHAPTER 2

Lesions of the Red Nucleus - Observations of Gross Behavioural Effects

Early attempts to determine the functions of the mammalian red nucleus consisted largely of removal of large areas of tissue during studies of decerebrate rigidity. Rademaker (1923;25;26 summarized by Massion 1967) perhaps conducted the most extensive investigation by these means and concluded that the r.n. is the centre responsible for inhibition of extensor muscles, destruction of which produced the characteristic decerebrate rigidity. Such crude methods, involving staged sections, are however unsatisfactory, involving as they do, removal of large amounts of tissue other than just the r.n. Localised lesions of the r.n. were first carried out successfully by Mussen (1967), in the cat, who found that lesions of the posterior poles i.e. the large celled area, produced only a slight unsteadiness of gait which lasted two days. On the other hand, unilateral lesions of the rostral areas (small celled area) produced curvature of neck, body and tail towards the contralateral side, a loss of contralateral righting reflexes, loss of sense of position and general hypotonus. The animal made a complete recovery within three weeks. Later work by Ingram et al. (1934) using smaller lesions, again in the cat, essentially confirmed these findings but demonstrated that righting reflexes were not disturbed. McNew (1968) listed a number of effects of r.n. lesions in the rat including aphagia and adipsia, impaired postural adjustment, absence of pupillo-constrictor response, canting of the head laterally, locomotor circling, awkwardness of gait, absence of vibrissae movement, paucity of exploratory behaviour and resistance to handling. Again, the symptoms were transitory, disappearing within 14-21 days. Papaioannou (1971), on the other hand, found that small, unilateral, radio frequency lesions in the rat, produced little effect on the animals' turning tendencies, facility in using limbs, righting reflexes, or walking. The only observed effect was one of hyperkinesis which however, on the basis of control lesions to the brachium conjunctivum, the author believes was due to destruction of cerebellar fibres ending in the r.n. Other workers (cited in Massion 1967) demonstrated that lesion effects produce a slight temporary hypertonus, coupled with unsteadiness of gait and a loss in the tactile placing reaction. Such symptoms were short lasting. Massion argues that any hypotonus is due to destruction of cerebellar fibres which course through the brachium conjunctivum, which decussates slightly caudal to the r.n. In the Rhesus monkey, Carpenter (1956) found that bilateral lesions produced little effect on muscle tone. The most striking effects found were hypokinesis, which he claims was long lasting and torticollis, in which the head is tilted and rotated to one side, resulting from asymmetric or unilaterally placed lesions of the r.n. - the tilt and rotation being to the contralateral side of the lesion. Symmetrically placed lesions did not produce this effect. Carpenter's finding of torticollis, is in agreement with Mussen's results in the cat.

If, as stimulation studies show, the r.n. exerts a facilatatory effect on contralateral flexors and an inhibition of contralateral extensors, one would expect to find that lesions of the r.n. would produce a hypertonus in extensor muscles and a degree of rigidity. However, the mammalian lesion studies do not produce these symptoms. Some experiments show a hypertonus and others hypotonus. All authors report that the effects are transitory, the animal recovering fully in a period of days. It will be recalled that staged sections of the brain (Rademaker 1923 et seq.) show that removal of the r.n. produces decerebrate rigidity. These facts appear to argue clearly that in the mammal the classical pyramidal motor system compensates for insult to the r.n. and rubro-spinal tract and this was indeed the opinion of Evans and Ingram (1939). In staged sections the motor areas and pyramidal tract would of course be destroyed before the r.n. area.

Lesions of the avian r.n. have not been investigated but arise occasionally as incidentals to lesions of other areas. Durkovic and Cohen (1969) in lesions of rostral midbrain areas, obtained a temporary torticollis or hypotonus of neck musculature and a transient loss of tone in leg extensor muscles. Such lesions involved r.n. areas and the similarity to mammalian lesion results leads one to suspect a similarity of function.

The experiment outlined in this chapter was completed before the Durkovic and Cohen publication. The intention was to lesion the r.n. bilaterally and to carefully observe gross changes which might follow.

THE LESION EXPERIMENT

Subjects

The subjects were three apparently normal pigeons of mixed strain.

Surgery

The animals were anaesthetised by pectoral muscle injection of Equithesin according to bodyweight and placed in a Kopf stereotaxic instrument. The skull was exposed over a midline area approximately where the lesioning electrode was to be driven and scraped free of periosteum. The desired point of entry of the electrode was determined from the Hodos and Karten (1967) stereotaxic atlas and the skull was trephined. The lesioning electrode was driven down to 0.5 mm above the r.n. position and connected to recording leads. The electrode was then advanced until unit spikes were apparent on the oscilloscope, the recording leads were disconnected and a lesion produced by R.F. electrocoagulation.* If single unit spikes were not apparent during attempted location of the r.n. the electrode was withdrawn, resited in a more anterior or posterior position and the procedure repeated. (A description of the rationale underlying the method of electrode positioning is given in Ch. 4). Following electrode withdrawal the skull fenestra was filled with an absorbent gelatine plug, and the skin sutured.

Electrodes

Lesioning electrodes were made from stainless steel insect pins by dipping them mechanically in Shenvar 31 lacquer.* Three or four coats of varnish were given, heat drying between each coat and the tip scraped bare of insulation for 1 mm with a scalpel blade. Each electrode was tested for leakage by bubbling in a 10% saline solution Delius (1966).

Apparatus and Procedure

Each bird was housed in a cage 2' x 2' x 2'. The three cages were removed from the general animal house and kept in the experimenter's study. From the day following surgery written records were made of general behaviour and tests of flight and placing reflexes. Daily photographic records were made of the birds until recovery from lesion effects was considered complete.

Histological Procedure

Some time following completion of the experiment, the birds were sacrificed by an overdose of Nembutal, the brains perfused through the carotid arteries successsively with saline and 10% formalin. The brains of two birds were embedded in paraffin wax and serially sectioned at 15 μ. Alternate sections through the lesioned area were stained with cresyl violet and haematoxylin

---

* See appendix 4

respectively. The brain of the third bird was sectioned on a freezing microtome and stained with cresyl violet.

RESULTS

Histology

Each of the three birds suffered damage to one or both r.n.

Bird 5.12 - suffered an estimated 75% destruction of left r.n. and suffered an estimated 25% destruction of right r.n.

Bird 7 - bilateral 100% destruction.

Bird 2 - 100% destruction of right r.n., minimal damage left.
(The estimate for the left r.n. was difficult due to an infection which eventually had destroyed a large area of midbrain tissue).

Other Areas Destroyed by the Lesions

Bird 5.12 - the lesions were small and involved no damage to the major pathways. The left lesion extended posteriorly to the level of the brachium conjunctivum with some slight involvement of the vestibular mesencephalic tract on the left. The right side lesion was small and limited to the r.n. with a slight involvement of medial reticular substance.

Bird 7 - lesions were extensive. The right lesion extended to 0.5 mm anterior to the rostral pole of the r.n. That of the left extended to 0.25 mm anterior to the rostral pole, with some damage to the stratum cellulare externum just lateral to the midline. Complete destruction of both r.n. was accompanied by bilateral destruction of the third nerve and medial reticular substance. Damage to the left mesencephalic vestibular tract was suffered.

Bird 2 - the right lesion produced total destruction of the third nerve with involvement of medial reticular substance dorsal to the r.n., as well as the interstitial nucleus of Cajal slightly ventral to the r.n.; there was some damage to the right mesencephalic vestibular tract.

The left lesion extent was difficult to determine because of an infection sustained at the site of the electrode which appeared to develop some time after the completion of the study. Assuming the lesion to have been the same size as that on the right, the lesion would have probably included destruction of the tractus occipito-mesencephalicus and ansa lenticularis (tractus strio-tegmentalis et strio cerebellaris) [illegible]; stratum cellulare extermum, medial reticular substance and part involvement of the mesencephalic vestibular tract.

OBSERVATIONS

Torticollis

Birds 5.12 and 2 demonstrated the most dramatic symptom of torticollis, in which the head and neck were rotated through an angle of almost 180° to left and right respectively (see Figs.2·1A and 2·2A) Bird 7 (Fig.2·3A) did not manifest this extreme rotation but there was a slight tilt to the left. Recovery of correct head and neck position took place gradually over 5 - 7 days - see Figs.2·1 and 2.2. Bird 7 however showed little recovery of vertical position and continued to tilt its head to the left some 14 days after surgery. Birds 5.12 and 2, from the day following surgery could get their heads into a relatively normal position in a slow and jerky series of movements but relapsed to the rotated position as soon as movement ceased.

Movement and Leg Muscle Tone

Birds 2 and 7 initially manifested abnormalities in leg muscle tone (see Figs.2·2 and 2·3) Bird 7 tended to take a resting position with legs bent, apparently suffering from loss of extensor tone,

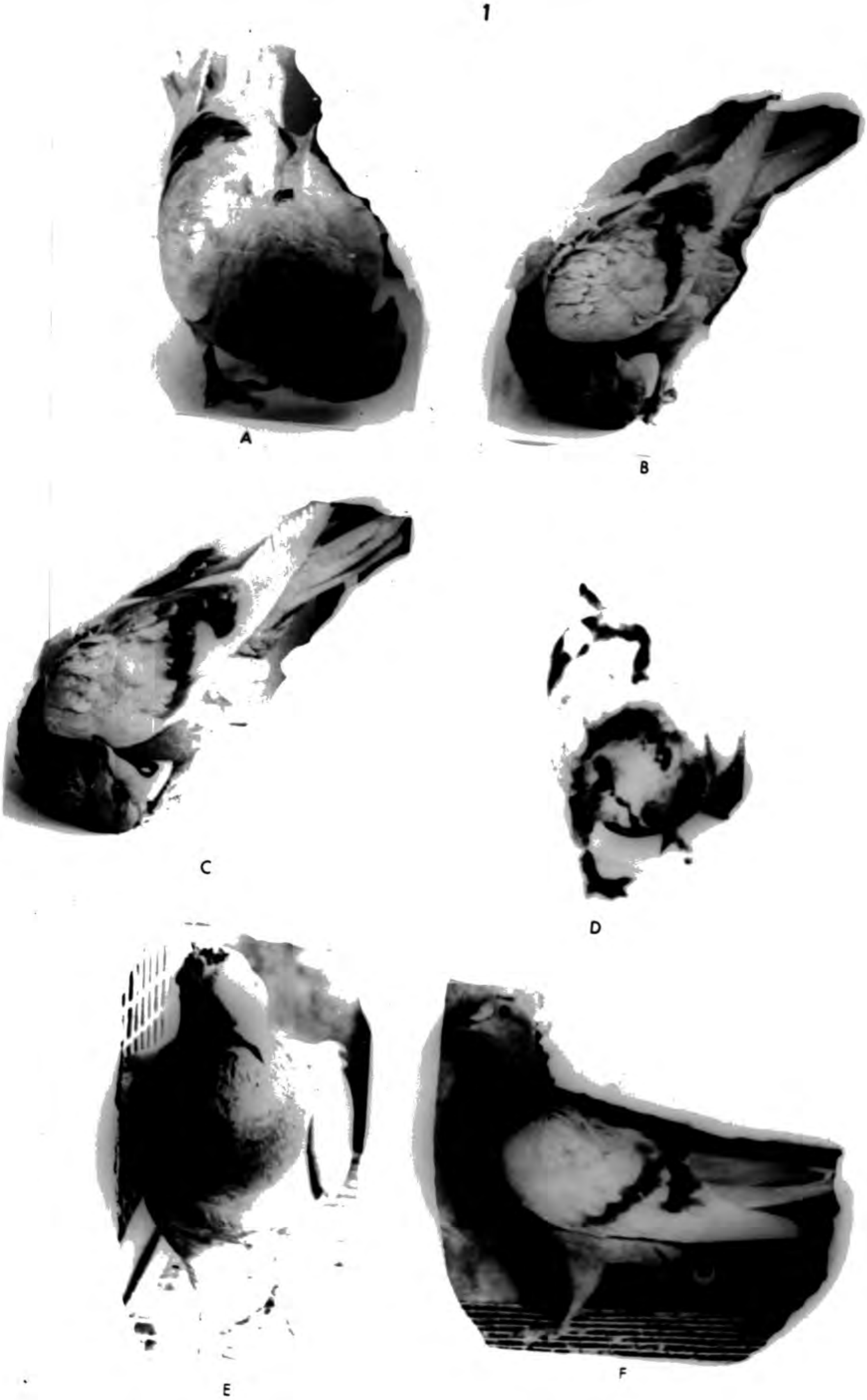


Fig.21. Bird 5.12, effects of bilateral r.n. lesions. Course of recovery on six consecutive days. Note torticollis in A, B, and C and recovery in D, E and F. No abnormality of muscle tone.

2

Fig.2·2 Bird 2, effects of bilateral r.n. lesions. Course of recovery on five consecutive days. Note torticollis and the effects on leg musculature.

3

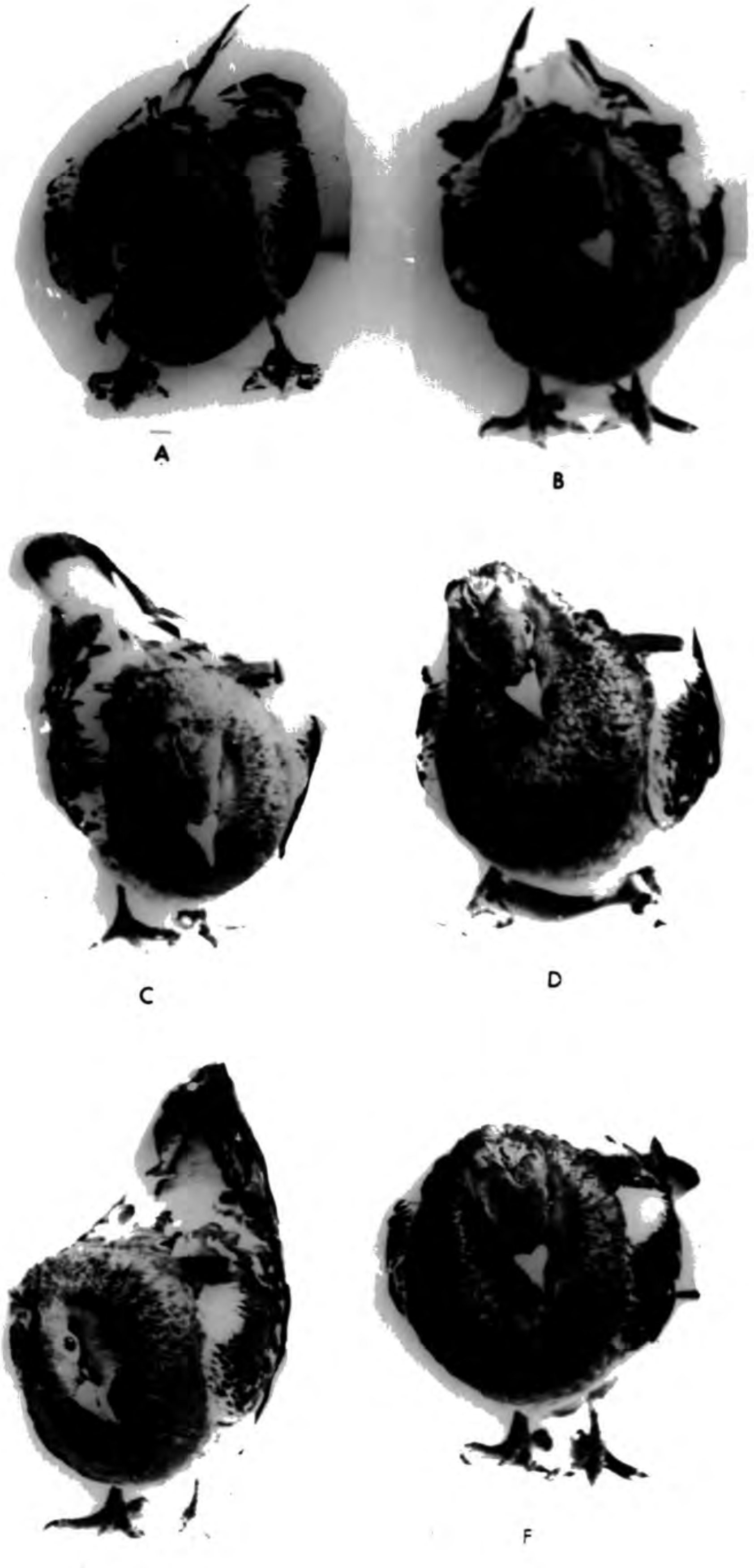


Fig2.3 Bird 7, effects of bilateral r.n. lesions. Course of recovery on six consecutive days. Note slight torticollis.

although this was partially corrected when it moved. Bird 2 appeared to suffer from hypertonus of extensors in the right leg and hypotonus/hypertonus of left extensors/flexors (see Figs. 2.2A and 2.2E.) Bird 5.12 showed no abnormality in leg musculature. Birds 5.12 and 2 tended to walk in circles in the direction that their heads and neck were rotated, whereas Bird 7, when it moved, which was rare, was able to move in a forward direction.

All birds demonstrated a weakened claw grasp relative to normals when placed on the experimenter's finger. In each case the weakened grasp was asymmetric; Birds 5.12 and 2 being initially poor at grasping with the right claw and Bird 7 with it's left. None of the birds were able to perch. Again, each animal improved in ability to stand, move and perch over 5 - 7 days.

Reactivity

All birds were hyporeactive to external stimuli and could be freely handled without display of escape tendencies. Light flashes, sudden noises and touching of feathers failed to elicit much response. Birds 5.12 and 2 gradually became more responsive and were essentially normal at the end of 5 - 7 days. Bird 7 showed little improvement in reactivity within the 21 days up to sacrifice.

Flight Reflexes

Flight reflexes were tested by tilting the animal forward, backward and sideways and observing reflex movement of wings and tail. No deficits were observable.

Feeding and Drinking

Each bird showed no interest in feeding and was force fed. Birds 5.12 and 2 began to show interest in feeding on day 4 but found difficulty due to their abnormal head position. They were able to mandibulate after 5 - 7 days. Bird 7 remained aphagic throughout the observation period. Each animal was able to drink when its bill was held in the water by the experimenter. Bird 2 was hyperdypsic and drank greedily until it vomited. Again Birds 5.12 and 2 exhibited improvement in self drinking during the first 7 days, whereas Bird 7 was unable to drink unaided throughout the period that it was observed.

Eye Movements and Pupillary Response

Eye movements were tested by holding the birds head in a stationary position and moving a hand parallel to its head. Birds 7 and 5.12 appeared to have normal eye movements but Bird 2, whilst able to move its eyes, seemed to have difficulty in tracking movements. Each bird manifested asymmetric, abnormal pupil dilation and absence of pupillary reflex. Bird 7 was deficient in its left pupil, Bird 2 in the right and Bird 5.12 in its left. Each bird recovered pupillary reflex to light during the observation period but asymmetric dilation persisted.

DISCUSSION

The degree of similarity between the findings of this experiment and those derived from mammalian work is near enough to provide further argument in favour of an analogous function of the avian r.n.

Asymmetry in lesion placement in Birds 5.12 and 2 resulting in torticollis, has also been observed in mammalian studies. Carpenter (1956) asserts that asymmetric lesions of the r.n. in the Rhesus monkey produces head tilt and rotation to the side contralateral to the side of maximal r.n. destruction and the present findings confirm this in the pigeon (see Figs.2·1A and2·2A). To obviate possible misunderstanding, reference to Bird 5.12 (Fig.2·1A) which suffered greater destruction of the left r.n., although having its head and neck positioned to the left of its body, the rotation is actually to the right. The left side positioning is simply a result of maximal rotation to the right.

In terms of muscle tone, it would seem that the torticollis results from an excess of extensor tone on the right and/or an excess of flexor tone on the left. This would be in agreement with mammalian findings that the r.n. exerts a facilitation of contralateral flexors and an inhibition of contralateral extensors. Hence, lesions of the left r.n. should produce a muscular imbalance in which right side extensor tone is greater than left and left side flexor tone is greater than right. Bird 2, which suffered a greater lesion in the right r.n., did of course produce converse symptoms (Fig.2·2A). It is interesting to note that Bird 7, which suffered symmetrical destruction, did not have the extreme head/neck rotation (Fig.2·3A).

It could be argued that the torticollis phenomenon is due to lesions of the mesencephalic vestibular tract but whilst this possibility can not be ruled out, Bird 7 sustained a unilateral destruction of the left mesencephalic vestibular tract and on this hypothesis one would expect similar symptoms to Birds 5.12 and 2. Nevertheless, Bird 7 did manifest a slight head tilt to the right - see Figures 2·3B and 2·3D. Clearly further experiments, in which the vestibular tract is lesioned would be necessary to clarify this point.

The effects on leg musculature were not wholly definitive. Bird 7 initially suffered from what was an apparent loss of tone in both legs. Bird 2 appeared to have an excess of extensor tone in the right leg and an absence of tone in the left - see Figs, whilst Birds 5.12 showed no apparent deficit. The symptoms produced by Bird 2 do not fit the findings of mammalian workers in as much as a lesion of the right r.n. would be expected to produce a tendency to extensor rigidity in the left leg. By the same token Bird 7 would be expected to demonstrate an increase of extensor tonus, relative to flexor, in both legs. Furthermore, the absence of any effect on wing muscles poses problems. Clearly the results are confusing and perhaps allow only a conclusion of an effect on leg muscle tone. Lack of such effect in Bird 5.12 may be due to the fact that only partial r.n. destruction was achieved.

The unimpaired flight reflexes are not surprising for these would be under cerebellar control.

The effects on pupil dilation and eye movements resulting from destruction of the oculomotor nerve were predictable. Hyporeactivity to external stimuli was the most noticeable behavioural effect of the lesions and has some relevance to later chapters of this thesis.

Later lesions, produced in the course of a behavioural study - see Ch. 7 and 8 - effected similar results to those described, although, because the lesions were smaller, the effects were even more transitory than described above.

CHAPTER 3

Electrical Stimulation of the Red Nucleus and Some Other Sites

Electrical stimulation of the mammalian r.n. has been carried out many times with largely similar results. Given that the major output of the nucleus is via the rubrospinal tract, it is hardly surprising that the studies have tended to concentrate on motor effects of stimulation. It has been fairly conclusively established that stimulation of the mammalian r.n. produces a facilitation of contralateral flexor muscles and an inhibition of contralateral extensors (Massion 1967). The only reported case of stimulation in the free moving animal is that of Delgado (1965), who obtained, in the Rhesus monkey, a sequence of contralateral head turning, contralateral circling, walking and climbing. Massion (1967) suggests that such complicated behaviour is a result of stimulation of other more complex neuron circuits. However, whilst the animals were naturally asleep, Delgado demonstrated only the head turning as a response to stimulation. Because only simple motor patterns of flexion were obtained by other workers, when in all cases the animal was either restrained or anaesthetised, does not necessarily mean that Delgado's results, in a free moving animal are due to secondary stimulation of other systems.

Electrical stimulation of the avian brain has been carried out by several authors, prominently, Phillips (1964), Akerman (1966) and Maley (1969). None of these specifically refer to the r.n. in their published work. Akerman's studies were restricted to forebrain regions, Phillips and Maley were solely concerned with agonistic patterns of behaviour. Only Putkonen (1967) gives a comprehensive coverage to responses from telencephalon, diencephalon and mesencephalon but does not include r.n. stimulation.

The experiment to be described was conducted on relatively free moving birds with implanted electrodes. This was considered desirable to stimulation of anaesthetised or restrained animals in order to be of more relevance to the behavioural studies outlined in later chapters.

In addition to the implantation of electrodes in the r.n., placement was also made in forebrain and cerebellar nuclei areas which have been linked to the nucleus under investigation.

THE STIMULATION EXPERIMENT

Subjects

Six adult pigeons of mixed strain weighing between 250 - 300 gms were used as experimental animals.

Surgery

The birds were anaesthetised by administration of Equithesin according to body weight and placed in a Kopf stereotaxic instrument. The skull was exposed through a midline incision, scraped free of periosteum and dried. The desired location of the point of insertion of the electrodes was determined by means of the Hodos and Karten stereotaxic atlas and the skull was trephined with a dental burr. The dura was pierced, the electrode driven to the required depth and fixed in place by a small blob of acrylic cement. Six electrodes were implanted in each bird, two in the r.n. bilaterally, two in cerebellar nuclei and two in various forebrain areas. Individual leads were soldered to the terminals of the female miniature socket, which was permanently mounted on the skull by means of stainless steel anchoring screws and acrylic cement. The indifferent electrode was a loop of uninsulated stainless steel wire tucked under the skin sutured around the socket.

Electrode Manufacture

Electrodes were manufactured in accordance with Delius (1966) and essentially consisted of a length of straightened stainless steel wire (36 swg) insulated by six coats of Schenvar 31 varnish, the tip of which was ground to a paraboloid shape by a fine emery disc driven by a high speed electric motor. Fine soft tinned copper wire was soldered to the electrode at the point where it would be level with the skull surface to link the electrode to the female socket.

Stimulation Technique

A constant voltage stimulator was used to deliver a negative square wave pulse of 50 pulses/sec., with a pulse duration of 2 m. sec.* The current was determined by measuring the potential drop across a 1000 Ω resistance. Fine wire leads conveyed the current to the electrodes. The wires were suspended from a counterbalanced arm which allowed the animal relatively free movement around the experimental cage (see Fig.3.1).

The cage measured 2½' x 2½' x 2½'. The birds were observed by means of a closed circuit television arrangement in order to obviate contamination of any stimulated behavioural sequence by emotional responses to the experimenters presence. A wide angle lens fitted to the camera allowed complete coverage of the experimental cage. Stimulus trains varied from 1 - 5 secs. and current from .01 ma - .3 ma. The stimulus train and current were monitored on a Tektronix 502 oscilloscope.

Written records and drawings were made of behavioural responses to stimulation. In addition, photographs of reliably reproducible responses were made (unfortunately the film was later accidentally destroyed). No attempt was made to monitor any autonomic responses. Stimulation through two electrodes simultaneously was carried out

* See appendix 4.

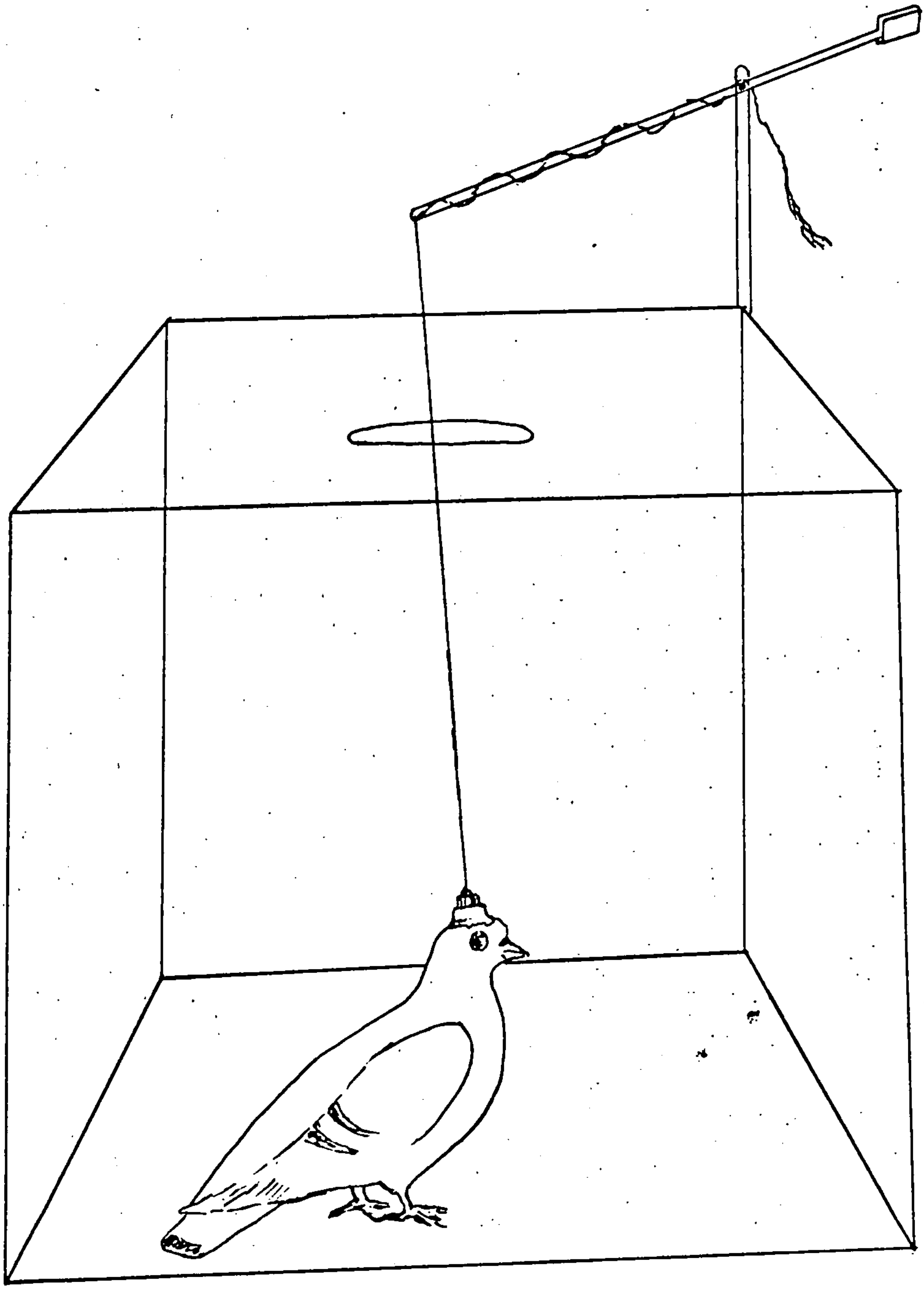


Fig31, Drawing of the experimental cage used in the stimulation experiment. Note the counterbalanced arm carrying the electrode leads which allowed relatively free movement.

where the calculated impedance of the electrodes concerned and the current necessary to elicit a response were similar. This was simply done by doubling the current needed to obtain a response during stimulation through the single electrode, hence administering a current value to each site approximately equal to the already established threshold current.

Histological Procedure

At the conclusion of the experiment, the birds were sacrificed by an overdose of Nembutal and the location of the electrode tip was marked by a small radio frequency (R.F.) lesion. Immediately following death, the animal was decapitated and the brain perfused through the carotid arteries successively with saline and 10% formalin and fixed in 10% formalin. The brains were then sectioned on a freezing microtome at 40 µ. Sections including electrode tracks and tip lesions were stained with cresyl violet.

RESULTS

Electrode Position

Unfortunately the twelve attempted r.n. implants were unsuccessful in all but two cases. Often the attempted and actual implanted positions varied by up to 2 mm. Cerebellar implants were more successful. Table 3·1 gives the locations of electrodes obtained from examination of the histological material.

Stimulation Results

The observations, electrode sites and stimulus parameters are shown in Tables 3·1 and 3·1a, Table 3·2 summarises the responses observed.

The two electrodes sited in the r.n. produced consistent contraversive rotation (circling on the spot) and in addition both elicited leg muscle effects in which the bird appeared to stumble whilst turning. Circling movements were also produced from neostriatal areas near the tractus fronto archistriatalis,

Table 3-1a

Stimulation sites from which no discernible effects were obtained

Bird	Electrode Location
1	paleostriatum primitivum
2 *	archistriatum dorsale paleostriatum augmentatum(anterior region)
3	archistriatum ventrale
4	paleostriatum primitivum
5	paleostriatum augmentatum(anterior region)

* The terminals of two electrodes were broken

TABLE 3·1

Bird	Electrode Location	Stimulus	Observations
1	N. lateralis	.05 ma	Immediate head and neck tilt to contralateral side.
	Ectostriatum	up to .3 ma	No effect.
	Paleostriatum (augment)	.3 ma	Head bow and circle contralaterally.
	Tr. dorsolat. thal.	.08 ma	Head turn to contralat. side, held after stim. cessation for 2 sec.
	Red nucleus	.08 ma	Contralat. rotation, signs of ataxia.
3	N. medialis	.04	Head lower.
		.06	Head lower and squat
	Post commissure	.08	Head lower and ipsiversive circle.
	Tr. fronto-arch.	.3	Ipsiversive circle.
4	N. medialis	.04	Head lower.
	Tr. fronto-arch.	.1	Ipsiversive head turn and squat immobile.
	Neostriatum	.1	Head shake, ipsiversive circling.
5	N. internus	.08	Contralat. body lean.
		.1	Topple onto contralat side.
	N. medialis	.05	Head lower.
	Tr. fronto-arch.	.1	Head lower and occasional ipsiversive circle.
6	Neostriatum	.3	Immobility?
	N. basalis	.08	Head shake, regurgitation.
	N. rotundus	.2	Alert, preen ipsi. side followed by contra.
	Tr.occip.-mes. or tr. strio-tegmentalis et strio-cerebellaris	.05	Contra. rotation.
2	Red nucleus	.02	Contralateral rotate ataxic walking.

TABLE 3.2

Contra head turn	Contra circle	Ipsi circle	Bow	Lean	Head shake	Electrode location
				*		N.lateralis
	*		*			Paleostriatum
*						Tr.dorsolat. thal.
			*			N. medialis
		*	*			Post comm.
		*				Tr.fron.-arch.
		*			*	Neostriatum
					*	N.basalis
	*					Tr.occip. mes or tr. st.teg et str.cereb.
	* (and ataxia)					Red nucleus

Summary of responses obtained following stimulation.

* denotes a response falling within a listed category.

paleostriatum augmentatum, posterior commissure and tractus strio-tegmentalis et strio-cerebellaris or occipitomesencephalicus, although only the latter was contraversive. The pattern produced by stimulation of the r.n. and the tractus occipito-mesencephalicus was quite distinctive and consisted of a rotation rather than a circling movement, with, in the case of r.n. stimulation, the apparent muscular discoordination superimposed.

The cerebellar nuclei-nucleus lateralis, medialis and internus were all successfully stimulated and produced distinctly different effects. The nucleus lateralis effected a head and neck tilt of 45° to the contralateral side, nucleus medialis a head lowering describable as a bowing movement and nucleus internus, a dramatic body lean to the unstimulated side, which, with an increase in current, caused the bird to fall stiffly onto its side.

The nucleus basalis of the telencephalon produced head shaking and regurgitation and neostriatal areas immediately adjacent to the basalis and fronto archistriatalis produced a complex pattern of head shaking and ipsiversive head turning and circling.

DISCUSSION

The major essay of this experiment was to stimulate the r.n. and although only successful in two of twelve electrodes the results are reasonably definitive. Both electrodes produced a contraversive rotation and obvious signs of muscular discoordination. Circling and rotatory movements have been described by Phillips (1966) and Putkonen (1967). Putkonen, moreover (personal communication) asserts that such movements are extremely common, especially in diencephalic and mesencephalic areas. Indeed, it seems only reasonable, that where the effect of stimulation is simply to create an increased level of arousal, an asymmetrical response would be expected, probably manifested in circling locomotory behaviour.

The muscular discoordination (ataxia) effected by r.n. stimulation does however point to a more specific effect, probably mediated by the rubro-spinal tract. It may be that the circling pattern is due to stimulus spread to adjacent reticular substance.

The contraversive circling obtained from the occipito-mesencephalic tract or tractus strio-tegmentalis et strio-cerebellaris, fits the data provided by Putkonen only if one assumes the stimulated tract to be the latter, for he obtains only ipsiversive circling from the former at diencephalic levels.

Head shaking and regurgitation was also found in similar forebrain areas by Putkonen.

Stimulation of the cerebellar nuclei-lateralis, medialis and internus, produced what appeared to be conspicuously vestibular responses of leaning, bowing and head tilting. This would be in accord with the findings of Groebbels (1929 - cited in Pearson 1972) that the avian cerebellar nuclei are involved in vestibular activities. The author had been expecting a response from one of the three cerebellar nuclei, in view of the well established cerebellar-r.n. connections in mammals, which would contain some elements of the r.n. response, but clearly the effects appear to have no common components. (The reader is, however, referred to the experiments described in Ch. 4 in which a connection with the nucleus lateralis is established).

Interaction effects of stimulating the r.n. concurrent with other sites, was successfully carried out in one bird only but proved to be interesting. Stimulation of the r.n. and ipsilateral ectostriatum simultaneously, resulted in a slight head turn to the contralateral side, followed by a walk forward. When stimulation of the ectostriatum was terminated the bird immediately began to rotate contraversively in response to r.n. stimulation. As no noticeable response had been obtained to ectostriatal stimulation alone, this result suggests that the ectostriatum may have been exerting an inhibitory effect on the r.n. response. Stimulation of r.n. and contralateral paleostriatum produced no diminution of the rotating effect, however the response elicited by the paleostriatal electrode alone, of bowing and contralateral circling, did not appear. This could indicate an inhibition of the paleostriatum by the r.n. or that the r.n. response masked any others.

In conclusion, the r.n. response of circling is in accord with the Delgado (1965) circling in free moving monkeys and certainly the ataxia displayed by the birds during stimulation suggests that the avian r.n. contributes to muscle control in accordance with findings in mammalian studies.

CHAPTER 4

Afferent Rubral Connections of the Avian Brain

Introduction

A great deal of work has been done in recent years, using both anatomical and electro-physiological techniques, in the elucidation of connections of the mammalian r.n. with other areas, whereas the linkages of the avian supposed homologue has been but little explored since the anatomical work reported in Ariens Kappers et al. (1960).

It will be recalled, that whilst the major intention of the work reported in this thesis was to provide comparative data, it was also felt that a study of the avian r.n. might serve to shed some light on the problem posed by the findings of Thompson et al (1964, 1967, 1969) which implicate the mammalian r.n. in learning and memory. To this end a thorough mapping of afferent connections of the pigeon r.n. was felt to be necessary not only for comparative reasons but because connections with other than what could be described as motor components might be disclosed.

In consequence of the paucity of data regarding the avian r.n. the background material to be cited hereunder will be of mammalian origin and whilst there are many similarities between mammalian and avian species in anatomical structures of diencephalon and mesencephalon, the telencephalon may well be dissimilar. The review of the literature will therefore be concentrated on midbrain and brainstem areas, with mention of forebrain links in so far as is necessary to clarify major functional and anatomical connections in the mammal. The number of publications devoted to the mammalian r.n. and anatomically related structures, is so large as to preclude a comprehensive coverage in this thesis and therefore only the major, well confirmed links will be initially discussed.

The Mammalian Red Nucleus

The Rubro-spinal System

The r.n. and its direct efferent tract the rubro-spinal, has been known for many years and investigation of it has produced a great deal of data in analysis of the means by which motor control is effected. It has been confirmed many times that the r.n. exerts a facilitatory action on contralateral flexors and an inhibitory action on contralateral extensors (e.g. Pompeiano 1958, Sasaki et al 1960). The precise means by which such control is exerted is not wholly clear, although Appelberg (1967A and 1967B) has shown that the gamma motor system is involved as well as the alpha. In terms of the contribution of the r.n. to overall motor control, the very elegant work by Lawrence and Kuypers (1968) demonstrated that the r.n. and rubro-spinal tract is responsible for control of the distal musculature - hand, feet and arm movements, although the cortical motor system and pyramidal pathways duplicate this and are, in addition, responsible for fine finger movements. As stated, the precise mechanisms are not yet known but are in any case irrelevant to the main theme of this thesis.

Cerebellar-rubral Links

Although efferent fibres from cortical areas 4 and 6 project ipsilaterally to the magno-cellular portion of the r.n. (Rinvik and Walberg 1963), Tsukahara and Kosaka (1966) have shown that the input from the cerebellar nucleus interpositus is more efficient in terms of its synaptic connections. This fact would appear to suggest that cerebellar influence is of greater importance in r.n. function than that of the cerebral cortical areas. The probable interaction process between the cortico-spinal and rubro-spinal motor systems is suggested by Eccles et al (1967) in their excellent book. They show that when the r.n. is facilitated by fibres from the interpositus nucleus of the cerebellum, an inhibitory post synaptic potential can be recorded in the ventrolateral thalamus. As the ventro-lateral thalamus inhibits the cerebral cortical areas from which the pyramidal tract projects it would seem that facilitation of the r.n. would be accompanied by a diminution of the pyramidal tract activity. Furthermore, Eccles et al (1967) point out that stimulation of the medullary pyramid effects an inhibition of the r.n. presumably by axon collaterals of the pyramidal tract. Fig.4.1 schematises the possible relation between the respective motor pathways.

Other Afferent Sources

In addition to the input from the nucleus interpositus of the cerebellum and areas 4 and 6 of the cerebral cortex, there would appear to be afferent tracts from the basal-ganglia, although there is some confusion as to the precise source of the input; Massion (1967) concludes his review of experimental findings "....we can say that in carnivores and primates, whenever experimental lesions have been carried out, it seems established that no fibres pass from either the lentiform nucleus or from the caudate to the magno-cellular part of the r.n. A small pallidorubral pathway does probably exist and it, again, probably ends in the magno-cellular part of the r.n. ....."

Papez and Stotler (1940) and Martin (1969) describe a direct visual projection from the superior colliculus to the r.n. Ariens Kappers et al (1960) describe a similar tecto-rubral projection in birds.

In addition to the direct visual pathway, sensory projections of an indirect nature, probably via the cerebellum, have been demonstrated by Massion and Albe-Fessard (1963) from somaesthetic and auditory stimulation using the evoked potential recording

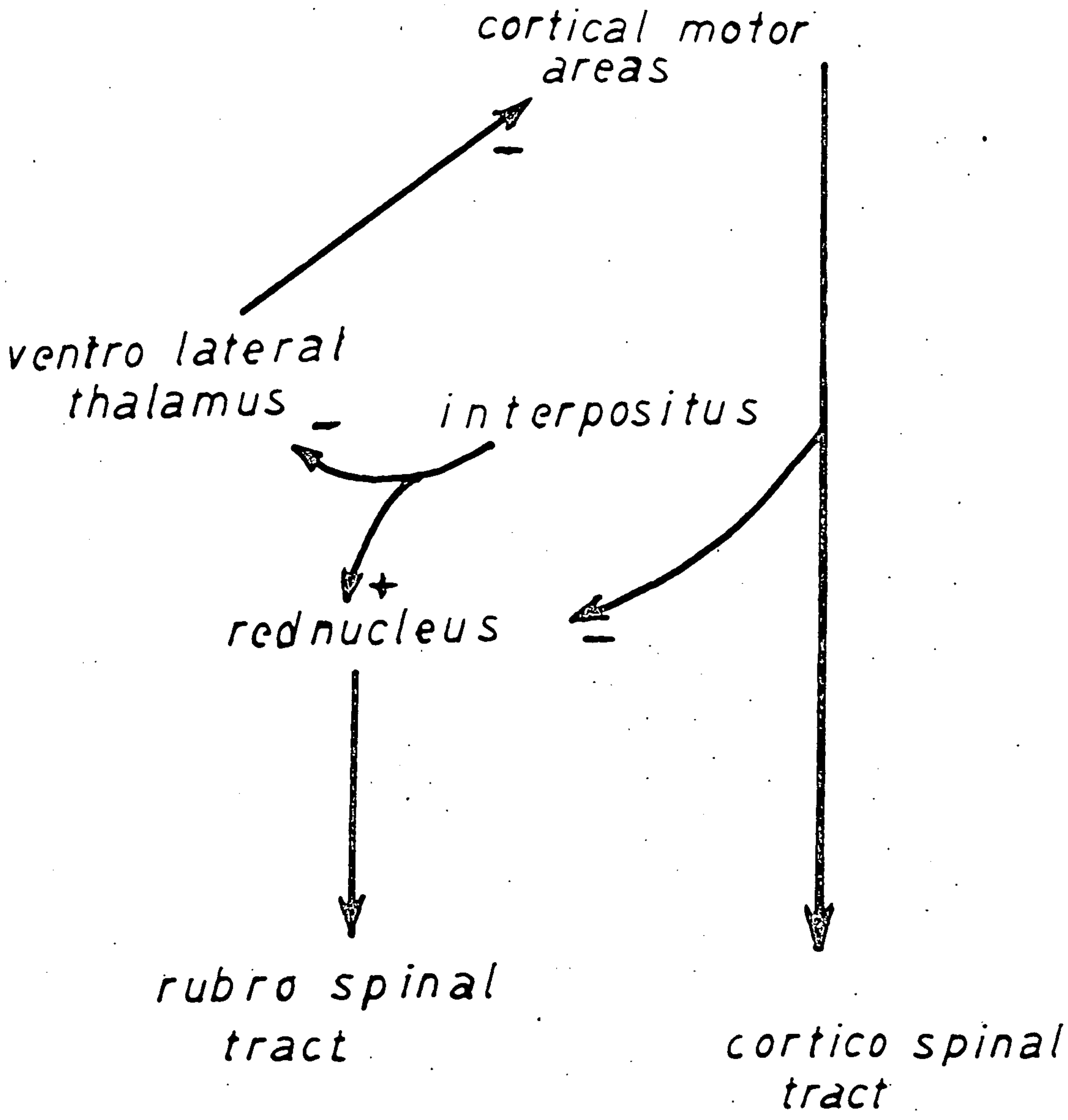


Fig.4.1. Probable process of interaction between red nucleus and cortical motor systems. Positive and negative signs denote excitatory and inhibitory linkages respectively. The diagram illustrates that activation of the r.n. by the nucleus interpositus of the cerebellum is paralleled by a reduction of activity in cortical areas and activation of the cortico-spinal system effects an inhibition of the r.n.

technique. In addition, Ermolaeva and Chernigovsky (1964) have shown visceral afferents following stimulation of the splanchnic nerve whilst Manni et al. (1965) have recorded potentials in the r.n. arising from vestibular stimulation,

Although other afferent projections have been shown (see Fig.4.2) those outlined above represent what are probably of major importance.

Other Efferent Links

In addition to the rubro-spinal tract, ipsilateral efferent connections have been shown to exist with the subthalamus and globus pallidus (Carpenter 1956). Stimulation of the r.n. has also suggested indirect efferents affecting autonomic function. Thayer et al. (1966) found that electrical stimulation of the rostral r.n., in the Macaque, produced a significant increase in gastric secretion and Lewin (1967), also using electrical stimulation, demonstrated an inhibitory effect on spontaneous contractions of the urinary bladder in the cat. Sano et al. (1967) found that electrical stimulation of the r.n. in man produced parasympathetic reactions such as lowered blood pressure and pupil constriction. Clearly there would appear to be an across species similarity in autonomic response produced by r.n. stimulation. Monnier (1968) desribes a hypothalamic-r.n. link which is ergotrophic in function (page 207) and another which is trophotrophic (page 223). This double innervation may well account for the contradictory findings of Lewin (1967) and Thayer et al. (1966) of urinary inhibition and facilitation of gastric secretion respectively, the former being ergotrophic and the latter trophotrophic. It will be recalled that Ermolaeva and Chernigovsky (1964) demonstrated an afferent link between the splanchnic nerve and the r.n., the former being intimately concerned in autonomic nervous system regulation

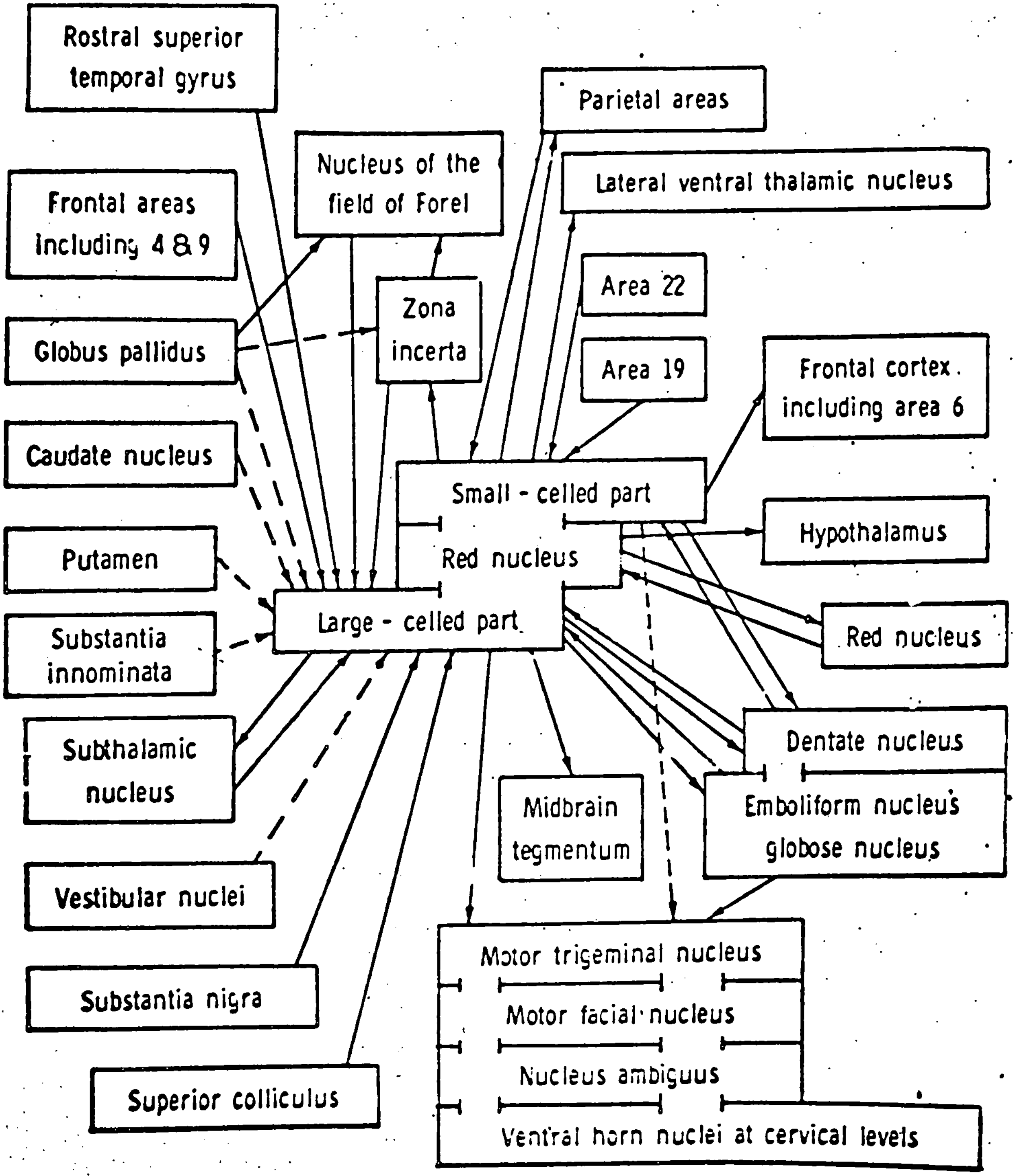


Fig.4.2 Afferent and efferent connections of the r.n. Solid lines linking areas denote confirmed links, broken lines indicate as yet unconfirmed findings.
(from Rapoport et al. 1968)

(Grossman 1967). Thayer et al. find their results "..... surprising, because this region has been traditionally associated with motor activity rather than autonomic regulation". It seems only reasonable however to expect autonomic effects from stimulation of a motor system, either by way of direct or indirect efferents or from feedback from muscle systems.

The foregoing very brief outline of findings pertaining to the mammalian r.n. by no means covers all the published work. The reader is directed to the review by Massion (1967) for a more comprehensive coverage. The magnitude of the task of unravelling the connectivity of the r.n. is perhaps best illustrated by renewed reference to Fig.4.2 which outlines many more of the reported connections than have been discussed above.

Given the lack of research on the avian r.n. it was felt necessary to determine connections in order to ascertain:-

(1) Whether the avian r.n. was analogous to that of the mammal, in terms of afferent links.

and (2) What comparative differences, if any, exist.

THE MAPPING EXPERIMENT

Experiments were performed on 29 pigeons of mixed strain, obtained from a dealer, and selected on the basis of weight - all birds weighed more than 300 gms. The lower weight restriction was found to be necessary in the interests of accuracy of electrode location given that the stereotaxic atlas used, that of Karten and Hodos (1967) was based on Carneaux pigeons which are a standard breed weighing between 400-600 gms. Early work by the author showed that the use of animals weighing less than 300 gms. tended to produce inaccurate electrode placement probably due to immaturity in skull size.

Electrodes

Recording and stimulating electrodes were fine, stainless steel insect pins. The electrodes were insulated by six thin coats of Shenvar varnish, achieved by mechanical dipping, with each coat being "baked" at 130°C for five hours. A 0.5 mm tip was then scraped bare under a low power binocular lens and the electrode tested for insulation leakage by bubbling in a saline solution (Delius 1966). Stimulating electrodes were fixed in parallel pairs and those for recording purposes in threes by means of an acrylic dental cement. The tip separation of the electrodes was between 0.50 - 0.75 mm.

Stimulation and Recording Procedure

Electrical stimulation of the brain, achieved by means of a Nuclear (Chicago) constant current stimulator, consisted of a biphasic rectangular pulse of 0.5 m. sec. and 0.01 - 0.2 ma. Amplification of r.n. activity following stimulation of brain areas was by means of a Tectronix 122 preamplifier using a low frequency cut off of 5 cycles/sec. and a high frequency cut off of 250 cycles/sec. The resultant activity was displayed either on a dual trace storage oscilloscope or averaged on a Biomac 500 averaging computer.

Location of the recording electrode in the r.n. was achieved both by the stereotaxic co-ordinates and also by a phenomenon discovered early in the series of experiments. It was found that the coated insect pins were sufficient to record the spike activity of single or groups of the large cells of the r.n. whilst being too coarse to record from the units of surrounding areas. More accurate placement of the recording electrode was therefore obtained by filtering out the low frequencies associated with mass potentials and observing unit activity by using the oscilloscope and a sound amplification system. It was thus possible to gain both visual and auditory confirmation of the successful location of the r.n. area.

Procedure

The animal was anaesthetised by intra-muscular injection of Equithesin according to body weight (2.5 cc per k.g.) and placed in a Kopf stereotaxic instrument. The skull, above the electrode sites, determined from the atlas, was trephined and the dura incised. The recording electrode was driven downward until the tip was approximately 1 mm rostral to the r.n. area according to the atlas co-ordinates and the filtering and amplification system adjusted to record single units. The electrode was then drivedn down by a microdrive until spike activity became apparent. In the absence of such activity it was assumed that location was inaccurate and the electrode was withdrawn and re-located.

Several points regarding electrode location should be made at this juncture:-

(a) Stereotaxic co-ordinates alone were not reliable enough - often being up to .75 - 1 mm discrepant.

(b) Skull markings were found to be unreliable as a means of location.

(c) Single units were not present or at least not recordable with the coarse electrodes used, whilst the animal was deeply anaesthetised.

The stimulating electrode was lowered so that the tips just penetrated the tissue at the chosen site. The recording equipment was reset to record mass potentials and stimulation was then begun.

At each stimulation site the parameters of the electrical stimulation were varied. Pulses were delivered at intervals of 1 per second to 1 per five seconds.** Amplitude varied from 0.01 -

---

** During early experimentation it was found that the r.n. response became rapidly attenuated if stimulation exceeded 15 - 20 pulses per second.

0.2 ma. The onset of each stimulus also triggered the storage oscilloscope or Biomac sweep thereby providing a synchronised recording of any induced potential in the r.n. Photographic records were made of any resultant activity. Following completion of the stimulation sequence the stimulating electrode was advanced 0.5 - 1.0 mm and the procedure repeated until the limits of the electrode track, determined from the stereotaxic atlas, were reached. The lower limits of each electrode track was marked by means of a small radio frequency lesion, the electrode withdrawn and a further site explored using the same method. No more than three stimulating tracks were made in any one animal in order to minimise the effects of electrode damage to the brain tissue. As the stimulation and recording procedure was lengthy, especially where stimulation sites produced indications of induced potentials in the r.n., the animals were often anaesthetised for prolonged periods before sacrifice. In view of this the condition of the animal, in terms of central response to stimulation, was often felt to be such that more than a single track was inadvisable.

Following completion of each experiment the animal was sacrificed by means of an overdose of Nembutal, the brain perfused through the carotid arteries with 5% saline followed by 10% formalin and fixed in 10% formalin for several days. Serial sections of 40 µ were taken on a freezing microtome and each section showing the electrode track or terminal lesion, stained with cresyl violet.

Throughout the series of experiments stimulation was largely restricted to the homolateral side of the brain to the r.n. from which the recordings were made, on the assumption that, as in mammals, the vast majority of r.n. connections rostral to that nucleus would be uncrossed. The only instances in which contralateral stimulation was attempted involved the cerebellar nuclei, again following what is known of mammalian connections.

Sites which produced potentials in the r.n. were occasionally checked in other preparations.

RESULTS

Histology

A total of 43 electrode tracks was used in the mapping experiment - see Fig.4·3.

Stimulation sites, on examination of the histological material, were often found to be up to 1 mm discrepant from those attempted. Recording electrodes, on the other hand, were always in or near enough to the r.n. to be regarded as satisfactory.

Figs 4·4 - 16 show the areas of the brain from which potentials arose. The drawings are of trans-sections in the same vertical plane used for stereotaxic purposes. Electrode tracks are shown by dotted, vertical lines.

Recordings

Latencies to the first deflection of the induced potential varied greatly - the shortest being 1 m. sec. and the longest 35 m. sec. The majority of recordings were obtained from stimulation of telencephalic areas with the exception of two sites in the diencephalon and mesencephalon and two in the cerebellar nuclei. It is possible that other mesencephalic areas may have connections with the r.n., it being found impossible to reduce the very large stimulus artifact, which would have obscured any potentials, when attempting to stimulate near the recording electrode. For this reason it was found to be impracticable to attempt to establish the existence of rubro-rubral connections.

Telencephalic Areas

The forebrain areas, which on stimulation produced r.n. evoked

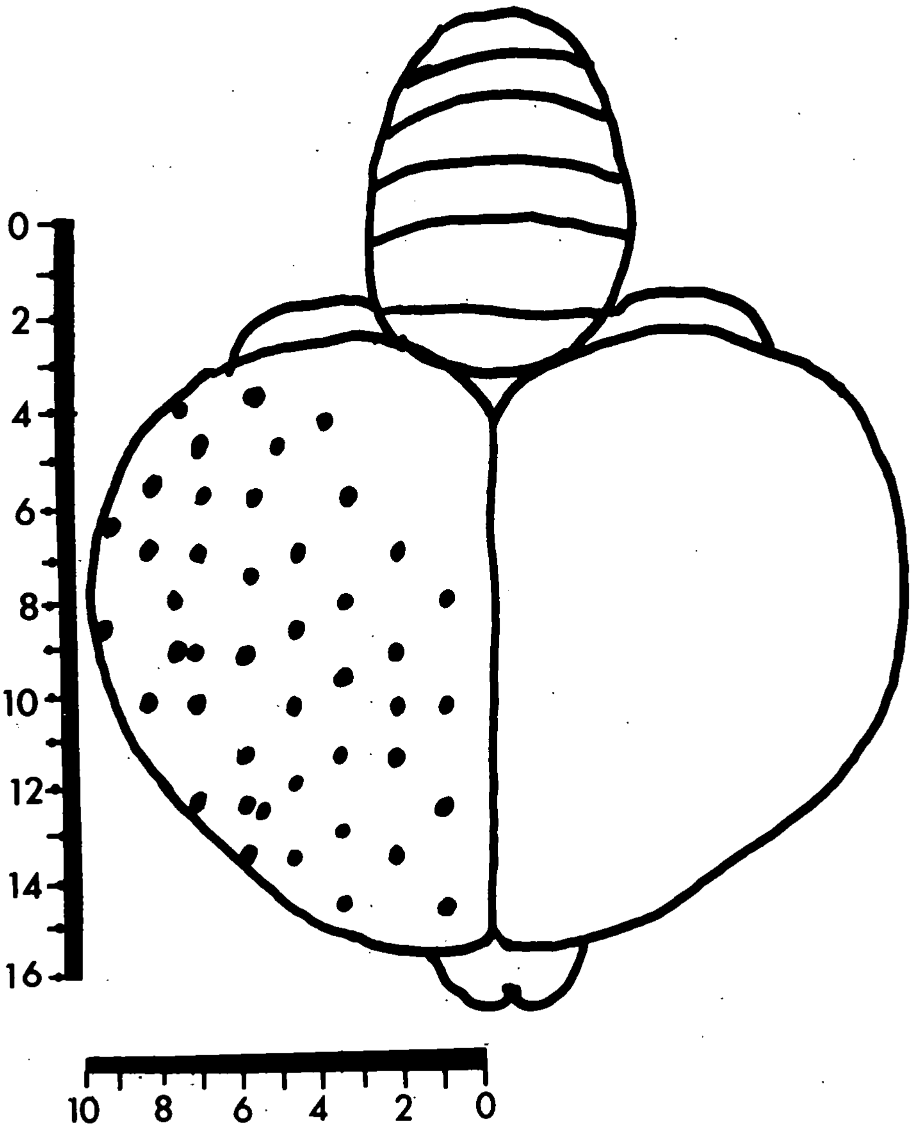


Fig.4.3. The distribution of electrode penetrations shown on a drawing of the dorsal surface of the pigeon brain. Scale in mm.

potentials are shown in Figs.4·4, ·5, ·6, ·7, ·8, ·9, ·10, ·11 and ·14. The shortest latencies (hereinafter used as meaning the time period to the first deflection of the induced potential trace) were obtained from the tractus occipito-mesencephalicus (3 m. sec.) and archistriatum medialis (4 m. sec.) see Figs4·11 and4·14 respectively.

List of Abbreviations Used in Figs.14 - 16

AD	archistriatum dorsale
Aid	archistriatum intermedium pars dorsalis
Ai	archistriatum intermedium
AL	ansa lenticularis
Am	archistriatum mediale
AV	archistriatum ventrale
Bas	nucleus basalis
Bc	brachium conjunctivum
BO	bulbus olfactorius (olfactory bulb)
CA	commissura anterior
Cb	cerebellum
Cbi	nucleus cerebellaris internus
CbL	nucleus cerebellaris lateralis
CbM	nucleus cerebellaris medialis
E	ectostriatum
FA	tractus fronto- archistriatalis
FLM	fasciculus longitudinalis medialis
FPL	fasciculus prosencephali lateralis (lateral forebrain bundle)
FPM	fasciculus prosencephali medialis (medial forebrain bundle)
HA	hyperstriatum accessorium
HD	hyperstriatum dorsale
HIS	hyperstriatum intercalatus superior
Hp	hippocampus
Hv	hyperstriatum ventrale
HVvv	hyperstriatum ventrale ventro-ventrale
Lhy	lateral hypothalamus
LPO	lobus parolfactorius
N	neostriatum
OM	tractus occipito-mesencephalicus
PA	paleostriatum augmentatum
PP	paleostriatum primitivum
QF	tractus quintofrontalis
Rt	nucleus rotundus
TeO	tectum opticum
TrO	tractus opticus
TSM	tractus septomesencephalicus
V	ventriculus
VeL	nucleus vestibularis lateralis
VeM	nucleus vestibularis medialis

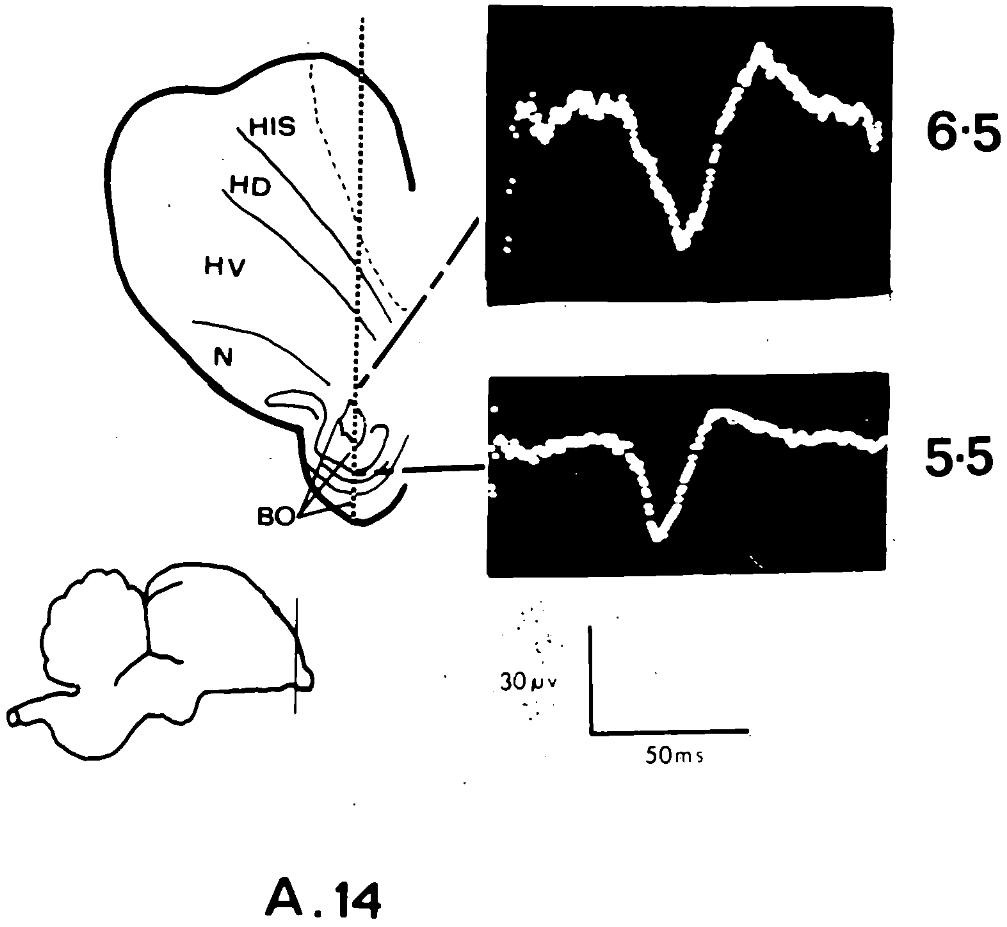


Fig. 4.4 Evoked potential recordings from the r.n. following stimulation of the ipsilateral olfactory bulb. Stereotaxic atlas coordinate (Atlas of Karten and Hodos) anterior 14. Numbers to right of traces indicate stereotaxic vertical coordinates of the stimulating electrode. Stimulus pulse (.5msec., .05ma.) occurred at the extreme left of the trace. Latency 35 - 40msec.

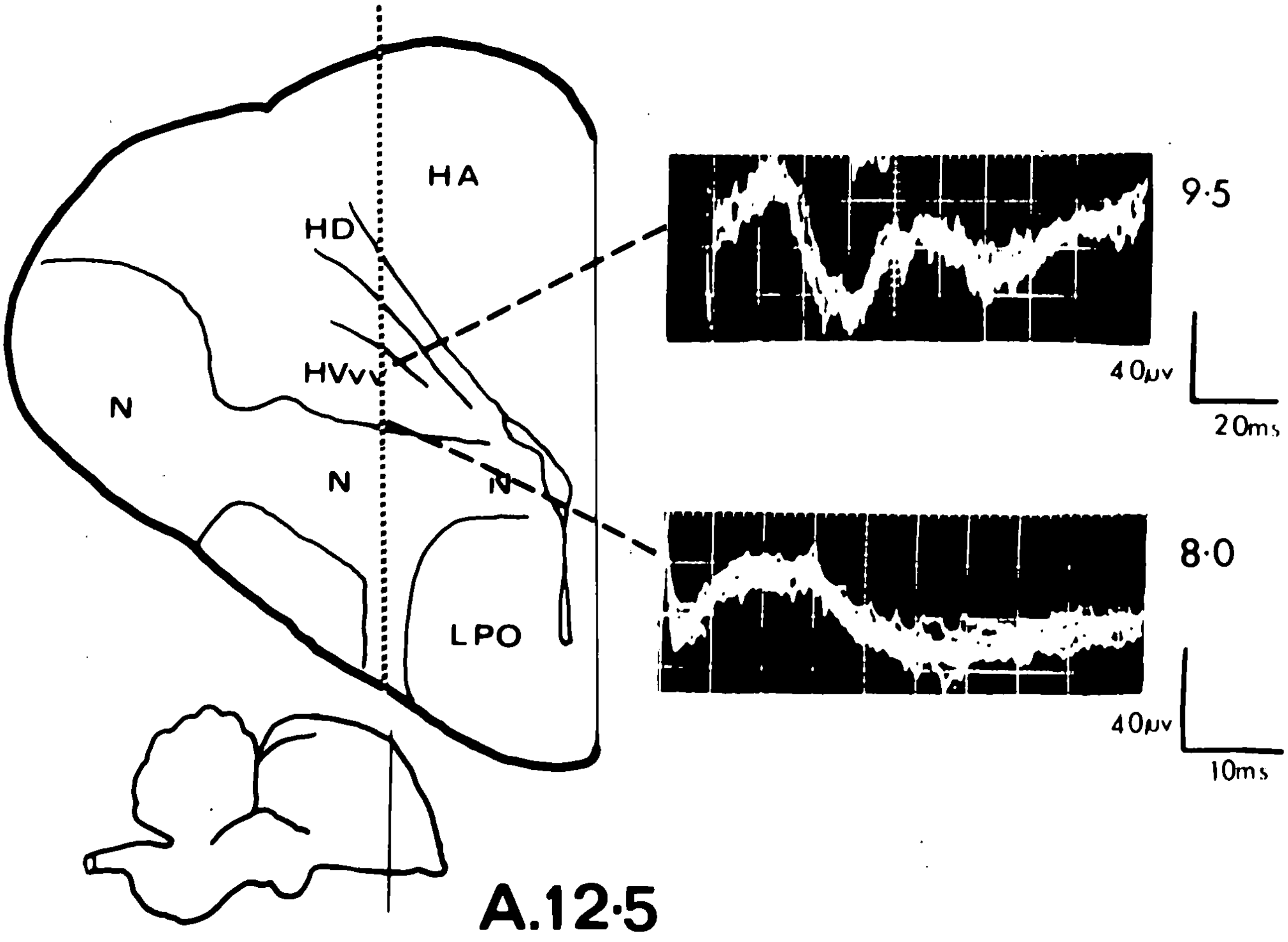


Fig.45. Evoked potential recordings obtained from the r.n. following stimulation of ipsilateral hyperstriatum ventrale ventro-ventrale. Anterior 12.5, vertical 9.5 - 8.0 indicate coordinates of stimulating electrode. Stimulus pulse occurred at the extreme left of the trace. Latencies, for these traces estimated at 18msec., varied both within a single stimulating electrode penetration and between different penetrations from 15 - 40msec.

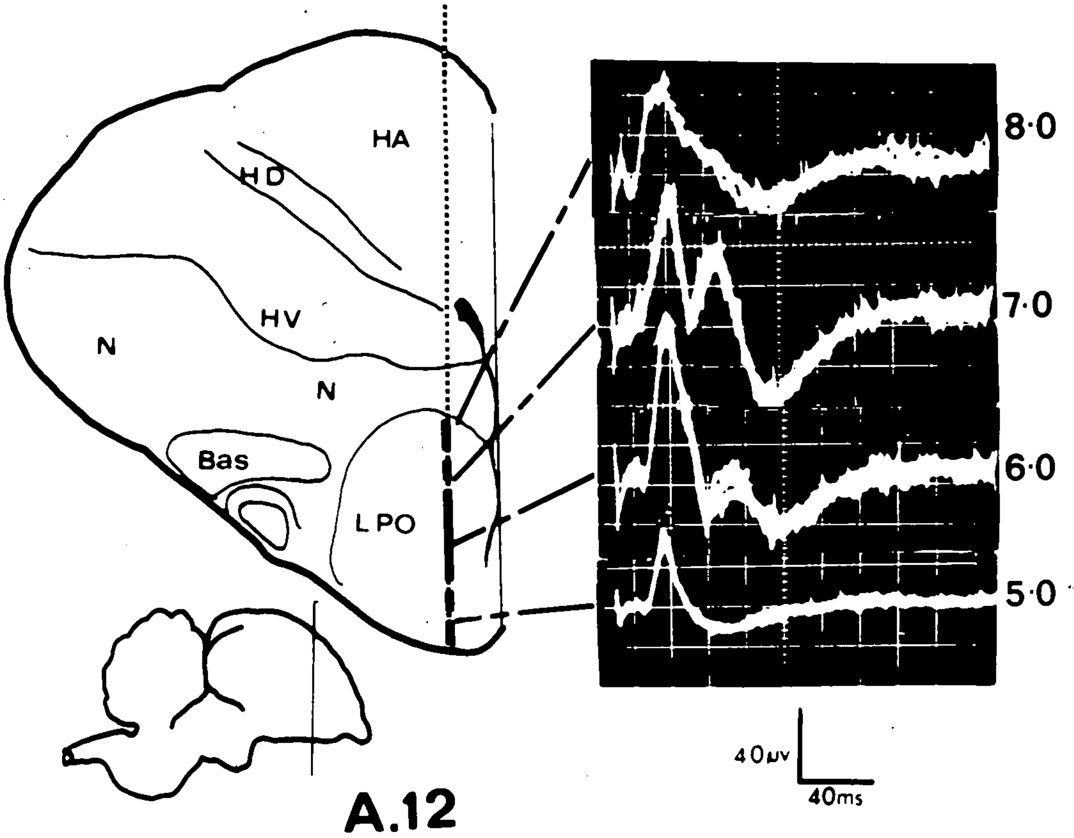


Fig.4·6 Evoked potential recordings obtained from the r.n. following stimulation of the ipsilateral parolfactory lobe. Anterior 12, vertical 8.0 - 5.0 indicate coordinates of stimulating electrode. Stimulus pulse occurred at the extreme left of the trace. Latency estimated at 15msec.

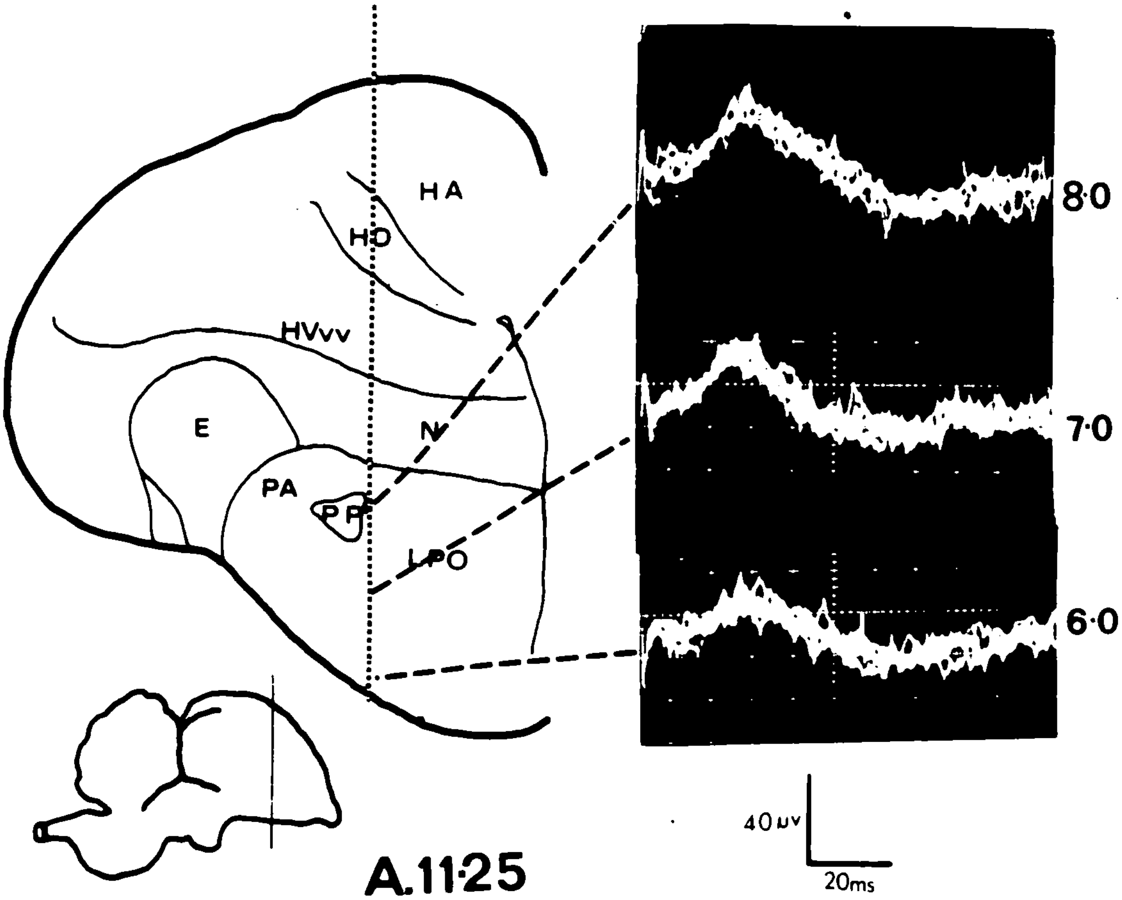


Fig.4·7 Evoked potential recording obtained from r.n. following stimulation of the ipsilateral parolfactory lobe. Anterior 11.25, vertical 8.0 - 6.0 indicate coordinates of stimulating electrode. Stimulus pulse occurred at the extreme left of traces. Latency estimated at 15msec.

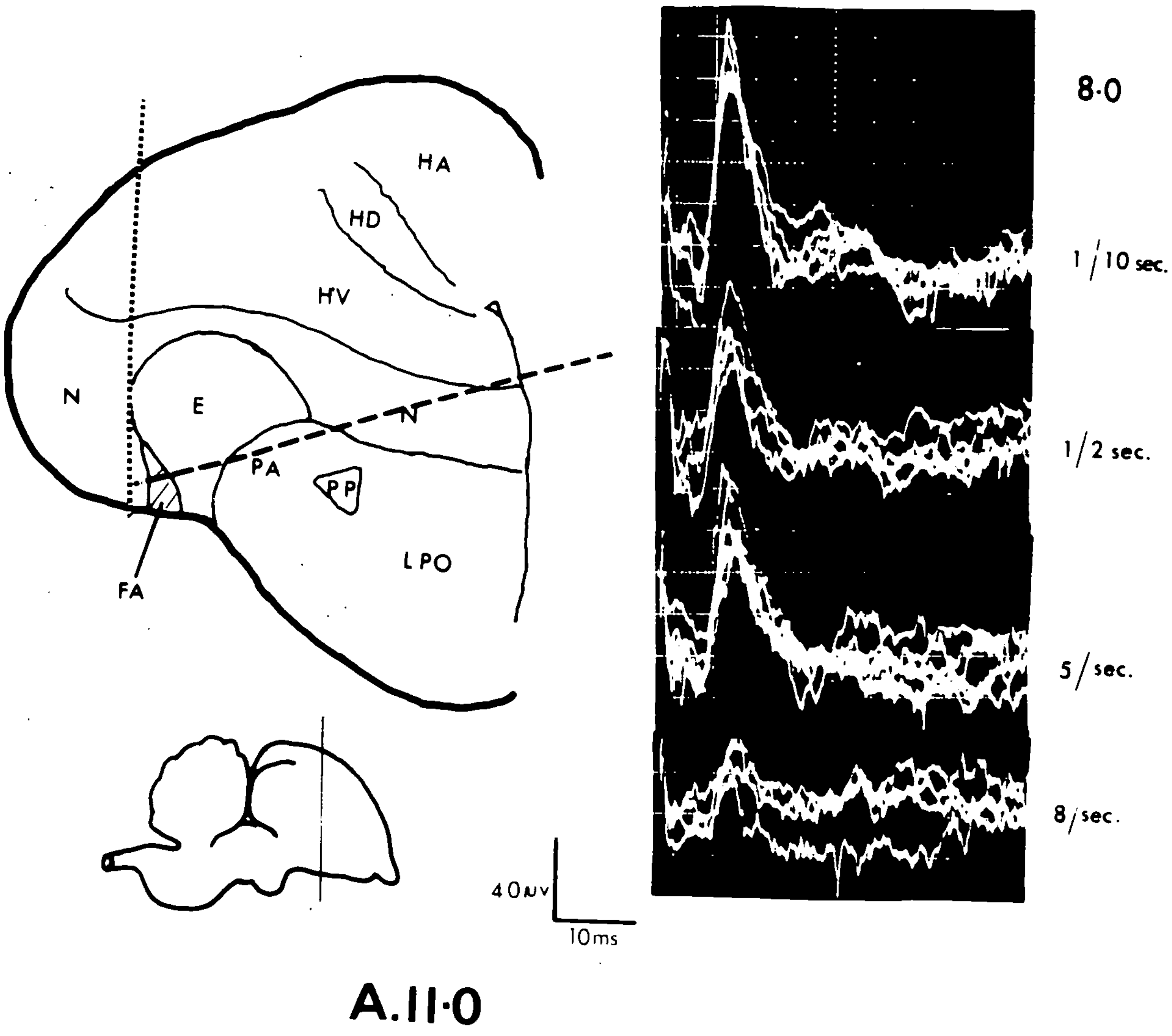


Fig.4·8. Series of evoked potential recordings obtained from the r.n. following stimulation at a single point, probably of the ipsilateral tractus fronto-archistriatalis. Anterior 11.0, verical 8.0 indicate coordinates of stimulating electrode. Traces show recordings following stimulation rates of 1 per 10 secs., 1 per 2 sec., 5 per sec., 8 per sec. Note the reduced aplitude at 8 per sec. Latency estimated at 5msec.

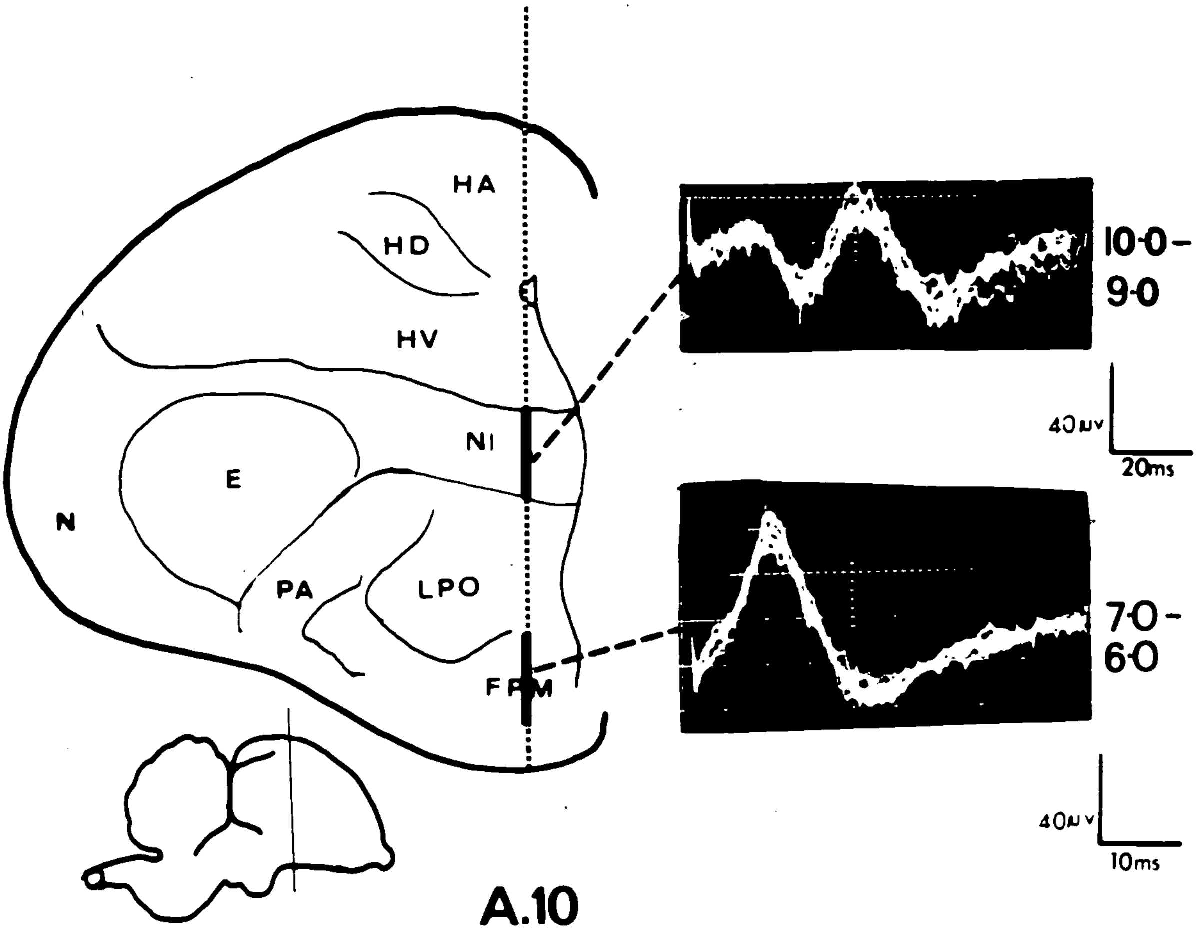


Fig.4·9. Evoked potential recordings of two distinct potentials obtained from the r.n. during a single penetration, following stimulation of the ipsilateral neostriatum intermedium and medial forebrain bundle. Anterior 10 and vertical 10.0 - 9.0 and 7.0 - 6.0 indicate coordinates of stimulating electrodes. Stimulus pulse occurred at extreme left of the traces. Latencies from stimulation of neostriatum intermedium, estimated at 20msec. for the above trace, varied from 12 - 20 msec. Latency from medial forebrain bundle was consistent: estimated at 4msec.

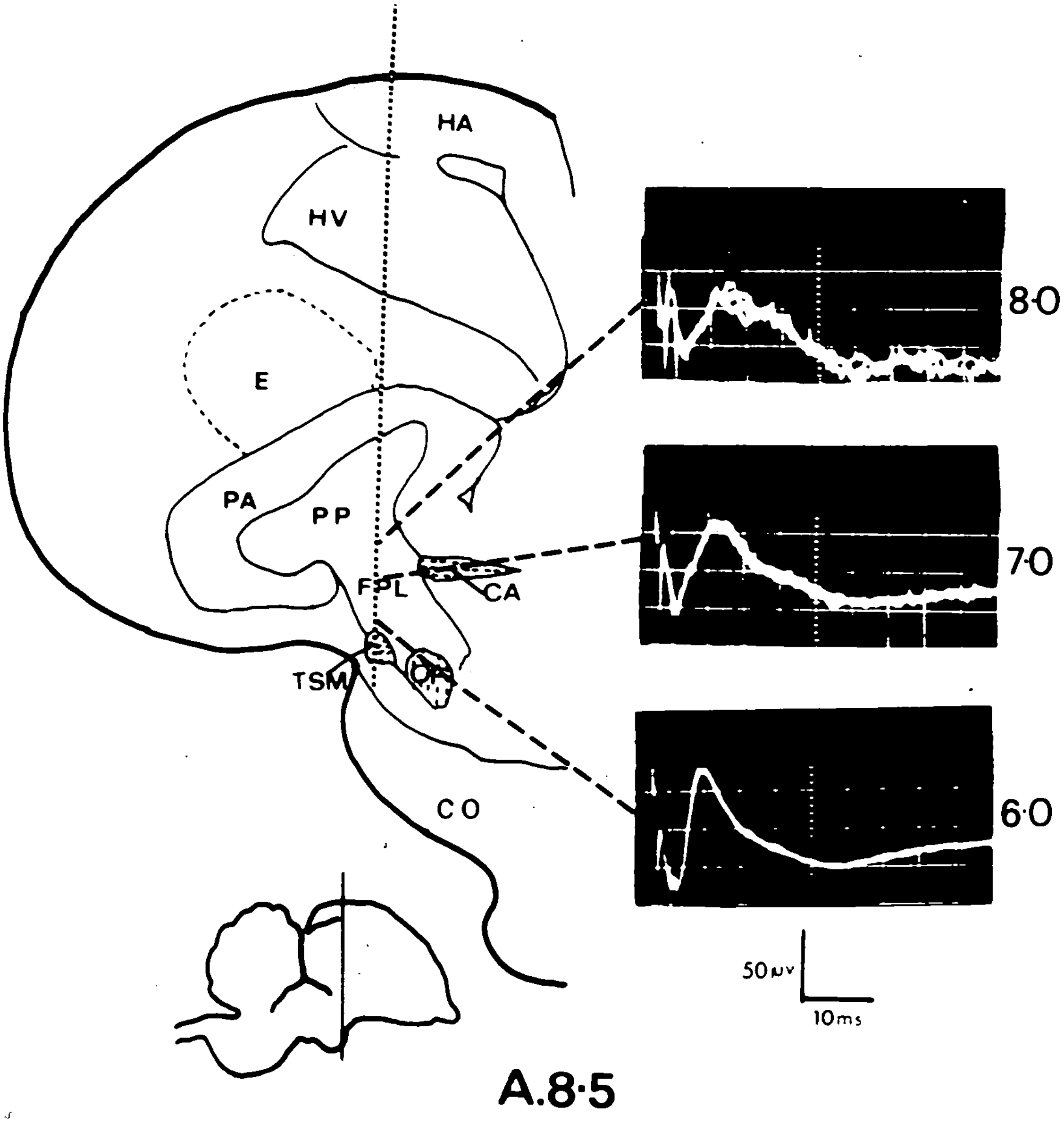


Fig.4·10 Evoked potential recording obtained from the r.n. following stimulation of the ipsilateral lateral forebrain bundle. Anterior 8.5 vertical 8.0 - 6.0 indicate coordinates of stimulating electrodes. Stimulus pulse occurred at the extreme left of the trace. Latency extimated at 5msec.

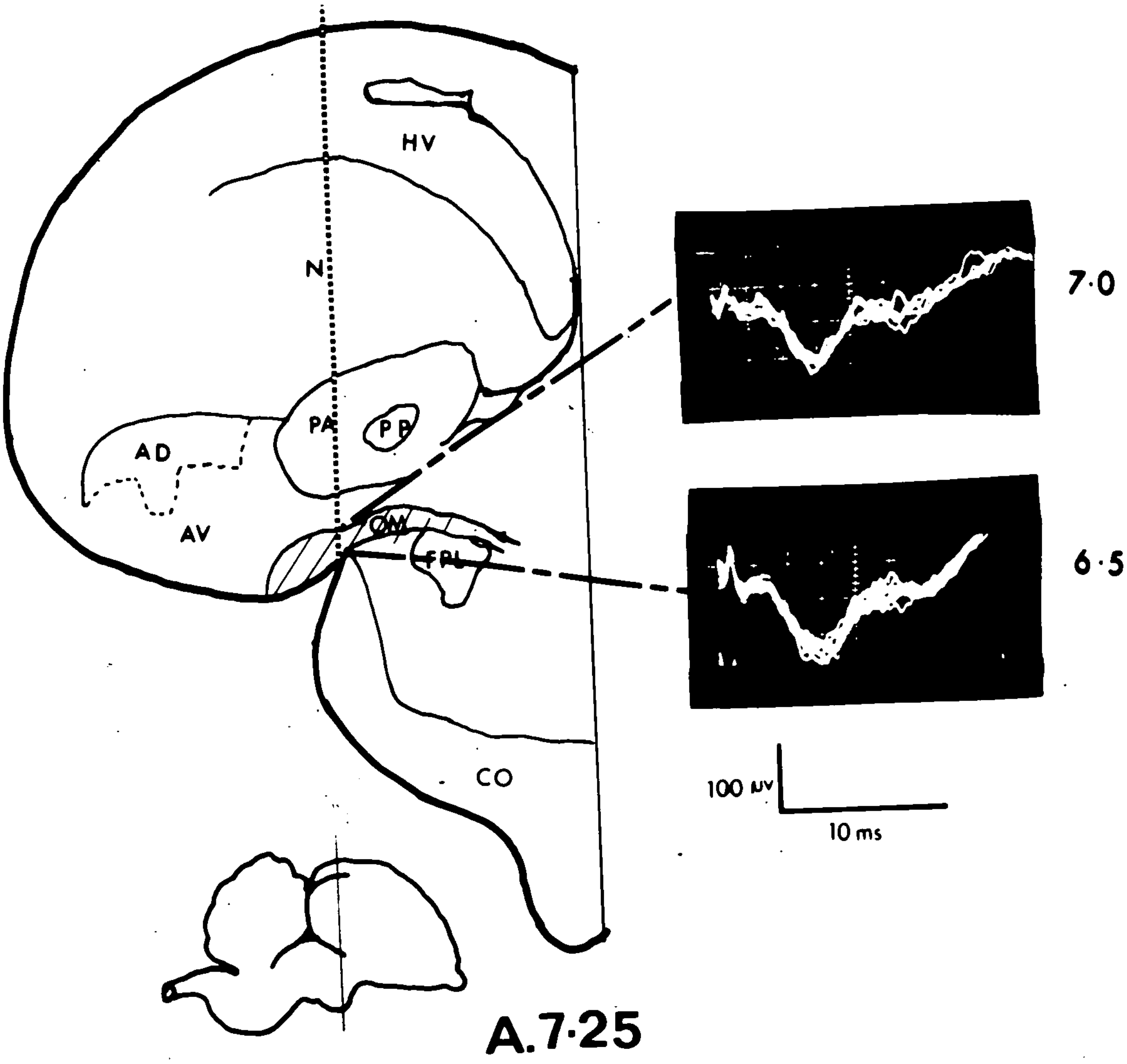


Fig.4·11. Evoked potential recording from the r.n. following stimulation of ipsilateral tractus occipito - mesencephalicus. Anterior 7.25, vertical 7.0 - 6.5 indicate coordinates of stimulating electrode. Stimulus pulse occurred at extreme left of trace. Latency estimated at 3msec.

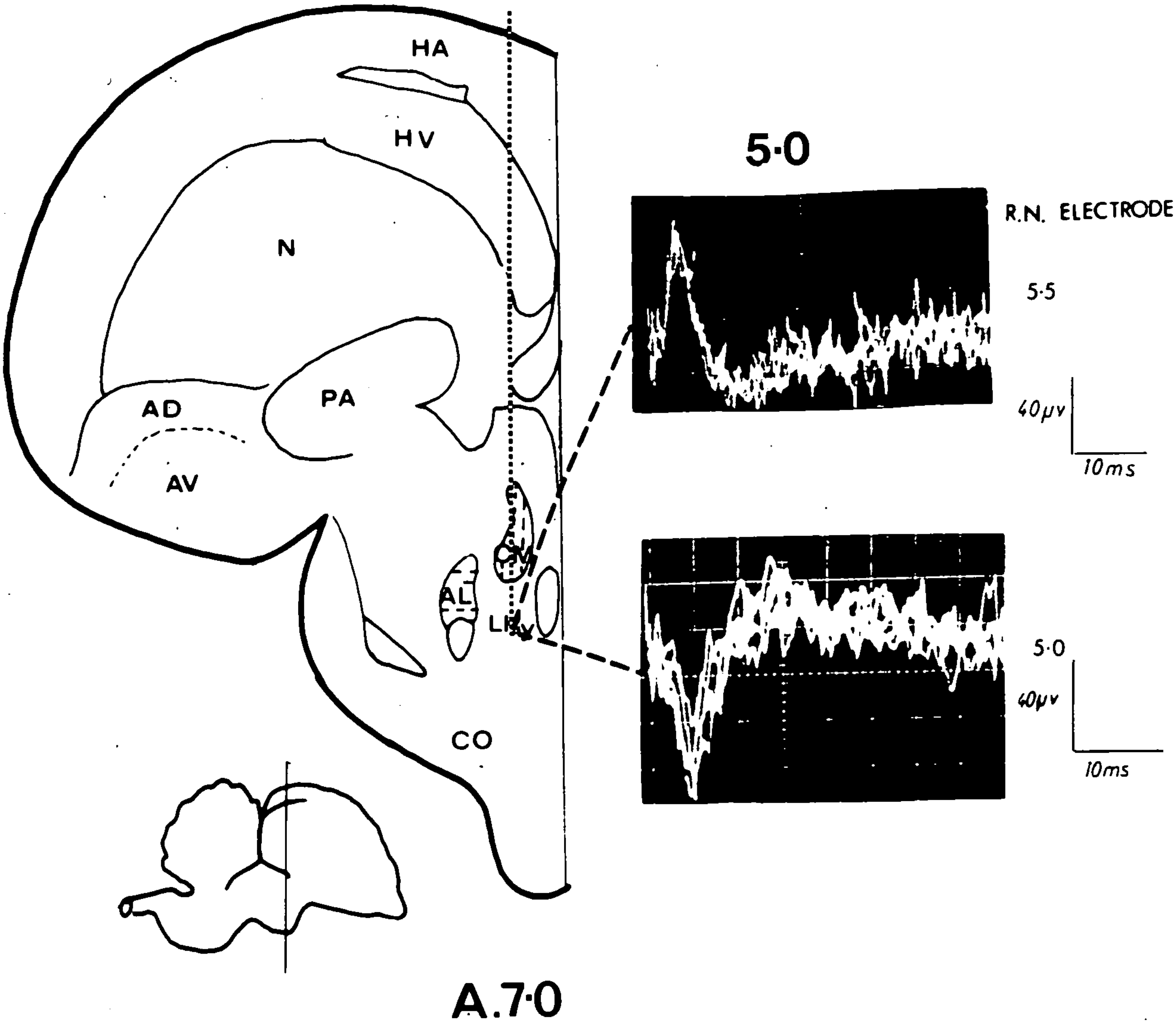


Fig.4·12. Evoked potential recordings from the r.n. following stimulation at a single point in the ipsilateral lateral hypothalamus. Anterior 7.0 vertical 5.0 indicate coordinates of stimulating electrodes. The two traces are recorded from points 0.5mm apart dorso-ventrally in the r.n. The reversal of the potential indicates that the centre of the source of the potential lay between the two electrode positions and thus was unlikely to originate from outside of the r.n. Latency estimated at 2msec.

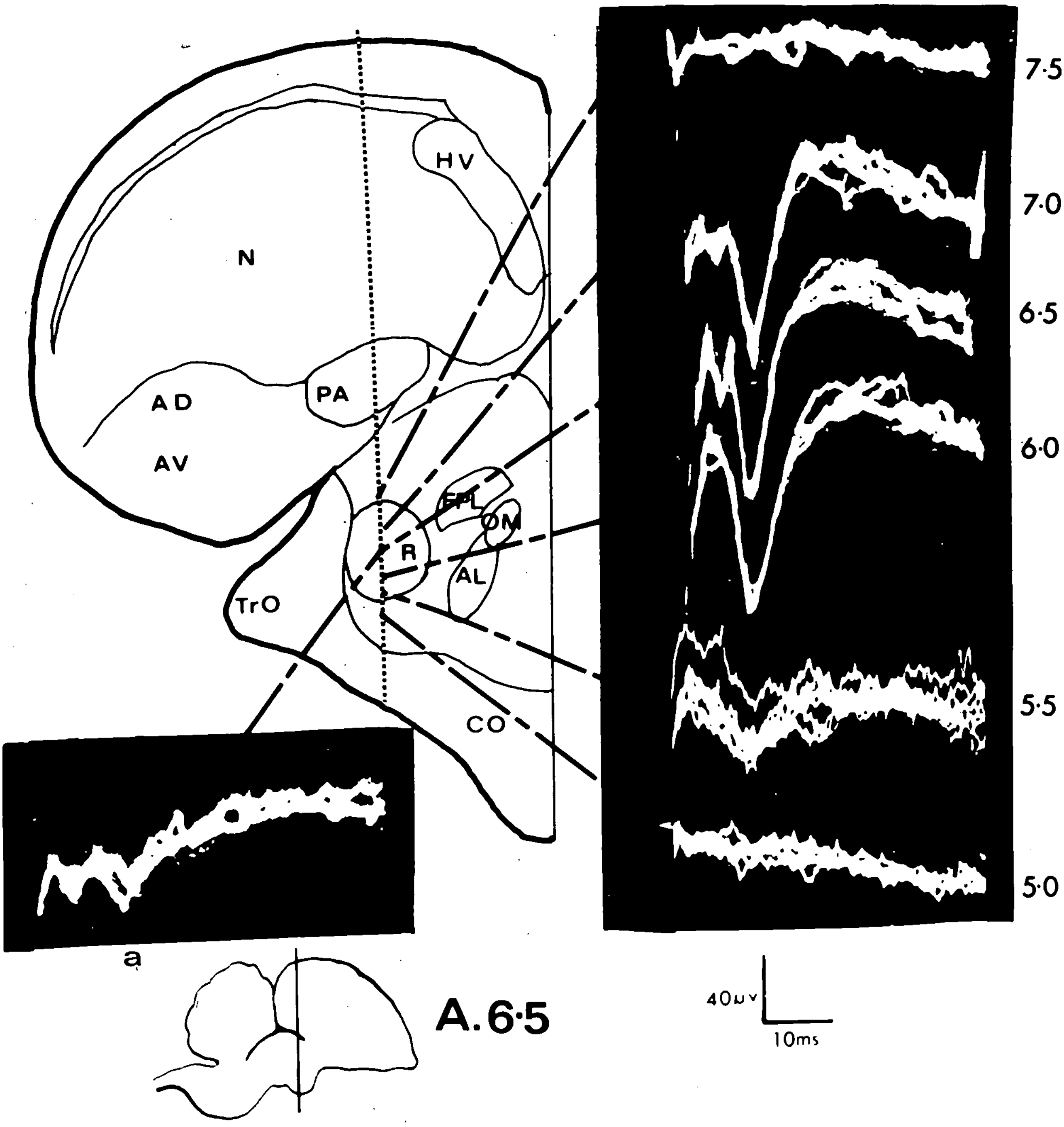


Fig.4·13. Series of evoked potential recordings obtained from the r.n. as the stimulating electrode traversed the ipsilateral nucleus rotundus. Rate of stimulation 1 per sec. Anterior 6.5, vertical 7.5 - 5.0 indicate coordinates of stimulating electrodes. Latency estimated at 6msec. Inset (a) demonstrates the effect of stimulating at 10 per sec. and as explained in the text provides evidence that the evoked potential resulted from orthodromic conduction.

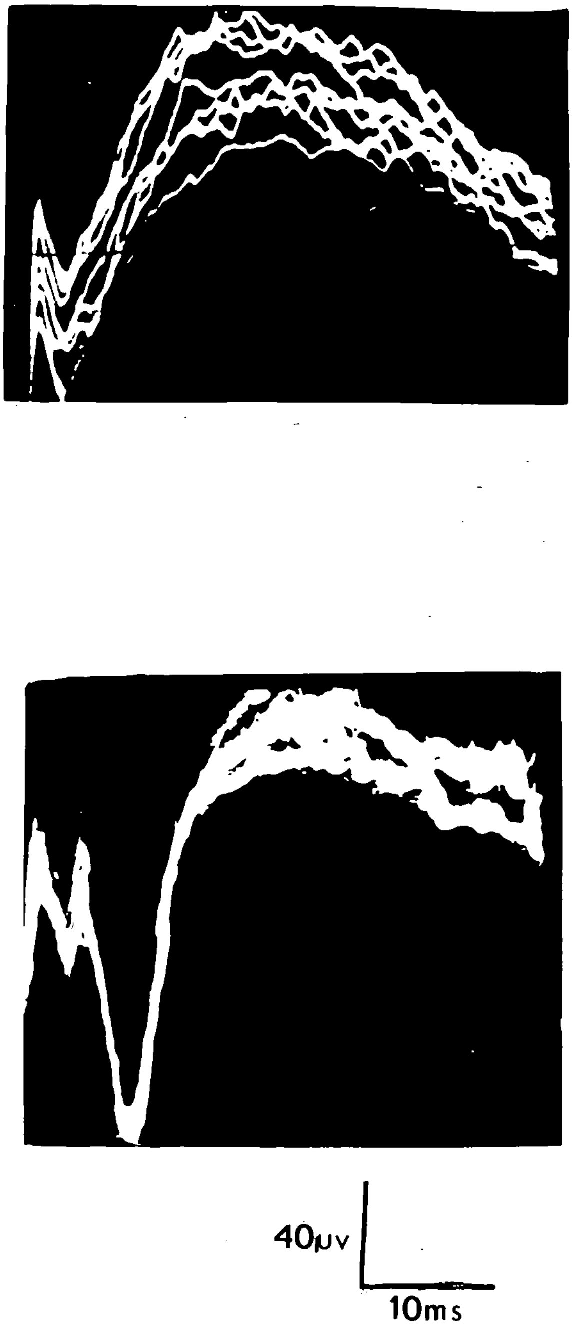


Fig.4·13a. Upper trace is a recording from the nucleus rotundus following stimulation of the r.n. Lower trace a recording from the r.n. following stimulation of the nucleus rotundus. The upper potential was still present at stimulation rates of 50 per sec. whereas the lower became attenuated at 10 per sec. (not shown but see inset (a) in Fig.4·13). Notice the small oscillation prior to the large deflection in the lower trace which may represent presynaptic activity. The suggestion is made that both findings indicate a rotundus- red nucleus rather than a red nucleus -rotundus connection.

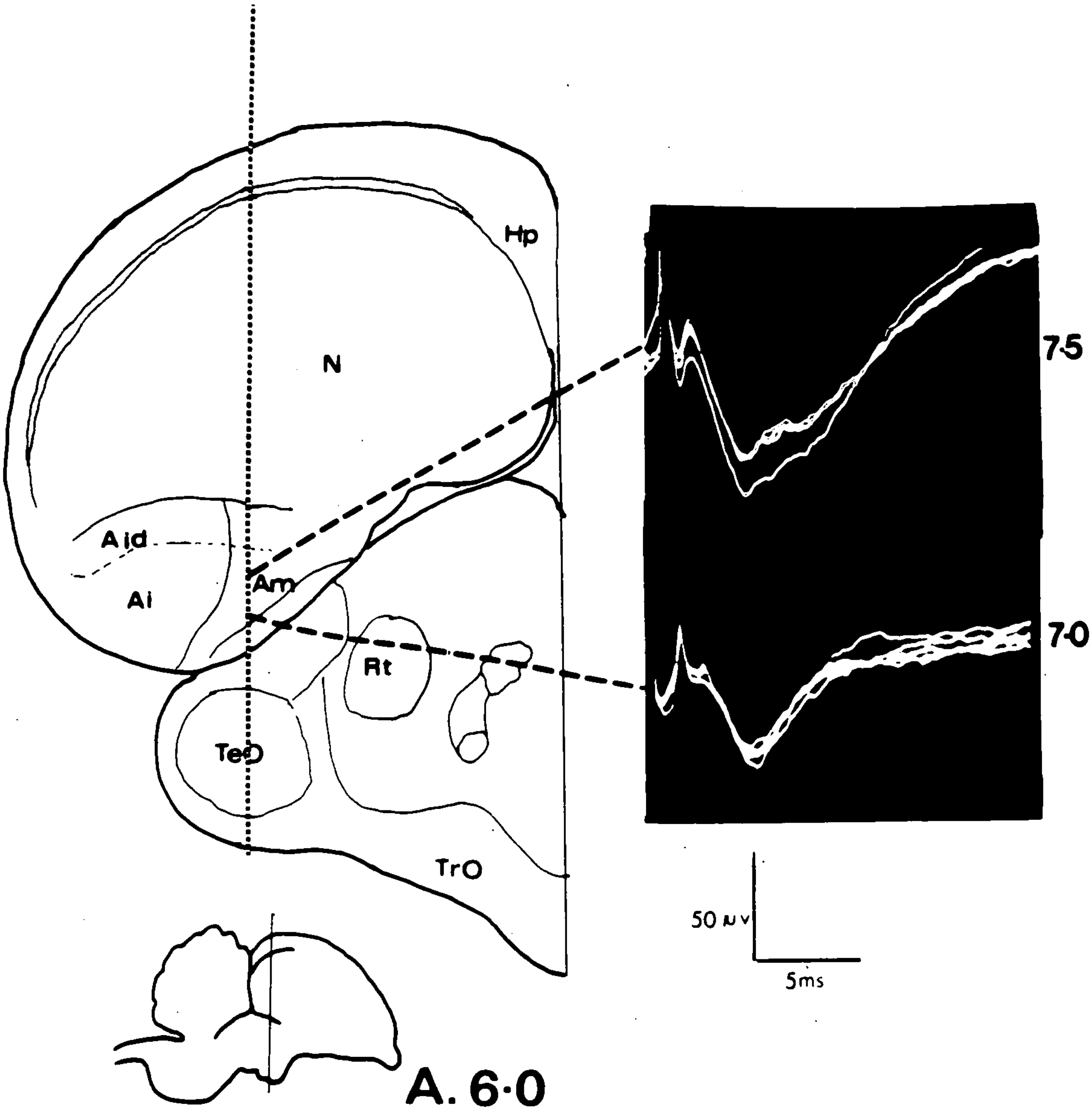


Fig.4·14. Evoked potential recording obtained from the r.n. following stimulation of the ipsilateral archistriatum medialis. Anterior 6.0, vertical 7.5-7.0 indicate coordinates of the stimulating electrode. Stimulus pulse occurred at the extreme left of the trace. Latency estimated at 7msec.

Stimulation of medial and lateral forebrain bundles (Figs.4.9 and 4.10) and tractus fronto-archistriatalis (Fig.4.8) produced latencies of 4, 5 and 5 m. sec. respectively.

Other sites, namely the parolfactory lobe (Figs.4.6 and 4.7), hyperstriatum ventrale ventro-ventrale (Fig.4.5), neostriatum intermedium (Fig.4.9) and olfactory bulb (Fig.4.4), produced latencies of 15, 15 - 40, 12 - 20 and 35 m. sec. respectively.

<u>Diencephalic and Cerebellar Areas</u>

The two diencephalic sites from which potentials were evoked in the r.n. were the nucleus rotundus, with a latency of 6 m. sec. (Fig.4.13) and the lateral hypothalamus, at 2 m. sec. (Fig.4.12). The former was carefully investigated because it was unexpected in view of the findings of Revzin and Karten (1966) (see Discussion). In particular, an attempt was made to determine whether the projection was afferent or efferent. Inset (a) in Fig.4.13 demonstrates the effect of stimulating the nucleus rotundus at 10 per sec. Fig.4.13 (a) gives the potential recorded in the same nucleus following a reversal of the stimulation and recording leads, i.e. stimulation of the r.n. and recording from the nucleus rotundus. The absence of the oscillations in the trace prior to the large deflection in the latter case (the oscillations probably represent presynaptic activity) coupled with the fact that the potential was still present at a stimulation rate of 50 per sec. tends to suggest an antidromic potential. It would therefore seem that the projection is from the nucleus rotundus to the r.n. Although the stimulation and recording leads were not reversed for any of the other sites, the fact that the response became attenuated at stimulation rates of 10 - 20 per sec. was considered to be sufficient evidence that the connection with the r.n. was afferent. Allowing an absolute refractory period of 2.0 m. sec. as representing the longest in the C.N.S. (Grossman 1967) it should be possible to record an antidromic potential at stimulation rates up to 500 per sec.

Potentials were recorded following stimulation of the nucleus lateralis (Fig.4.16) and nucleus medialis and/or internus (Fig.4.15) of the cerebellum, with latencies of 1 and 5 m. sec. respectively, although the author has doubts about the latter recording (see Discussion).

DISCUSSION

Given that the r.n. is an important motor control centre, the apparent paucity of connections demonstrated in this experiment is surprising, especially when one considers the large number of reported links in the mammal. (see Fig.4.2).

The telencephalic induced potentials are divisible into two distinct categories on the basis of the latency of the recorded potential. It would seem that the tractus fronto-archistriatalis, lateral forebrain bundle, medial forebrain bundle and tractus occipito-mesencephalicus, each prominent tracts in the avian brain, with latencies of 5, 5, 4 and 3 m. sec. respectively, conduct more or less directly to the r.n. On the other hand the olfactory bulb, parolfactory lobe, neostriatum intermedium and hyperstriatum ventrale ventro-ventrale with latencies of 35, 15, 12 - 20 and 15 - 40 m. sec. respectively must involve many synapses. The only 'area' as distinct from a tract which produced a short latency response was the archistriatum medialis, stimulation of which produced a potential of 4 m. sec. latency which ties in with the short latency response (3 m. sec) of the occipito-mesencephalic tract which has been shown to originate in the archistriatum (Huber and Crosby 1929, Zeier and Karten 1971).

The other latencies present problems. Given the short latency potentials induced by stimulation of the lateral and medial

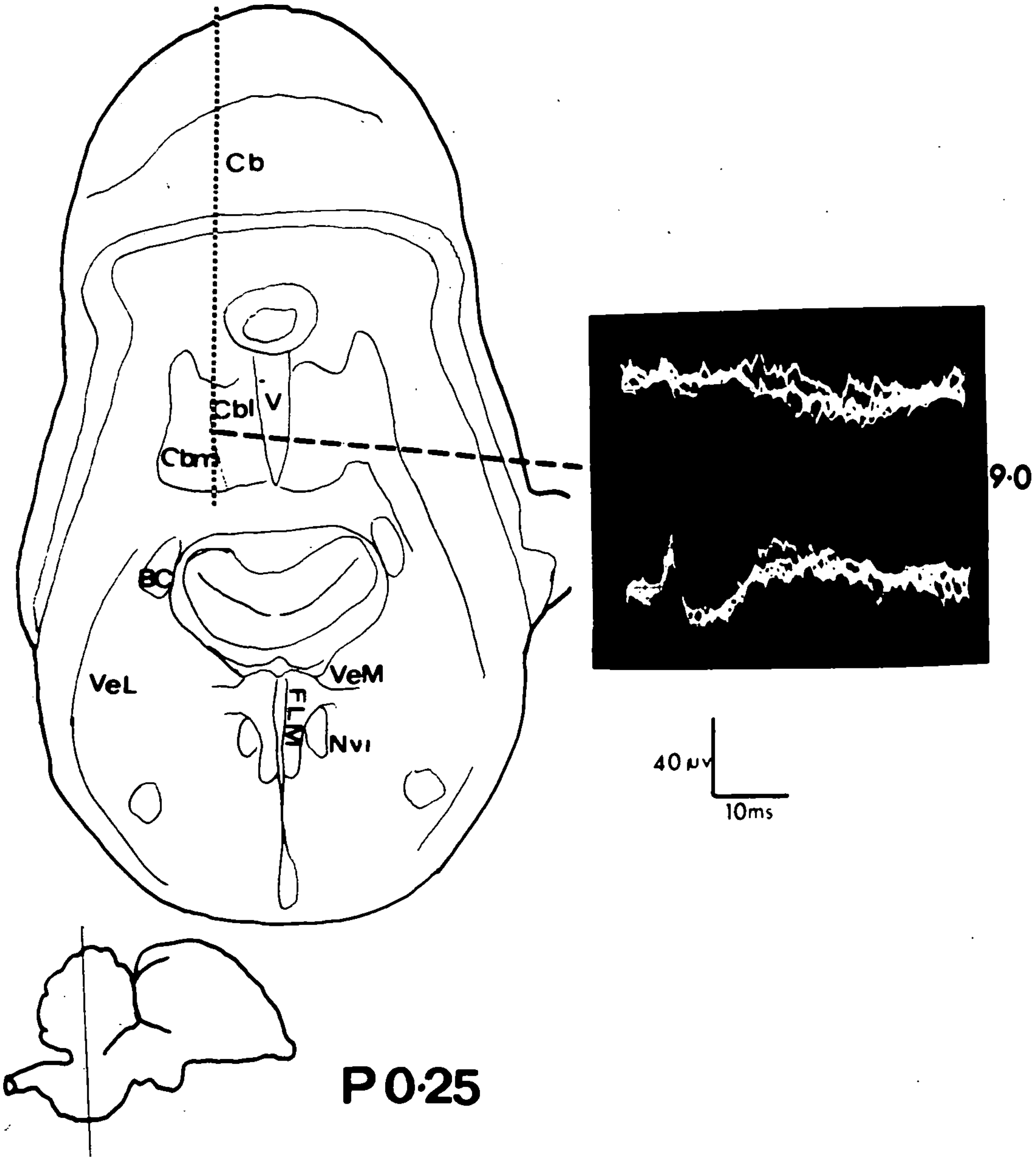


Fig.4.15. Evoked potential recording obtained following stimulation of the contralateral nucleus medialis and/or internus of the cerebellum. On the basis that the potential was recorded from only one of the electrodes, as shown, (see text for full explanation) it was assumed that it arose in the brachium conjuntivum.

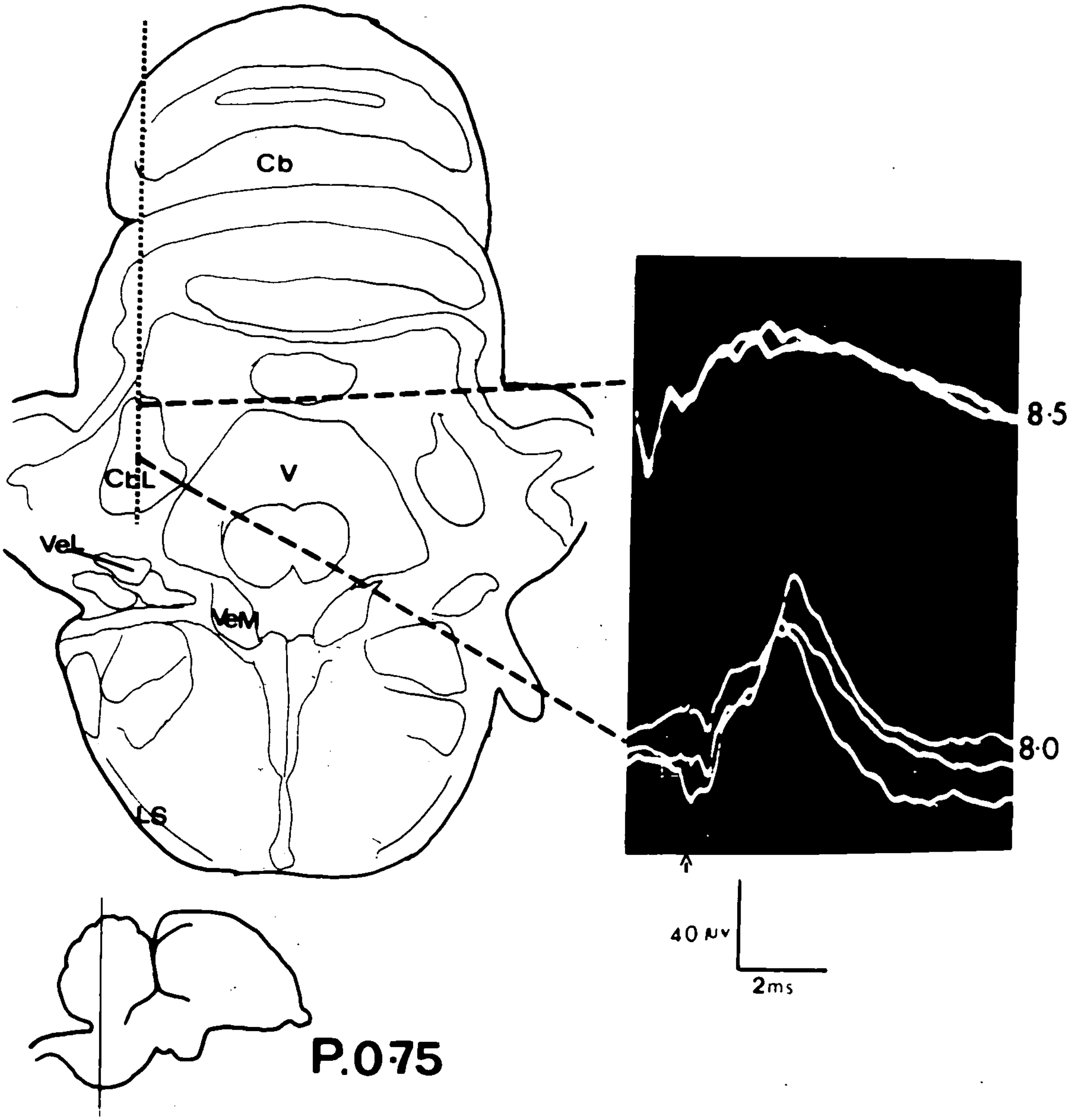


Fig.4·16. Evoked potential recording obtained from the r.n. following stimulation of the contralateral nucleus lateralis of the cerebellum. Posterior 0.75, vertical 8.5-8.0 indicate the coordinates of the stimulating electrodes. The arrow in the lower trace marks the point at which the stimulus pulse occurred; in the upper trace the pulse occurred at the extreme left edge of the trace. Latency extimated at 1msec.

forebrain bundles of 5 and 4 m. sec., one would expect to find areas giving rise to potentials at 7 or 8 m. sec., allowing at least one synapse. If however, there are several synapses within areas, then one would expect to find different latencies within the same area, depending upon the synaptic connections stimulated. Certainly the results from the neostriatum and hyperstriatum, both of which displayed variability, tend to bear out this hypothesis.

Avian Red Nucleus Connections suggested by the Findings

A synthesis of the findings into probable pathways is possible. Two routes connecting higher centres with the r.n. can be distinguished on the basis of latencies which fit existing anatomical data.

(1) The olfactory bulb is linked to the archistriatum via the lateral olfactory tract and the tractus fronto-archistriaticus. (Huber and Crosby 1929). The archistriatum is linked to many diencephalic and mesencephalic centres by the tractus occipito-mesencephalicus and occipito-mesencephalicus pars hypothalamus (Zeier and Karten 1971). It is clear from the findings of the latter authors, that the tractus occipito-mesencephalicus does not project directly to the r.n., although they do distinguish a pars hypothalami which projects from the medial areas of the archistriatum (the region from which the potential was obtained in the present study) to the medial hypothalamus. Unfortunately the medial hypothalamus was not stimulated in the course of mapping and a pathway from there to the r.n. can only be hypothesised, although such a projection was demonstrated from the lateral hypothalamus. The neostriatum intermedium and hyperstriatum ventrale ventro-ventrale project

to the tractus fronto-archistriatalis. (Huber and Crosby 1929) Pathway (1) is diagrammed in Fig.4.17.

(2) The parolfactory area is linked via the medial forebrain bundle to the lateral hypothalamus (Karten and Dubbeldam 1973) and thence to the r.n. Pathway (2) is given in Fig.4.18.

Two potentials provide some difficulty. That recorded from stimulation of the lateral forebrain bundle (5m. sec.), by virtue of the complex nature of the tract, containing as it does many fibre tracts serving a great number of areas (Huber and Crosby 1929) and the potential arising from stimulation of the nucleus rotundus (6m. sec.). The nucleus rotundus has been extensively investigated by Revzin and Karten (1966/67) and Karten and Revzin (1966), who find that the nucleus is an important way station in visual projection to the ectostriatum of the forebrain. If this is correct, the rotundo-rubral projection must carry visual information. Reference to Chapter 5 of this thesis confirms a visual projection to the r.n. although the latency obtained by electrical stimulation of the optic tract (3m. sec) - see Fig.5.2c Ch. 5 - is too short to enable the rotundal projection (6m. sec.) to be responsible for, at least, the early phase of the evoked visual response. The exclusively visual function of the rotundus claimed by Karten and Revzin (1966) has however been questioned by Baker-Cohen (1968) viz:- ".....it has been repeatedly emphasised by Huber and Crosby that a characteristic pattern in the reptilian brain is the convergence of somatic sensory impulses from spinal, brainstem and medullary centres upon the highly differentiated correlation centre developed in the optic tectum. After synapse many of the impulses are projected forward upon the dorsal thalamus; the nucleus rotundus is one of the chief recipients of the large tecto-thalamic

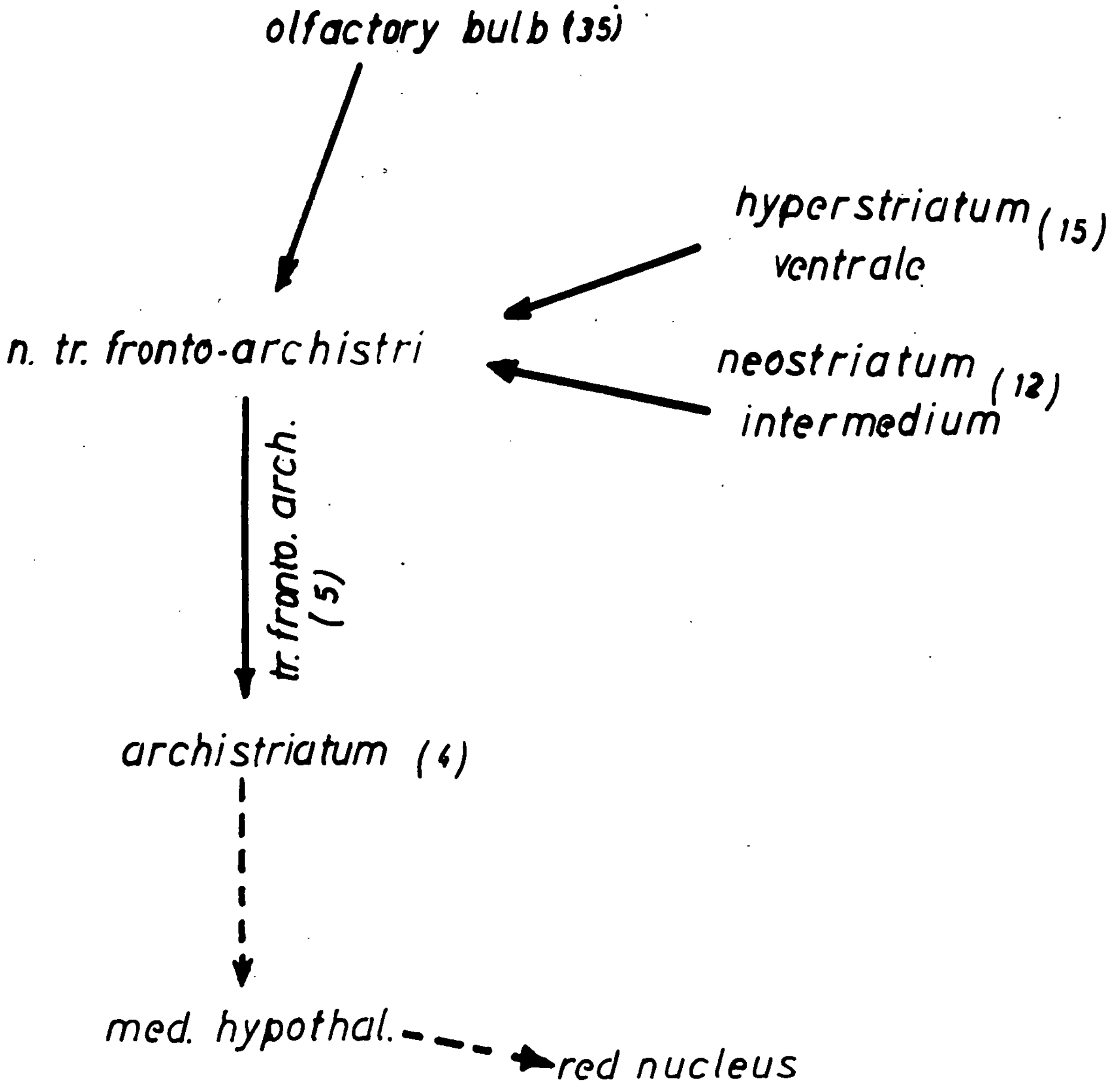


Fig.4.17. Pathway 1. Hypothesized pathway linking olfactory bulb, hyperstriatum ventrale and neostriatum intermedium with the r.n. of the pigeon, based on potentials evoked in the r.n. by electrical stimulation of the areas cited. Numbers in parenthesis indicate latencies, in milliseconds, of the evoked potentials following stimulation of respective areas

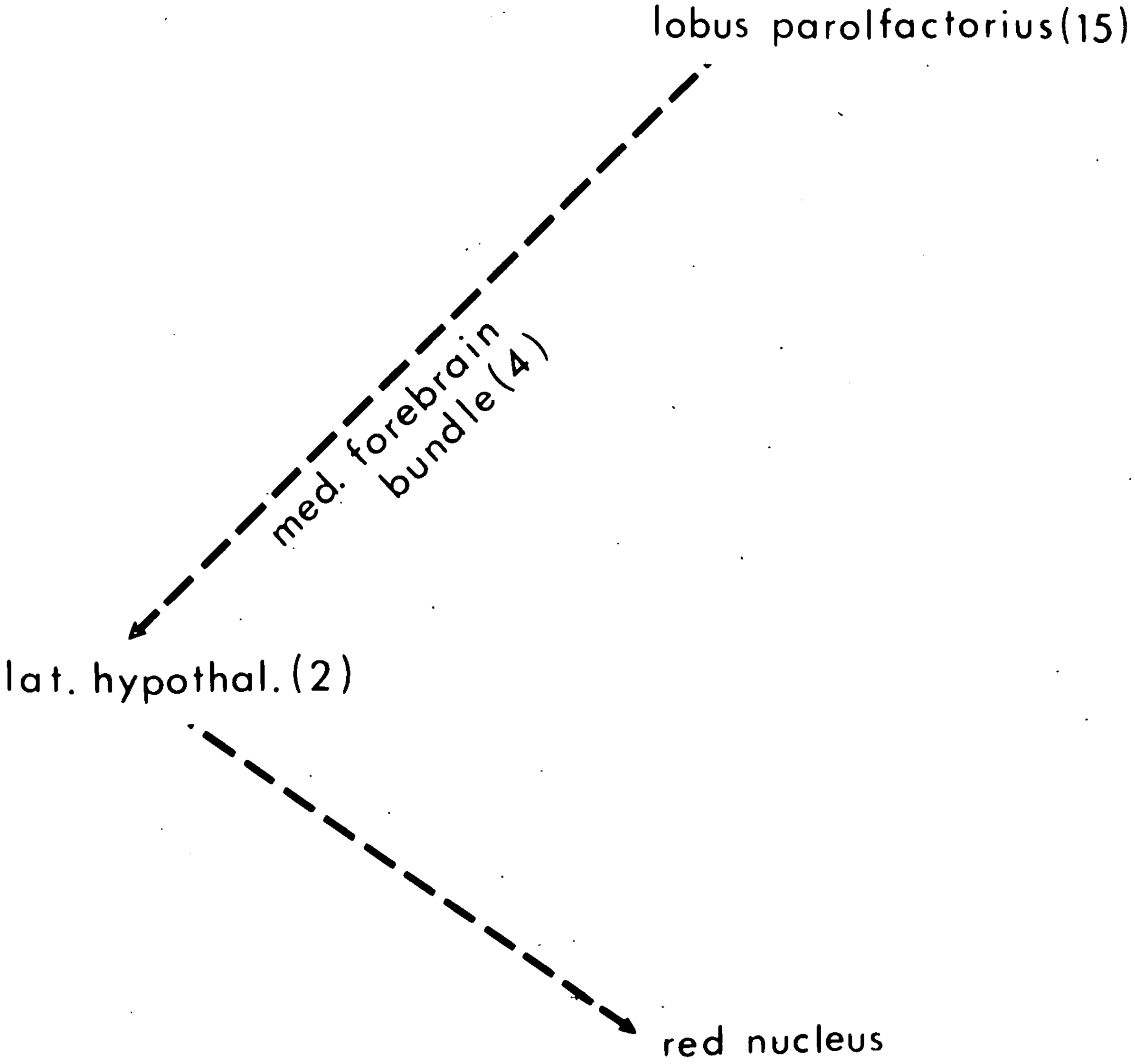


Fig.4.18. Pathway 2. Hypothesized pathway linking the parolfactory area with the r.n. based on evoked potentials arising in the r.n. following electrical stimulation of the areas cited. Numbers in parenthesis indicate the recorded latencies of the potential.

tracts. Tecto thalamic and thalamotectal connections in birds are similar to those of the reptile." In other words, although Karten and Revzin, by lesioning lower centres and looking for degeneration in the rotundus have demonstrated a lack of direct somaesthetic projections, it is possible that indirect pathways exist. In support of Karten and Revzin's conclusion however, Erulkar (1955) and unpublished work by Delius and Benetto in this laboratory, have failed to find evoked rotundal responses to somaesthetic stimulation.

The potentials obtained from the contralateral cerebellar nuclei (Figs.4.15 and 4.16) were expected on the basis of the many mammalian studies and the fact that the baseline activity of the r.n. is dependent upon a constant input from the cerebellum (Eccles et al 1967). The obtained potentials are quite emphatic. That from stimulation of the contralateral, cerebellar nucleus lateralis (Fig.4.16), with a latency of 1 m. sec., the fastest obtained in the present study, clearly shows the importance of this link. The potential obtained by stimulation of the nucleus medialis and/or internus - latency 5 m. sec. - Fig.4.15 - is probably not derived from the r.n. It will be recalled that the recording electrode consisted of three electrodes cemented together. The recording leads were arranged such that two separate traces were displayed i.e. one from the anterior and central electrode and the other from the posterior and central electrode. In situations where activity was recorded on one trace only it follows that the potential must be centred near one of the outer electrodes. The potential under discussion represents such a case. Examination of the histological material indicated that the electrode from which the recording was made was sited caudally to the r.n. and the recorded activity probably originated from the brachium conjuctivum, the major mesencephalic and diencephalic efferent pathway of the cerebellum.

An expected pathway, outlined by Huber and Crosby (1929), from the paleostriatum to the r.n. area by way of the tractus striotegmentalis, was not confirmed in the present study, although the paleostriatum was stimulated on several occasions in different regions. A recently published paper by Karten and Dubbeldam (1973) confirms this result anatomically. Although the tract in question lies at the antero-lateral margin of the r.n. they found it to be "... apparently unrelated to it."

The connections and hypothesised pathways discussed above are shown in Fig.4.19.

COMPARISON WITH MAMMALIAN CONNECTIONS

The major problem in comparing findings in mammalian and sub-mammalian species is that little is known about the functional properties of avian and reptilian forebrain areas. It is generally agreed that the paleostriatum of the bird includes the globus pallidus and putamen caudate complex of the mammal. This conclusion is based on histochemical grounds (Baker-Cohen 1968 and Karten and Dubbeldam (1973) and electron microscope studies (Fox et al. 1965). It will be remembered that the mammalian r.n. has links with the globus pallidus and possibly the putamen and caudate. If the paleostriatum is homologous to these mammalian areas then this study has failed to demonstrate any projection to the r.n. However, the area immediately adjacent to the paleostriatum, designated lobus parolfactorius by Karten and Hodus (1967) is far more extensive than the septal (parolfactory) area outlined by Huber and Cresby (1929) which is confined to a narrow strip parallel to the midline. It could therefore be, that the potentials obtained from parolfactory areas are proper to paleostriatal regions. However, potentials were obtained from parolfactory areas too anterior to be regarded as paleostriatal, see Fig.46 and furthermore, always arose from tissue

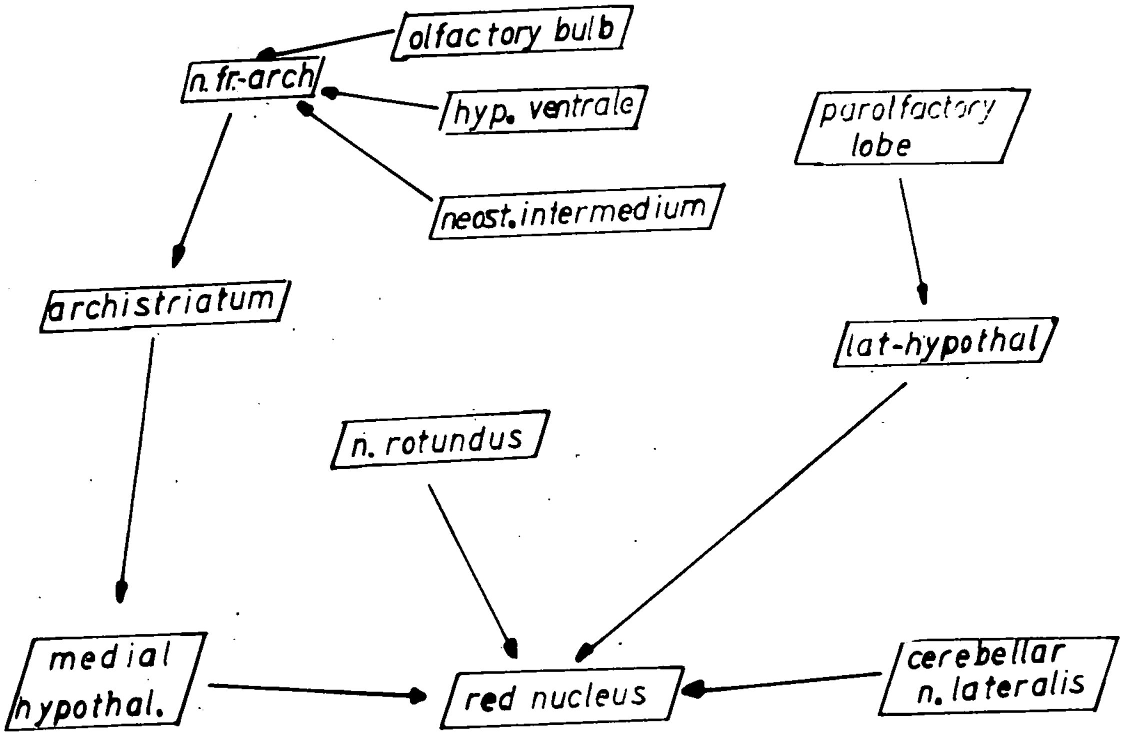


Fig.4.19. Composite schema of afferent pathways of the pigeon red nucleus determined from potentials evoked in the nucleus by electrical stimulation of the ipsilateral telencephalon, diencephalon and cerebellar nuclei.

designated as parolfactory in the Karten and Hodos atlas. No potentials were obtained by stimulation of paleostriatal regions lateral to the lobus parolfactorius. The writer is of the opinion therefore, that the induced potentials did arise from the parolfactory areas. Karten and Dubbeldam (1973) have recently provided confirmatory evidence, using anatomical methods, that the area designated as lobus parolfactorius in the atlas of Karten and Hodos has a distinctly different projection to that of the paleostriatum. If then the parolfactory region is homologous to the mammalian septum, one is immediately faced with the problem that, to the author's knowledge, no connection has been demonstrated in the mammal between the septum and r.n. The mammalian limbic system is however extremely complex and connections do exist between septum, hippocampus, hypothalamus and subthalamus (Grossman 1967), the latter nucleus being reciprocally linked to the r.n. (Carpenter 1956). If a similar projection system exists in the bird it may serve to explain the difference in latency between the parolfactory area (15 m. sec.) and the medial forebrain bundle (5 m. sec.). In the mammal the latter fibre tract links the limbic regions to the diencephalon, especially the hypothalamus (Grossman 1967). Karten and Dubbeldam (1973) have shown that the parolfactory region of the bird is linked to the rostral lateral hypothalamus through the medial forebrain bundle. Clearly, distinct similarities are present between the avian and mammalian species. Finally, this experiment has indicated a projection from lateral hypothalamus (latency 2 m. sec.) to the r.n. which ties in with the 5 m. sec. latency of the medial forebrain bundle. It is unfortunate that the stimulating electrode which traversed the avian hippocampus (if such it be) failed to evoke a potential in the r.n., although due to stereotaxic errors the track was sited at the anterior limit of the hippocampus.

Further investigation of this possibility would seem to be required.

The archistriatal area of submammals is, in part, regarded as being homologous to the mammalian amygdaloid complex. Again no direct link between amygdala and r.n. has been shown in the mammal although the former has, like the septum, connections with the hypothalamus (Grossman 1967). Zeier and Karten (1971) have shown that the medial area of the archistriatum is linked to the medial hypothalamus through the tractus occipito-mesencephalicus. The potential evoked in the r.n. in the present study, by stimulation of the medial archistriatum, with a latency of 4 m. sec., indicates a link from hypothalamus to r.n.

As in the mammal, a major input is derived from the cerebellar nuclei. Surprisingly, the results of the present study point to the avian nucleus lateralis as the afferent source whereas, in terms of positional similarity to the mammalian nucleus interpositus, one might have expected the nucleus internus to be involved.

The input from the nucleus rotundus has, to the authors knowledge, no mammalian homologue unless it be assumed to be a sensory projection for which ample mammalian evidence exists (see Ch. 5). On the other hand the nucleus rotundus appears to be specific to the avian species (Karten and Revzin 1966).

Homologues for the potentials derived from stimulation of the neostriatum intermedium and hyperstriatum ventrale ventro-ventrale are, in light of the lack of knowledge regarding the functional properties of these areas, impossible to suggest. It may be that they are the avian equivalents of the sensory-motor regions of the mammalian brain. Zeier and Karten (1971) believe that the non-hypothalamic part of the occipito mesencephalicus is equivalent to a variant of the mammalian pyramidal tract. The neostriatal and hyperstriatal regions in question, project to the archistriatal areas, from which this part of the occipito-mesencephalicus is derived, through the tractus fronto-archistriatalis.

In the mammal the pyramidal tract projects to the r.n. through collaterals, and whilst Zeier and Karten found no degeneration in the r.n. following lesions of the archistriatum, there are many possibilities for indirect action through adjacent reticular nuclei which they found do receive fibres from the tractus occipito-mesencephalicus.

The study reported in this chapter did not investigate efferent connections and clearly this is required. Such a study may well indicate an avian homologue of the mammalian rubro-ventrolateral thalamic projection. It has been assumed throughout that the avian rubro-spinal tract is motor in function and stimulation experiments reported in Ch. 3 appear to provide some evidence of this.

The behavioural ramifications of the connections outlined above and summarised in Fig.4.19 are discussed in the concluding chapter following Section 2.

In conclusion, further evidence that the avian and mammalian r.n. are homologous in some respects has been obtained. The most surprising findings were the links with the archistriatum and parolfactory lobes which are regarded as homologous to the mammalian amygdala and septum.

CHAPTER 5

SENSORY EVOKED POTENTIALS AND SINGLE UNIT RECORDS

There have been many studies in which potentials have been evoked in the mammalian r.n. by sensory stimulation (see Massion 1967). Massion and his co-workers (1963, 1968) have perhaps carried out the most extensive investigation. In the cat, they found, in agreement with other workers, that potentials could be evoked in the r.n. by somaesthetic, visual and auditory stimulation. Manni et al. (1965) demonstrated activation of the r.n. following vestibular stimulation in the guinea pig and Ermolaeva and Chernigovsky (1965) showed induced rubral potentials following stimulation of the splanchnic nerve in the cat.

In a thorough micro-electrode study Massion and Albe-Fessard (1963) demonstrated a degree of convergence of somaesthetic, visual and auditory fibres at the level of the single unit. They point out that the receptive fields of these multi input units are very large in contrast to the localised fields of the thalamus. They did however find that convergence was less noticeable in the cells of the magnocellular portion than in those of the small celled area. Furthermore they demonstrated two distinct responses dependent upon whether the recording was from the parvocellular or magnocellular portion. In the large celled region the response to sensory stimulation consisted of a short latency facilitation followed by a phase of inhibition and a later phase of prolonged activation. The response of cells in the parvocellular region was initially similar to that of the large cells but the late phase of prolonged activation was absent. They ascertained that the inhibitory phase and the period of late facilitation is produced by an indirect pathway through the cerebellum. The same authors found that a sharp tap was necessary to evoke a somaesthetic response. This finding is contradicted by that of Nakahama et al. (1969) who demonstrated a reaction to very light hair bending. The difference may well be due to anaesthetic effects.** One interesting finding, Massion and Urbano (1968), was that stimulation of group 1 muscle afferents (annulo-spiral endings of muscle spindles and Golgi tendon organs) do not appear to produce responses in the r.n. This absence of feedback from receptors denoting the state of the muscle they find surprising given that the r.n. contributes to control of muscle tone. They were, in the same study, able to demonstrate a contribution by Group 2 fibres (flower spray endings of muscle spindles and receptors sensitive to mechanical deformation of the skin) to r.n. control. If however, one considers the contribution of Groups 1, 2 and 3 fibres to overall muscle control through the gamma and alpha motor systems, then the absence of Group 1 afferents to the r.n. is understandable.

Firstly, the result of activation of Group 2 fibres is to lead to an inhibition of extensors and a facilitation of flexors throughout the limb; (precisely what stimulation of the r.n. produces). In terms of overall muscle effect, in conjunction with Group 1 effects, which result in faciliatation of both extensors and flexors in synergistic muscles and inhibition of antagonistic muscles, activation of Group 2 fibres would lead to an opposition of any Group 1 effected extension and a supplementation of any Group 1 effected flexion. In a sense, feedback from Group 2 fibres increases the readiness for withdrawal reflexes i.e. defensive reflexes.

** In a recent paper Nishioka and Nakahama (1973) have in fact shown that this is so. Under chloralose anaesthesia a sharp tap was necessary, under gallamine triethrodide very light hair bending was sufficient.

Secondly, the r.n. has been shown to take its effect partly through the gamma motor system (Appelberg 1967a and 1967b) which is responsible for contraction of muscle spindle fibres, which in turn stretch and activate the spindle afferent fibres which result in the operation of the alpha motor system. The sufficient stimuli for operation of the gamma system are mediated by Group 2 and 3 fibres i.e. light touch, pressure and pain. Group 1 afferents do not appear to influence the gamma system (Thompson 1967). Hence it is <u>not</u> surprising that Group 1 fibres do not project to the r.n. for they are not necessary, at least for the gamma system components of r.n. influence.

The action of the r.n. is attained through the inter-neurones of reflex pathways which facilitate the reflex action. In addition it has been shown that the r.n. exerts an inhibition of primary afferents at the spinal cord level. (Thompson 1967). Hence the r.n. effects a complicated action on spinal reflexes and motor organisation which presumably would require inputs from many sensory sources and reciprocal linkages with other systems involved in motor control.

The object of the study reported in this chapter was again principally of a comparative nature and was restricted to the recording of potentials evoked in the r.n. by peripheral, visual, somaesthetic and acoustic stimulation and by electrical stimulation of olfactory and vagus nerves. A limited study of single unit responses was also undertaken.

METHOD

Subjects

Fifteen pigeons of mixed strain, three of which were used for unit recording experiments.

Procedure

The animal was anaesthetised by injection of Equithesin into the pectoral muscle and placed in the stereotaxic instrument. The surgical procedure and method of electrode positioning has been described fully in Chapter 4.

Evoked potential and some single unit recordings were made to auditory, visual and somaesthetic stimulation. Visual stimulation was achieved by a Dawe model 1201E stroboscope positioned 30 cms. from the birds eye. The output of the stroboscope was a 40 microsecond, 90 joule flash. Simultaneous triggering of the stroboscope and a Tektronix 564 storage oscilloscope allowed a visual record of any activity in the r.n. ensuant upon stimulation. Permanent records were obtained by photographing the oscilloscope displays. Auditory stimulation was achieved by means of a small hearing aid loud speaker, of unknown make and characteristics, which produced a click stimulus by feeding in a square pulse of 1 m. sec. Three methods of somaesthetic stimulation were used:-

(1) Mechanical tapping with a blunt 3 mm diameter rod attached to a pair of springleaf mounted contacts serving for triggering purposes.

(2) 1 m. sec. biphasic electric shock of 1 - 5 v from a Grass S4 stimulator delivered through a pair of stainless steel insect pins implanted in the skin or muscle.

and

(3) 1 m. sec. duration biphasic shocks of 1 - 5 v delivered to the surgically isolated ischiadic nerve through a pair of silver wire electrodes mounted on a small probe made of acrylic resin.

In all cases the storage oscilloscope trace, or occasionally the Biomac 500 averaging computer, was triggered simultaneously with administration of the stimulus and a photographic record made.

In addition two experiments were carried out in which the olfactory and vagus nerves were surgically isolated, the former by removing the sponge-like bone of the nasal cavities, and the latter by exposing the nerve in the birds neck where it runs between the oesophagus and carotids, ~~and~~ Both were stimulated electrically using the method described above for stimulation of the ischiadic nerve.

Single unit recording was attempted using fine wire concentric electrodes. (see below).

In order to obtain satisfactory auditory stimulation, the recording electrode was cemented into position, using an acrylic dental cement and the ear bars of the stereotaxic instument removed. At the completion of recording, the position of the electrode tip was marked by a small radio frequency lesion and the bird sacrificed by an overdose of Nembutal. Histological procedure was as described in Chapter 4.

Electrodes

Recording electrodes for evoked potentials were as described in Chapter 4 for the mapping experiment. Electrodes used for single unit recording were constructed from fine gauge hypodermic tubing and fine stainless steel wire (swg 44) forming outer and inner electrodes respectively of a concentric electrode. Both components were first coated in insulating varnish and tested for leakage in accordance with the method outlined in Chapter 4. Following insertion of the inner electrode through a small hole filed in the outer tubing so that the inner electrode protruded about 1 mm., the two components were cemented together with Araldite at the point of entry and exit of the inner fine wire electrode. The tip was then ground to a fine point using a miniature emery disc. The net result was to leave about $\frac{1}{2}$ mm of the outer insulation free and a very small uninsulated tip to the inner recording electrode. Whilst such an electrode is relatively crude in its construction, it was found to be very highly effective in recording from the large r.n. units. See Fig5·1 for method of construction.

RESULTS

Histology

Of fifteen attempts to locate an electrode in the r.n., twelve were successful.

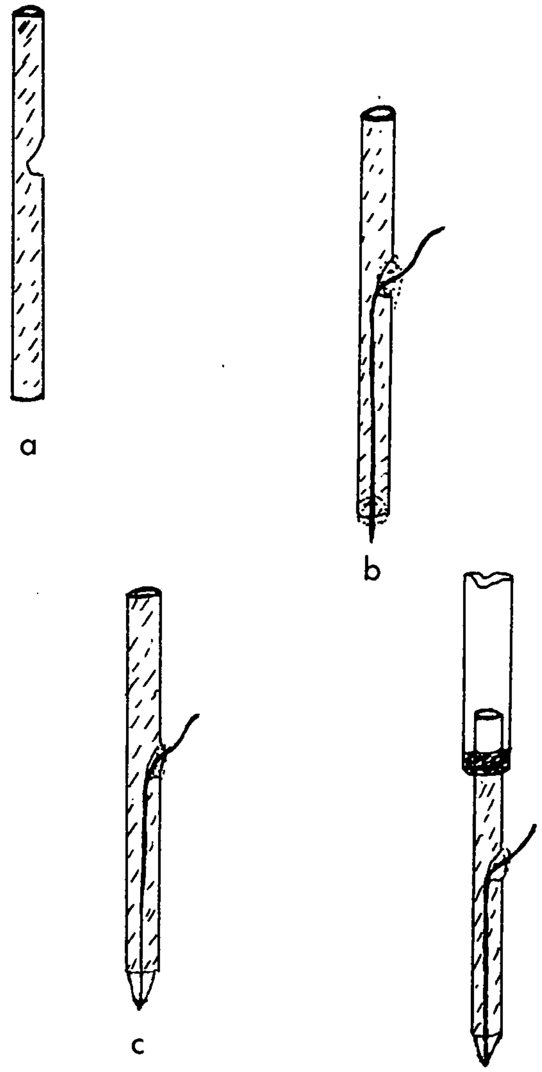


Fig 51. Method of construction of the micro-electrodes.

a) short length of insulated fine gauge hypodermic tubing showing cut filed in wall.

b) length of insulated fine stainless steel wire (swg 44) inserted and secured with Araldite at point of entry and exit.

c) the tip ground to a fine point using a miniature emery disc.

d) length of coarse gauge hypodermic tubing soldered to the electrode to facilitate securement in the chuck of the stereotaxic instrument

Evoked Potentials

(1) Visual: See Figs5·2a and ·2b.

Of all the sensory evoked potentials, the visually evoked proved to be the most reliable and the largest in terms of amplitude. The latency to the first inflection varied little from 23/24 m. sec., with the large downward deflection, beginning at 40 m. sec. and ending at 50-52 m. sec. A recording obtained direct from the optic tract during the mapping experiment shown in Fig.5·2c gives a latency of 3 m. sec. to the first inflection.

(2) Somaesthetic

The results from somaesthetic stimulation are given in Fig.5·3. The potentials tended to vary somewhat from one recording session to another within each method of stimulation. The most reliable recordings arose from stimulation of the isolated ischiadic nerve - see Fig.5·3a. The latency to first inflection began 8 m. sec. after stimulation and the large deflection at 16 m. sec. Mechanical stimulation tended not to produce highly reliable potentials, unless administered to wing or leg, and variation in the shape of the recording was common, although the latency to first deflection fell within 8 - 12 m. sec. in all cases. Fig.5·3b provides an interesting record of unit activity superimposed on the evoked potential. Fig.5·3c, a recording following stimulation of the deltoid muscle of the wing sufficient to produce a twitch, is fairly representative of the potential shape produced by such electric stimulation to, or near, muscle.

(3) Auditory: Fig.5·4

Auditory evoked potentials proved difficult methodologically for the electrode had to be cemented into place before stimulation began to enable the earplugs of the stereotaxic instrument to be removed. Potentials that were recorded tended to be small, between

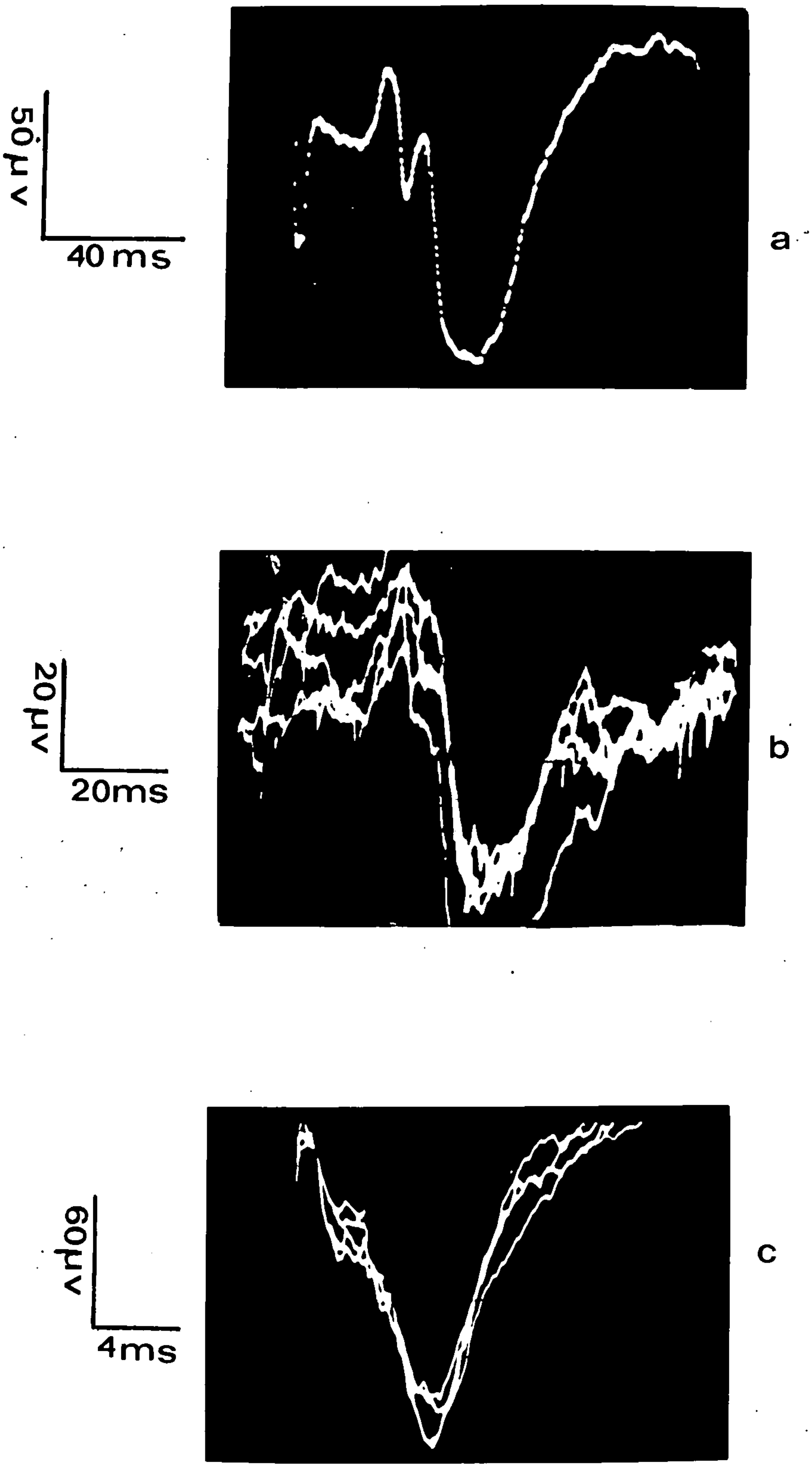


Fig.5·2 Evoked potentials recorded from the r.n. following stroboscopic flash stimulation of the contralateral eye - a and b. Latency estimated at 23-24msec. Trace c - evoked potential resulting from electrical stimulation of the optic tract, estimated latency 3msec., included to show the delay occurring prior to the optic tract. Stimulus in each case occurred at extreme left of trace.

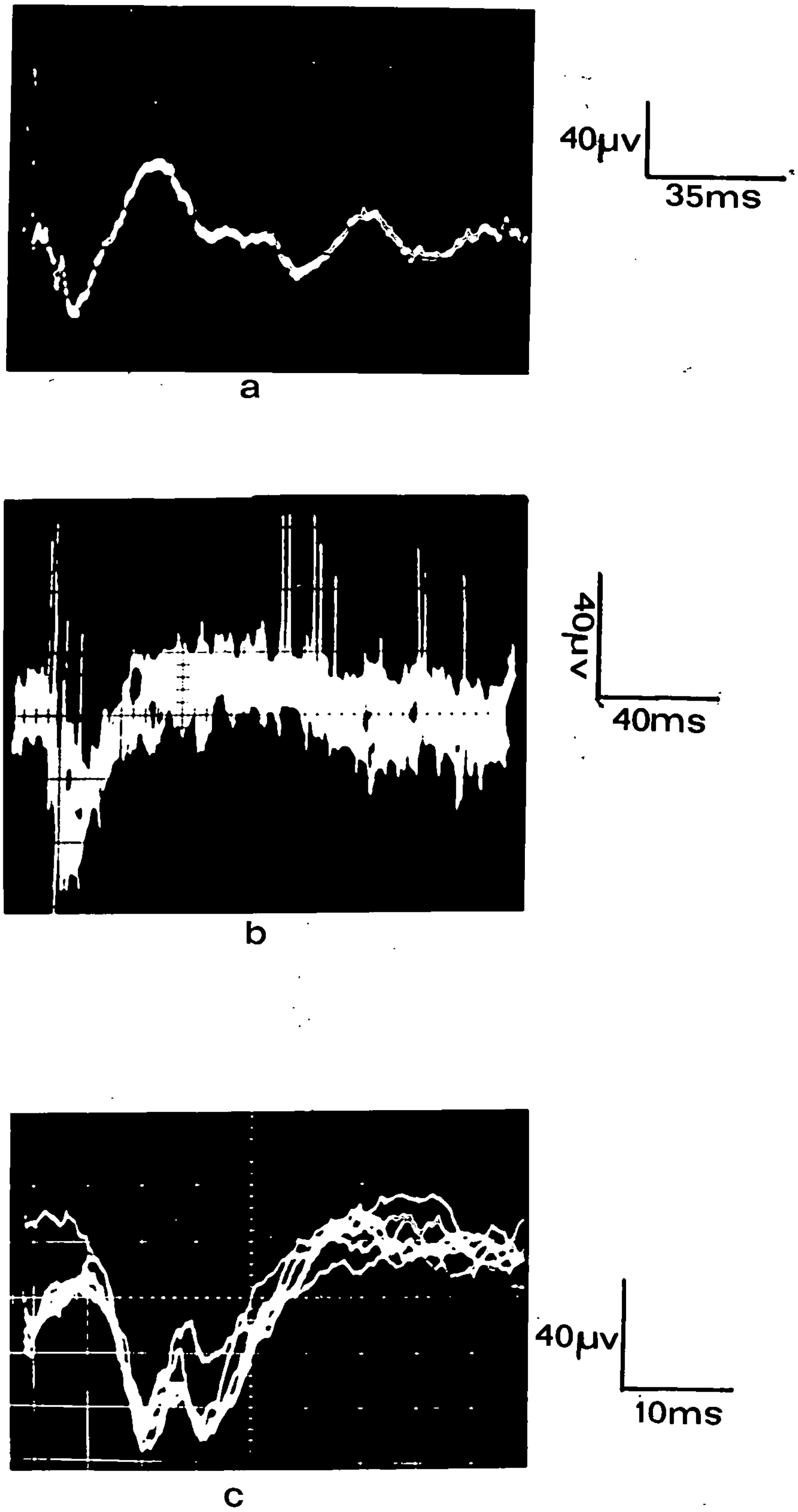
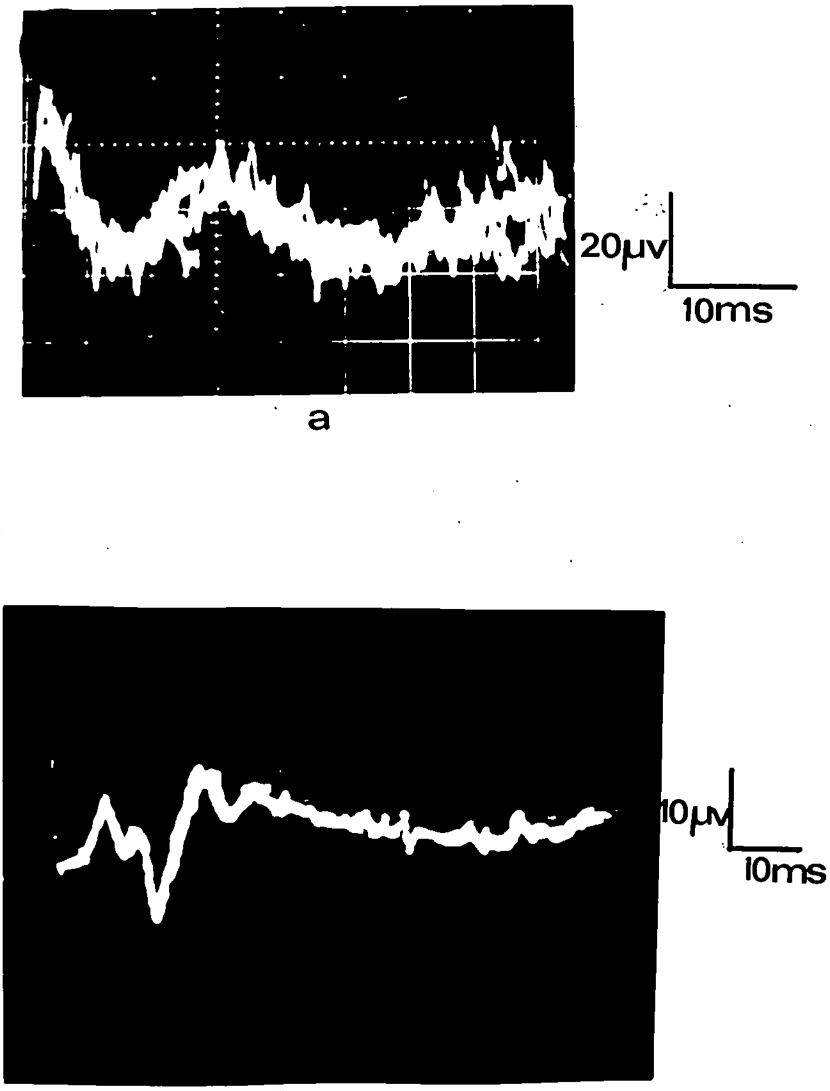


Fig.5.3. Evoked potentials recorded from the r.n. Somaesthetic stimulation.
Trace a - electrical stimulation of contralateral ischiadic nerve. Stimulus applied at extreme left of trace. Estimated latency 8msec.
Trace b - mechanical stimulation of contralateral wing. Stimulus applied at extreme left of trace. Estimated latencies for this potential 12msec., varied between 8-12msec. Note the single unit response and the period of inhibition.
Trace c - electrical stimulation of deltoid muscle contralateral wing. Stimulus applied at extreme left of trace. Latency estimated at 8msec. Note the double peak which was characteristic of shock applied to wing or leg muscle.



a

b

Fig.5.4. Evoked potentials recorded from the r.n. following auditory stimulation. Click stimulus occurred at extreme left of trace in both cases. Latencies, of the traces shown estimated at 8msec.,varied between 8-10msec. Amplitude of auditory evoked responses were small falling between 20 - 40µ v.

20 - 40 µv although the shape was fairly consistent. Latency to the first inflection was 8 - 10 m. sec. No unit records were obtained because of the earbar problem, i.e. the electrode had to be cemented into place before any recording could be obtained, so that it was impossible to determine whether the cell was responsive to auditory stimulation before it was permanently fixed.

(4) Olfactory Nerve Stimulation: Fig.5·5

Results from olfactory nerve stimulation, in terms of size and shape of potential, were varied. Fig.5·5a gives a typical result with a latency of 36m. sec. Fig. 5·5b gives the result of stimulation of the homolateral nerve again with a latency of 36 m. sec. It was found that each time the stimulus parameters were varied the amplitude of the potential increased and then attenuated over the next 4 - 5 stimulations.

(5) Vagal Stimulation: Fig.5·6

Fig.5·6 gives a typical recording following stimulation of the vagus nerve. The latency to first inflection was 25 m. sec. with the large deflection beginning at 30 m. sec.

MICRO-ELECTRODE STUDIES

The micro-electrode work carried out was limited to three birds with an attempt to record from another under free moving conditions with implanted electrodes - see Chapter 3 for method of implantation and equipment for allowing the animal relatively free movement. Unfortunately the latter was only partially successful probably due to electrode movement resulting from a method of plug mounting which although good enough for stimulation seemed to be inadequate in obviating the very small electrode movement sufficient to 'lose' a single unit.

In all fourteen units were examined, of which two were responsive to flash only, five to somaesthetic stimulation only, two to both

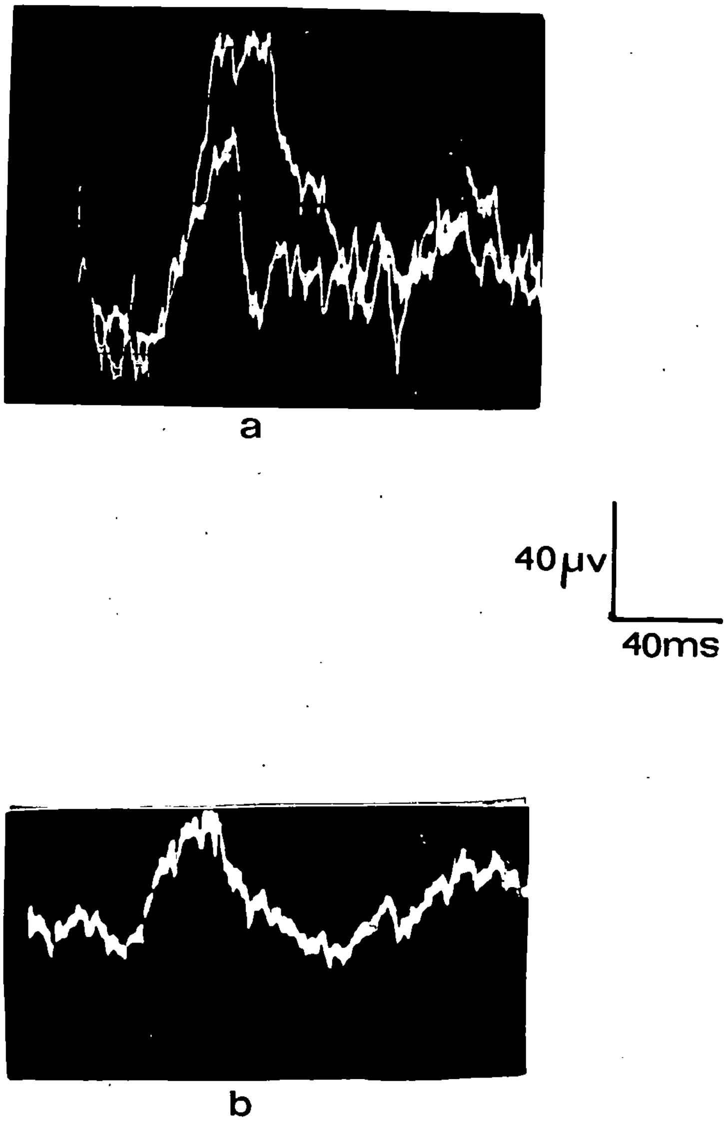


Fig. 5.5 Evoked potential recordings from the r.n. following electrical stimulation of contralateral - a and homolateral - b olfactory nerves. Latencies estimated at 36msec. Stimulus occurred at extreme left of traces. Size and shape of potentials varied - see a - the smaller of the two traces followed the larger in temporal order, note the reduction in amplitude: it is suggested that this may indicate habituation (see text).

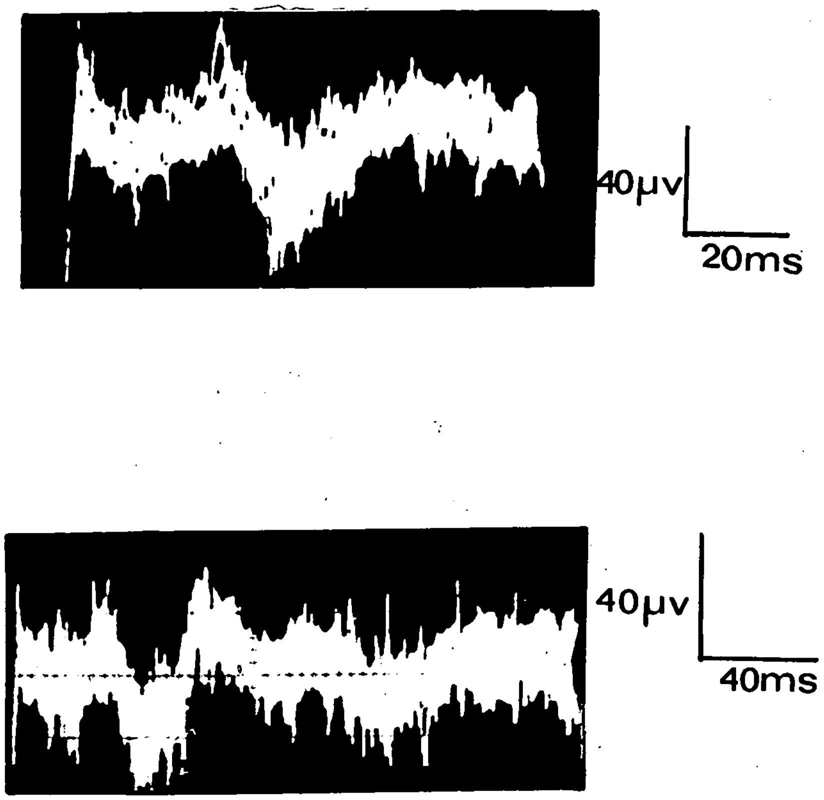


Fig.5·6. Evoked potential recordings from the r.n. following electrical stimulation of the contralateral vagus nerve. Stimulus occurred at the extreme left of both traces. Latency extimated at 25msec.

light and somaesthetic and five non-responsive to either visual, somaesthetic or vestibular stimulation, although the latter was a relatively crude test involving tilting and rotating the stereotaxic platform. Figs.5·7 - 11 give the results obtained, the upper trace showing the unit, or group of units, response to stimulation, the lower trace in each case being the unstimulated activity during a similar time period.

The typical response to stimulation falls into one of two categories:-

(a) A period of inhibition - see Fig.5·7

and (b) A period of inhibition followed by a burst of activity - see Fig.5·8.

Of the solely visual units both responded with a period of inhibition beginning at 24 - 25 m. sec. and lasting 48 - 50 m. sec. Of the solely somaesthetic units all responded with a period of inhibition followed by a burst of activity - see Fig.5·8, the inhibition at 20 - 25 m. sec. and the burst of activity at 60 - 80 m. sec. following stimulation.

The two units responsive to both visual and somaesthetic driving produced a phase of inhibition followed by a burst of firing - see Fig.5·9, although the activity burst was not so pronounced as the solely somaesthetic unit - see Fig.5·8. Of the five cells found to be non-responsive to stimulation, the recorded activity was typical of that shown in Fig.5·10 in which the cells gave repetitive bursts of activity with a fixed interval between bursts which shifted in time to longer or shorter intervals, probably in accordance with depth of anaesthesia. These apparent repetitive bursts were not correlated with a concurrently monitored heart rate or with breathing.

Fig.5·11, the only successful record from an implanted electrode in a free moving bird, shows the record of such a repetitive cell in the awake animal. Fig.5·11a shows the regular firing of the cell

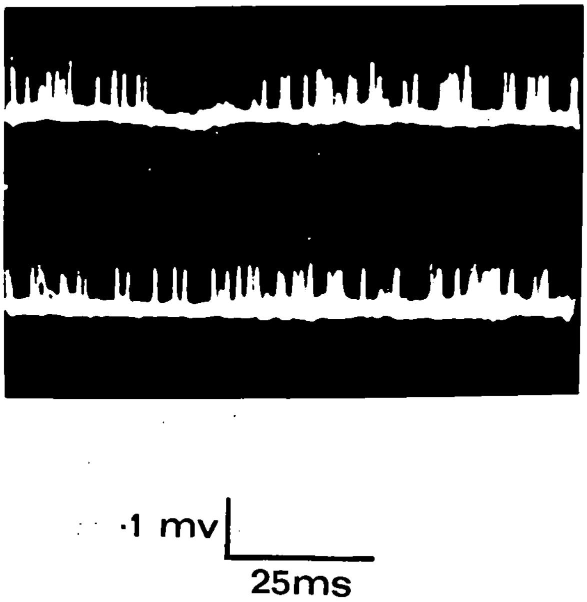


Fig.5.7. Single unit recording derived from the r.n. following visual stimulation at 1 per sec. 25 superimposed traces. Lower trace shows the distribution of unit activity during an identical period without stimulation. Note the period of inhibition of firing beginning at 24msec. No response obtained from somaesthetic stimulation.

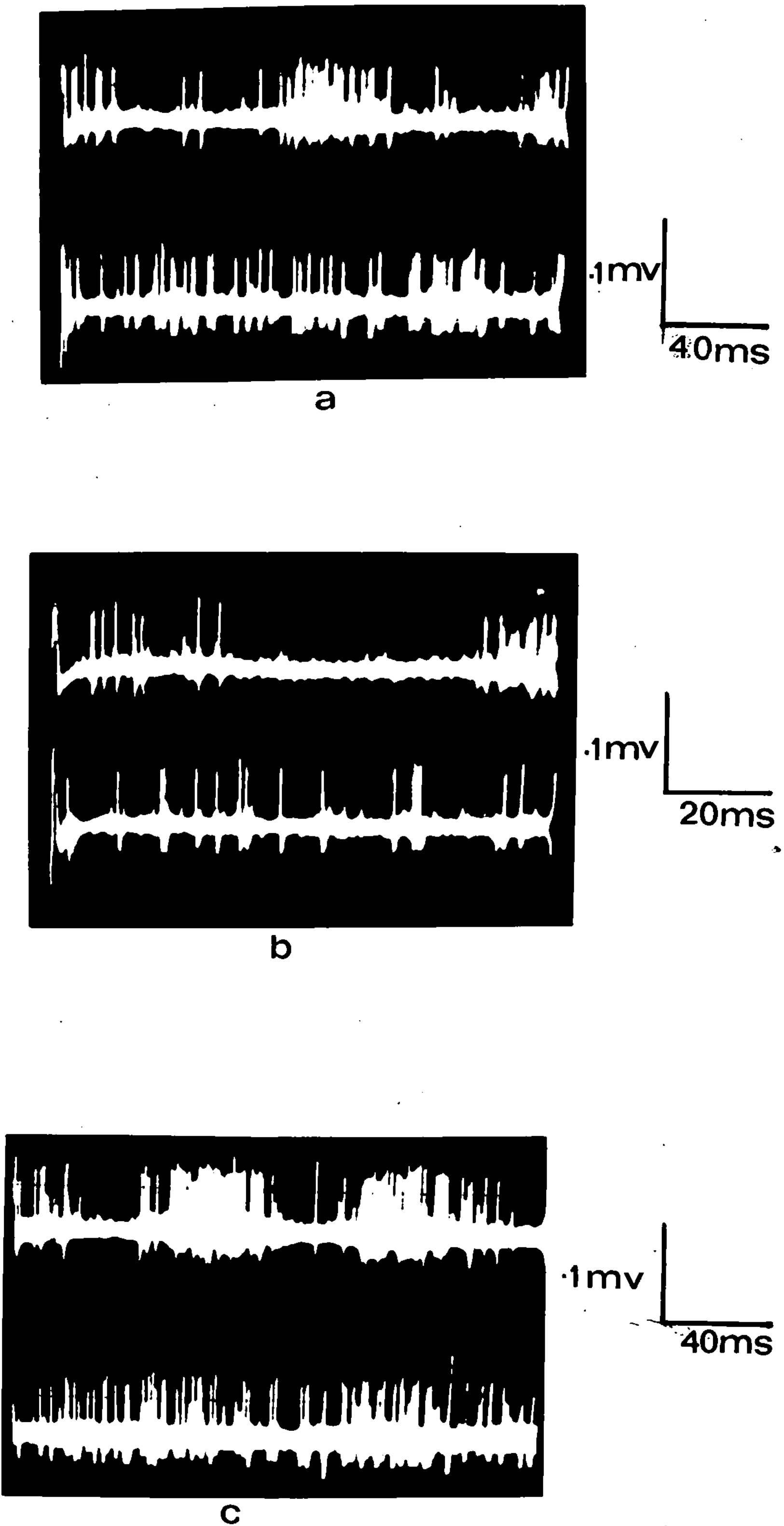


Fig.5·8 Single unit recordings derived from the r.n. a and b - electrical stimulation of ischiadic nerve at 1 per sec. 20 superimposed traces. Lower traces in each case show the distribution of unstimulated activity during an identical period. Note the period of inhibition at 20-25 msec. followed by a burst of activity at 80msec. c - electrical stimulation of leg muscle. Note the two periods of inhibition and activity. (see also Fig5·3c) These units were not responsive to visual or vestibular stimulation.

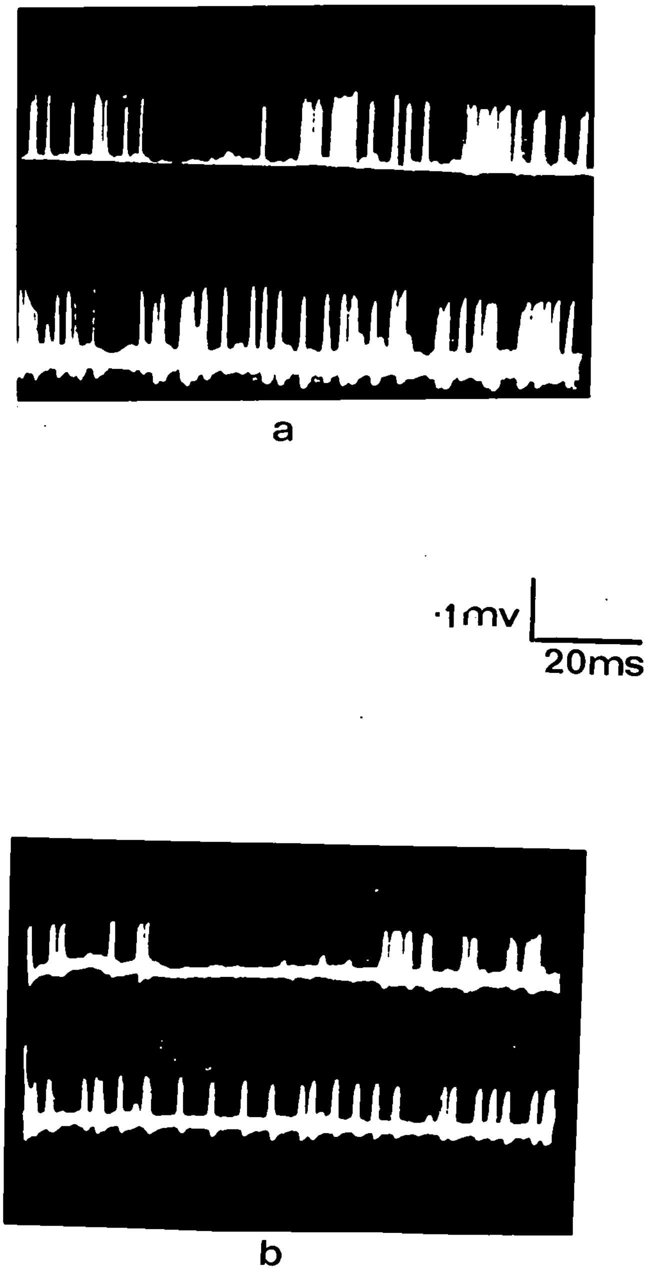


Fig.5.9. Single unit records derived from the same unit in the r.n. a - visual stimulation, b - electrical stimulation of the ischiadic nerve. Stimulus occurred at the extreme left of each trace. 20 superimposed traces. Lower trace in each case shows the distribution of unstimulated activity during an identical period. Note the period of inhibition beginning at 24msec., more prolonged for the somaesthetic response than the visual, and the tendency towards an increased burst of firing following the inhibitory period.

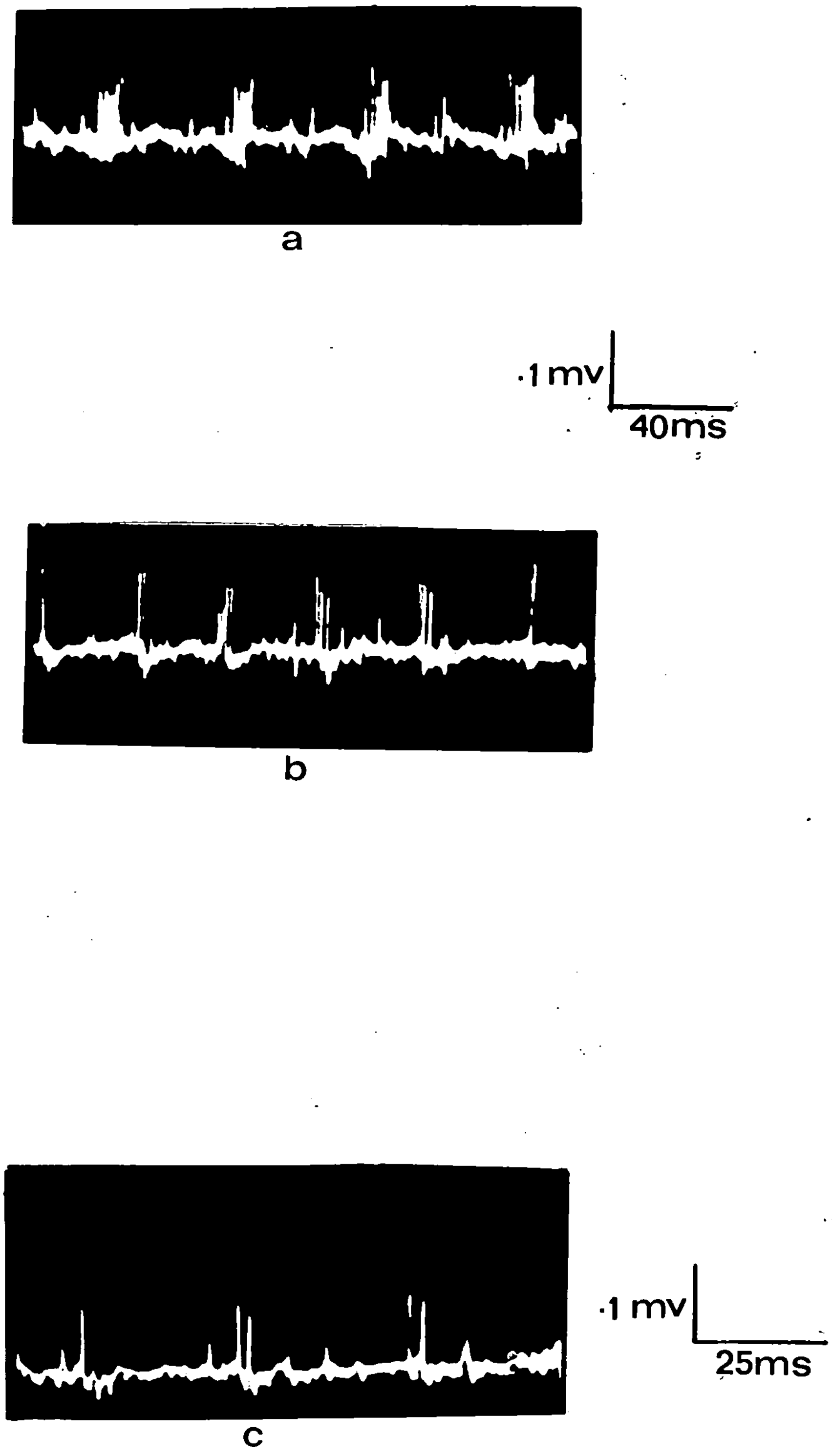


Fig.5·10. Single unit recordings derived from the r.n. Typical firing pattern of the units non-responsive to visual, somaesthetic or vestibular stimulation. Note the repetitive firing. a - five spikes every 40msec. b - same cell, 5 mins. later: note the repetitive firing at a faster rate.
c - five minutes following b
Firing not correlated to heart rate or breathing.

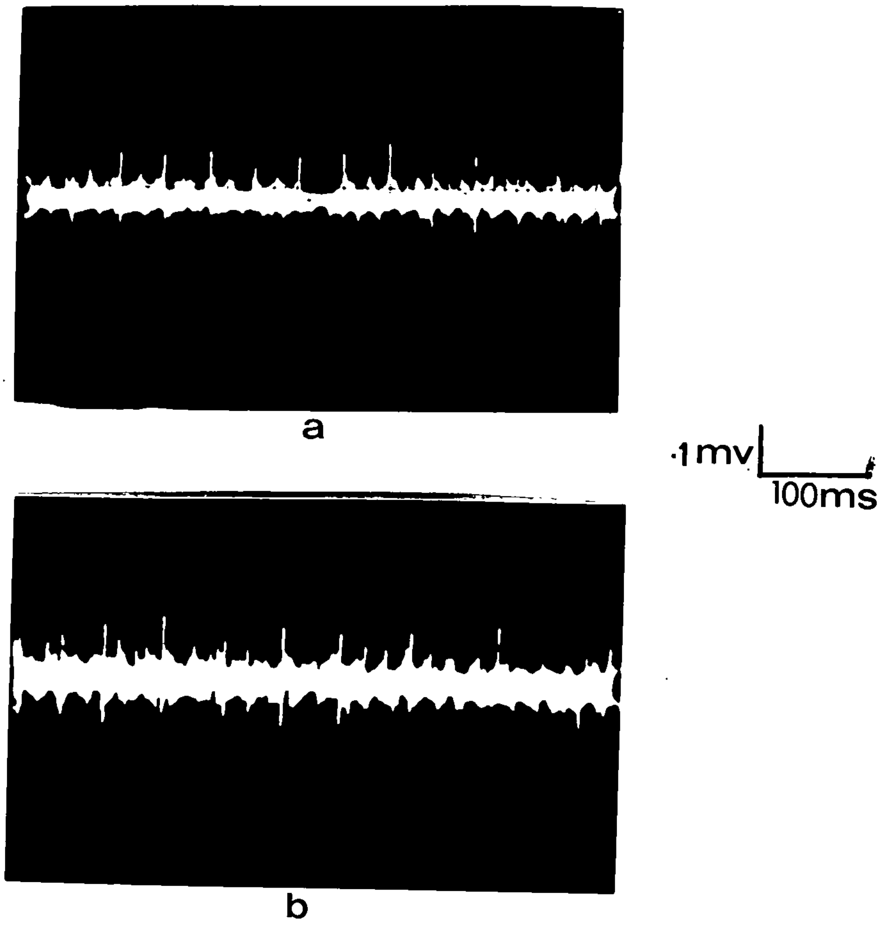


Fig. 5·11. Single unit activity recorded from the r.n. in an awake implanted bird. Unit could not be driven by visual, somaesthetic or auditory stimulation.

a - firing pattern in darkness; note regular firing.

b - same unit with room lights switched on; note that the firing pattern becomes more irregular.

every 40 m. sec. when the bird was awake but stationary in a dark room and 11b the same cell when the room lights were switched on. Clearly the regularity of firing disappears. The cell was nevertheless non-responsive to stroboscopic flash.

DISCUSSION

The findings outlined above are in essence, further evidence for an analogous function between the r.n. of mammals and birds.

Clearly the avian r.n. receives inputs from most, if not all, sensory modalities. The results indicate in addition that the action of the input is largely one of disruption of a fairly regular firing pattern, at least at a unit level.

Compared to the mammalian studies conducted by Massion and Albe-Fessard (1963), one distinct difference does however emerge; they found that inhibitory periods in single unit records and evoked potentials although commencing at very similar time intervals following stimulation, last rarely less than 100 m. sec. and usually exceed 200 m. sec. Reference to Figs.5·2 -5·9 shows that the longest period of inhibition, that for a somaesthetically driven unit is less than 50 m. sec. This difference may be interpreted as an indication of a functional disparity between the mammalian and avian r.n. or may simply reflect a methodological difference. Firstly, on the basis that the mammalian nucleus is structurally more clearly delineated and morphologically divisible into large and small celled areas, unlike that of the bird, one may well expect some functional differences. On the other hand it has been shown that the r.n. is highly susceptible to anaesthetics - (Gogolak et al. 1969). It was shown that barbiturate drugs produced"....a very regular, quasi-sinusoidal rhythm with a frequency that is related to the depth of anaesthesia". Massion and Albe-Fessard did in fact show that in the cat maintained awake under local anaesthetic, "....somatic stimulation can evoke the same unit reactions as in the chloralose anaesthetised cat; but this same stimulus can also induce a prolonged unit activation not preceded by a phase of inhibition". It would seem that in the absence of recordings obtained under similar anaesthetic conditions, little importance can be attached to variations in response characteristics. By the same token, the apparent absence of the fast unit response preceding the period of inhibition, which they found to be typical of the large cells, cannot be meaningfully discussed in terms of possible species difference. On the other hand the absence of the short latency response in the present records may be due to the small sample of cells from which recordings were made.

The most frequently encountered type of unit was not responsive to sensory stimulation under the anaesthetic conditions of the experiment - Fig.5.10. Even in the awake, implanted, bird these cells, characterised by a repetitive burst of firing every 30 - 40 m. sec. could not be driven. However, reference to Fig.5.11 shows that the baseline activity of such a unit is nevertheless determined by sensory conditions. Precisely what could be determining the baseline firing pattern of these cells is difficult to see unless it be internally generated.

Massion and Albe-Fessard (1963) found about 21% of the cells they encountered could not be driven, but make no reference to the characteristics outlined above. They do however refer to occasions when they obtained highly repetitive responses which they state were synchronous with heart rate and assume this to have been due to penetration of glial cells or electrode location in the wall of small blood vessels.

On the occasion that the writer first encountered a repetitively firing cell, so like a heart record was it, that the same assumption was made. After several such occurrences heart rate was monitored concurrently with the cell's activity but no obvious relationship could be found, nor was there any correlation with breathing although this was only established by visual observation.

Of the units encountered, only one, - that shown in Fig.5·9, was responsive to more than one sensory modality. It is highly probable, in view of the relatively coarse electrodes used for unit recording, that the cells from which activity was recorded were large cells only, which would perhaps explain the lack of sensory convergence, for Massion and Albe-Fessard (1963) found convergence to be less marked in the magnocellular portion than in the small celled area.

That the nucleus of the bird, in concordance with that of the mammal, is responsive to inputs from several sensory modalities has been shown by the evoked potentials given in Figs.5·2 -5·7. Recordings following visual stimulation were reliable both in terms of the shape of the recorded potential and the latencies to deflections of the waveform and fit, almost to the millisecond, the periods of inhibition and excitation recorded from single units.

Comparison of the results obtained in the present series of experiments with those of other workers proves to be interesting. In an extensive anatomical study of the avian visual system, Karten (1969) described two major visual projections. One projection proceeds from the optic tectum via the thalamic nucleus rotundus to the ectostriatum of the telencephalon and the other from the optic tract to the dorsolateral anterior thalamic complex and thence to the hyperstriatum via the lateral forebrain bundle. Parker (1971) and Parker and Delius (1972) in an evoked potential study of the visual system in the pigeon found latencies of 15 - 16 m. sec. at the optic tract, 17 m. sec. in the dorsolateral anterior thalamus and 50 m. sec. in the nucleus rotundus. As the latency at the r.n. disclosed in the present work, was 23 - 24 m. sec., the projection to the nucleus under study can not be via the nucleus rotundus, but must derive either direct from the optic tract or from the dorsolateral anterior thalamus or perhaps from the surface layers of the optic tectum. Fig.5·2c shows a potential evoked at the r.n. by electrical stimulation of the optic tract, during the mapping experiment described in Chapter 4, with a latency of 3 m. sec., which, when considered in conjuction with the latency of the potential evoked at the r.n. of 23 - 24 m. sec. allows a transmission period prior to the optic tract of 20 - 24 m. sec. This is clearly at variance with the optic tract latency of 15 - 16 m. sec. found by Parker (1971). The difference may be explained however by a greater efficacy of electrical stimulation over sensory in the pathway between optic tract and r.n.

Karten (1967) in an anatomical study of the avian auditory system describes the major projection from the avian homologue of the inferior colliculus (nucleus mesencephali lateralis pars dorsalis) to the nucleus ovoidalis. Harman and Phillips (1967) describe an auditory evoked potential in the nucleus ovoidalis with a latency of 8 - 12 m. sec. The latency of the potential evoked in the r.n. in the present study of 8 - 10 m. sec. suggests that the projection to the r.n. bypasses the nucleus ovoidalis. It should be pointed out however that the amplitude of the recorded response, was at 20 - 40 µv, the smallest of any obtained and as the nucleus ovoidalis is situated 1.5 mm anterior and 1.5 mm dorsal to the r.n. it is not impossible that I was in fact recording activity from the former nucleus. However, Biederman-Thorson (1970) has shown that auditory evoked potentials in the ovoid nucleus itself are not only small but unreliable.

Delius and Bennetto (1972) have investigated somaesthetic projections in the pigeon and found a potential evoked by cutaneous stimulation in the dorsal thalamus with a latency of 9 - 10 m. sec.

which ties in well with the potential found in the r.n., with a latency of 8 m. sec.

The recordings following olfactory nerve stimulation were highly variable in amplitude and outline; (Fig.5·5a shows two traces,) although the latencies to first deflection were reliable. An interesting finding was that not only would the r.n. apparently rapidly habituate or fatigue to stimulation rates of more than one per second, which was found to be a characteristic of the nucleus irrespective of sensory modality, but in the case of olfactory nerve stimulation habituation was also noticeable to the particular parameters, that is to say, a change in rate of stimulation from, for example, one per sec. to one per three secs. would effect an increase in the amplitude of the potential as would a voltage change. That this was habituation in the accepted sense of the word was demonstrated by the fact that the same amplitude increase followed a change to a higher rate of stimulation (provided it did not exceed 8 per second) or to a lower voltage. Clearly this habituation was occurring within the olfactory system for similar effects were not found in other modalities. In so far as the author is aware no previous workers have demonstrated an olfactory input to the r.n.

As would be expected, somaesthetic stimulation produced varying waveforms and amplitudes depending on the means of stimulation (see Fig.5·3). By and large, electrical stimulation near muscle tended to produce a waveform similar to5·3c, with a double inhibitory deflection (see also Fig.5·8) whereas mechanical and ischiadic nerve stimulation produced a single deflection.

The probable reason for the different shapes of the potentials is dependent upon the number and characteristics of the fibres stimulated by the different methods.

That stimulation of the vagus nerve evoked a potential in the r.n. is not surprising in view of the finding by Ermolaeva and Chernigovsky (1965) of evoked potentials following splanchnic nerve stimulation for both nerves are involved in the control of involuntary processes. Presumably the state of internal organs could be of some importance to a system involved in motor control.

PART 2

CHAPTER 6

THE BEHAVIOURAL EXPERIMENTS

Introduction

The role of the r.n. in motor control, in mammals, has been firmly established over many years in a variety of physiological methods (see Part 1, Chapters 2, 3, 4 and 5 of this thesis). Experiments outlined by the author in Part 1, in which the r.n. of the pigeon was lesioned (Ch. 2) and, in a separate essay, electrically stimulated (Ch. 3) indicate a similar motor function in the avian species.

In recent years the work of Thompson and his collaborators (1960, 1964, 1967, 1969) has however implicated the r.n. in visual learning in the rat. Whilst it may seem surprising that a system playing an important role in motor control should also be implicated in other behavioural processes, it should be pointed out that in recent years studies of the classically defined extra-pyramidal motor systems have indicated that they may subserve other than only motor functions:- Kitsikis and Rougeul (1968) found that electrical Stimulation of the caudate nucleus in the Nemestrina monkey produced a disruption of conditioned bar-pressing; Ursin et al. (1969) demonstrated that high frequency stimulation of the caudate nucleus in the cat produced an orienting response which they claim to be indistinguishable from that produced by novel, acoustic stimulation and by electrical stimulation of reticular centres known to produce orienting reactions. The same authors also noted that the response evoked by caudate stimulation was subject to habituation in the same manner as to peripheral sensory events. Perhaps more specific to learning phenomena, Rosvold and Delgado (1956) reported that the post operative performance of the monkey, on a delayed alternation task, was impaired by lesions of the caudate. Clearly, the role of what is described as part of a motor system is more complex than hitherto believed. Given these findings the result obtained from lesions of the r.n. appear less surprising.

The major findings, all involving the rat, providing the impetus to this section are as follows:-

(1) Bilateral destruction of the r.n. significantly interfered with the relearning of a brightness discrimination (Thompson, Rich and Langer 1964).

(2) Bilateral lesions of the r.n. significantly impaired retention of a pattern (vertical v horizontal black and white stripes and a white cross v a white disc) discrimination (Thompson 1969).

(3) Bilateral destruction of the r.n. produced impaired performance on a previously learned size discrimination problem (4" dia. white circle v 1$\frac{3}{4}$" dia. white circle on black backgrounds). (McNew 1968).

(4) Lesions of the ventral mesencephalon, including three animals in which the lesions were restricted to the r.n. bilaterally, produced a greater deficit on a pattern (vertical v horizontal back and white stripes) discrimination than on a brightness (black and white) discrimination (Thompson,Lukaszewska et al. (1967).

That the results listed above can not be due to a peripheral motor disturbance is illustrated by the observations of each author, that no signs of motor impairment were present but perhaps more succinctly, that if the results were due to motor impairment one would not expect greater deficits on some tests than on others.

The studies cited above appear to implicate the r.n. or r.n. area in the performance of visual habits and that the destruction of the said nucleus impairs the learned behaviour.

Thompson (1969) interprets his findings in terms of a highly localised cortico-subcortical system subserving visual discrimination habits, involving the occipital (visual) cortex, the posterior thalamus and the ventral midbrain (r.n. area). The importance he ascribes to the r.n. is examplified in a quote from his monograph (1969) - viz "....there is evidence that the size of the lesion within a given critical area directly influences the magnitude of the retention deficit, particularly with reference to the posterior thalamus and ventral midbrain. This effect was also seen in the current study, but was reflected mainly in the number of visual habits that were impaired. For example, extensive damage to the r.n. led to defective retention of all three problems, whereas slight rubral damage failed to impair retention of more than one problem"...

In addition to the lesion studies of visual learning, Thompson and his co-workers have shown retention deficits in learning situations involving other sensory modalities:-

(5) Rats trained to make a jumping response to the onset of a 1,000 cycles per second tone to avoid footshock, manifested impaired retention after bilateral destruction of the r.n. (Thompson, Rich and Langer 1964).

(6) Following bilateral r.n. lesions, a retention loss was obtained in a task utilising 'kinaesthetic' cues:- rats were trained to discriminate between an upward and downward sloping runway, following enucleation of the eyes and trimming of the vibrissae. In this study the animals not only displayed a learning deficit but also an inability to relearn the task (Thompson, Lukaszewska et al. 1967).

At this juncture, the experimental method habitually used by the 'Thompson school' requires some elaboration. With very few exceptions (see 5 above) the apparatus and procedures used, which I summarize below, is as described by Thompson and Massopust (1960).

The apparatus, a two choice discrimination box, consists of a start box, choice chamber and goal box; start box and choice chamber containing a grid floor. To enter the goal box the rat pushes aside one of two stimulus cards placed behind windows in the choice box. Initially the rat is trained to enter the goal box to escape/avoid electric shock. During the ensuing discrimination training the animal is required:- (a) to leave the start box within five seconds and (b) to respond to the stimulus card within 30 seconds. Failure to accomplish (a) or (b) results in the administration of footshock, as does a response to the incorrect stimulus.

Two points are worthy of note.

(1) The complexity of this experimental method, containing a mixture of punishment and discriminated avoidance may well be a factor accounting for Thompson's results.

(2) In all his experiments the animal must learn to escape/avoid shock in order to reach the learning criterion.

It would seem desirable to attempt to break down the experimental method by utilising what appear to be behaviourally more simple situations. For example, the results may be largely attributable to an interference with avoidance learning rather than the 'memory' of the visual discriminanda; a possibility which is easily testable in the traditional runway situation, in which the animal learns to avoid shock by running from a start chamber to a goal box at the onset of a sound or light stimulus; failure to reach the goal within a prescribed period resulting in the administration of shock. In this situation, no sensory discriminations are required other than stimulus on/off.

It would also seem necessary to ascertain whether the observed deficits hold true in a non-avoidance situation, by for example, using positive reinforcement. Use of the latter could test the possibility that negative reinforcement per se is contributing to the Thompson results.

Thompson is not unaware of such possibilities:- "In all probability divergent results would be obtained if thirst motivation were used or if no punishment were given for errors .... however it is reasonable to assume that the occipital cortex, the posterior thalamus and the ventromedial midbrain (the neuronal circuit he believes to subserve visual memory which he has mapped out by observing the effects of lesions on learned discriminations) would still be found to be critical for retention and that any discrepancies arising from a change in methodology would be reflected in the extent to which other cortical and/or sub-cortical structures would be involved" (Thompson 1969).

Thompson, Rich and Langer (1964) have in fact carried out an experiment into the effects of r.n. lesions on a learned jumping response to avoid footshock at the onset of a visual or auditory stimulus (see (5) above). They found that the lesions had effected a decrement in performance. However, the small number of animals involved - three in the visual task and two in the auditory and the size of the lesions - about one-fifth of the area of the transverse midbrain section through the r.n. seem to require additional experimentation.

Sporadic work from other laboratories are relevant at this juncture.

(7) Smith (1970) (published after completion of the work to be reported in the following two chapters), in a traditional one-way avoidance situation, found that the rats which had suffered bilateral lesions of the r.n. -

(a) were slower to escape shock.

(b) took more trials to reach criterion than controls,

and (c) were deficient in ability to learn horizontal v vertical stripe discriminated avoidance but displayed no loss of ability to learn a black v white discrimination in the same situation.

It is interesting to note that Smith's animals were lesioned prior to training and hence indicate a loss of ability to learn rather than a loss in already learned abilities; although such a phenomenon is indicated by the fact that in the Thompson experiments animals often take longer to relearn a discrimination, following lesioning, than they did on original learning. (See for example McNew 1968 and Thompson, Rich and Langer 1964). Smith's interpretation of his results is that the degree of deficit is a function of stimulus complexity and/or orientational cues inherent in the situation viz:-

"A comparison of the simultaneous discrimination tasks with the successive discrimination task reveals that with simultaneous discrimination the positive stimulus indicates, by virtue of its position, the direction of approach for the animal. In the successive disc learning however (see (9) below) the locus of the discriminative stimulus gives no indication of the direction of approach...."

There are three relevant published experiments utilising positive reinforcement.

(8) Ellen, Wilson and Powell (1964) found that rats with r.n. lesions were able to learn a bar pressing response under fixed interval reinforcement, producing response records similar to normal control animals. With the assumption that the discriminative dimension of fixed interval behaviour reflects temporal discrimination, they conclude that lesions of the r.n. do not produce any significant alteration in attention.

Again lesions effected prior to original learning are not strictly relevant to an analysis of the Thompson findings.

(9) Smith (1970), using a T maze situation and food reinforcement, found that rats lesioned prior to training showed no learning difficulties in a left/right position discrimination but considerable difficulty with a black/white successive discrimination.

Prima facie such a result is in agreement with his stimulus complexity/orientation analysis outlined earlier. However, as the position task is probably non-visual it does not clarify the problem of the effect of positive reinforcement in visual learning situations and again the lesions were effected prior to learning.

(10) Myers (1964) found, in the cat, a deficit in retention of a triangularity discrimination following lesions to the midbrain which also involved the area of the r.n. However, the lesions were accomplished with suction aspiration or leucotome and as a result were both large and irregular. His conclusion however....." It is however possible that to some degree the alterations in visual response that result from tegmental lesions may be due to a surgical dissociation of the visual interpretive mechanisms of the forebrain from basic motor mechanisms resident in the brain stem".... would appear to indicate that he implicates mid-brain systems/pathways in the observed phenomena.

All in all it seems that the r.n. or r.n. area is in some way implicated in learning performance. The work summarized in this introduction appears to suggest that the nucleus is involved in the retrieval of already learned information rather than in the laying down of new learning, for lesions prior to learning have minimal effect on subsequent performance relative to lesions effected following initial learning.

It is of course difficult to be more precise than this given the lack of knowledge regarding the encoding and retrieval necessarily involved in learning performance.

Without exception the lesions in all the studies outlined above were large, often destroying up to $\frac{1}{4}$ of the area of the transverse midbrain section at the level of the r.n. (see for example Thompson, Rich and Langer 1964). The reader is referred to Fig.6.1 which illustrates the incredible multitude and complexity of fibres in this region of the brain. It seems clear that extensive lesions to the

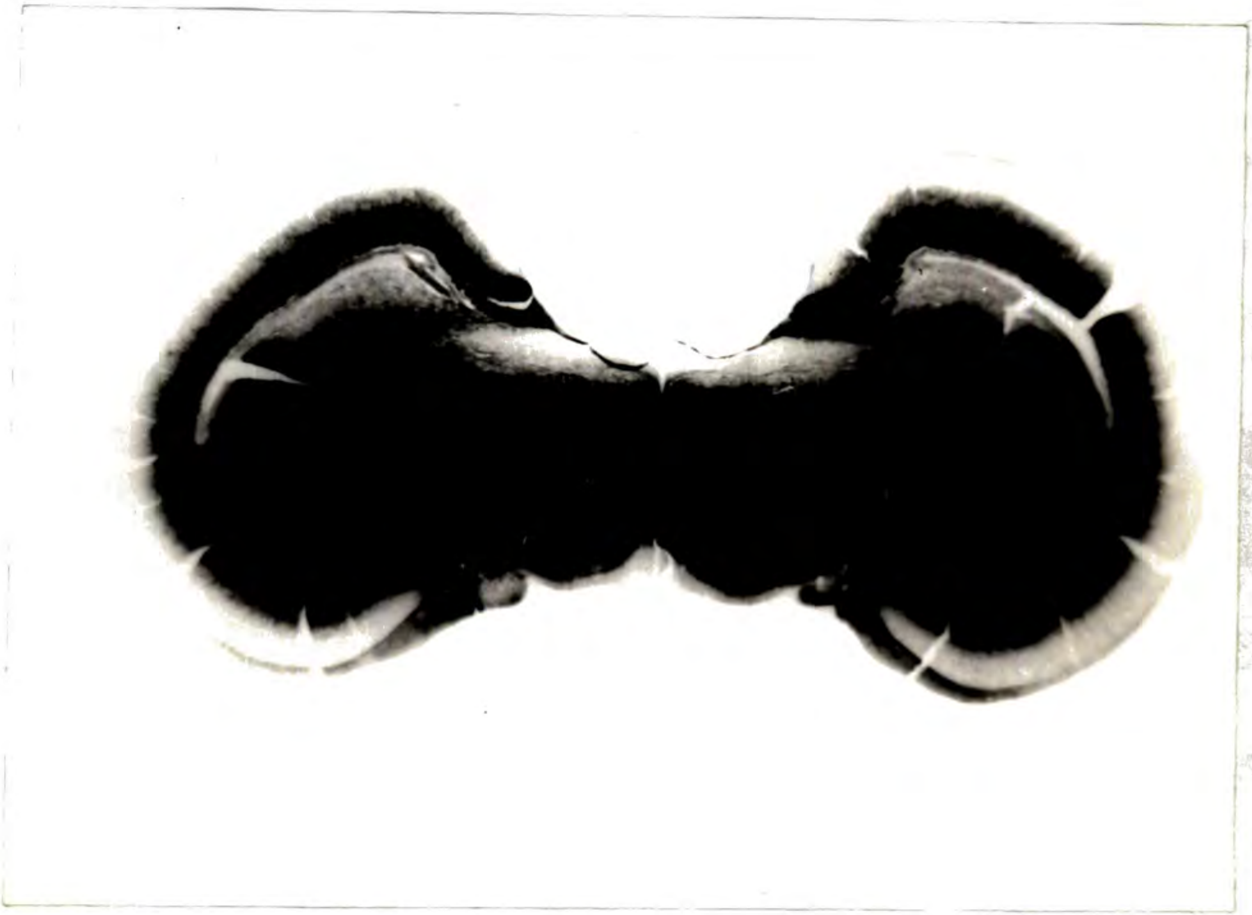


Fig. 6·1 Photomicrograph of a tranverse section through the mesencephalon of the pigeon at the level of the r.n. Bielschowsky-Agduhr silver stain for nerve fibres. Note the great complexity of fibres around the r.n. area.

area in question would render statements about the functional significance of relatively small systems open to methodological criticism. In addition, with the exception of Myers (1964) study, lesions were accomplished by means of D.C. coagulation, which is regarded with suspicion by some researchers because of the possibility of abnormal activity in structures bordering the lesion produced by the deposition of metallic ions. It seems that tissue destruction using very high frequency alternating current (R.F. method) allows better control of lesion extent and is virtually free from electrolytic deposits. (Reynolds, 1965).

Given the above criticisms of the lesion techniques employed by the authors cited and the questions asked earlier of the experimental method used by the 'Thompson school', my own experiments, outlined in the following chapters, were designed with four objectives in mind:-

(1) To study the effects of r.n. lesions on established simple avoidance behaviour.

(2) To ascertain the effects of positive reinforcement and/or task complexity on any learning deficit produced by r.n. lesions.

(3) To conduct a comparative study in the pigeon.

(4) To attempt to isolate r.n. function by restricting tissue destruction through R.F. coagulation.

CHAPTER 7

THE AVOIDANCE EXPERIMENT

The experiments to be reported in this and the following chapter were devised with the objective outlined previously.

Before the description of the experiment it is deemed necessary to include a section on the establishment of avoidance conditioning in the pigeon.

(1) A note on the difficulty of establishing avoidance conditioning in the pigeon.

The author approached the problem with some trepidation having been advised by colleagues that it was difficult, if not impossible, to establish and maintain in the pigeon, a reliable avoidance behaviour i.e. in which the animal must execute a response within a prescribed period of time in order to avoid a noxious stimulus. A cursory review of available literature at that time suggested this was indeed the case (Rachlin and Hineline 1967, Hoffman and Fleshler 1959). The latter authors achieved limited success with one bird only after an extremely protracted training. Rachlin and Hineline succeeded in establishing escape behaviour (i.e. behaviour in which the animal learns to execute a response which terminates a noxious stimulus) but did not report any attempt to condition avoidance behaviour. They report that the sensitivity of individual birds to electric shock varies and recommend a shock train of increasing intensity.

It seemed probable that if reliable conditioned avoidance was to be obtained, then the behaviour to be conditioned must, in part, already be present as a native response to a noxious stimulus. It required no great deductive power to emerge with the conclusion that as the natural response of most birds to a noxious stimulus is one of flying, then the behaviour to be conditioned must, in part, involve flight. Such an approach suggested that the difficulties encountered by Hoffman and Fleshler (1959) using a head lift as a conditioned response and Rachlin and Hineline (1967) using key pecking, may have been methodological rather than inherent in the pigeon itself.

EXPERIMENT 1

An attempt to Establish Conditioned Avoidance in the Pigeon

Apparatus

Given the preceding analysis, the apparatus constructed required the pigeon to fly upward and alight on a perch to escape/avoid shock. (see Fig.7.1). The base of the box measured 24" square and the height was 30". Three of the walls were constructed from clear perspex, the back plate containing stimulus bulb and perch mechanism was of polished aluminium. The retractable perch was situated 22" from the floor of the box. The onset of the conditioned stimulus (C.S.), shock and insertion and withdrawal of the perch, was controlled automatically by electro-mechanical programming. Shock intensities used were in 0.5 ma steps from 0.5 ma to 2.5 ma.* Shock was delivered through flexible leads running through a hole in the roof of the box. A counterweighed arm, to which the leads were attached, allowed the animal unrestricted movement with minimal lead entanglement. The C.S. consisted of the illumination of a 110 v., 10 watts bulb coincident with the insertion of the perch. A small viewing hole was inserted into the chipboard container which covered the apparatus.

Subjects

Five adult pigeons (Columba livia) were used. Each bird was implanted with stainless steel wire electrodes under the pubis bones in accordance with Azrin (1959). The electrodes were joined to a miniature plug secured to the animals back by a broad elastic band looped around the wings allowing virtually unimpeded wing movements whilst giving a degree of damping to sudden jerks of the leads.

* See appendix 4

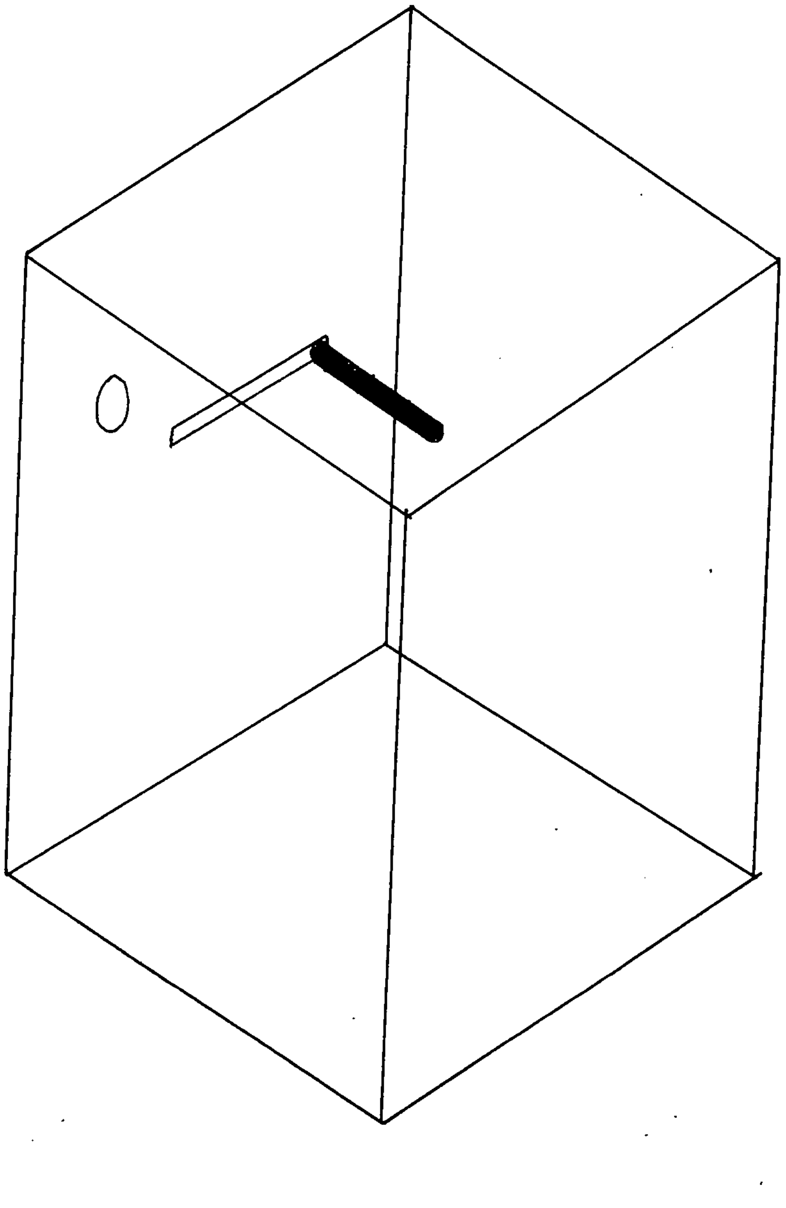


Fig.7·1 Perch Box, sketch of the apparatus used in the initial attempt to condition avoidance behaviour. The pigeon was required to fly up to the retractable perch to avoid shock administered through electrodes implanted under the pubis bones. Eventually abandoned in favour of shuttle box training.

Procedure

Before the first trial each bird was tested to ascertain the minimum shock level required to produce obvious signs of discomfort. This level was found to vary between birds from 0.5 - 1.5 ma presumably dependent upon differing electrode/tissue resistances.

At the start of each trial the C.S. (light + perch insertion) was presented for 5 secs. followed by continuous shock (at the determined minimum level) until the bird flew up to land on the perch which turned off the light and aversive stimulus. After 20 secs. the perch withdrew slowly and the bird returned to the floor of the box. The sequence of events was as follows:-

(1) C.S. on and perch introduced into box,

(2) 5 secs. after (1) continuous shock was administered until

(3) the bird alighted on the perch thereby terminating shock and light;

(4) 20 secs. later the perch was withdrawn and the bird returned to the floor,

(5) 10 secs. later (1) - (4) repeated.

Successful avoidance required the animal to alight on the perch within 5 secs. of C.S. presentation, whereupon (2) would be omitted. Twenty trials per day were given. Before each block of twenty trials minimum shock levels were re-tested and increased where necessary.

Results

The experiment was terminated after 300 trials when it became clear that none of the birds had begun to display avoidance behaviour.

Discussion

Failure was almost certainly due to the design of the 'perch box'. Problems arose because of the sensitivity of the perch to accidental vibration and buffeting as the bird began to fly. This occurred more often than not within 2 secs. of shock administration. At no time was it apparent that the behaviour was directed towards the perch and hence could not be regarded as genuine escape behaviour. Clearly a larger box was necessary in order to obviate the 'accidental' escape.

At that time, McPhail (1968) described a means of successfully establishing avoidance behaviour in the pigeon, utilizing a shuttle box and rather than reconstruct the 'perch box' which may have proved, in the end, to be unsuccessful in establishing the desired behaviour, it was decided to adopt his procedure, somewhat modified, in the remainder of the experiments.

EXPERIMENT 2

The Effects of Red Nucleus Lesions on Previously Established Avoidance Behaviour in the Pigeon

Subjects

The subjects were fourteen pigeons (Columba livia). Each bird was implanted with electrodes as described in Experiment 1.

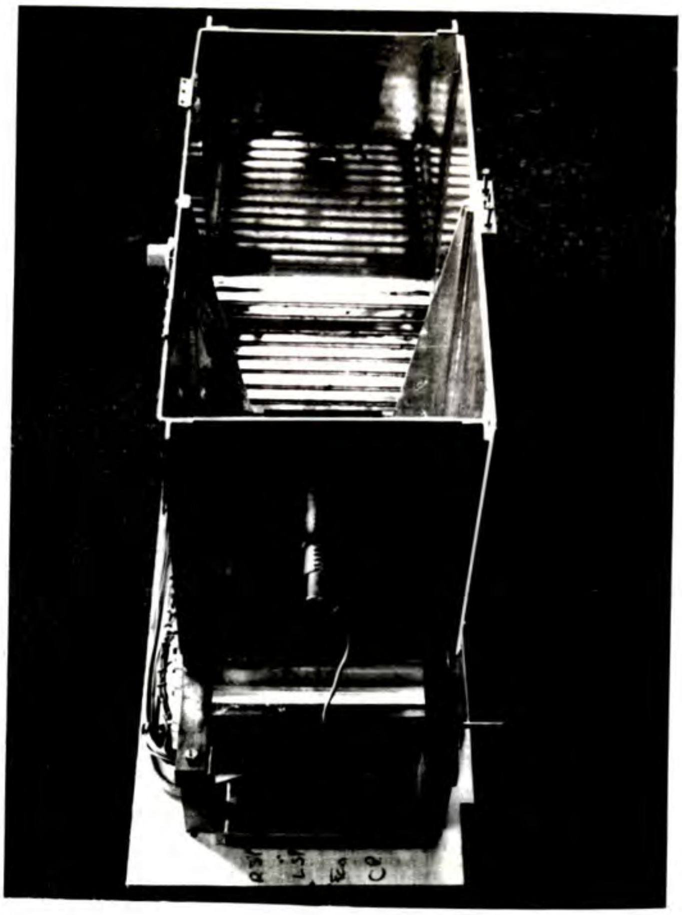
Apparatus

The apparatus used was a modified, two way shuttle box* previously used for rats (Figs.7·2 and 7·3). The dividing wall was cut into a V shape to obviate snagging of the electrode leads as the animal moved from one compartment to the other. The floors of both compartments were independently pivoted and linked to micro-switches which were tripped under the animal's weight as it moved across the dividing wall, thus locating the bird's position in the box. A counter-balanced arm allowed free movement, whilst taking up slack, to eliminate possible entanglement of the leads which entered the box through a hole cut in the roof.

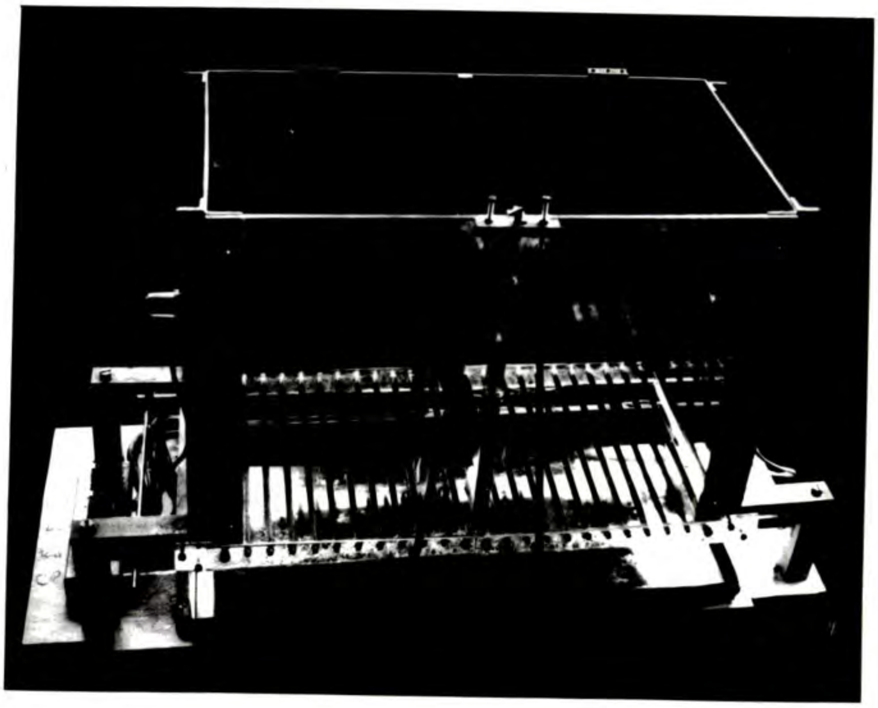
The C.S. consisted of illumination of the 110 v. 10 watt bulb

---

* King et al. (1961)



2



3

Figs.7·2 and7·3.Shuttle Box used in the avoidance experiment.
Dividing wall and stimulus lights shown in
both Figs. Note the roller arranged between
the two compartments to obviate the bird
avoiding shock by perching on the divider.

in the compartment in which the animal stood. Shock intensities were as described in Experiment 1. A continuous, masking, white noise was utilised to obscure any extraneous sounds of programming apparatus etc. which may have served as an auditory C.S.

The experimental box was unlit. A peephole fitted with a wide angle lens was inserted into an enclosing chipboard soundproofing. The whole procedure was automatically programmed by means of electro-mechanical apparatus.

Procedure

Each animal was tested before each block of 20 trials to ascertain minimal shock sensitivity. Minimal values fell within 0.5 - 1.5 ma.

After a five minute period to allow the bird to habituate, the programme was activated. Each trial began with C.S. presentation which dimly illuminated the compartment in which the animal stood. After five seconds, unless the bird had crossed to the other compartment, continuous shock was given until escape was accomplished, whereupon both shock and light were terminated, If the animal crossed into the other compartment within five seconds of C.S. presentation the light was switched off, the shock sequence omitted and the trial was scored as a successful avoidance. A time out period of thirty seconds was given between each trial. Each bird was given twenty trials per day and trained to a criterion of seventeen successful responses on each of three consecutive days.

Having reached criterion, the experimental birds were lesioned bilaterally in the r.n. as described in Chapter 2. The control group was sham operated by adopting the exact surgical procedure except for insertion of the lesioning electrode.

After a recovery period of twenty-one days, the birds were tested for retention of the avoidance habit. The identical procedure utilised in training was adopted and testing was terminated when criterion was reached.

The experimental subjects were sacrificed with an overdose of Nembutal on completion of testing, perfused through the carotid arteries with 5% saline and 10% formalin. The brain removed and fixed for two days in 10% formalin. Following the fixing period each brain was sectioned transversely at 40 µ on a freezing microtome. Every fifth section through the lesioned area was stained with cresyl violet.

RESULTS

Of the fourteen birds, six showed no sign of avoidance behaviour by the twelfth day of training (240 trials) and were consequently discarded. The experimental and control groups therefore each comprised four animals.

Histology

Histological procedures showed that each of the four experimental birds had suffered some destruction of the r.n. Despite the attempt to hold placement and lesion size constant there were differences in both symmetry and extent in all animals.

In all probability differences in electrode resistance accounted for the variability in lesion size.

Determination of the extent of r.n. destruction was accomplished by a large cell count using the method outlined in Chapter 1. The estimated percentage destruction of r.n. tissue for each bird is shown in Table 7.1.

TABLE 7·1

Estimated %age Destruction of r.n. Tissue

Bird	Right	Left
1	75	50
2	100	100
3	100	50
4	50	50

Bird 1

Partial destruction of both r.n.:- 50% left and 75% right with some involvement of medial reticular substance bilaterally. There was no apparent damage to the tractus occipito-mesencephalicus or tractus quinto-frontalis, the major tracts adjacent to the r.n.

Bird 2

100% bilateral destruction was achieved. The lesion also invaded medial reticular substance, nucleus interstitialis of Cajal, tractus quinto-frontalis and possibly part of the occipito-mesencephalic tract. These lesions were more extensive than any achieved using the R.F. (radio frequency) technique and were probably due to a procedural error.

Bird 3

Again the lesions were asymmetrical - 100% destruction of right r.n. with slight involvement of the medial reticular formation. The lesion of the left r.n. was rather ventro-laterally placed with an estimated 50% destruction but almost certainly involving the tractus quinto-frontalis, tractus ansa-lenticularis and perhaps the ecto mamillary nucleus.

Bird 4

Unfortunately the sections for this animal were inadvertently destroyed in the staining procedure, but observations made at the time of sectioning suggested that the lesions were small and restricted to the r.n. bilaterally. In the absence of the cell count procedure a 50% bilateral destruction is estimated. The observations made at the time of sectioning indicated little or no involvement of other structures.

Despite the asymmetry of most of the lesions, the area of destruction was less than that usually achieved by D.C. lesion techniques. Even what has been described as extensive tissue destruction in Bird 2, is estimated to be less than that displayed in the majority of animals utilised by the experimenters discussed in the introduction to this section. However it must be remembered that these workers used the rat or the cat, thereby rendering a comparison of lesion size rather difficult.

Tests

The data derived from the tests is tabulated in Tables 7·2 and ·3 in terms of % savings:-

$$\left.\frac{\text{Training Trials - Test Trials}}{\text{Training Trials}} \times 100\right\} \quad \text{in trials to criterion}$$

and % savings in errors to criterion respectively. A 't' test (Edwards 1965) gives a highly significant difference between the mean savings of experimental and control birds in both Tables ($p < .005$).

Fig.7·4 illustrates the difference in errors to reach criterion on testing. Fig.7·5 is a graph of mean errors to reach criterion before and after lesion. The smooth learning curve prior to lesion and the flatter, relatively discontinuous curve after lesioning should be noticed. Although relearning obviously occurs, the rate of re-acquisition is reduced despite the savings manifested in the first four days.

Table 7·4 shows the mean running times on the last block of twenty trials in training and testing. The mean of the differences was significant ($p < \cdot 025$).

Lesion Extent and Performance

The lesion suffered by Bird 2, being the most extensive, involving total destruction of both r.n., produced a learning deficit, in terms of error savings of -56% (see Table 7·3). Reference to the graphical presentation (Fig.7·4) demonstrates not only the overall lower performance in terms of errors but the greatest discontinuity in the learning curve. The lesion did however also encompass some medial reticular substance.

In terms of r.n. involvement, the next most severe was Bird 3 (100% and 50%). This animal manifested the greatest

TABLE 7·2

Percent Savings in Trials to Criterion

Experimental	Training Trials to Criterion	Test Trials to Criterion	% Savings
Bird 2	140	240	-71
Bird 3	120	240	-100
Bird 4	120	120	0
Bird 1	140	180	-40
		mean	-53%
Control			
Bird 5	160	20	88
Bird 6	160	20	88
Bird 7	120	0	100
Bird 8	360	20	81
		mean	89%

$t = 3.926$ d.f. 6 $P < .005$ *

* See appendix 2.

TABLE 7·3

Percent Savings in Errors to Criterion

Experimental	Training Errors	Test Errors	% Savings
Bird 1	105	106	-1
Bird 2	116	180	-56
Bird 3	107	180	-68
Bird 4	92	58	38
		mean	-22%
Control			
Bird 5	125	15	92
Bird 6	118	9	92
Bird 7	92	6	93
Bird 8	260	14	95
		mean	93%

$t = 4.472$ d.f. 6 $P < .005$ *

TABLE 7·4

Mean Running Times in the Last Twenty Trials

Experimental	Training	Testing	Difference
Bird 1	3.01 secs.	3.21 secs.	.20 secs.
Bird 2	1.86 "	2.18 "	.32 "
Bird 3	2.38 "	2.63 "	.25 "
Bird 4	2.48 "	2.97 "	.49 "
		mean	.315 "
Control			
Bird 5	2.71	2.83	.12
Bird 6	2.50	2.71	.21
Bird 7	2.76	2.81	.05
Bird 8	2.46	2.32	-.14
		mean	.06

$t = 2.5$ d.f. 6 $P < .025$ *

* See appendix 2.

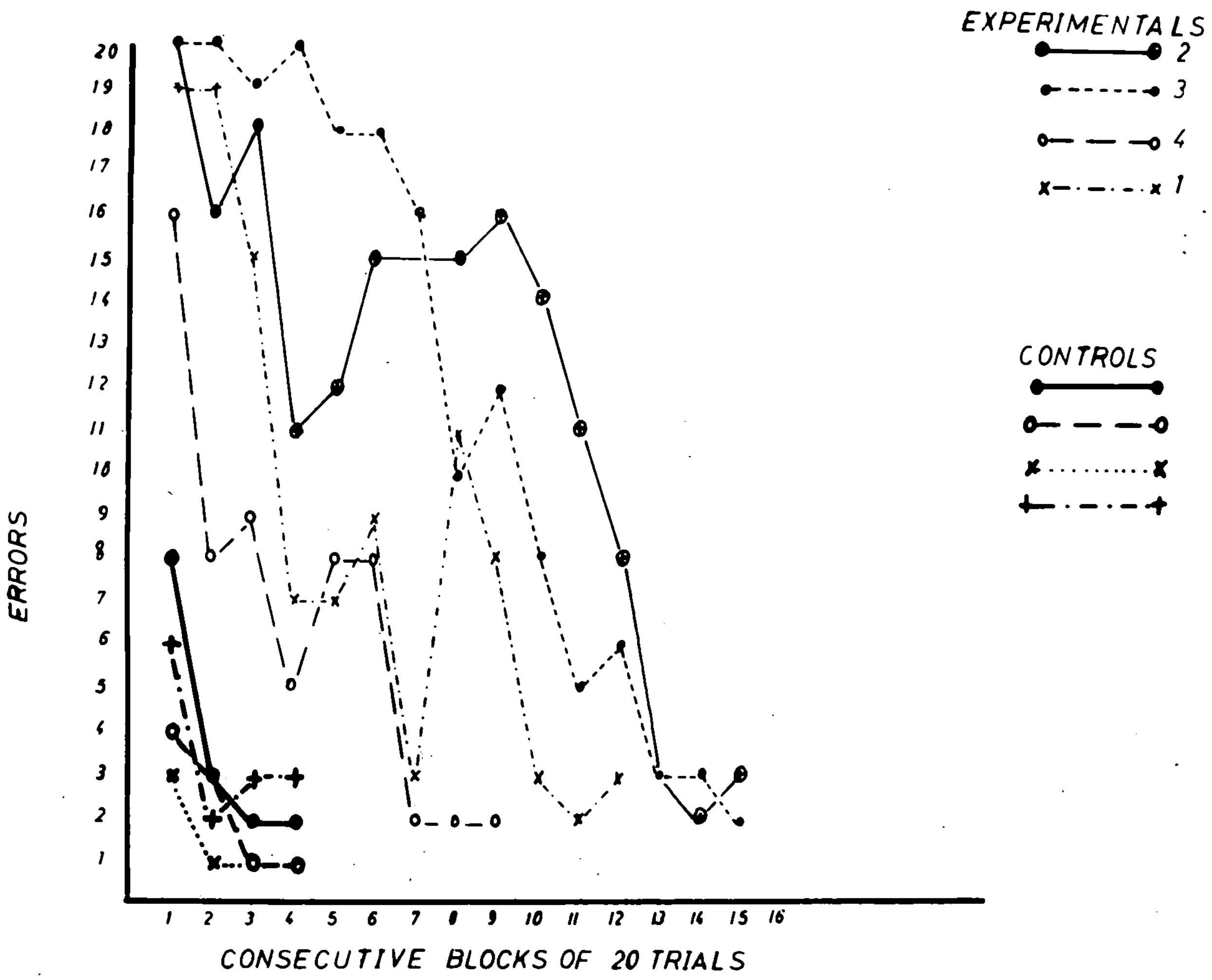


Fig74. Graphical presentation of the number of errors to reach criterion during testing in each block of 20 trials. Notice that the control animals all reached criterion on day four, whereas experimental animals, having suffered r.n. lesions following initial training, took 9-15 days.

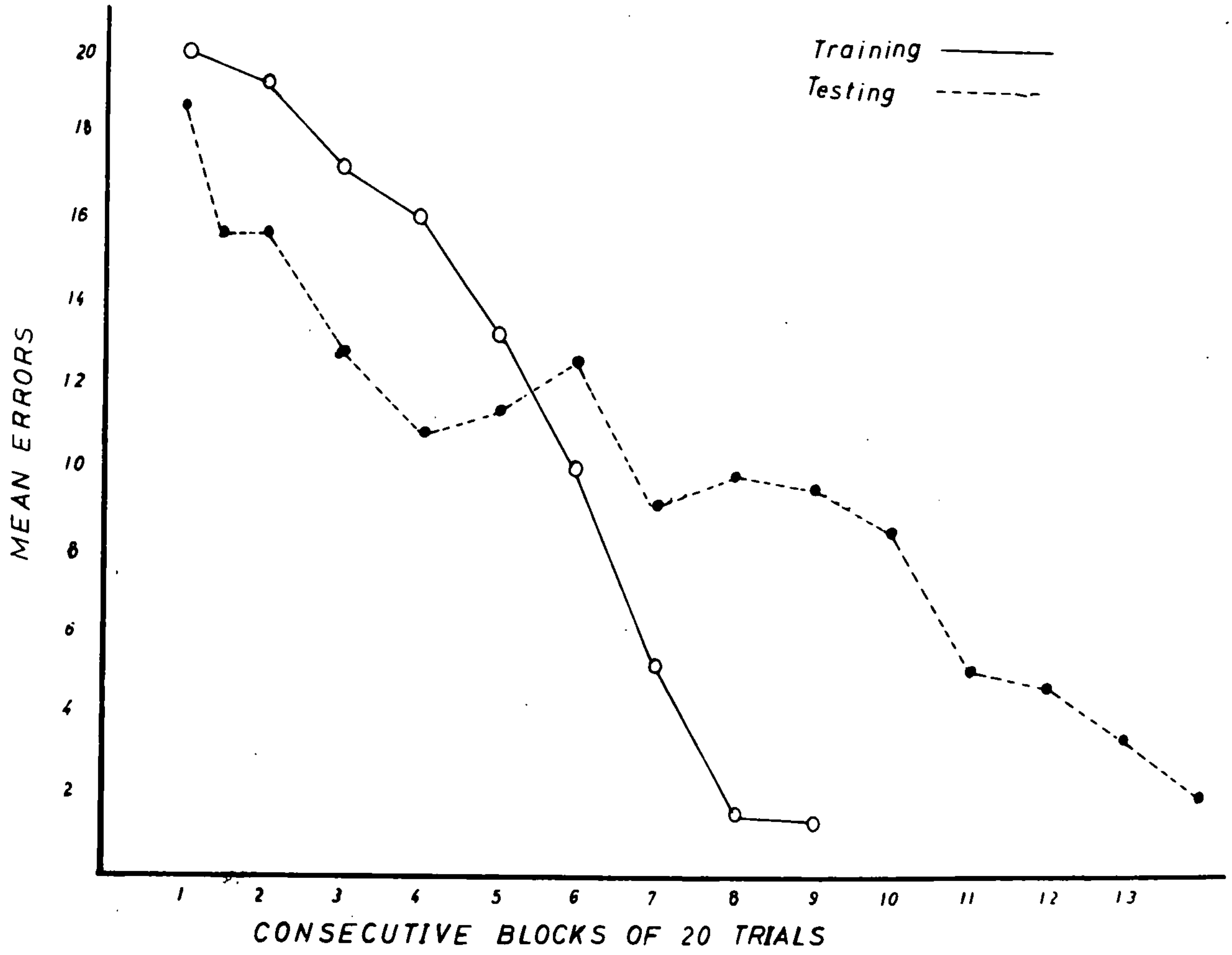


Fig.7·5. Mean errors by experimental group in each block of 20 trials in training and testing. Note the relatively flatter and more discontinuous curve generated in testing.

error savings deficit of -68%.

Birds 1 and 4 suffered the same approximate percentage destruction, (although the loss of tissue sections for Bird 4 makes exact comparisons impossible) in both cases being less than Birds 2 and 3. The mean error savings were -1% and 38% respectively. The individual relearning curves are roughly comparable up to day 7 whereafter the curve generated by Bird 1 displays the discontinuous function typical of Birds 2 and 3.

DISCUSSION

The results of this experiment are surprisingly similar to those produced by Thompson, Rich and Langer (1964), bearing in mind the different species involved. Clearly the r.n. or r.n. area is critically involved in the maintenance of learned avoidance behaviour.

The increase in running times of the experimental group at post-lesion criterion relative to that of the control group, indicates a performance deficit. Whilst it is not impossible that the impairment in running time is indicative of some peripheral motor disturbance, no obvious disability was discerned at the time of post-lesion testing. It may be that Smith's (1970) conclusion, of an impaired ability, in rats, to initiate a conditioned movement after r.n. lesions may also hold true in the pigeon. On the other hand, the graphical presentation of mean learning curves of the experimental animal (Fig.7.5) may indicate a different learning process. The training learning curve is typical of learning curves produced in psychological laboratories (see e.g. Hilgard and Marquis 1961 p115) whereas the post lesion curve is flatter and more irregular. If one accepts that the r.n. is part of a specific system involved in the performance of a learned habit, it follows that if the animal relearns that habit, following lesions to the r.n., then some other system must take over the function of the lesioned area. It may be that the different nature of the learning curve after lesioning is indicative of the substitution of a system with different characteristics and efficiency.

It could be argued that the re-learning curve is not a function of a system involved in the learning of a conditioned avoidance but rather is representative of the gradual recovery of motor systems involved in the performance of such activity. Several points argue against such an hypothesis. Firstly that successful avoidance responses, when executed, usually took about 3 secs. following the onset of the C.S. and this varied little irrespective of the stage of re-training until criterion levels of performance began to be approached when the avoidance time usually reduced slightly. In other words even early in retraining the animal possessed the motor ability to clearly avoid shock. Secondly, were the motor deficit hypothesis true one would anticipate that avoidance times would, early in retraining, be very close to the five second point at which shock would occur, getting faster as the deficit improved. Again, as aforementioned, the animals avoidance responses were well within the five seconds allowed for execution and fairly clearly represented a different form of response to trials in which escape occurred. Other possible reasons for the deficit, including motivational and shock sensitivity effects, are discussed in the overall conclusion to this work (Ch. 9).

Whilst it is not possible to state categorically that destruction of the r.n. alone accounts for the obtained results, due to the involvement of other structures, it is clear that lesions around this area are critical to the memory deficit Clearly a further experiment including a control group with lesions adjacent to but avoiding destruction of r.n. tissue is indicated.

In conclusion the Behavioural Experiment 2 has shown that:-

(1) Lesions to the r.n. or r.n. area effect a significant decrement in the performance of a previously learned avoidance task.

(2) r.n. lesions produce an increase in the mean time taken by the bird to avoid electric shock (or produce an increase in the time necessary to initiate a conditioned movement - Smith 1970).

CHAPTER 8

THE EFFECTS OF R.N. LESIONS ON PREVIOUSLY ESTABLISHED KEY PECKING BEHAVIOUR FOR POSITIVE REINFORCEMENT

It will be recalled that the author has previously discussed the experimental procedure utilised by Thompson and his co-workers (1960, 1964, 1967, 1969) and has suggested that his (Thompson's) results may well be attributable to an interference with conditioned avoidance behaviour rather than to an amnesic effect on the visual discriminanda and associated behaviour, being the view posited by Thompson.

The only directly relevant publication, that of Myers (1964), who demonstrated a deficit in retention of a triangularity discrimination following lesions to the r.n. area was criticised on the grounds that the lesions were large, asymmetrical and crudely produced. Findings by Smith (1970), in the rat, of a deficient ability to learn a black/white discrimination and of Ellen et al. (1964) of no effect on bar pressing behaviour under a fixed interval reinforcement schedule do not contribute to the problem under consideration i.e. the effect of r.n. lesions on previously learned behaviour in a positive reinforcement situation.

Experiments were therefore designed

(a) to test the effects of r.n. lesions on behaviour previously established by means of positive reinforcement,

(b) to test the role of stimulus complexity/task difficulty on any amnesic effects following r.n. lesions,

and (c) to attempt to more clearly delineate critical tissue areas through the production of small lesions by R.F. coagulation.

The positive reinforcement situation considered suitable was the traditional Skinner box in which the bird is trained to peck a disc to obtain food. Such a situation is free from any aversive stimulation unless one regards the omission of food (reinforcement) contingent upon an incorrect response as being aversive. Whilst it is not impossible that food omission is aversive it is likely that there is a qualitative rather than quantitative difference between the consequences of non-reinforcement and the application of electric shock contingent upon an incorrect response.

APPARATUS

The apparatus consisted of a two key Skinner box 18" x 18" x 18". The keys were transluscent, allowing the projection of discriminanda from behind, and outside of the experimental chamber. The stimuli were projected by means of micro-projectors. A correct response resulted in the immediate activation of a grain hopper allowing access to food for a predetermined interval. Three walls and the ceiling of the experimental chamber were constructed from clear perspex; the back plate, containing the keys and grain hopper, was of aluminium. Illumination inside the box was by means of a 15 watt bulb placed over the centre of the perspex ceiling. The keys, projectors and food hopper were automatically operated by means of electro-mechanical programming. Extraneous noise from relays etc. was masked by continuous white noise.

SUBJECTS

The subjects were twenty pigeons of mixed strain (Columba livia).

PROCEDURE

Twenty one days before the commencement of training four birds were randomly selected to serve as a small group lesioned prior to training (P.L. group). Under Equithesin anaesthetisation the skull was trephined and bilateral lesions of the r.n. attempted by R.F. coagulation (see Chapter 2 for detailed description of the surgical and lesioning technique).

Four birds were assigned to a non-lesioned control group (N.L.) and twelve to the experimental group lesioned following initial training (P.T.L. group).

All animals were reduced to 80% ad libitum body weight prior to the commencement of 'shaping' and held at that weight throughout the experiment. Before training was begun each bird was given a period of a few hours in the experimental chamber to habituate. Shaping was accomplished in the usual manner by means of an external key operated by the experimenter. In order to obviate undesirable position preferences care was taken to reinforce approximately the same number of responses to each key at this stage. During the shaping of pecking responses both keys were illuminated by a white light with a brightness midway between the values of the illumination used in the brightness discrimination, again to avoid the creation of preferences.

Following shaping, the birds were introduced to the first of the three discrimination problems utilised. The tasks were, in order of completion,

1. Brightness
2. Colour
3. Pattern

Task 1 consisted of both keys being illuminated by white light, the negative i.e. the non-reinforced key, being the brighter of the two. The brightness levels were arranged such that the level of brightness used during shaping was judged, by the experimenter, to be of intermediate value.

Task 2 The positive key was illuminated with red and the negative with green light. The brightness level of both colours was randomly varied to obviate its utilisation as a cue.

Task 3 The positive stimulus was an outline square of $\frac{1}{2}$" and the negative an outline triangle with base and height measuring $\frac{1}{2}$".

In each task the stimuli were randomly switched across keys using the Gellerman random series (Gellermann,1933).

Animals were given 20 discrete training trials per day on the brightness task until a criterion level of 18 correct responses on three consecutive days was reached, whereupon they were given a further 40 overtraining trials to ensure reliable conditioning. Following the overtraining trials, the birds were introduced to the colour discrimination problem with the same technique being utilised, and finally to the 'pattern' discrimination.

A correct response, a peck on the key illuminated by the positive stimulus, removed the stimuli and simultaneously activated the grain hopper giving five seconds access to grain. This was followed by a fifteen second 'time-out' interval before presentation of the next stimulus pair. An incorrect response, to the negative key, removed the stimuli and was followed by a twenty second time-out interval.

Paper print-outs recorded the nature and latencies of responses. Immediately following the attainment of criterion plus forty overtraining trials on the pattern task, the non-lesion (N.L.) and post training lesion (P.T.L.) groups were anaesthetised and subjected to surgery. (see Chapter 2 for lesioning method). Group (N.L.) was subjected to an identical procedure apart from the insertion of lesioning electrodes and coagulation. The four birds which had sustained lesions prior to training, (P.L.) group, were returned to their home cages, where they remained until testing began.

Twenty one days following surgery all animals were tested for retention of the previously learned behaviour. An identical procedure to that used in training was adopted, i.e. where the bird did not immediately attain the criterion level it was retrained until criterion was reached. In order to retain an identical methodology forty additional trials were given following each test.

Immediately following completion of the retention testing, groups P.L. and P.T.L. were sacrificed by an overdose of Nembutal and perfused through the carotid arteries with saline solution followed by 10% formalin. Each brain was sectioned transversely at 40 µ on a freezing microtome. Sections through the lesioned area were stained with cresyl violet and examined for placement and extent of lesion. The extent of the r.n. lesion was calculated as a percentage destruction on the basis of a cell count using the method outlined in Chapter One.

RESULTS

One of the P.T.L. birds died during surgery reducing the number in that group to 11.

Histology

Histological analysis revealed that all of the P.T.L. group and three of the four P.L. animals suffered some damage to the r.n. ranging from complete bilateral destruction to birds 15 and 20 to minimal unilateral destruction in birds 19 and 20. Table 8·1 shows the estimated percentage destruction of the r.n. both left and right.

A procedural error whereby the brain of bird 3 was left in saline instead of formalin prior to sectioning, made the preparation of stained sections impossible and estimation of lesion extent was made from the unstained transverse section remaining on the microtome freezing module and a sketch made. The percentage destruction shown for bird 3 is therefore not as reliable as that for other birds. It was clear from the transverse section that 100% bilateral r.n. destruction had been achieved, although other adjacent structures had most certainly been involved.

Fig.8·1 shows the maximal and minimal extent of lesions in the

P.T.L. and P.L. groups.

Examination of the brain of bird 1 of the P.L. group failed to disclose any recognisable signs of a lesion, due one assumes to either a procedural error or apparatus malfunction.

Test Results

Table 8·2 shows the percentage error savings for each of the three tests. [Table 8·2a gives the raw data]

$$\left(\text{error savings} = \frac{\text{Training errors} - \text{Test errors}}{\text{Training errors}} \times 100\right)$$

Statistical examination by 't' test revealed that the only significant difference ($P < .05$) lay between the P.T.L.

TABLE 8·1

PERCENTAGE DESTRUCTION OF RED NUCLEI

Group	Bird	Right	Left	Mean
Prior Lesion	1	none	none	-
(P.L.)	4	100	5	52.5
	5	100	50	75.0
	12	50	75	62.5
Post Training Lesion	3	100?	100?	100.0 (see text)
(P.T.L.)	7	100	55	77.5
	9	50	85	67.5
	15	100	100	100.0
	17	70	70	70.0
	18	85	60	72.5
	19	10	none	5.0
	20	100	100	100.0
	21	20	none	10.0
	23	70	50	60.0
	24	70	70	70.0

TABLE 8·2 *

PERCENTAGE ERROR SAVINGS

$$\left(\frac{\text{Training errors - Test errors}}{\text{Training errors}} \times 100\right)$$

Group	Bird	Brightness	* Colour	Pattern
Prior lesion	1+	76%+	100%+	93%+
(P.L.)	4	62	100	99
	5	85	100	89
	12	86	97	86
		mean = 77.7	mean = 99.0	mean = 91.3
Non-lesioned	6	90	100	91
(N.L.)	8	87	75	90
	13	96	98	92
	16	92	96	89
		mean = 91.25	mean = 91.0	mean = 90.5
Post-Training-Lesion	3	81	80	35
	7	80	69	70
(P.T.L.)	9	81	95	76
	15	82	73	-52
	17	85	100	70
	18	64	95	70
	19+	98+	100+	91+
	20	95	94	9
	21+	98+	100+	97+
	23	84	95	70
	24	87	100	73
		mean = 82.1	mean = 89.0	mean = 46.8

+ Excluded from calculations (see text)

Only the difference between the means of N.L. and P.T.L. and P.L. and P.T.L. on the pattern test were significant. $P < .05$

* See appendix 3.

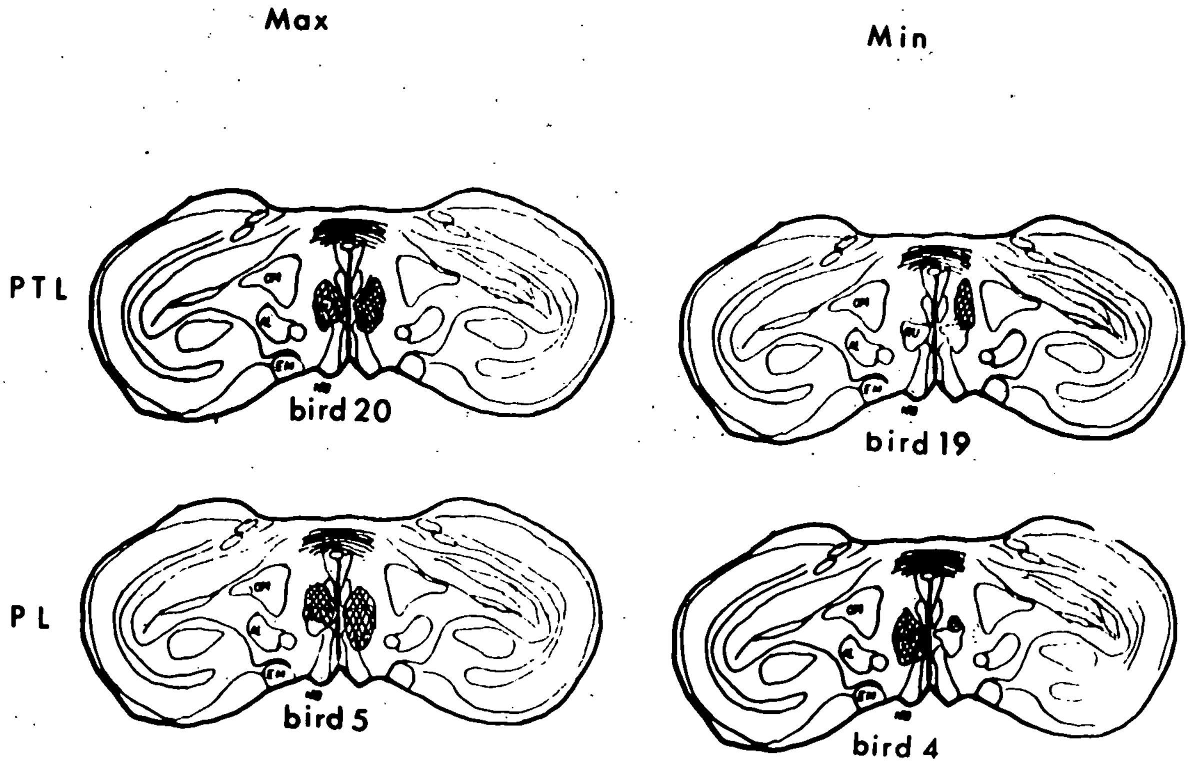


Fig. 8·1. Maximal and minimal extent of lesions in P.T.L. and P.L. groups.

group and the two control groups on the pattern test. Only the results of those birds having suffered bilateral destruction of the r.n. were included in the statistical treatment, it being clear that the omitted results of birds 19 and 21 were quite atypical of the experimental group.

Fig.8·2 displays the error savings on the pattern and colour tests as a function of mean percentage destruction of both r.n., brighness being omitted in the interests of graphical clarity, it being clear that no relationship existed (see the correlation coefficients in Table 8·4 discussed below).

Table 8·3 shows the response latencies, i.e. the period between the occurrence of the stimuli and the birds' response, presented as a difference between the mean latencies of training and testing during the last twenty correct trials for each task, where it could be assumed that latencies would be close to asymptote. The P.T.L. group was not significantly different from the N.L. within any of the tests.

Spearman rank-difference correlation coefficients (Table 8·4) (McNemar 1962) were computed for percentage destruction and saving scores, percentage destruction and latency difference, performance on each test, and within each test between savings and latencies. Significant coefficients were found for percentage destruction and both colour and pattern savings of .72 and .83 respectively, between colour and pattern savings of .64 and between percentage destruction and response latency on the brightness task of -.68.

DISCUSSION

It is of course impossible to analyse the findings

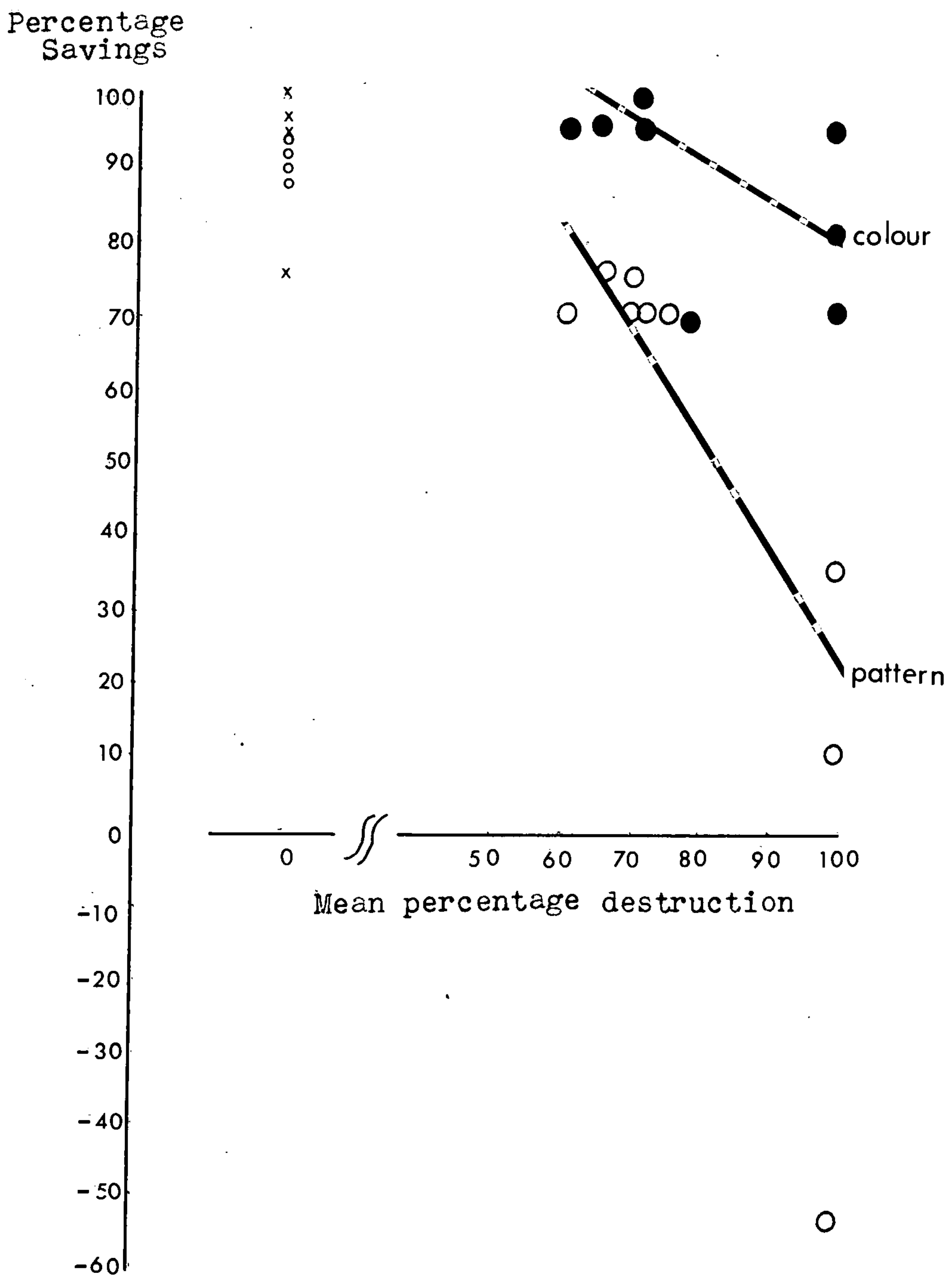


Fig8·2. Percentage error savings on pattern and colour tests as a function of mean percentage destruction of the r.n. Note the two tentative functions drawn for colour and pattern tests. Brightness results are not included (see text). Performance of control birds are shown above the 0% destruction point.

x= colour (controls) o= pattern(controls)

TABLE 8·3

MEAN RESPONSE LATENCY DIFFERENCE (mean test-latency-mean training latency) OF THE LAST-20 CORRECT TRIALS OF TRAINING AND TESTING

Group	Brightness	Colour	Pattern
P.L. 1+	- .13 secs.	.05 secs.	- .44 secs.
4	- .03	.07	- .38
5	.03	.05	.27
12	.04	.08	- .07
	mean .01	mean .07	mean - .06
N.L. 6	- .12	.00	- .07
8	.04	.07	.04
13	.02	- .08	.01
16	.12	- .03	.05
	mean .02	mean .01	mean .01
P.T.L.3	- .04	.11	.29
7	.06	- .06	.04
9	.12	.07	.31
15	.05	.01	- .02
17	- .08	.07	- .03
18	.04	- .04	- .13
19	.05+	.05+	.47+
20	- .12	.06	.09
21	- .19+	.08+	.17+
23	.15	.03	- .02
24	.00	.03	.22
	mean .02	mean .03	mean .08

+ Excluded from calculations (see text)

The differences between mean latency differences within each task are not significant ($P > .05$)

TABLE 8·4

SPEARMAN RANK DIFFERENCE CORRELATION COEFFICIENTS

Bird	D	Bs	B1	Cs	C1	Ps	P1
3	100(2)	81(3.5)	-.04(7)	80(3)	.11(1)	35(3)	.29(2)
15	100(2)	82(5)	.05(4)	73(2)	.01(7)	-52(1)	-.02(6.5)
20	100(2)	95(9)	-.12(9)	94(4)	.06(4)	9(2)	.09(4)
7	77.5(4)	80(2)	.06(3)	69(1)	-.06(9)	70(5.5)	.04(5)
18	72.5(5)	64(1)	.04(5)	95(6.3)	-.04(8)	70(5.5)	-.13(9)
24	70(6.5)	87(8)	.00(6)	100(8.5)	.03(5.5)	73(8)	.22(3)
17	70(6.5)	85(7)	-.08(8)	100(8.5)	.07(2.5)	70(5.5)	-.03(8)
9	67.5(8)	81(3.5)	.12(2)	95(6.3)	.07(2.5)	76(9)	.31(1)
23	60(9)	84(6)	.15(1)	95(6.3)	.03(5.5)	70(5.5)	-.02(6.5)

KEY

D = mean r.n. destruction

Bs = Brightness savings

B1 = latency difference on brightness task

Cs = Colour savings

C1 = latency difference on colour task

Ps = Pattern savings

P1 = latency difference on pattern task

Correlation	P	Sig.Value
D/Bs	.07	N.S.
D/B1	-.68	P < .025
D/Cs	.72	P < .01
D/C1	-.07	N.S.
D/Ps	.83	P < .01
D/P1	.08	N.S.
Bs/B1	.54	N.S.
Cs/C1	-.30	N.S.
Ps/P1	-.20	N.S.
Bs/Cs	.39	N.S.
Bs/Ps	.12	N.S.
Cs/Ps	.64	P < .025

of these experiments in terms of specific deficits and to compare the findings with those of the 'Thompson school' due to the species differences involved. Results derived from lesions of the r.n. in the rat showed retention deficits in:-

(1) Brightness discrimination - (Thompson et al.1964, and 1967, McNew 1968)

(2) Pattern discrimination - (Thompson 1969, McNew 1968)

(3) Size discrimination (McNew 1968).

The results of the present experiments do not disclose any retention deficit in the pigeon, following r.n. lesions, on either brightness or colour discriminations, the latter not of course being possible in the rat, and the former probably a more difficult discrimination for the rat, being essentially a nocturnal animal, than for the pigeon. However in common with rats, a deficit has been shown in the retention of a 'pattern' discrimination which suggests that the avian r.n. may have similar functions. It is however clear that lesions of the r.n. in the pigeon do not obliterate recall of all learned visual discriminations.

It could be posited that the deficit on the pattern task is due to the fact that the same order of task presentation, i.e. brightness-colour-pattern, was used throughout and that the result is due to some form of proactive interference or negative transfer. Were this so, one would anticipate similar effects in the control birds, which did not occur, or if an interaction between lesion and proactive interference was hypothesized, an effect on the colour task would be predicted, which again did not occur. The proactive interference/lesion interaction hypothesis would also suggest that the P.L. group should be deficient in the original learning of the problems relative to normals. Inspection of the figures presented below (Table 35) do not substantiate this.

TABLE 8·5*

ERRORS TO CRITERION IN INITIAL LEARNING

Group	Brightness	Colour	Pattern
P.L.1+	34	58	234
4	45	7	194
5	67	42	98
12	30 m=47	35 m=28	482m=258
N.L.6	26	40	268
8	57	29	193
13	57	48	364
16	53 m=48	30 m=37	297 m=280

+ Excluded from calculations (no lesion sustained).

Differences not significant P > .05

The non-significance of the latency difference between P.T.L. and N.L. groups (Table8·3) and the correlation coefficient of .08 between mean r.n. destruction and latency differences on the pattern test (Table8·4) suggests that the savings deficit on the pattern problem is not attributable to a motor disturbance.

The correlation coefficients of .83 and .72 relating lesion extent and performance on the pattern and colour tests respectively, suggest, as Thompson (1969) found in the rat, that the extent of r.n. destruction directly influences the magnitude of the deficit. The latter coefficient suggests that larger lesions may have produced a deficit on the colour task as does the .64 correlation between pattern and colour savings. If this is the case, it is difficult to see why there is no correlation (.07) between brightness savings and r.n. destruction, unless performance of learned brightness discriminations is determined by other systems.

The author can offer no explanation for the significant negative

* See appendix I.

correlation (-.68) between mean r.n. destruction and latency difference on the brightness task, for this would mean that large lesions tend to be associated with reduced testing latencies. It must however be realised that the correlations were a 'post hoc' exercise and spurious significances would not be totally unexpected.

The essential difference between the results obtained by the workers previously cited, in the rat and the cat, and those of the present study is largely one of degree. Thompson and his co-workers (1960, 1964, 1967 and 1969) and Myers (1964) obtained a far more definitive effect on retention that that shown in this series of experiments. Once again bearing in mind the dangers of comparing across species, two points appear relevant. Firstly, the lesions utilised by the aforementioned authors were much larger than those of the present study in which on only three occasions was 100% bilateral destruction achieved, the remainder being largely restricted within the r.n. Secondly, the tests used by these authors would, it seems, fall within the category of difficult visual discriminations, (size, verticality, cross v disç,brightness and triangularity) whereas the colour and brightness problems used by the present author fall within what could be called 'easy' discriminations for the pigeon (c.f. mean errors to criterion in Training, 48 and 37 for brightness and colour respectively and 280 for pattern - Table8.5).

If then the avian r.n. is functionally similar to that of the mammal and certainly the physiological studies outlined in Part 1 of this thesis suggest a close similarity, it may be that in the rat and cat lesions restricted to the r.n. would effect a disruption of only difficult discriminations.

In terms of the findings of the experiments reported in this Chapter, two hypotheses can be posited:-

(a) Lesions of the r.n. in the Pigeon affect retention of difficult problems only,

and (b) lesions of the r.n. in the pigeon effect a deficit only of learned 'pattern' discriminations.

In retrospect, it is clear that an experiment utilising a 'difficult' task, not involving 'pattern' is necessary to clarify the issue. Hypothesis (a) agrees with Smith's (1970) complexity thesis.

Neither hypothesis posits any physiological mechanism, both being entirely descriptive of the data. The electro-physiological results outlined in Part 1, together with the results of other workers on the physiological basis of behaviour, suggest a tentative hypothesis which is offered in the overall conclusion to the work described in this thesis (see Chapter 9).

CHAPTER 9

CONCLUSIONS

The physiological experiments and observations described in Part 1 of this thesis have shown that in many respects the r.n. of the pigeon and presumably that of the avian species generally, bears a close resemblance to that of the mammal.

It was shown in Ch. 1 that the pigeon r.n. is richly supplied with fine blood capillaries and is made up of not only the distinctive large cells but also consists of medium and small cells. Both high vascularity and large cells are characteristic of the mammalian r.n. The former feature may be simply due to the metabolic requirements of the area or may possibly indicate the operation of hormonal processes. In this context Kishida et al (1960) found that chemical stimulation of the r.n., in the rabbit, produced mastication, salivation, vomiting, chattering of teeth, increased respiration and ataxic walking, which essentially replicated the effects of peripheral injection, and could not be obtained elsewhere in the C.N.S.

The lesion and stimulation studies reported in Chs. 2 and 3 were consistent with mammalian work with regard to transitory abnormalities in leg and neck muscle tone in the former and contralateral rotation and ataxia in the latter.

The 'mapping' experiment reported in Ch. 4 in which the pigeon brain was systematically stimulated electrically and recordings made from the r.n. indicated that, as in the mammal, the major input is from the cerebellar nuclei. Comparable links were also shown with the hypothalamus. The surprising finding from this experiment was the connections with the assumed avian homologues of the mammalian septum and amygdala, which have not been shown to be present in mammals although, as was pointed out, indirect pathways may possibly exist.

It was shown in Ch. 5 that both species possess inputs from the major sensory modalities and in addition the cells of the r.n. of the bird and mammal respond to sensory stimulation with a characteristic period of inhibition.

The results of the behavioural experiments reported in Part Two demonstrate strong similarities to those produced in mammalian work in that lesions of the r.n. produce apparent disruption of previously learned behaviour. An attempt was made to analyse the findings of the 'Thompson school' by breaking down their experimental procedure. The suggestion is made that if, as in the pigeon, an important effect of r.n. lesions in the rat is the disruption of previously established avoidance behaviour, then to use the procedural method adopted by Thompson and his co-workers, involving avoidance, in the investigation of visual discrimination, renders any results meaningless. R.N. lesions in the pigeon were however shown to produce a significant deficit in 'pattern' discrimination established through positive reinforcement but not of brightness or colour.

Other suggestions could be made to explain the observed behavioural phenomena:-

(1) A reduction in motivation.

(2) A motor performance deficit.

(3) A reduction in sensory sensitivity.

Dealing with the avoidance experiment first, a motivational reduction would presumably mean a reduction in fear. The lesioned animals were however indistinguishable from normals in that the former in re-training and the latter in initial training, when at the 'escape' stage of learning both manifested the same response to the light C.S. of cessation of movement and orientation of the body towards the 'goal' compartment. In addition, the observations in Ch. 2 indicate a recovery of general emotional behaviour to the presence of the experimenter. It is suggested that this behaviour pattern indicates fear. Whilst it is possible that the animals did suffer some motor impairment, it was pointed out in Ch. 7 that an examination of the avoidance responses that did occur well before criterion was reached, disclose that successful reactions were well within the 5 second period before shock onset. Clearly the motor ability for avoidance was present. It will be recalled, that before each block of trials the animals' sensitivity to shock was tested and the level set to produce obvious signs of discomfort. In general, each animal, both experimental and control, required increases in shock intensity during the period of the experiment, probably due to increased shock/electrode/tissue resistance. It is suggested that the setting of shock values before each block of trials would counteract any reduction in pain sensitivity produced by r.n. lesions.

Referring to the positive reinforcement experiments, it seems unlikely that motivation reduction could be responsible for the deficit, for the animals disclosed a savings loss on only one of the three tests in which, one assumes, motivation was a constant. With much the same reasoning a motor performance deficit is ruled out for an identical motor pattern was required in each of the three tests.

Whilst it is not impossible that the lesions of the r.n. may effect visual ability through damage to the adjacent third nerve and consequent deficit in eye movement control, the results of the birds lesioned prior to initial training suggest that this could not be a determining factor of the deficit on pattern discrimination (see Table 8·5[illegible]). The only hypothesis, in terms of a visual loss, which could explain the large savings loss of the experimental group in testing, would be if the lesion effects on vision were such that the animal was forced to utilise different cues to solve the square v triangle test. In other words, lesions prior to training may produce little effect on ability to learn the discrimination, although the animal may utilise different cues, whereas lesions following initial training would necessitate the bird relearning the discrimination due to the loss of previously available visual information. This possibility could be examined by attempting to restrict lesions to the third nerve. The mammalian third nerve is responsible for eye movements, accommodation, and sphincter contraction (Davson 1962) and the observed effects, listed in Ch. 2, of r.n. lesions which damaged this nerve included pupil dilation and paucity of eye movements suggesting that much the same function is served by the avian third nerve. If the third nerve of both species subserve similar functions, it is possible that the lesions of the mammalian r.n., which also would involve third nerve damage - indeed McNew (1967b) cites abnormal pupil dilation as one of the results of r.n. lesions, - may be an explanation of the results obtained by Thompson and his co-workers. However, McNew (1967b) considered the possibility of a perceptual deficit arising from r.n. lesions and found that although rats with r.n. lesions were deficient relative to normal controls, in a size threshold discrimination, in which a 4" circle had to be discriminated from 1$\frac{3}{4}$", 2", 2$\frac{1}{2}$" and 3" diameter circles, they were not significantly different from animals which had suffered midbrain lesions slightly dorsal to the r.n. Whilst it is clear from his photographic material that in all cases damage was probably sustained to either the third nerve or its nucleus, in a test of retention of size and 'pattern' discriminations the r.n. lesioned animals showed a significantly greater loss than the rats with lesions dorsal to the r.n. In other words, although involvement of the oculomotor nerve may be responsible for some loss in visual ability such damage is insufficient to account for the whole of the retention loss. Bearing in mind that we are here concerned with the avian as opposed to mammalian species, sufficient evidence for a high degree of physiological similarity has been presented to warrant the assumption that similar results would obtain in the pigeon, although experimental support is obviously necessary.

The integration of physiological with behavioural data is often difficult especially in the avian species for which brain mechanisms and particularly forebrain mechanisms are largely unexplored. However, the links demonstrated between the medial archistriatum and r.n. reported in Ch. 4 and the behavioural finding (Ch. 7) of a significant deficit in the retention of a two-way avoidance task received some support from the work of Phillips (1964, 1968). Phillips showed that lesions of the medial archistriatum produced greatly reduced escape or avoidance responses in the Mallard (1964) and in the Peach-Faced Lovebird (1968) and also that stimulation of the same area, in the former study, produced escape behaviour, although the latter finding was not confirmed by either Putkonen (1966) or Maley (1969) both of whom found that electrical stimulation elicited patterns of attack. However, as Putkonen (1967) points out, the difference may well lie in the interpretation of the reactions or in different environmental conditions during stimulation. The latter author, in the same report, describes fear-like responses from some septal (parolfactory) areas. It will be recalled that in Ch. 4 I present electrophysiological evidence of a pathway linking this region to the r.n. In general, I have shown r.n. links, in the pigeon, with what Putkonen (1967) describes as the axis for emotional reactions, of archistriatum - tractus occipito mesencephalicus - area septalis.

It is however clear that the role of the r.n. in this system is not simply that of controlling the motor manifestation of fear/escape/ avoidance emotions, for, as stated earlier, the lesioned animals were not apparently deficient in overt emotional responses at the time of behavioural testing although they did suffer a transient hyporeactivity during the first fourteen days following surgery. This point will be discussed later in this chapter.

The olfactory bulb connections with the r.n. reported in Ch. 4 may simply indicate a sensory input in addition to those reported from visual, auditory and somaesthetic sources. However, the olfactory bulb is included, at least in the mammal, in the limbic system which includes septal, hippocampal and amygdaloid regions, and may well also be the case in the avian species. Lesions of the olfactory bulb and olfactory nerve section in the pigeon have been carried out by Wenzel and Salzman (1968a) and Wenzel et al (1968b) who found that such lesions produced retardation in the birds' ability to learn a simple key pecking behaviour for food and that the deficit appeared to lie in the animals' poor orientation towards the food hopper and light. They also found that the heart rate response to the light did not habituate unlike that of the control birds. A recent publication by Thomas (1973) reports that olfactory bulb ablation in the rat produced a disruption of one way and a facilitation of two way active avoidance. This finding will be discussed later in this chapter.

The major problem of interpretation of the results of the behavioural tests remains that of linking the r.n. in a functional system, disruption of which would produce the observed phenomena, bearing in mind that the major caudal efferent tract, the rubrospinal, is involved in motor control.

Vanderwolf (1971) postulates that voluntary and involuntary behaviour are distinguishable by the structures involved in their initiation and performance in that the former depends on the activation of an ascending pathway from the diencephalon to the hippocampus and neocortex which is not necessary for the performance of automatic behaviours. He believes the pattern of motor activity to be determined by forebrain structures including the coupling of the relevant drive state, the overt behaviour resulting when this stored programme is activated by a trigger mechanism largely located in the diencephalon. He implicates the medial thalamus in the triggering mechanism on the basis of a number of experimental observations viz:- Following lesions of the medial thalamus, rats were unable to avoid shock in an avoidance situation although escape behaviour was relatively intact. The effect was apparent whether the lesions were made before or after training, or if they were tested in a one or two way avoidance situation. Furthermore the effect did not disappear with time, the animals still being unable to avoid as long as a year after surgery. In a very careful analysis of the phenomenon Vanderwolf concludes that "....the defect appears to lie on the initiation of movement, in dysfunction of a mechanism linking perceptual, mnemonic, and motivational systems to the voluntary motor system. There may be two distinct phases in higher level motor control. First, a programming mechanism must select from a large number of possible movements those which are appropriate in a given situation. The central representations of these movements must be maintained in a subthreshold state of excitation for some time in such a way that they can be activated in a particular order. Then, at an appropriate moment, a trigger mechanism fires off whatever motor activities have been programmed....."

In many ways the behaviour of the medial thalamic lesioned rats is similar to r.n. lesioned rats described by Thompson and his collaborators (see e.g. McNew, 1967b) and also bears a partial resemblance to that of the lesioned birds described in Chs. 2 and 7. All animals were initially incapable of feeding or drinking without assistance, were hypoactive, suffered from defects in muscular tone but recovered such that within 14-21 days they were indistinguishable from normal animals. All animals were deficient in tests of, or involving, avoidance of electric shock. The major difference between the effects of medial thalamic lesions and r.n. lesions is that the animals are capable of relearning the task in the latter case but not in the former. Medial thalamic lesions do not affect simple appetitive tests such as bar pressing for food or water and this is also true of r.n. lesions (Ellen et al. 1964). Unfortunately, the present author can find no report of a test of medial thalamic lesions on 'complex' discrimination tasks which, as has been shown, are affected by lesions of the r.n. in the pigeon (see Ch. 8) and in the cat (Myers, 1964).

In short, the effect of lesions to both areas is to produce, by and large, very similar behavioural symptoms except for the inability of medial thalamic lesioned animals to relearn an avoidance task. It is possible, if Vanderwolf's theory is correct, that the r.n. is an integral part of the system involved in the carrying out of learned motor acts and although no direct links have been shown to exist between it and the medial thalamus, indirect pathways may well exist perhaps by way of the rubral projection to the ventro-lateral thalamus (the efferent and afferent projections of much of the medial area of the thalamus are largely unknown (Grossman, 1967). The findings of Smith, (1970) in the rat, and myself, in the pigeon, that r.n. lesions effect an increase in the time necessary to initiate a conditioned response suggests that it (the r.n.) could form part of Vanderwolf's triggering mechanism.

The alternative hypothesis, offered by Thompson (1969) is that the r.n. or r.n. area is involved in ".... a highly localised cortico-subcortical system mediating visual discrimination habits in the rodent", along with the posterior thalamus and occipital cortex. Although the present author has shown, albeit in a different species, that Thompson's results may be largely attributable to the fact that his methodology includes an avoidance task, which as is shown in Ch. 6 is greatly disrupted by r.n. lesions, it may well be that the occipital cortex and posterior thalamus would prove to be involved in the performance of such behaviour, perhaps specifically where the task is coupled with a visual discrimination.

It will be recalled that it was shown in Ch. 4 that the avian r.n. is linked to the assumed homologues of the mammalian septum and amygdala. Both of these structures form part of the limbic system believed to be involved in the regulation of emotional behaviour (Grossman,1967). Lesions of the septal area in the mammal effects a variety of behavioural phenomena such as impairment of learned passive avoidance in which the animal must omit a response in order to avoid shock (McCleary,1961), either a facilitation of or impairment of two way active avoidance, according to whether the lesions are produced before or after initial training respectively (see Grossman,1967 for a review), but an impairment of one way avoidance behaviour whether the lesions are produced before or after initial training (Kenyon,1962, Rich and Thompson,1965).

Zucker (1965) cast some doubt on the latter result by suggesting that the impairment when lesions were produced before training is due to the animals' aversion to being handled in the one way situation and went on to show that where handling does not occur, septal lesions effect a facilitation of one way avoidance. In positive reinforcement situations septal lesions increased the number of perseverative errors in a discriminated operant task (Schwartzbaum et al. 1964) and also produced a deficit in a simple position habit by similar perseveration (McCleary, 1965). The latter author suggests that the perseveration may be due to the loss of a mechanism responsible for the inhibition of non-reinforced or punished responses. This would explain the various impairments resulting from septal lesions but can not explain a facilitation of initial training in active avoidance unless due to a hyperactivity arising from irritibility of tissue surrounding the lesioned area.

In addition to the Phillip's (1964) report of reduced avoidance following lesions of the supposed avian homologue of the amygdala, Horvath (1963) found that lesions of the amygdala in the rat, impaired the retention and acquisition of active avoidance, both one and two way, whilst Schwartzbaum et al. (1964) demonstrated no effect on either acquisition or retention of an auditory discrimination. By and large the major effect of amygdalectomy appears to be that of the Kluver-Bucy syndrome in which animals become tame, displaying little sign of fear or anger.

It would seem in general that septal, amygdaloid and r.n. lesions in the mammal effect similar impairments in retention of active avoidance and septal and r.n. lesions tend to produce impaired retention of habits acquired under positive reinforcement conditions (perhaps restricted to difficult discriminations). I have shown much the same effects in the pigeon following r.n. lesions and Phillips (1964) has demonstrated impairment of avoidance reactions ensuant upon lesions of the archistriatum, the assumed avian homologue of the amygdala. It is by no means wild speculation to predict that lesions of the parolfactory lobe (assumed equivalent of the mammalian septum) of the bird would also have an effect on avoidance behaviour. The suggestion is made that septum, amygdala, r.n. and probably other limbic structures including the olfactory bulb (lesion of which has recently been shown to produce similar effects on avoidance conditioning - Thomas,1973), are components of a mechanism involved in learned motor behaviour.

The physiological validity for linking these areas has been given, at least for the pigeon, in Ch.4 based on an extensive mapping of connections of the r.n.

Problems arise with the integration of some of the other projections outlined. The efferent link with the avian nucleus rotundus may be simply a further visual input, if the rotundus, as Karten (1969) believes, is exclusively visual. However as pointed out in Ch. 4, Baker-Cohen (1968) has criticised Karten's thesis on methodological grounds and the suspicion must remain that the nucleus rotundus may well have functions other than as a visual way station. Bearing this in mind, the visual discrimination deficit following lesions of the rotundus (Hodos and Karten,1966) may well represent a learning/memory deficit rather than a perceptual loss. This point is reinforced by the fact that the degree of retention deficit appears to be related to difficulty of discrimination, a fact reminiscent of r.n. lesions. It is not impossible that the nucleus rotundus is functionally comparable to areas of the mammalian thalamus. As has been described earlier, lesions of the mammalian medial thalamus (Vanderwolf,1971) and posterior thalamic lesions (Thompson,1969) (although as these structures are adjacent it is doubtful whether in either study,damage to both areas was avoided) produce impaired retention of avoidance behaviour and in the Thompson experiment, impaired retention of visual discrimination with the severity of the deficit being related to the difficulty of the discrimination. It would be interesting to determine the effects of lesions of the nucleus rotundus on previously established avoidance behaviour.

By way of conclusion, in the avian species, the r.n. appears to be involved in the performance of learned avoidance behaviour and difficult visual discriminations established under positive reinforcement. It is suggested that basal forebrain structures including septum and amygdala may be involved. The precise mode of action of the r.n. in the performance of certain learned behaviours is not known, although it has been tentatively hypothesised that it may form part of a triggering system of voluntary behaviour (it seeming only logical that the motor effects of the nucleus must play some part in the learning phenomena) along the lines suggested by Vanderwolf (1971). It is not impossible that the inputs from the major sensory modalities (see Ch. 5) coupled with feedback from muscle may be involved in the selection of behaviour perhaps by providing postural/spatial information necessary for the mnemonic coding of appropriate motor acts.

ADDENDUM

A very recent publication by Routtenberg and Holzman (July 1973) in which electrical stimulation of the substantia nigra in the rat, while they were learning a passive avoidance task, produced impaired retention, whereas stimulation of adjacent structures including, so they claim, the red nucleus, was without effect. Were this so, then some doubt would be cast upon the conclusions that I offered above. However, examination of their figures showing stimulation sites, clearly shows that only once was an electrode sited in the r.n. and even this was located at the anterior dorsal junction with the midbrain reticular formation. I would suggest that this is insufficient evidence for their suggestion of no effect on retention following r.n. stimulation. The fact that they obtain a disruption of retention following stimulation of the substantia nigra may in fact provide confirmatory evidence for the suggested role of the r.n. in the performance of learned behaviour, for the substantia nigra is closely linked to the r.n. (Carpenter and Stevens, 1957) see Fig. 2 Ch. 4.

BIBLIOGRAPHY

Akerman, B. Behavioural effects of electrical stimulation in the forebrain of the pigeon - 1 Reproductive behaviour. Behaviour 26, 328-338 (1966).

Akerman, B. Behavioural effects of electrical stimulation in the forebrain of the pigeon 11. Protective behaviour. Behaviour 26, 339-350 (1966).

Appelberg, C. and Molander, C.A. rubro olivary pathway - 1 Identification of a descending system for control of the dynamic sensitivity of muscle spindles. Exp. Brain Res. 3, 372 - 381 (1967a).

Appelberg, B. A rubro olivary pathway - 11 Simultaneous action on dynamic fusimotor neurones and the activity of the posterior lobe of the cerebellar cortex. Exp. Brain Res. 3, 382-390 (1967b).

Appelberg, B. and Jeneskog, T. A dorsolateral spinal pathway mediating information from the mesencephalon to dynamic fusimotor neurones. Acta Phys. Scand. 77, 159-171 (1969).

Ariens-Kappers, C.U., Huber, G.C. and Crosby, E.C. The comparative anatomy of the nervous system of vertebrates. Hafner, 1960.

Azrin, N.H. A technique for delivering shock to pigeons. J. Exp. Anal. Behav. 2, 161-163 (1959).

Baker-Cohen, K.F. Comparative enzyme histochemical observations on sub-mammalian brains: 1 Striatal structures in reptiles and birds. 11 Basal structures of the brainstem in reptiles and birds. Ergebnisse Anat. 40, 1-70 (1968).

Bierderman-Thorson, M. Auditory evoked responses in the cerebrum (Field L) and ovoid nucleus of the Ring Dove. Brain Res. 24, 235-45 (1970).

Brodal, A. and Gogstad, A.C. Rubro-cerebellar connections: An experimental study in the cat. Anat. Rec. 118, 455-486 (1954).

Cajal, S. Ramon Histologie du systeme nerveux de l'homme et des vertebrates. Quoted in Massion (1967).

Carpenter, M.B. A study of the red nucleus in the rhesus monkey. J. Comp. Neurol. 105, 195-249 (1956).

Carpenter, M.B. and Stevens, G.H. Structural and functional relationships between the deep cerebellar nuclei and brachium conjunctivum in the rhesus monkey. J. Comp. Neurol. 107, 109 -164, 1957.

Davson, H. The Eye. Vol. 3 - Muscular mechanisms Acad. Press, New York and London (1962).

Delgado, J.MR. Sequential behaviour induced by stimulation of the red nucleus in free monkeys. Science 148, 1361-1363 (1965).

Delius J.D. Some techniques for the electrical brain stimulation of small unrestrained animals. Med. and Biol. Engng. 4, 393-397 (1966).

Delius J.D. and Bennetto, K. Cutaneous sensory projections to the avian forebrain. Brain. Res. 37, 205-221 (1972).

Durkovic, R.G. and Cohen, D.H. Effects of rostral midbrain lesions on conditioning of heart and respiratory responses in pigeons. J. Comp. Physiol. Psychol. 68, 2, 184-192 (1969).

Eccles, J.C., Ito, M. and Szentagothai, J. The cerebellum as a neuronal machine. Springer-Verlag, 1967.

Edwards, A.L. Experimental design in psychological research. Holt, Rinehart and Winston (1965).

Ellen, P. Wilson, A.S. and Powell, E. Motivational changes in the rat following lesions of the Mesencephalic Reticular formation Exp. Neurol. 11, 334-340 1965.

Ermolaeva, V. and Chernigovsky, V.N. Induced potentials in the nucleus ruber and tractus tegmentalis of the cat in splanchnic nerve stimulation. Biull, E.K.S.P. Biol. med 60, 3-6 (1965).

Erulkar, S.D. Tactile and auditory areas in the brain of the pigeon. J. Comp. Neurol. 103, 421-457 (1955).

Evans, B.H. and Ingram, W.R. The effects of combined red nucleus and pyramidal lesions in cats. J. Comp. Neurol. 70, 461-476 (1939).

Fox, C.A. Hillman, D.E. Siegesmund A. and Sether, L.A. The primate globus pallidus and its feline and avian homologues. A golgi and electron microscopic study. In: Evolution of the forebrain (eds. Hassler and Stephan) Thieme, Stuttgart Germany (1965).

Fuse, G. Beitrage Zur Anatomie der Regiones rubra et oculomotoria bei den Saugetieren. Arb. Anat. Inst. Kaiserl. Japan. Univ. Sendai. 20: 115-121 (1937) (summarised in Carpenter M.B. A study of the red nucleus in the Rhesus monkey. J. Comp. Neurol. 105, 195-249 (1956).

Gellermann, L.W. Chance orders of alternating stimuli in visual discrimination learning. J. Genet. Psychol. 42, 206-208 (1933).

Glees, P. and Wall P.D. Fibre connections of the subthalamic region and the centro-median nucleus of the thalamus. Brain, 69, 195-208 (1946).

Gogolak, G. Liebeswar, G. and Stumpf C.H. Action of drugs in the electrical activity of the red nucleus. E.E.G. and Clin Neurophysiol. 27, 296-303 (1969).

Grofava, I. and Marsala, J. Nucleus ruber kocky Morfologie 9, 209-220. 1961 (summarised in Massion 1967).

Grossman, S.P. A textbook of physiological psychology. Wiley, New York. (1967).

Harman, A.L. and Phillips, R.E. Responses in the avian midbrain thalamus and forebrain evoked by click simuli. Exp. Neurol. 18, 3, 276-286 (1967).

Hatschek, R. Zur vergleichenden Anatomie des Nucleus ruber tegmenti. Arb. Neurol. Inst. Univ. Wien 15: 89-136, 1907 (summarised in Carpenter 1956).

Hilgard, E.R. and Marquis, D.G.M. Conditioning and learning. Methuen (1961).

*

Hoffman, H.S. and Fleshler, M. Aversive control with the pigeon. J. Exp. Anal. Behav. 2, 629-632 (1959).

Horvath, F.E. Effects of basolateral amygdalectomy on three types of avoidance behaviour. J. Comp. Physiol. Psychol. 56, 380-389 (1963).

Hough, H.B. and Wolff, H.G. The relative vascularity of subcortical ganglia of the cat's brain: the putamen, globus pallidus, substantia nigra, red nucleus and geniculate bodies. J. Comp. Neurol. 71, 427-436 (1939).

*

Hodos,W. and Karten, H.J. Brightness and pattern discrimination deficits in the pigeon after lesions of the nucleus rotundus. Exp.Brain Res. 2,151-167(1966)

Huber, G.C. and Crosby, E.C. The nuclei and fibre paths of the avian diencephalon with consideration of telencephalic and certain mesencephalic centres and connections. J. Comp. Neurol. 48, 1-225 (1929).

Ingram, W.R. Ranson, S.W. and Barris, R.W. The red nucleus, its relation to postural tonus and righting reactions. Arch. Neurol. Psychiat. 31, 768-786 (1934).

Karten, H.J. The organisation of the ascending auditory pathway in the pigeon: 1 Diencephalic projections of the inferior colliculus (nucleus mesencephali lateralis, pars dorsalis) Brain Res. 6, 409-527 (1967).

Karten, H.J. and Hodos, W. A stereotaxic atlas of the brain of the pigeon. John Hopkins Press, Baltimore (1967).

Karten, H.J. The organisation of the avian telencephalon and some speculations on the phylogeny of the amniote telencephalon. Ann. N.Y. Acad. Sci. 167, 164-179 (1969).

Karten, H.J. and Dubbeldam, J.L. The organisation of the pale-striatal complex in the pigeon. J. Comp. Neurol. 148, 61-90 (1973).

*1

Kenyon, J. The effects of septal lesions upon motivated behaviour in the cat. PhD dissertation McGill Univ. (cited in Grossman 1967).

*2

Kishida, T. Ota, H. Tsujurmura, K. Yasue, M. and Kato, V. Degenerescence du noyau rouge du lapin par l'extrait du 'Nejiki' C.R. Seanc. SOc. Biol. 154, 2411-2415 (1960).

---

[1] Karten, H.J. and Revzin, A.M. The afferent connections of the nucleus rotundus in the pigeon. Brain Res. 2, 368-377(1966)

[2] King, F.A., Achenbach, K.E. and Levine, S. A fully automated shuttle-box for avoidance conditioning. Psych.Rep. 9, 377-390(1961)

Kitsikis, A. and Rougeul, A. The effect of caudate stimulation on conditioned motor behaviour in mondays. Physiol and Behav. 3, 831-837 (1968).

Lawrence, D.G. and Kupers, H.G.J.M. The functional organisation of the motor system in the monday: 1 The effects of bilateral pyramidal lesions. Brain, 91, 1, 1-14 (1968).

Lawrence, D.G. and Kuypers, H.G.J.M. The functional organisation of the motor system in the monday: 11 The effect of lesions in the descending brain-stem pathways. Brain, 91, 1, 15-36 (1968).

Lewin, R.G. Dillard, G.V. and Porter, R.W. Extra pyramidal inhibition of the urinary bladder. Brain Res. 4, 301-307 (1967).

McLeary, R.A. Response specificity in the behavioural effects of limbic system lesions in the cat. J. Comp. Physiol. Psychol. 54, 605-613 (1961).

McManus, J.F.A. and Mowry, R.W. Staining methods. Hoeber International (1960).

MacNeilage, P.F. Motor control of serial ordering of speech. Psych. Rev. 77, 3, 182-196 (1970).

McNemar, Q. Psychological statistics. Wiley, New York (1962).

McNew, J.J. The role of the red nucleus in visually guided behaviour in the white rat. Unpublished doctoral thesis. Louisiana State University (1967).

McNew, J.J. Role of the red nucleus in visually guided behaviour in the rat. J. Comp. Physiol. Psych. 65,282-289 (1968).

McPhail, E.M. Avoidance responding in pigeons. J. Exp. Anal. Behav. 2, 629-632 (1968).

Maley, M.J. Electrical stimulation of agonistic behaviour in the Mallard. Behaviour 34, 3, 138-160 (1969).

Manni, E. Battista, G. Casey, H. and Dow, R.S. Influence of the labyrinth on unitary discharge of the oculomotor nucleus and some adjacent formations. Exp. Neurol. 12, 9-24 (1965).

Martin, G.F. Efferent tectal pathways of the opossum. J. Comp. Neurol. 135, 2, 209-224 (1969).

Massion, J. and Albe-Fessard, D. Dualite des voies sensorielles afferentes controlant l'activite du noyau rouge. E.E.G. and Clin. Neurophys. 15, 435-454(1963).

Massion, J. The mammalian red nucleus. Phys. Rev. 47, 3, 383-436 (1967).

Massion, J. and Urbano, A. Projections sur le noyau rouge par les colonnes dorsales. Arch. Ital. Biol. 106, 197-309 (1968).

Monnier, M. Functions of the nervous system - Vol. 1. Elsevier Amsterdam (1968).

Mussen, A.T. The cerebellum and red nucleus - Anatomy and Physiology. Hafner New York. (1967).

Myers, R.E. Visual deficits after lesions of the brain stem tegmentum in cats. Arch. Neurol. 11, 73-90 (1964).

Nakahama, H. Aikawa, S. and Nishioka, S. Somatic sensory properties of red nucleus neurones. Brain. Res. 12, 264-267 (1969).

Nishioka, S. and Nakahama, H. Peripheral somatic activation of neurones in the cat red nucleus. J. Neurophys. 34, 2, 296-307 (1973).

Olszewski, J. and Baxter, D. Cytoarchitecture of the human brain-stem. Karger, Bale (1954).

Otabe, J.S. and Horowitz, A. Morphology and cytoarchitecture of the red nucleus of the domestic pig. (Sus scrofa) J. Comp. Neurol. 138, 3, 373-390 (1970).

* Papez, J.W. and Stotler, W.A. Connections of the red nucleus. Arch. Neurol. Psychiat. 44, 776-791 (1940).

Parker, D.M. Electrophysiological and behavioural studies of vision in the pigeon. Unpub. PhD thesis Durham Univ. (1971).

Parker, D.M. and Delius, J.D. Visual evoked potentials in the forebrain of the pigeon. Exp. Brain Res. 14, 198-209 (1972).

Pearson, R. The Avian Brain. Academic Press (1972).

Phillips, R.E. Wildness in the mallard duck: effects of brain lesions and stimulation on escape behaviour and reproduction. J. Comp. Neurol. 122, 139-156 (1964).

Phillips, R.E. Approach withdrawal behaviour of peach faced lovebirds. Agapornis Roseicolis, and its modification by brain lesions. Behaviour, 31, 163-184 (1968).

Phillips, R.E. Evoked potential study of connections of avian archistriatum and caudal neostriatum. J. Comp. Neurol. 127, 1, 89-100 (1966).

*

Papaioannou,J.N. Rubral functions in the rat: a lesion study. Neuropsychologica 9,345-349(1971)

Pompeiano, O. Responses to electrical stimulation of the intermediate part of the cerebellar anterior lobe in the decerebrate cat. Arch. Ital. Biol. 96, 330-360 (1958).

Putkonen, P.T.S. Attack elicited by forebrain and hypothalamic stimulation in the chicken. Experientia, 22, 1-5 (1966).

Putkonen, P.T.S. Electrical stimulation of the avian brain. Annal. Acad. Scient. Fennicae. 130, 1-88 (1967).

Rachlin, H. and Hineline, P.N. Training and maintenance of keypecking in the pigeon by negative reinforcement. Science, 197, 954-955 (1967).

Rademaker, G.G. (1923, 1925 and 1926) cited in Massion (1967)

*1

Revzin, A.M. and Karten, H. Rostral projections of the optic tectum and nucleus rotundus in the pigeon.

*2

Brain Res. 3, 264-276 (1967).

Rich, I. and Thompson, R. Role of the hippocampo-septal system thalamus and hypothalamus in avoidance conditioning. J. Comp. Physiol. Psychol. 59, 66-72 (1965).

Rinvik, E. and Walberg, F. Demonstration of somato-topically arranged cortico-rubral projection in the cat. J. Comp. Neurol. 120, 393-408 (1963).

Rosvold, H.E. and Delgado, J.M.R. The effect on delayed alternation test performance of stimulating or destroying electrically structures within the frontal lobes of the monkeys brain. J. Comp. Physiol. Psychol. 49, 365-372 (1956).

---

*1

Rapoport,A.,Horvath,W.J.,Baldwin-Small,R.and Fox,S.S. The mammalian central nervous system as a network. Aerospace Medical Research publication AMRL-TR-67-187 (1968)

*2

Reynolds,R.W. An irritative hypothesis concerning the hypothalamic regulation of food intake. Psych.Rev.72,105-116(1

(1965)

Routtenberg, A. and Holzman, N. Memory disruption by electrical stimulation of substantia nigra, pars compacta. Science, 181, 83-85 (1973).

Sano, K. Yoshioka, M. Ogushiwa, M. Ishijuma, B. Ohye, C. Sekino, H. and Mayanagi, Y. Autonomic, somatomotor and EEG responses upon stimulation of the hypothalamus and the rostral brain stem in man. Confin. Neurol. 29, 257-261 (1967).

Sasaki, K. Namikawa, A, and Hashiramotor, S. The effect of midbrain stimulation upon alpha motoneurones in the lumbar spinal cord of the cat. Japan. J. Phys. 10, 303-316 (1960).

Schwartzbaum, J.S. Thopson, J.S. and Kellicutt, M.H. Auditory frequency discrimination and generalisation following lesion of the amygdaloid area in rats. J. Comp. Physiol. Psych. 57, 257-266 (1966).

Smith, A.M. Deficits in conditioned movement and visual discrimination following rubral area lesions in the rat. Physiol. and Behav. 5, 893-898 (1970).

Thomas, J.B. Some behavioural effects of olfactory bulb damage in the rat. J. Comp. Phys. Psych. 83, 1, 140-148 (1973).

Thayer, W.R. Poindezter, R. Winship, D. Spiro, H.M. and Delgado, J. Studies on the cerebral localization of gastric secretion in the mazaque. J. of Surg. Res. 6, 478-487 (1966).

Thompson, R. and Massopust, L.C. The effect of subcortical lesions on retention of a brightness discrimination in rats. J. Comp. Phys. Psych. 53, 488-496 (1960).

Thompson, R. Rich, I. and Langer, S.K. Lesion studies on the functional significance of the posterior thalamo-mesencephalic tract. J. Comp. Neurol. 123, 29-44 (1964).

Thompson, R. Lukaszewska, I. Schweigerdt, A. and McNew, J.J. Retention of visual and kinesthetic discrimination in rats following pretecto-diencephalic and ventral mesencephalic damage. J. Comp. Phys. Psych. 63,458-468 (1967).

Thompson, R. Localization of the visual memory system in the white rat. J. Comp. Phys. Psych. 69, 2, 1-29 (1969).

Thompson, R.F. Foundations of Physiological Psychology. Harper International New York (1967).

Tsukahara, N. and Kosaka, K. The mode of cerebral activation of red nucleus neurones. Experientia 22, 193-196 (1966).

Ursin, H. Sundberg, H. and Mensker, S. Habituation of the orienting response elicited by stimulation of the caudate nucleus in the cat. Neuropsychologica 7, 313-318 (1969).

Vanderwolf, C.H. Limbic-diencephalic mechanisms of voluntary movement. Psych. Rev.78, 2, 83-113 (1971)

Von Monskow, C. (1909) quoted in Carpenter (1956).

Wenzel, B.M. and Salzman, A. Olfactory bulb ablations or nerve section and behaviour of pigeons in non-olfactory learning. Exp. Neurol. 22, 472-479 (1968a).

Wenzel, B.M. Albruton, P.F. Salzman, A. and Oberjat, T.E. Behavioural changes in pigeons following olfactory nerve section or bulb ablation. Proc. 3rd. Int. Symp. on Olfaction and Taste (1968b).

Zeier, H. and Karten, H.J. The archistriatum of the pigeon: organisation of afferent and efferent connections. Brain Res. 31, 313-326 (1971).

Zucker, I. Effect of lesions of the septal-limbic area in the behaviour of rats. J. Comp. Phys. Psych. 60, 344-352 (1965).

APPENDIX 1

Table 8.5 has been expanded to include the errors to criterion, in initial learning, of the P.T.L. group, thus increasing the number of non-lesioned animals against which to compare group P.L.

Group	Brightness	Colour	Pattern
P.L.			
4	45	7	194
5	67	42	98
12	30	35	482
	mean 47	mean 28	mean 258
N.L.			
6	26	40	268
8	57	29	193
13	57	48	364
16	53	30	297
3	75	24	238
7	59	13	189
9	47	20	180
15	49	15	150
17	66	52	175
18	38	18	175
19	47	20	158
20	44	38	365
21	54	33	376
23	45	41	222
24	85	32	132
	mean 53	mean 30	mean 232

Differences between group P.L. and non-lesioned animals remain not significant on a 't' test $P > .05$ (two-tailed).

---

2. Additional 't' tests on the acquisition data given above disclose no significant difference between the means of the P.T.L. group and those of P.L. or N.L. ($P > .05$)

APPENDIX 2

The statistical significances given in Tables 7.2, 7.3 and 7.4 are based on a one-tailed 't' test. The reasons for so doing are in accordance with McNemar(1962) who states (p.62) ... ' Since the situation involving prediction is equivalent to taking .. the hypothesis that the difference between two population values is in a specified direction, it is not only defensible to use a one-tailed test but actually better in a sense that if there is a real difference in the predicted direction it will be more apt to be detected by a one-tailed than a two-tailed test.'

Concerning the data in Tables 7.2 and 7.3, it was predicted(based on the results of the Thompson 'school'), that lesions of the r.n. would produce a deficit in retention of avoidance behaviour and thus a one-tailed test was considered to be appropriate.(The results would however still be significant at a $P < .05$ level on a two-tailed 't' test)

Furthermore, it is suggested that the one-tailed test applied to the data in Table 7.4 is justified; firstly on the grounds(although not specifically mentioned) that it was obviously predicted that lesions of a nucleus known to be involved in motor control would produce a motor deficit which would be reflected in longer running times.(It would not be logical to predict an improved motor ability which would be necessary for the other tail of the distribution.) Secondly, a motor deficit hypothesis, requiring refutation, was set up as a possible explanation of the results. In fact, the use of the one-tailed test was favourable to the acceptance of the motor deficit hypothesis and thus contrary to the general explanation of the learning deficit advocated by the author.

APPENDIX 3

The appropriateness of 't' appears to be debatable. It seems that a Kruskal-Wallis one-way analysis of variance(McNemar,1962) is suitable in this situation.* The results obtained from the latter analysis are as follows:-

Brightness	$\chi^2 =$	1.6	d.f. 2	Not significant
Colour	" "	3.44	"	Not significant
Pattern	" "	11.18	"	$P < .01$

The results of this analysis in no way alter the development of the argument in the text apart from the improvement in significance level of the deficit on the pattern task.

* My appreciation is offered to Drs G.R.J. Hockey and D.C. Howell for their advice on this matter.

APPENDIX 4

Technical Data

1. Schenvar 31 lacquer is obtainable from:-
Schenectady Varnish Co.
Four Ashes,
Wolverhampton.

2. Electrode insulation - following each coat of lacquer, the electrode was 'baked' in a thermostatically controlled oven at 130°C for five hours.

3. Electric shock was delivered by a Grason Stadler shock generator, model E1064GS.

4. R.F.(radio frequency) parameters used in the lesion experiments described in Chs. 2.7 and 8:-

   approx 20-30ma, 100kHz, duration- 15secs. for lesions described in Ch.2 and 10secs. for those in Chs. 7and 8.

5. Stimulation arrangement consisted of a Grass S4 stimulator with stimulation isolation unit SI4 feeding through a 100KΩ resistor(giving approximate current constancy) and a 1KΩ resistor in series with the electrodes implanted in the subjects(impedance approx. 6KΩ). The current was monitored by displaying the voltage drop across the 1KΩ resistor through the differential input of a 502 Tektronix oscilloscope.